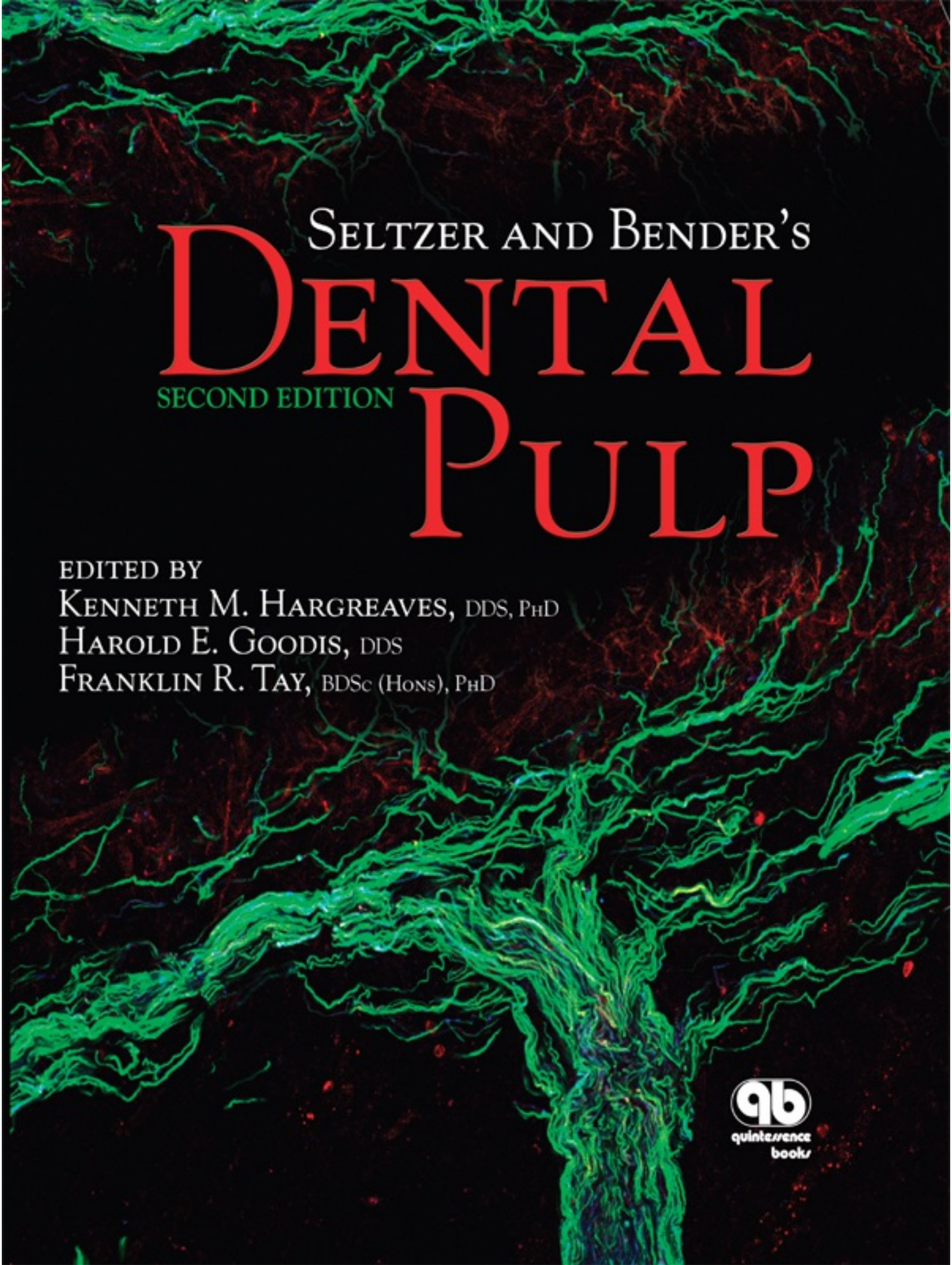
Seltzer and Bender's
Dental Pulp
SECOND EDITION
EDITED BY
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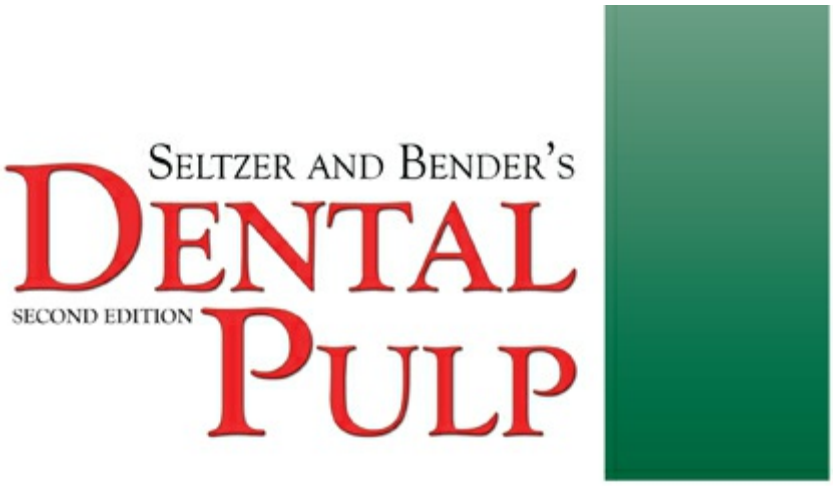
Seltzer and Bender's Dental Pulp, *Second Edition*

Cover image illustrating the innervation of normal human dental pulp using antibodies for neurons (N52, green; PGP, blue) and the receptor TRPA1 (red). Image courtesy of Michael A. Henry, DDS, PhD.



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Preface

Welcome to the second edition of *Seltzer and Bender's Dental Pulp*. Like the first edition, this book focuses on the dental pulp and its interaction with other tissues during health and disease, with each chapter providing the latest information on the biologic principles and the basis for clinical treatment procedures. As such, the book is ideally suited for practicing dentists as well as residents and dental students. This newly revised second edition includes entirely new topics (eg, regenerative endodontics) as well as greatly expanded reviews on dental implications of biofilms, immune interactions, pain mechanisms, the interactions between restorative dental procedures and pulpal health, and neuroanatomy, among other topics. We welcome many new and returning authors to this edition who have shared their incredible expertise with you, our reader.

The central theme of this book—a fundamental theme of dentistry in our opinion—is the critical role that pulp tissue plays in dental health. Both local (eg, caries, periodontitis) and systemic (AIDS, hyperparathyroidism) disease can contribute to pulpal pathosis. In turn, pulpal pathosis can contribute to both local (eg, root resorption, periodontitis) and systemic (eg, referred pain) conditions. The astute clinician needs this information to provide accurate diagnoses and effective treatment. Accordingly, we have focused on the biology of dental pulp and its interaction with other tissues during health and disease in order to provide comprehensive, biologically based clinical recommendations for practicing dentists.

We have been gratified by the support and encouragement generated from the first edition of this text, and we were thrilled that both I. B. Bender and Sam Seltzer lived to enjoy its publication. We have now lost many of the pioneering giants of endodontics and pulp biology. Their early contributions laid the foundation for generations of dentists to deliver biologically based dental care. In this age of gene arrays, signal transduction pathways, novel restorative materials, and computerized data retrieval, it is difficult to appreciate the magnitude of their contributions based entirely upon intellectual rigor and using relatively simple tools. To their memories, we dedicate this second edition.

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Development of the Pulpodentin Complex

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Dentin is a unique, avascular mineralized connective tissue that forms the bulk of the tooth. It underlies enamel in the crown and cementum in the roots, providing these tissues structural support and the tooth its resilience. In a mature tooth, dentin encloses a richly innervated and highly vascularized soft connective tissue, the dental pulp. Dentin and pulp are derived from the dental papilla, whose cells migrate to the first branchial arch from within the ectomesenchyme of the cranial neural crest. The tissues remain closely associated during development and throughout the life of an adult tooth and are hence most commonly referred to as the *pulpodentin complex*. It is this biologic intimacy that dictates the response of the pulpodentin complex to physiologic and pathologic stimuli.

Because the practice of endodontics involves manipulation of both the dentin and pulp, learning about the mechanisms that lead to their formation is crucial and will create a better understanding of the response to and treatment of pulpal injuries. The purpose of this chapter is to provide a background for the succeeding chapters that discuss the biology of the mature pulpodentin complex during health and disease and

in aging. The chapter has two goals: The first goal is to review classic and current knowledge of the events in tooth development that lead to odontoblast differentiation and to convey the excitement of this flourishing field. Attention is focused on the common themes that have emerged and what is known about the influence of tooth-signaling molecules and transcription factors on the development and homeostasis of the pulpodentin complex. The second goal of this chapter is to describe the general principles of dentin matrix formation, particularly the synthesis and secretion of extracellular matrix molecules and their postulated roles in the biomineralization of dentin. Examples of how basic information about the normal biology of the pulpodentin complex can be applied toward solving clinical problems are integrated throughout the chapter. The overarching goal is to emphasize how fundamental theories about development and homeostasis of differentiated and undifferentiated or stem cell populations can be translated to regenerative approaches targeted at restoring the integrity of the adult pulpodentin complex.

Tooth Development (Odontogenesis)

For several decades, the developing tooth organ has served as a valuable paradigm to study the fundamental processes involved in organogenesis. These processes are *(1)* the determination of position, through which the precise site of tooth initiation is established; *(2)* the determination of form or morphogenesis, through which the size and shape of the tooth organ are set; and *(3)* cell differentiation, through which organ-specific tissues are formed by defined cell populations, each with unique properties. The dental literature is enriched with excellent reviews on tooth development, and the reader is encouraged to study the topic in further detail.[1–4]

General features

Although the tooth is a unique organ, the principles that guide its development are shared in common with other organs such as the lung, kidney, heart, mammary glands, and hair follicles.[5,6] The most important among developmental events are those guiding epithelial-mesenchymal interactions, which involve a molecular

crosstalk between the ectoderm and mesenchyme, two tissues that have different origins. Although only vertebrates have teeth, their development involves genetic pathways that are also active in invertebrates. This conservation of a "molecular toolbox" for organogenesis throughout evolution proves that certain master regulatory molecules are critical to all tissue interactions during development. New studies have also shown that tooth-signaling molecules are repeatedly used at various stages of development. Both tooth morphogenesis and cell differentiation occur as a result of sequential interactions. Hence, it is not one biologic event involving a single molecule but a series of interactions involving several molecules that leads to the development of the pulpodentin complex.[3,4]

Importantly, signaling is reciprocal, whereby an exchange of information occurs in both directions, from dental epithelium to mesenchyme and from dental mesenchyme to epithelium. For example, in experiments where dental epithelium was separated from mesenchyme, cusp patterning failed to occur. Similarly, in the absence of dental epithelium, odontoblasts are unable to differentiate from dental mesenchyme.[7–9]

It is only logical to apply these basic developmental principles to the current understanding of how the adult or mature pulpodentin complex responds to injury and repair. The latter clearly involves a series of molecules that operate in concert to dictate the outcome of pulpal disease and therapies. In the adult situation, whether certain cells and molecules can mimic the inductive influence of dental epithelium during development has yet to be definitively proven and remains a subject of interest in pulpal biology research.

Stages of tooth development

Teeth develop in distinct stages that are easily recognizable at the microscopic level. These stages in odontogenesis, described by the histologic appearance of the tooth organ, are termed, from early to late, the *lamina*, *bud*, *cap*, and *bell* (*early* and *late*) stages of tooth development.[10–12] Although the following descriptions use these common terms, the modern literature uses functional terminology to describe odontogenesis in four phases: *(1) initiation*, *(2) morphogenesis*, *(3) cell differentiation* o r *cytodifferentiation*, and *(4) matrix apposition* (Fig 1-1). The photomicrographs in Fig 1-2 depict the morphologic stages of tooth development.

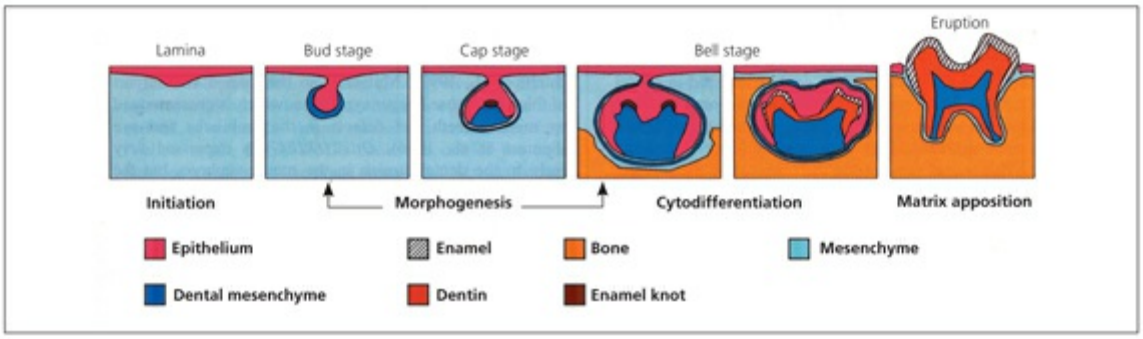

Fig 1-1 Stages of tooth development. Note the sequential transformation from the dental lamina to a distinctly shaped dental organ. The transient appearance of the enamel knot in the region of the forming cusp tips precedes the terminal differentiation of cells and the formation of specialized matrices. (Reprinted from Thesleff and Sharpe[12] with permission.)

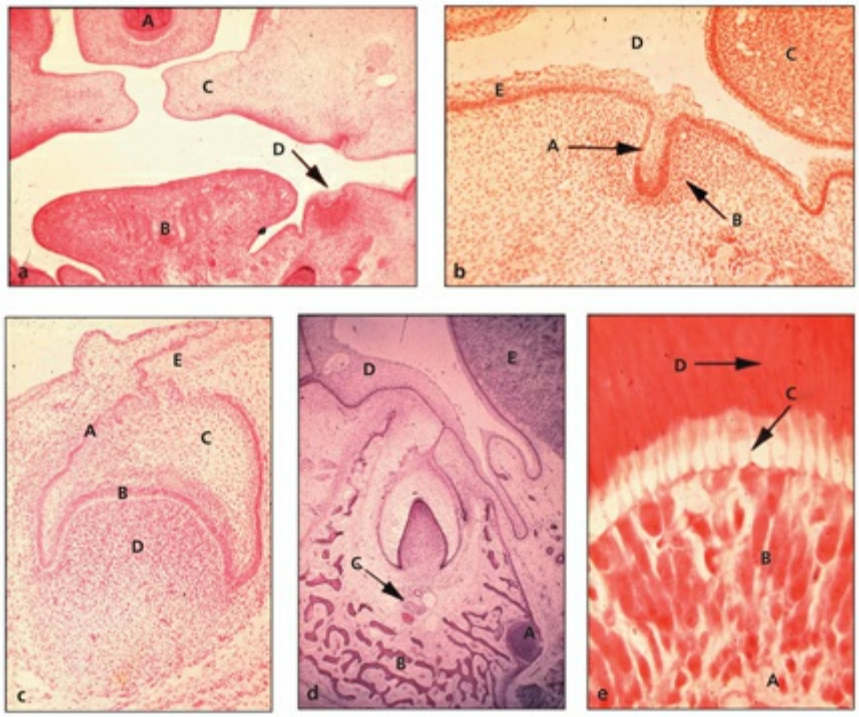

Fig 1-2 Histologic survey of odontogenesis in a pig embryo. (Courtesy of the University of Texas Health Science Center at Houston, Dental Branch.) *(a)* Lamina stage: A, nasal septum; B, tongue; C, palatal shelves; D, dental lamina (hematoxylin-eosin [H&E] stain; original magnification ×4). *(b)* Bud stage: A, ectodermal outgrowth; B, dental mesenchyme; C, tongue; D, oral cavity space; E, oral ectoderm (H&E stain; original magnification ×10). *(c)* Cap stage or transition to early bell stage: A, outer dental epithelium; B, internal dental epithelium; C, stellate reticulum; D, dental papilla ectomesenchyme; E, dental lamina (H&E stain; original magnification ×10). *(d)* Late bell stage: A, nerve bundle; B, alveolar bone; C, vas-culature; D, oral ectoderm; E, tongue. Note the extension of the dental lamina on the right aspect of the dental organ that will form the succeda-neous incisor (H&E stain; original magnification ×10). *(e)* Onset of dentinogenesis: A, dental pulp; B, cluster of odontoblasts that appear crowded at the tip; C, odontoblast process; D, dentin (H&E stain; original magnification ×20).

Lamina stage

The dental lamina is the first morphologic sign of tooth development and is visible around 5 weeks of human development and at embryonic day 11 (E11) in mouse gestation. This thickening of the oral epithelium lining the frontonasal, maxillary, and mandibular arches occurs only at sites where tooth organs will develop. At the lamina stage, cells in the dental epithelium and underlying ectomesenchyme are dividing at different rates, more rapidly in the latter. As explained later, the dental lamina has the full potential to induce tooth formation by dictating the fate of the underlying ectomesenchyme.[13]

Bud stage

As the dental lamina continues to grow and thicken to form a bud, cells of the ectomesenchyme proliferate and condense to form the dental papilla. At this stage, the inductive or tooth-forming potential is transferred from the dental epithelium to the dental papilla.

Cap stage

At this stage, the tooth bud assumes the shape of a cap that is surrounded by the dental papilla. The ectodermal compartment of the tooth organ is referred to as the *dental* or *enamel organ*. The enamel organ and dental papilla become encapsulated by another layer of mesenchymal cells, called the *dental follicle*, that separates the tooth organ papilla from the other connective tissues of the jaws.

The transition from the bud to the cap stage is an important step in tooth development because it marks the onset of crown formation. Recent studies have pointed to the role of the enamel knot as an important organizing center that initiates cuspal patterning.[14,15] Formally described as a transient structure with no ascribed functions, the enamel knot is formed by the only cells within the central region of the dental organ that fail to grow. As described later, the enamel knot expresses a unique set of signaling molecules that influence the shape of the crown as well as the development of the dental papilla. Similar to the fate of signaling centers in other organizing tissues, such as the developing limb bud, the enamel knot undergoes programmed cell death, or *apoptosis*, after cuspal patterning is completed at the onset of the early bell stage. In incisors, the enamel knot initiates the first folding of dental epithelium. Secondary enamel knots determine the site of new cusps in molars.

Early bell stage

The dental organ assumes the shape of a bell as cells continue to divide but at different rates. A single layer of cuboidal cells called the *external* or *outer dental epithelium* lines the periphery of the dental organ, while cells that border the dental papilla and are columnar in appearance form the *internal* or *inner dental epithelium*. The latter gives rise to the *ameloblasts*, cells responsible for enamel formation. Cells located in the center of the dental organ produce high levels of glycosaminoglycans that are able to sequester fluids as well as growth factors that lead to its expansion. This network of star-shaped cells is named the *stellate reticulum*. Interposed between the stellate reticulum and the internal dental epithelium is a narrow layer of flattened cells, termed the *stratum intermedium*, that express high levels of alkaline phosphatase. The stratum intermedium is believed to influence the biomineralization of enamel. In the region of the apical end of the tooth organ, the internal and external dental epithelial layers meet at a junction called the *cervical loop*.[16–18]

At the early bell stage, each layer of the dental organ has assumed special functions. The reciprocal exchange of molecular information between the dental organ and dental papilla influences the important events that lead to cell differentiation at the late bell stage.

Late bell stage

The dental lamina that connects the tooth organ to the oral epithelium gradually disintegrates at the late bell stage. The cells of the internal dental epithelium continue to divide at different rates to determine the precise shape of the crown. Shortly after, cells of the internal dental epithelium at the sites of the future cusp tips stop dividing and assume a columnar shape. The most peripheral cells of the dental papilla enlarge and become organized along the basement membrane at the tooth's epithelial-mesenchymal interface. These newly differentiated cells are called *odontoblasts*, cells that are responsible for the synthesis and secretion of dentin matrix. At this time, the dental papilla is termed the *dental pulp*.

After odontoblasts deposit the first layer of predentin matrix, cells of the internal dental epithelium receive their signal to differentiate further into ameloblasts, or enamel-producing cells. As enamel is deposited over dentin matrix, ameloblasts retreat to the external surface of the crown and are believed to undergo programmed cell death. In contrast, odontoblasts line the inner surface of dentin and remain metabolically active throughout the life of a tooth.

In summary, development of the tooth rudiment from the lamina to the late bell stages culminates in the formation of the tooth crown. As root formation proceeds, epithelial cells from the cervical loop proliferate apically and influence the differentiation of odontoblasts from the dental papilla as well as cementoblasts from follicle mesenchyme, leading to the deposition of root dentin and cementum, respectively. The dental follicle that gives rise to components of the periodontium, namely the periodontal ligament fibroblasts, the alveolar bone of the tooth socket, and the cementum, also plays a role during tooth eruption, which marks the end phase of odontogenesis.

Experimental Systems

In the last decade, basic understanding of the molecules that control the events that lead to odontoblast differentiation and the formation of dental pulp has advanced significantly.[19] Most of the contemporary experimental approaches used in these studies have taken advantage of the mouse model because of its availability and ease of accessibility. The development of dentition in mice closely parallels that in humans. Mice are the predominant system for genetic engineering approaches that have generated a volume of exciting data on tooth development.

Before the families of signaling molecules are discussed, it is important to understand modern experimental approaches and key techniques that are available for use in studies on tooth development. The intention is to provide a simple description of these modern scientific tools as the basic framework of reference for the dental student or endodontic resident interested in pursuing research in the area of pulpal biology.

Tooth organ culture systems

Over the years, researchers have utilized various approaches to study and manipulate developing tooth organs.[20–22] In vitro systems include whole mandibular and maxillary explants as well as individually dissected molar organs that can be cultured in enriched serum by means of a Trowell-type system. The system involves

placing the tooth organ in the correct orientation on a filter that is supported by a metal grid at the gas-liquid interface within a culture well[23] (Fig 1-3).

Another in vitro approach is the use of functional tooth organ recombination assays. Dental epithelium is separated from papilla mesenchyme using enzymes that degrade the basement membrane at the interface.[24,25] Isolated epithelium and mesenchyme can be cultured separately or recombined and then transplanted in vivo to study the effects on tooth development. Modifications of this approach include heterotypic recombinant cultures of epithelium and mesenchyme, each derived from a different organ system, and heterochronic recombinations where the tissues used are from the same organ system but at different stages in development[26] (Fig 1-4). Researchers interested in studying the effects of various molecules add these reagents in soluble form to the culture and then transplant the treated culture to the anterior chamber of the eye or the subcapsular region of the kidney in mice.[27] Overall, in vivo tooth organ explants that are cultured at in vivo ectopic sites advance farther than in vitro systems (Fig 1-5). In vivo culture systems are also better suited for tooth organ dissections and recombinations that are performed at early stages of development.

The availability of mice strains with spontaneous mutations and genetically engineered knockout mice has further refined the use of the bead implantation assay.[30,31] When the reactions of mutant dental mesenchyme and wild-type (normal) mesenchyme are compared, it is possible to determine whether a certain molecule is needed for the expression of a second gene. This approach has led to new information about the relationships of tooth-signaling molecules within a genetic pathway.[4,32]

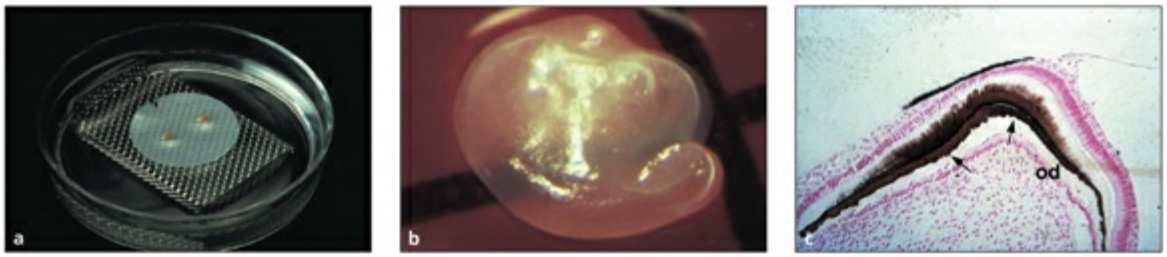

Fig 1-3 Tooth organ culture system. (Courtesy of Dr Richard Finkelman.) *(a)* Trowell method showing two molar organs at the early cap stage placed on a filter on top of a metal grid within a culture dish. *(b)* Molar organ after 12 days in culture. Note the formation of distinct cusps. *(c)* Histologic view of Fig 1-3b shows fully differentiated odontoblasts (od) and a layer of mineralized dentin *(arrows)* (Von Kossa stain; original magnification ×10).

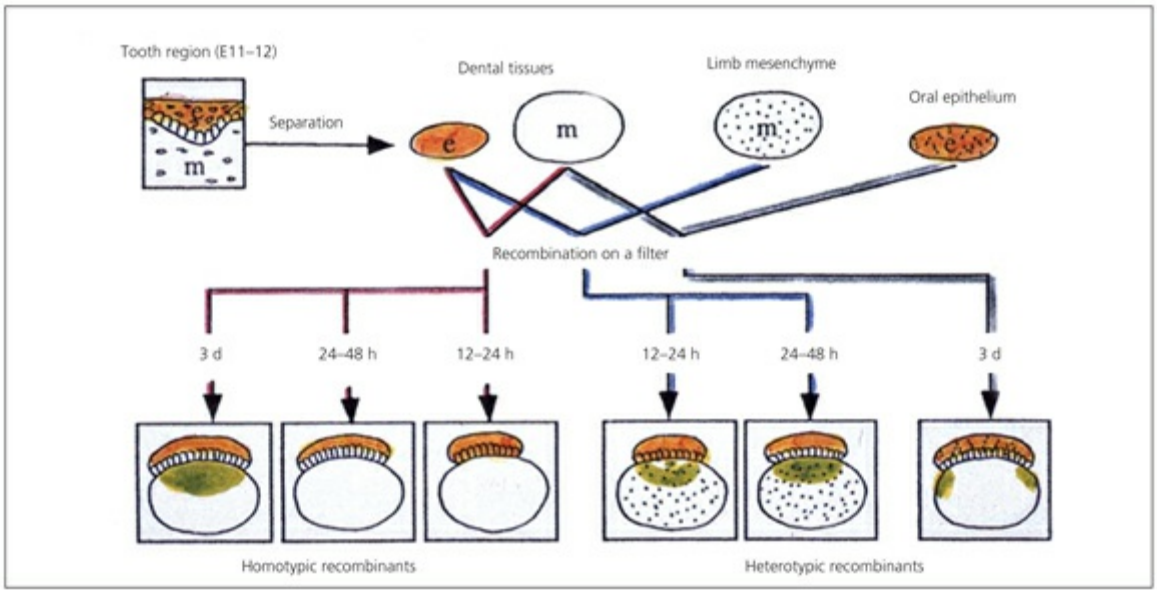

Fig 1-4 Strategy used for homotypic and heterotypic recombination assays. E11–12, days 11 to 12 of mouse embryonic development; e, epithelium; m, mesenchyme; h, hours in culture; d, days in culture. (Reprinted from Mitsiadis et al[26] with permission.)

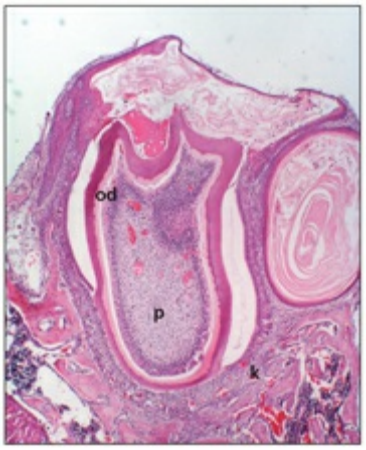

Fig 1-5 Microscopic view of a fully formed tooth that developed from the early cap stage after placement beneath the renal capsule. od, odontoblasts; p, dental pulp; k, kidney (H&E stain; original magnification ×4).

An elegant experimental approach that has yielded important information on the nature of the signaling interactions between tooth epithelium and mesenchyme is the use of bead implantation assays.[28] Briefly, either heparin or agarose beads that are soaked in a known concentration of a growth factor are placed on separated dental mesenchyme. After approximately 24 hours in culture, the mesenchyme is analyzed for changes in gene or protein expression in the region surrounding the bead[29] (Fig 1-6).

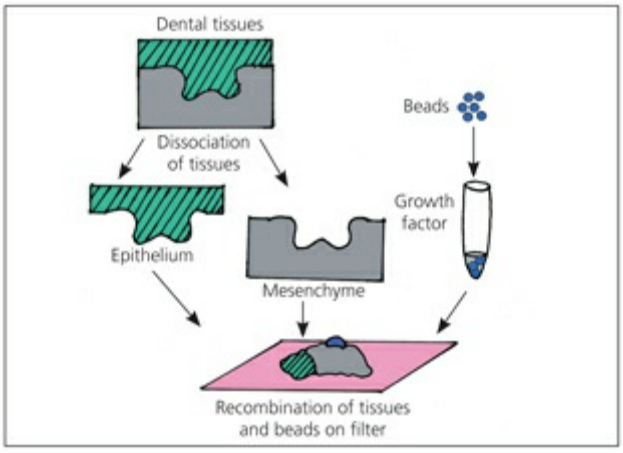

Fig 1-6 Principles of tooth tissue recombination and bead assays. (Modified from Thesleff and Sahlberg[29] with permission.)

Odontoblast and dental pulp cell lines

While tooth organ cultures have facilitated elegant studies of early tooth development, the recent availability of odontoblast and pulp cell culture systems have made it possible to study late events that involve cell differentiation and matrix synthesis. An interesting approach is to utilize hemisectioned human teeth from which dental pulp has been carefully extirpated. The remaining layer of intact odontoblasts can then be cultured within the native pulp chamber, to which nutrient media and various growth factors or cytokines are added. Thick slices of human teeth with the odontoblastic layer left intact offer another useful approach to study the behavior of odontoblasts under conditions that simulate dental caries.[33,34] The use of primary odontoblast cultures has been limited because intact cells are difficult to isolate in sufficient numbers and become phenotypically altered after several passages in culture. The recent development of cell-immortalization procedures has made it possible to generate two established odontoblast-like cell lines. The M06-G3 cell line was derived from an established murine odontoblast monolayer cell culture system that was infected with a temperature-sensitive simian virus 40 (SV40).[35] MDPC-23 cells are a spontaneously immortalized cell line derived from mouse embryonic dental papilla that expresses dentin matrix proteins.[36] Dental pulp clones, the RPC-C2A and RDP 4-1 cell lines, which exhibit characteristics ranging from pulpal fibroblasts to preodontoblasts, are also available.[37,38]

Tooth-derived stem cell lines

Central to the development of new regenerative strategies involving tissue engineering is the use of *stem cells*. These cells are classically defined as clonogenic and self-renewing cells that have the capacity to differentiate into multiple cell lineages. The two major types are embryonic stem cells and postnatal stem cells. Embryonic stem cells are obtained from embryos in the blastocyst stage of development. At this stage, these stem cells are pluripotent with the ability to differentiate into any cell type. In contrast, postnatal stem cells reside within niches in adult organs and retain the capacity to differentiate into a limited number of cell types.

Recently, stem cell characteristics were detected in cells isolated from primary[39] as well as permanent teeth,[40,41] periodontal ligament,[42,43] periapical follicle, and apical papilla mesenchyme.[43–45] Mesenchymal stem cells, which represent less than 10% of the total cell population, can be tagged with stem cell markers such as STRO-1 or CD 146 in fluorescence-activated cell-sorting analysis.[39] Stem cells isolated from dental tissues are capable of differentiating into adipocytes, neurons, and odontoblast-like cells.[39]

They form mineralized nodules in vitro[43] and are capable of creating boneor pulpodentin-like complexes after transplantation into immunocompromised mice.[39,41] These cells appear to reside in a perivascular niche and are likely to represent the population of cells that were shown by Fitzgerald et al[46,47] to migrate to the site of dentin injury to form reparative dentin. Tooth-derived stem cells are essential for the development and application of regenerative approaches to treat an injured pulpodentin complex (see chapter 5).

Figure 1-7 depicts the activity of two tooth-derived cell lines: stem cells from human exfoliated deciduous teeth (SHED) and periodontal ligament–derived stem cells (PDLSC). When seeded in a fibrin hydrogel system, both cell lines are capable of proliferation and further differentiation, as indicated by the deposition of a collagen-enriched matrix.

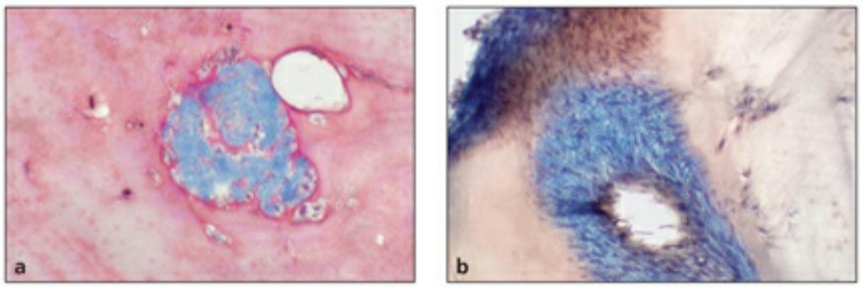

Fig 1-7 Osteogenic induction of stem cells increases the production of extracellular matrix such as collagen *(blue staining)*. *(a)* SHED cells in a fibrin patch, treated with bone morphogenetic protein 4 (BMP-4) (Masson trichrome stain; original magnification ×60). *(b)* PDLSCs (cells provided by Dr Songtao Shi) in fibrin patch, treated with BMP-4 (Masson trichrome stain; original magnification ×30).

Transgenic and knockout mice

The modern era of recombinant DNA technology and genetic engineering has made it possible to alter or mutate a gene of interest in vitro and then inject it into the pronucleus of a fertilized mouse egg.[48,49] The transgene, if successfully integrated into the host genome, can be transmitted through the germ line to the progeny. Transgenic and knockout mice thus offer a powerful means to study the role of molecules in their natural in vivo environment.

Transgenic mice generated using conventional technology can be designed to overexpress the gene of interest in cells or tissues where it is normally expressed[50] (Fig 1-8). When it is desirable to study the behavior of a gene at an ectopic site, the transgene of interest is placed behind the promoter of another gene that will drive expression in tissues where it is not normally expressed. In the case of a gene that is expressed in multiple tissues or organs, it is now possible to study activity at one particular site. This is achieved by driving the expression of the transgene using a tissue- or cell-specific promoter.

The following example illustrates the usefulness of a tissue-specific transgenic mouse model. As a means of assessing the precise role of transforming growth factor β1 (TGF-β1) in odontoblast differentiation, transgenic mice that overexpressed active TGF-β1 were generated. Because TGF-β1 is also highly expressed in bone and other tissues, TGF-β1 over-expression was restricted to odontoblasts alone by using the promoter for dentin sialophosphoprotein *(Dspp)*, a gene that is highly tooth specific. TGF-β1 overexpressors have defects in dentin that closely resemble dentinogenesis imperfecta (DI), an inherited disorder of dentin. This model

permitted the analysis of the direct role of TGF-β1 in dentin formation without confounding its effect in bone and other tissues.[51] The results indicated an important role for the growth factor in dentin formation, a subject that is discussed in further detail in chapter 2.

Knockout technology is used to generate lines of mice that lack a functional gene of interest throughout their life span. The targeted deletion of the gene is performed in embryonic stem cells that are derived from the inner cell mass of an early embryo. After these cells are cultured in a petri dish, to select for the desired deletion, they are implanted in the cavity of a fertilized egg to generate a percentage of offspring that inherit the mutation. Knockout mice offer a powerful means to assess the biologic roles of a molecule in the context of a normal mouse. Certain knockout strains appear completely unaffected, indicating that the functions of the gene that are eliminated in vivo can be shared by other genes within the family, a phenomenon of biologic (functional) redundancy.

Several knockout mice strains die during gestation or shortly after birth, indicating the importance of these genes in developmental processes. As a result, these mice have not proven to be informative about the role of the gene later in postnatal and adult life. Examples of this include the bone morphogenetic proteins 2 (*Bmp2*) and 4 (*Bmp4*) gene knockout mice that undergo embryonic lethality during early gestation. This problem is overcome by the use of conditional knockout technology, where the inactivation of the gene occurs at a specific time and location.

Of relevance to pulpal biology is the phenotype of the *Tgfβ1*-mutant mouse. Teeth develop fully in $Tgf\beta1^{-/-}$ mice and show no pathologic conditions at birth or through the first 7 to 10 days of postnatal life compared to those of $Tgf\beta1^{+/-}$ or $Tgf\beta1^{+/+}$ littermates. By the end of the second week, $Tgf\beta1^{-/-}$ mice develop a rapid wasting syndrome that is characterized by multifocal inflammatory lesions with dense infiltration of lymphocytes and macrophages in major organs such as the heart and lungs; these eventually lead to death by the third week of life. These observations support a vast volume of research documenting a critical role for TGF-β1 as a potent immunomodulator.

To study the state of the adult dentition in $Tgf\text{-}\beta1^{-/-}$ mice, their survival was prolonged with dexamethasone treatment. The absence of a functional *Tgfβ1* gene resulted in significant destruction of pulp and periradicular tissues as well as the hard tissues of the crown[52] (Fig 1-9). These data prove that no other TGF-β family members can substitute for the loss of TGF-β1. Clearly, the dual role of this growth factor as a key modulator of pulpal inflammation and its potent effects on

extracellular matrix (ECM) production are significant. Chapter 2 discusses in further detail the role of TGF-β1 in reparative dentinogenesis.

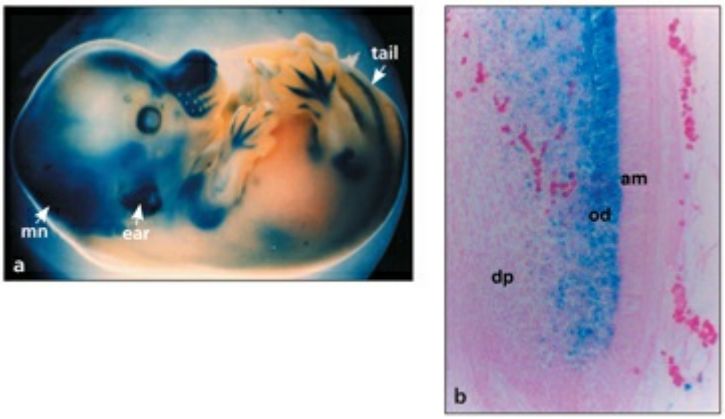

Fig 1-8 *(a)* Whole-mount view of a developing transgenic mouse embryo in which expression of the *LacZ* reporter gene *(blue staining)* is driven by a type I collagen promoter. Expression of ß-galactosidase is seen in all areas of the embryo that express type I collagen. mn, meninges (ß-gal stain). (Reprinted from Niederreither et al[50] with permission.) *(b)* Section through the developing incisor at the neonatal stage showing activity of the transgene in differentiating odontoblasts (od) and some cells of the dental pulp (dp). Note the complete absence of staining for type I collagen in ameloblasts (am) (H&E stain; original magnification ×10).

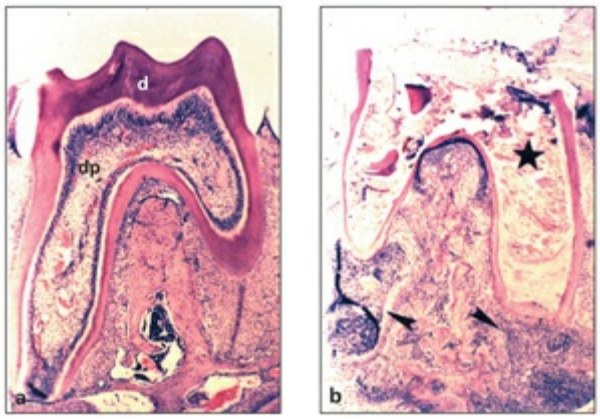

Fig 1-9 *(a)* Normal mandibular first molar in a *Tgfβ1*$^{+/+}$ mouse at 50 days of postnatal life. d, dentin; dp, dental pulp (H&E stain; original magnification ×10). (Reprinted from D'Souza et al[52] with permission.) *(b)* In a *Tgfβ1*$^{-/-}$ animal whose survival was prolonged with dexamethasone, there is extensive damage to the hard and soft tissues of the molar. *arrowheads*, periapical inflammatory infiltrates; *star*, calcification within the pulp chambers and canals (H&E stain, original magnification ×10). (Reprinted from D'Souza et al[52] with permission.)

Laser capture microdissection

Among the new in vivo approaches available to analyze the behavior of cells under normal and diseased conditions, laser capture technology stands out as being most innovative. Laser capture microdissection (LCM) was developed and applied in cancer biology to detect a mutant protein or gene in a single malignant cell[53] and to monitor in vivo differential gene expression levels in normal and malignant breast cell populations.[54] LCM is being used in the Cancer Genome Anatomy Project to catalog the genes that are expressed during solid tumor progression and to construct complementary DNA (cDNA) libraries from normal and premalignant cell populations. Microarray panels[55] containing these index genes are being used to obtain gene-expression patterns in human tissue biopsies. The fluctuation of expressed genes that correlate with a particular stage of disease is compared within or between individual patients. Such a fingerprint of gene-expression patterns will provide important clues about etiology and contribute to diagnostic decisions and therapy.

Applications in this area will be particularly useful for oral tissues, such as dental pulp, oral mucosa, and periodontal ligament, where individual cell populations are difficult to access. The progress in understanding odontoblast differentiation has been slow because of serious limitations inherent to both in vivo and in vitro approaches. Pure populations of differentiating or mature odontoblasts are technically difficult to obtain from heterogenous dental papilla and pulp. Furthermore, immortalized odontoblast-like cell lines fail to fully reflect the molecular events that occur in the complex milieu of the tooth organ from which they are derived. The terminology used to describe odontoblast differentiation is sketchy because it is unclear how morphologic change is reflected at the molecular cytogenetic level.

Initial studies of dentin ECM gene expression in differentiating odontoblasts have been promising (Fig 1-10). Data from the future use of LCM will provide a correlation between the morphologic changes and the expression of known ECM genes during odontoblast differentiation. Information generated from this approach will also be valuable in developing a nomenclature that can be consistently used by researchers. Moreover, known and unknown genes will be identified from the developmentally staged, odontoblast-specific cDNA libraries. Genes that are defined for each stage of primary dentin formation will provide important clues about temporal patterns of gene expression and the potential functions of encoded protein products in dentin mineralization. Such fundamental information will be useful in characterizing cells within the cell-rich zone of dental pulp, identifying the replacement population of pulpal cells involved in reparative dentin formation, and

developing vital pulp therapies aimed at hastening the healing of the injured pulpodentin complex.

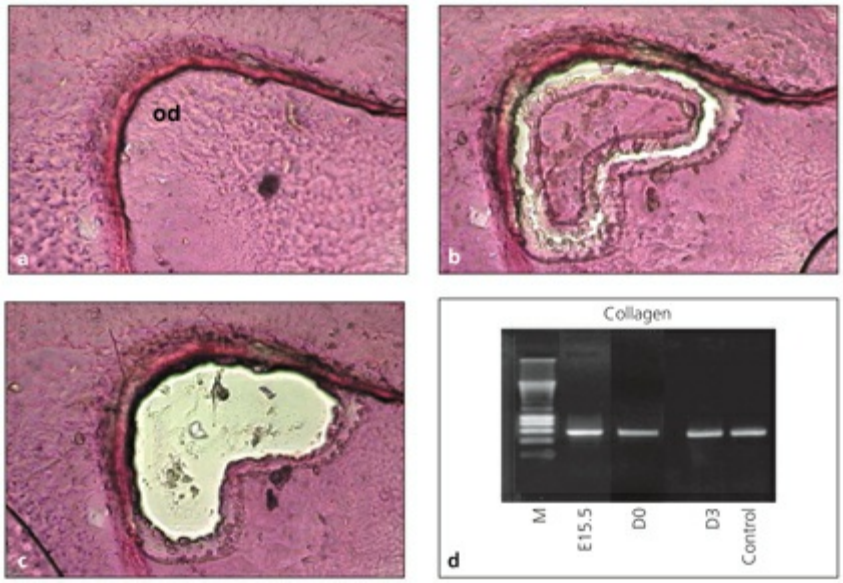

Fig 1-10 Preliminary reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of type I collagen from odontoblasts retrieved by laser capture microdissection. *(a)* Thick, stained frozen section through the mesial cusp tip of a first molar from a newborn mouse prior to laser capture. od, odontoblasts (H&E stain; original magnification ×10). *(b)* Outline of a zone of odontoblasts cut by the laser beam (H&E stain; original magnification ×10). *(c)* Hole created in tissue after catapulting of cells into PCR tube (H&E stain; original magnification ×10). *(d)* RT-PCR reaction showing type I collagen gene expression in odontoblasts at embryonic day 15.5 (E15.5), day 0 (D0), and day 3 (D3) of development. M, DNA markers; Control, MDPC-23 odontoblast-like cells.

Signaling Interactions that Influence Odontoblast Differentiation and Dental Pulp Formation

The combined use of conventional tooth organ culture and recombination techniques, as well as the application of modern molecular and genetic approaches, has significantly advanced current understanding of the genes responsible for tooth initiation and morphogenesis. Readers can access an informative internet site[56] for a current cataloging of all the molecules that are expressed in tooth organs.

The two principal groups of molecules that are involved in the reciprocal exchange of information between tooth epithelium and mesenchyme are transcription factors and growth factors. *Transcription factors* are proteins that bind to DNA near the start of transcription of a gene. They regulate gene expression by either facilitating or inhibiting the enzyme RNA polymerase in the initiation and

maintenance of transcription. Transcription factors are rarely found in high amounts and are not secreted outside the cell. In general, they perform critical cell or tissue-specific functions. Mutations involving transcription factors often result in defects of tooth formation.

Growth factors are secreted proteins that are capable of binding to specific receptors on the cell surface. Subsequent interaction with both membrane and cytoplasmic components leads to a complex series of intracellular events (signal transduction) that result in altered gene expression. These changes activate cell growth and differentiation. Most growth factors are synthesized at higher levels than transcription factors and perform versatile functions. In many instances, the functions of one growth factor overlap with those of a related family member so that loss of function can be compensated for by biologic redundancy.

Molecular changes in dental mesenchyme are affected by the following families of molecules (Fig 1-11): the bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and WNT family; sonic hedgehog (SHH) as well as transcriptional molecules such as the *Msx1* and *Msx2* homeobox genes; lymphoid enhancer-binding factor 1 *(Lef1)*; and *Pax9*, a member of the paired-box–containing transcription factor gene family. The actions and interactions of these molecules are complex and described eloquently in recent reviews.[1,4,12] The following discussion captures selected highlights.

The BMPs are among the best-characterized signals in tooth development. In addition to directly influencing morphogenesis of the enamel organ (see the discussion on enamel knots later in the chapter), epithelial BMP-2 and BMP-4 are able to induce expression of *Msx1*, *Msx2*, and *Lef1* in dental mesenchyme, as shown in bead implantation assays.[28,30,57,58] The shift in *Bmp4* expression from epithelium to mesenchyme occurs around E12 and is coincident with the transfer of inductive potential from dental epithelium to mesenchyme.[28] In mesenchyme, BMP-4 in turn requires *Msx1* to induce its own expression.[30]Figure 1-12 summarizes the experiments performed on the role of the BMPs in dental mesenchyme.

The FGFs, in general, are potent stimulators of cell proliferation and division both in dental mesenchyme and epithelium. Expression of FGF-2, -4, -8, and -9 is restricted to dental epithelium and can stimulate *Msx1* but not *Msx2* expression in underlying mesenchyme. *Fgf8* is expressed early in odontogenesis (E10.5 to E11.5), in presumptive dental epithelium, and can induce the expression of *Pax9* in underlying mesenchyme. Interestingly, BMP-4 prevents this induction and may share an antagonistic relationship with the FGFs similar to that observed in limb

development.[59]

The expression of SHH, a member of the vertebrate family of hedgehog signaling proteins, is limited to presumptive dental epithelium. Recent studies by Hardcastle et al[60] have shown that SHH protein in beads cannot induce *Pax9*, *Msx1*, or *Bmp4* expression in dental mesenchyme but is able to stimulate other genes that encode patched (Ptc), a transmembrane protein, and Gli1, a zinc finger transcription factor. Because neither FGF-8 nor BMP-4 can stimulate the genes *Ptc* or *Gli1*, it can be assumed at the present time that the SHH signaling pathway is independent of the BMP and FGF pathways during tooth development.[60]

Several *Wnt* genes are expressed during tooth development and may be required for the formation of the tooth bud.[12] These genes are believed to play a role in activating the intracellular pathway involving frizzled receptors, β-catenin, and nuclear transport of LEF-1. Other signaling molecules, including the *Notch* genes and the epidermal growth factor, hepatocyte growth factor, and platelet-derived growth factor families, may also influence tooth development, although the exact nature of their involvement remains to be elucidated.[61,62]

Mice genetically engineered with targeted mutations in transcription factor genes such as *Msx1*, *Lef1*, and *Pax9,* as well as activin-βA, a member of the TGF-β superfamily, have revealed important information. Knockouts of *Bmp2*, *Bmp4*, and *Shh* have proven less informative, largely because death occurs in utero prior to the onset of tooth development. In *Msx1*-, *Lef1*-, *Pax9*-, and *activin-βA*–mutant strains, tooth development fails to advance beyond the bud stage. Thus, these molecules are important in directing the fate of the dental mesenchyme and its ability to influence the progress of epithelial morphogenesis to the cap stage.[63–66] Exciting discoveries in the field of human genetics have shown that mutations in *MSX1* and *PAX9* are associated with premolar and molar agenesis, respectively[67–70] (Frazier-Bowers and D'Souza, unpublished data; Fig 1-13). These findings illustrate the importance of animal models in studies of human disease (Figs 1-14 and 1-15).

More recently, mice lacking an important osteoblast-specific transcription factor, Runx2 (formerly known as *core binding factor a*[1][*Cbfa1*]), were shown to completely lack osteoblast differentiation and bone formation.[71,72] Of interest to clinicians is the fact that mutations in *Runx2* cause cleidocra-nial dysplasia, an inherited disorder in humans that is characterized by open fontanels in the skull, defective clavicles, multiple supernumerary teeth that fail to erupt, and various tooth matrix defects.[73] *Runx2*$^{-/-}$ molar organs arrest at the late cap to early bell stage of development and appear hypoplastic and misshapen. *Runx2*-mutant incisors showed

defective odontoblasts and highly dysplastic dentin[74] (see Fig 1-14).

One theory is that this transcription factor serves multiple functions in mineralizing tissue organs.[75–77] In addition to playing an essential role in osteoblast differentiation, it is likely that Runx2 conditions the dental papilla mesenchyme to become responsive to epithelial signals. Once the molecular trigger from the enamel organ reaches the peripheral zone of dental papilla cells, Runx2 is downregulated in dental papilla and odontoblast differentiation ensues. Thus, the presence of Runx2 in dental papilla can be viewed as a limiting factor of odontoblast differentiation; its presence in dental papilla at the bud and cap stages of odontogenesis has an osteogenic-like influence, while its removal from dental papilla triggers terminal events in odontoblast differentiation.

More recent studies have shown that the ability of Runx2 to activate osteoblast differentiation is regulated by an antagonistic partner, Twist-1, a basic helix-loop-helix–containing nuclear protein factor.[78] Twist-1 functions as a cell survival factor and an inhibitor of cell differentiation, mineralization, and apoptosis.[79,80] Through its interaction with Runx2, a cell differentiation factor, Twist-1 controls the onset of osteoblast differentiation.[81] Decreased levels of Twist-1 in mice result in premature odontoblast differentiation as measured by the earlier onset of expression of ECM gene markers and the formation of dentin matrix (see Fig 1-15).

Our results also indicate that Twist-1 deficiency distinctly affects odontoblast-like cells, a population known to exist in adult dental pulps. These cells become more responsive to Runx2 when Twist-1 levels are decreased, leading to the formation of pulp stone–like deposits within the pulp core. In mice that lack both Runx2 and Twist-1 concurrently, the pulp appears free of ectopic calcifications (see Fig 1-15). Taken together, these findings support the hypothesis that Twist-1 is important in the control of the terminal events that lead to odontoblast differentiation and in maintaining homeostasis in dental pulp.

Multiples lines of evidence suggest that the process of terminal differentiation of odontoblasts is dependent on a vertical gradient of positional cues from overlying dental epithelium. Hence a morphogenetic gradient exists, so that the most differentiated cells align at the tooth epithelial-mesenchymal interface and less-differentiated cells toward the core of the papilla mesenchyme. This theory can explain why adult dental papilla cells have the capacity to differentiate into mineralizing cells when provided with the appropriate signal.

While the precise nature of the molecular events leading to the terminal differentiation of odontoblasts remains unknown, data from dentin repair studies

have shown that the matrix deposited after injury to dentinal tubules and pulp resembles osteoid rather than tubular dentin. This can be explained by the fact that cells of the dental papilla share a common developmental niche with surrounding osteogenic mesenchyme and that the presence of specific dental epithelial signals results in further lineage diversification of osteoblast-like cells into odontoblasts. Because the absence of the permissive influences required for differentiation of "true" odontoblasts is lacking in an adult tooth, cells engaged in the process of reparative dentin formation retain the osteoblastic phenotype and secrete a matrix that more closely resembles bone than dentin.[82,83]

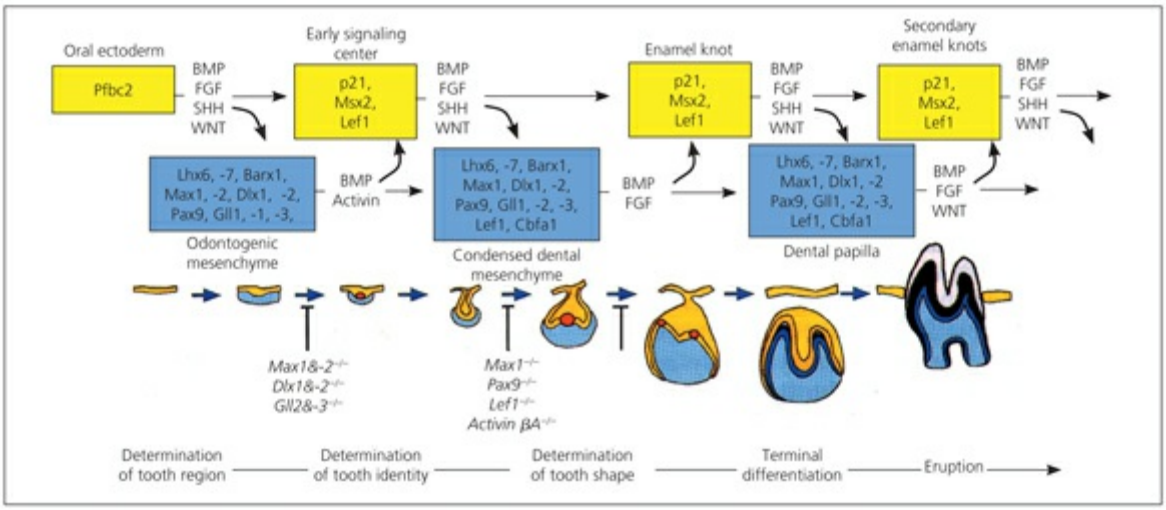

Fig 1-11 Molecules (transcription factors, growth factors, and other proteins) involved in epithelial-mesenchymal signaling interactions during tooth development. Little is known about molecules that influence the latest stages of terminal differentiation and tooth eruption. Note the time of arrest of tooth development in knockout mice that lack important transcription factors. (Reprinted from Jernvall and Thesleff[4] with permission.)

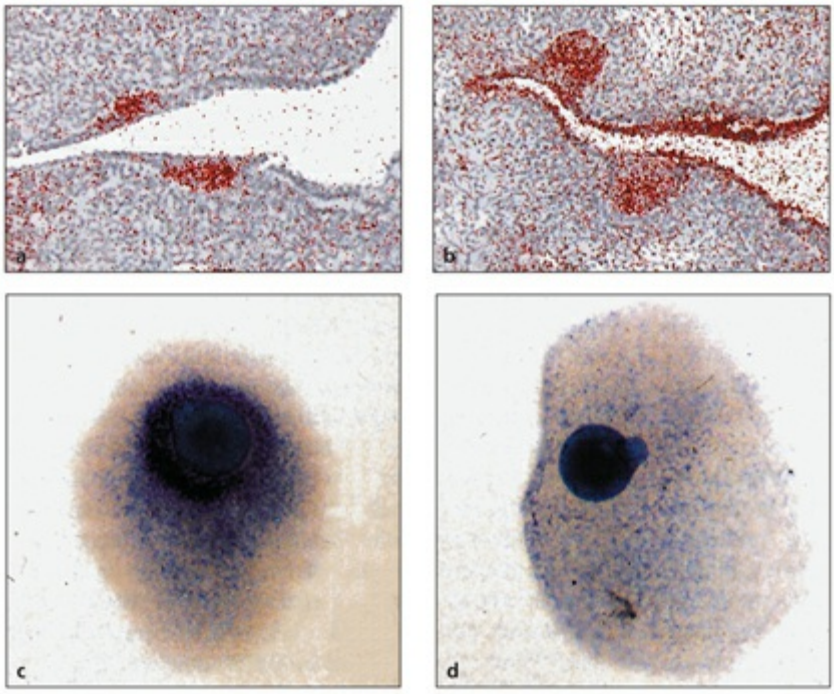

Fig 1-12 BMP, as shown by expression within the developing tooth organ in situ hybridization pictures that have been digitized and processed. Red dots represent messenger RNA transcripts. (Reprinted from Thesleff and Sharpe[12] with permission.) *(a) Bmp2* gene expression is highly restricted to the dental lamina. *(b) Bmp7* is coexpressed in the thickened dental epithelium. *(c)* A bead that releases BMP-2 protein is capable of stimulating *Msx1* expression in dental mesenchyme. *(d)* A control bead that has been soaked in bovine serum albumin is not capable of stimulating *Msx1* expression.

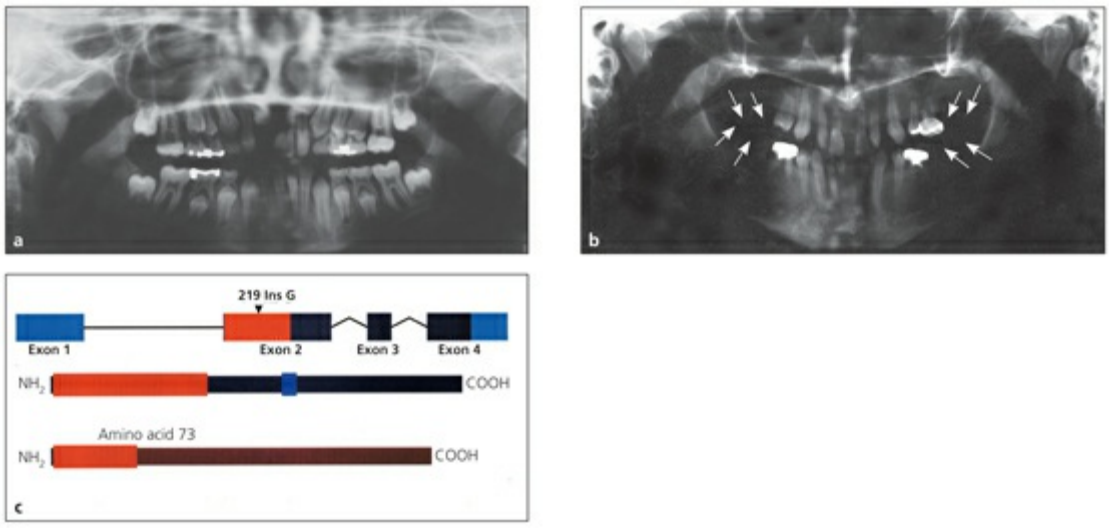

Fig 1-13 Role of *PAX9* in the formation of the posterior human dentition. *(a)* Panoramic radiograph of a normal dentition in an unaffected family member. *(b)* Panoramic radiograph of an affected individual who is missing molars *(arrows)*. *(c)* Insertion mutation of a single nucleotide, guanine, at residue 219. This defect caused a frameshift and a premature truncation site that resulted in a defective protein (compare the bottom drawing to the normal protein in the center) that could not function like a normal PAX9 protein.

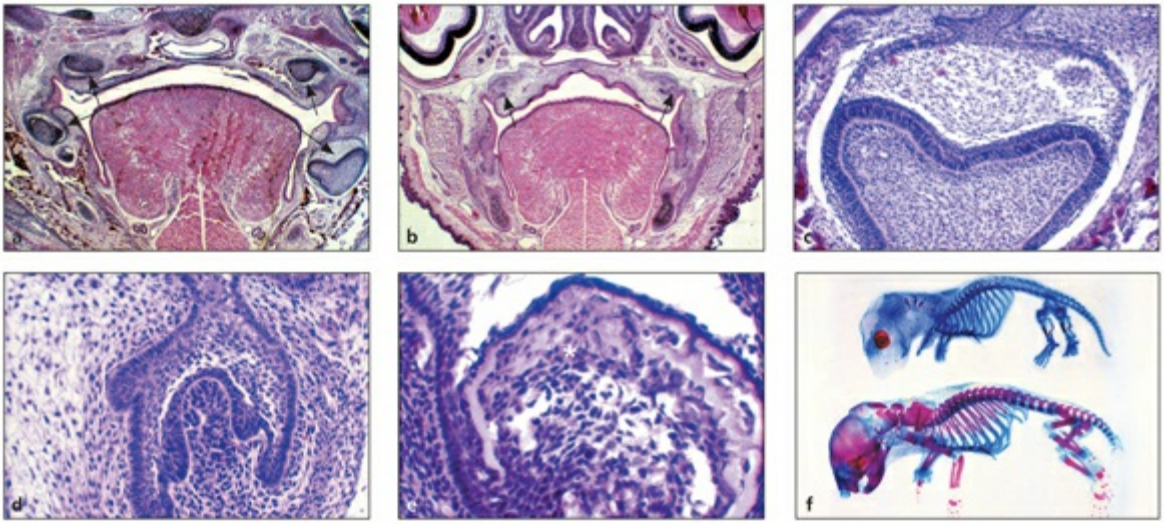

Fig 1-14 Bone and tooth phenotype in mice genetically engineered to lack a functional *Runx2 (Cbfa1)* gene. *(a)* Coronal section through the molar region of a $Cbfa1^{+/+}$ newborn mouse, revealing normal molar development *(arrows)* and formation of the alveolus (H&E stain; original magnification ×4). *(b)* Extremely hypoplastic molar organs and a complete lack of bone in a $Cbfa1^{-/-}$ mouse. *arrows*, knockout tooth organs (H&E stain; original magnification ×4). *(c)* High-magnification view of a normal $Cbfa1^{+/+}$ first molar (H&E stain; original magnification ×10). *(d)* High-magnification view of a *Cbfa1*-mutant molar, which lacks normal cusp formation (H&E stain; original magnification ×10). *(e)* Newborn $Cbfa1^{-/-}$ mouse with incisor organs that show defects in odontoblast differentiation and dentin formation. The dentin *(asterisk)* resembles an osteodentin matrix (H&E stain; original magnification ×10). *(f)* Whole-mount staining reveals the absence of bone *(red staining)* in the mutant *(top)* compared to a normal littermate *(bottom)* (alizarin red stain for bone; alcian blue stain for cartilage; original magnification ×2). (Reprinted from the cover of *Cell*, volume 89, May 1997, with permission.)

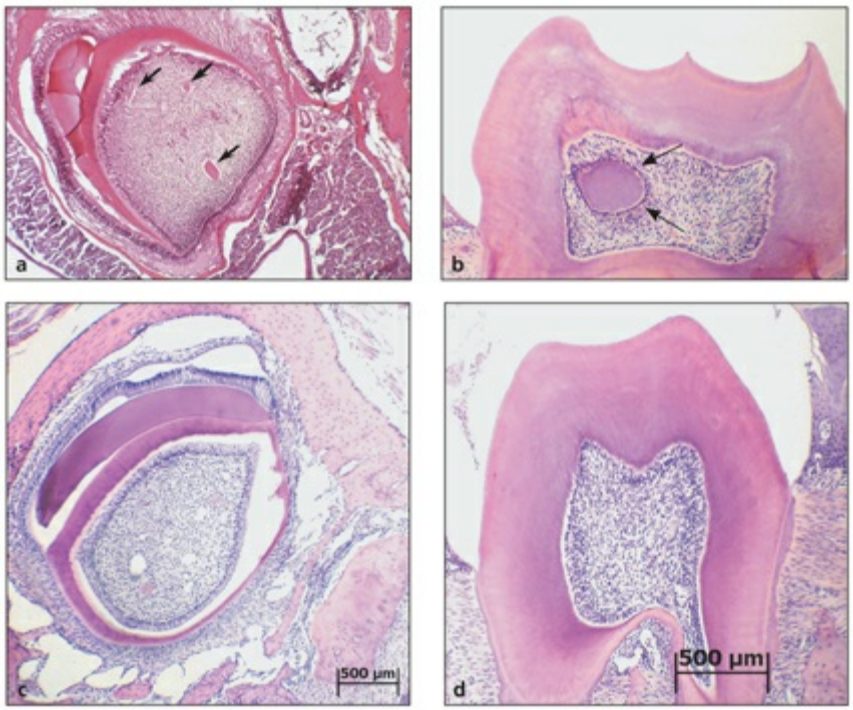

Fig 1-15 Genetic rescue experiments where *Twist*$^{+/-}$ animals at later stages show matrix deposits *(arrows)* and dentin-like structures within the pulpal tissues in incisors *(a)* and molars *(b)*; these cannot be found in *Twist*$^{+/-}$ × *Runx2*$^{+/-}$ compound double heterozygous animals in incisors *(c)* and molars *(d)* (H&E stain; original magnification ×10). (Reprinted from Galler et al[71] with permission.)

Enamel knots as signaling centers for cuspal morphogenesis

For more than a century, *enamel knots* were described as histologically distinct clusters of epithelial cells located first in the center of cap stage tooth organs (primary) and then at the sites of future cusp tips (secondary). For years, it was speculated that these structures, appearing only transiently in odontogenesis, controlled the folding of the dental epithelium and hence cuspal morphogenesis. Recently, the morphologic, cellular, and molecular events leading to the formation and disappearance of the enamel knot have been described, thus linking its role to that of an organizing center for tooth morphogenesis. [15,84,85]

The primary enamel knot appears at the late bud stage, grows in size as the cap stage is reached, and is no longer visible at the early bell stage (Fig 1-16). Cells of the enamel knot are the only cells within the enamel organ that stop proliferating[14] and eventually undergo programmed cell death.[86] Another intriguing finding has linked p[21], a cyclin-dependent kinase inhibitor that is associated with terminal differentiation events, to apoptosis of the enamel knot.[85]

Although morphogens such as BMP-2, -4, and -7,[74] FGF-9,[87] and SHH are expressed variantly throughout tooth morphogenesis, their colocalization within the primary enamel knot is strongly suggestive of its role as an organizing center for tooth morphogenesis. Notably, *Fgf4* is exclusively expressed in the enamel knot,[14,88] either singly or in concert with *Fgf9*, to influence patterning or to regulate expression of downstream genes such as *Msx1* in underlying papilla mesenchyme. Because the instructive signaling influence lies with the dental mesenchyme prior to the development of the primary enamel knot, it is reasonable to assume that the dental mesenchyme is involved in the regulation of enamel knot formation. It is highly likely that signals from the enamel knot area influence gene expression in an autocrine and paracrine fashion, thus influencing the fate of the enamel organ and the dental papilla.

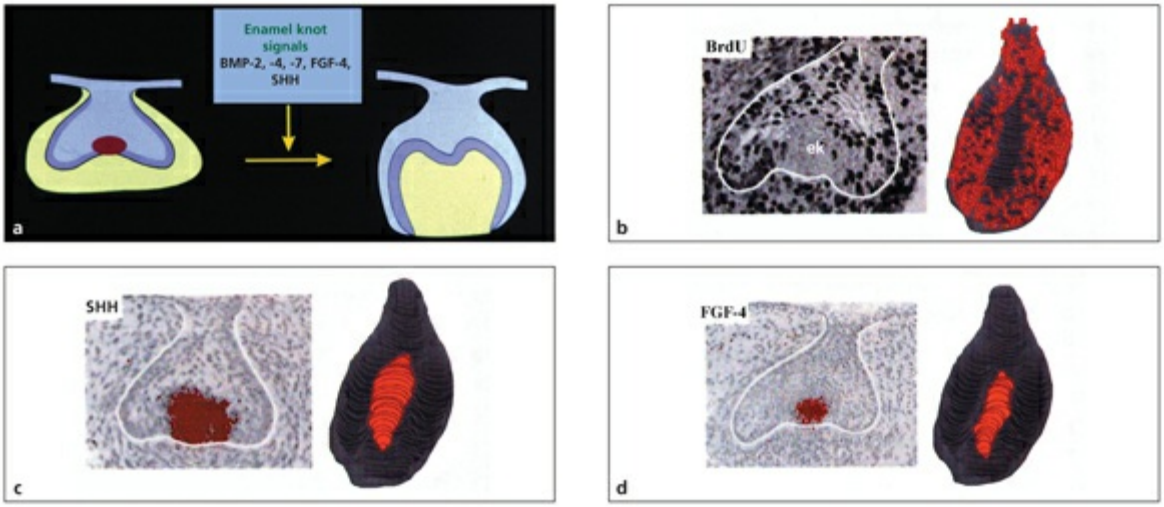

Fig 1-16 *(a)* Role of the enamel knot as a signaling center for morphogenesis of the tooth cusp. (Courtesy of Dr I. Thesleff.) *(b)* 5-Bromo-2'-deoxyuridine (BrdU) labeling identifies cells in the putative enamel knot (ek) that are negative for the stain and have left the cell cycle. (Reprinted from Thesleff and Sharpe[12] with permission.) *(c)* Same cell within the enamel knot expresses high levels of SHH. (Reprinted from Thesleff and Sharpe[12] with permission.) *(d)* Same cell within the enamel knot expresses high levels of FGF-4. Three-dimensional reconstructions of serial sections *(b to d)* illustrate the shape of the enamel knot. (Reprinted from Thesleff and Sharpe[12] with permission.)

Role of the extracellular matrix in tooth morphogenesis and cytodifferentiation

Remodeling of the ECM is an important feature of epithelial morphogenesis, especially in branching organs such as salivary and mammary glands.[5,89] The ECM also regulates morphogenetic functions in a variety of craniofacial tissues.[90] Results of several functional in vitro studies have shown that the integrity of the ECM, in particular the basement membrane, influences the budding and folding of dental epithelium during morphogenesis and the spatial ordering of cells that undergo terminal differentiation.[9,91,92] Molecules such as collagen types I, III, and IV, along with laminin and various proteoglycans, are differentially expressed in the basement membrane at the epithelial-mesenchymal interface of the tooth.[9,93,94] The precise nature of the molecular interactions that influence morphogenesis at this dynamic interface are unknown.

The presence of matrix metalloproteinases (MMPs) has been linked with the morphogenesis of several epithelial-mesenchymal organs, including teeth.[95,96]

Studies by Sahlberg et al[97] showed that gelatinase A (ie, MMP-2), an MMP that cleaves type IV collagen and increases in odontoblasts shortly after cuspal morphogenesis, contributes to the degradation of the basement membrane. The expression of protease inhibitors, tissue inhibitors of metalloproteinases (TIMPs) 1, 2, and 3, also correlates with tooth morphogenesis.[98]

Odontoblast Differentiation

Odontoblast differentiation is initiated at the cusp tip in the most peripheral layer of dental papilla cells that align the epithelial-mesenchymal interface and follows three steps: *(1)* induction, *(2)* competence, and *(3)* terminal differentiation. Inductive signals from the internal epithelial cells most likely involve members of the TGF-β family (BMP-2, BMP-4, and TGF-β1) that become partially sequestered in the basal lamina to which peripheral cells of the dental papilla become aligned. Competence is achieved after a predetermined number of cell divisions are completed and cells express specific growth factor receptors. In the final round of cell division, only the most peripheral layer of cells subjacent to the basal lamina responds to the signals from the internal dental epithelium to become fully differentiated into odontoblasts. The subodontoblastic layer of cells thus represents dental papilla cells that are competent cells exposed to the same inductive signals as differentiated odontoblasts except the final one (Fig 1-17).

Based on the information presented so far (see Fig 1-11), it is clear that considerable progress has been made in understanding the molecular events preceding the terminal differentiation of odontoblasts. However, the final determinants of odontoblast differentiation remain to be characterized.

As is well documented in the literature, fully differentiated odontoblasts are postmitotic cells that are morphologically distinct from cells of the dental pulp. As differentiation proceeds in an apical direction, these cells change their shape, which ranges from round to cuboidal, to a tall columnar appearance. On the subcellular level, cells acquire a synthetic and secretory apparatus by developing an extensive rough endoplasmic reticulum and Golgi apparatus along with numerous lysosomes. To accommodate these organelles and to prepare for the secretion of dentin matrix components in an apical and unidirectional manner, the nucleus moves to the opposite pole of the cell in a position opposite to the inner dental epithelial cells. Nuclear repolarization is one of the hallmarks of terminal odontoblast

differentiation.

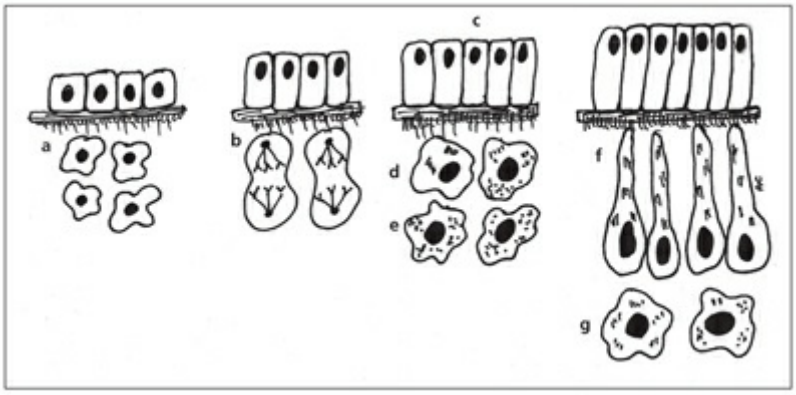

Fig 1-17 Terminal events in odontoblast differentiation. *(a)* Undifferentiated mesenchymal cells. *(b)* Committed dental mesenchymal cells that are in a state of mitosis or cell division. *(c)* Inner dental epithelium, which is important for driving the differentiation of cells nearest the basement membrane. *(d)* Basement membrane. *(e)* Daughter cells that are competent to become odontoblasts remain in the peripheral zone of the dental papilla. *(f)* Differentiated odontoblasts with a polarized nucleus and cytoplasmic extensions. *(g)* Subodontoblastic cells.

Dentin Matrix Proteins and the Biomineralization of Dentin

The formation of dentin follows the same principles that guide the formation of other hard connective tissues in the body, namely, cementum and bone. In dentinogenesis, the first requirement is the presence of highly specialized cells, termed *odontoblasts*, that are capable of synthesizing and secreting type I collagen–rich unmineralized ECM, referred to as *predentin*, which is subsequently mineralized when apatite crystals are deposited. As the odontoblasts build this type I collagen–rich fibrillar matrix, they recede in a pulpal direction and leave behind odontoblastic processes, through which these cells remain connected to the mineralized matrix.

The formation of predentin and its transformation to dentin as mineralization takes place are highly controlled, orderly processes. For example, under normal conditions of growth, the predentin width is rather uniform, not random, indicating that the rates of synthesis of unmineralized matrix and its conversion to dentin at the predentin-dentin border must be equal (Fig 1-18). On the other hand, in pathologic conditions such as DI, the widening and disorganization of the predentin layer indicate an incapacitation of this process.

The overall process of dentinogenesis involves a series of events that apparently begin at the boundary of the odontoblast cell body with the matrix and continue until the mineralization process is complete. In the past five decades, researchers in the biomineralization field have attempted to answer the following questions:

- What is the exact composition of dentin matrix and what biochemical features distinguish dentin from bone and cementum?
- Are there dentin-specific markers and can they be used to characterize the nature of the replacement cells responsible for forming reparative dentin?
- How do the physical features and conformational structures of ECM molecules facilitate the calcification of dentin?
- Do these macromolecules interact with each other during the mineralization of dentin?
- Do these macromolecules form supramolecular complexes that promote the deposition of hydroxyapatite crystals?
- What is the nature of the ECM molecules that modulate the initiation, rate, and extent of dentin deposition?
- What is the nature of the genes that encode for dentin ECM molecules?
- Are defects in these genes responsible for the inherited dentin disorders, namely DI and dentin dysplasia?
- What genes regulate the expression of key dentin ECM molecules?

Based on new research advances made in understanding dentinogenesis, valuable insights have been gained about the unique roles of these proteins in controlling the process of biomineralization. For example, ultrastructural studies on the collagen fibrils formed by rat incisor odontoblasts demonstrated that collagen fibrils progressively thicken from the time they are secreted until they are mineralized at the predentin-dentin border[100]; these observations along with many others[101–103] indicate that the transition from the zone of predentin immediately outside the bodies of the odontoblasts to the area adjacent to dentin represents a gradient of events, a maturation process that is dynamic. Another example is the important discovery that mutations in dentin sialophosphoprotein (*DSPP*) genes are responsible for the underlying dentinal defects seen in DI type II and DI type III.[104–107] These conditions affect a large number of individuals worldwide and are characterized by severe defects in dentin mineralization in both primary and permanent dentitions (Fig 1-19).

More than 90% of the organic component in dentin matrix is type I collagen (Fig 1-20). The importance of the correct collagen structure in dentin formation is clearly

seen in patients with DI type I, caused by mutations in the type I collagen gene,[108] which clinically resemble DI types II and III, caused by *DSPP* mutations.

In addition to type I collagen, the ECM of dentin contains a number of noncollagenous proteins (NCPs) and proteoglycans (Table 1-1). One category of the NCPs that are principally found in dentin and bone and are secreted into the ECM during the formation and mineralization of these tissues is termed the *small integrin-binding ligand, N-linked glycoprotein* (*SIBLING*) family, which includes dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), osteopontin (OPN), and matrix extracellular phosphoglycoprotein (MEPE).[109] Data obtained in the last several decades have demonstrated that these polyanionic SIBLING proteins are actively involved in the mineralization of collagen fibers and crystal growth within predentin when this tissue is converted to dentin.[110,111] The following sections provide an updated summary of the SIBLING proteins, their proteoglycan forms, and their postulated roles in the mineralization of dentin, with particular emphasis on DSPP and DMP1, two prominent SIBLING proteins in the dentin ECM that have been proven to be critical for dentin mineralization.

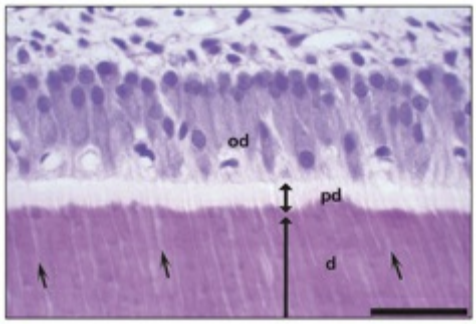

Fig 1-18 Buccolingual section from a mandibular incisor of a 5-week-old rat. Odontoblasts (od) secrete unmineralized predentin (pd, *double-headed arrow*), which is subsequently mineralized and becomes dentin (d, *long arrow*) when apatite crystals are deposited. The long columnar odontoblasts form processes that extend into dentin. *short arrows*, dental tubules that house odontoblast processes (H&E stain; bar =40 μm). (Reprinted from Qin et al[99] with permission.)

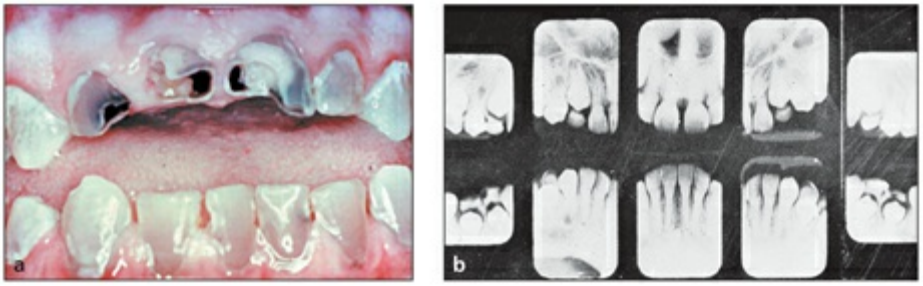

Fig 1-19 DI resulting from mutations in the *DSPP* gene demonstrates profound defects in dentin formation. (Courtesy of Dr Nadarajah Vigneswaran.) *(a)* Patient afflicted with DI. Note the

discoloration and extensive loss of tooth structure. *(b)* Periapical radiographs of the affected teeth, revealing bulbous crowns, obliterated pulp chambers, and narrowed root canals.

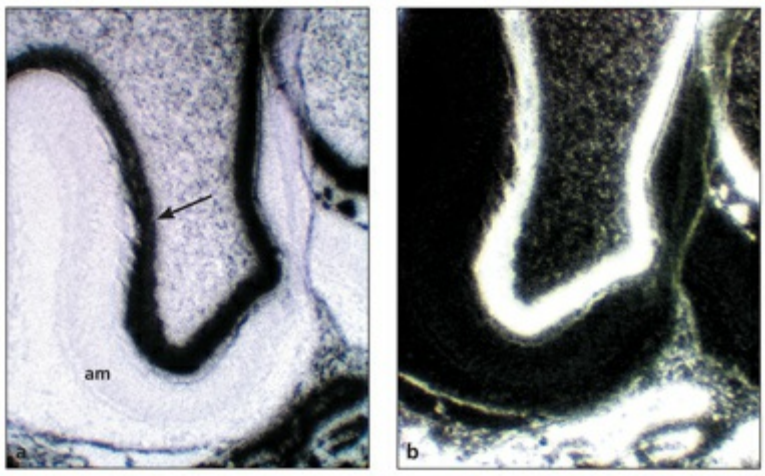

Fig 1-20 Type I collagen in odontoblasts. *(a)* Brightfield view showing high level of type I collagen messenger RNA transcripts in odontoblasts *(arrow)*. Opposing ameloblasts (am) are clearly negative, confirming the specificity of the α1(I) collagen probe (H&E stain; original magnification ×10). *(b)* Darkfield view of type I collagen messenger RNA transcripts in odontoblasts (H&E stain; original magnification ×10).

Table 1-1 **Noncollagenous proteins and proteoglycans present in the ECM of dentin**

Component	Category*	Quantity†	Potential functions in dentin‡
DSP§	SIBLING	Major	Unclear
DPP§	SIBLING	Major	Initiates and regulates mineralization
DMP1, 37 kDa‖	SIBLING	Moderate	Unclear
DMP1, 57 kDa‖	SIBLING	Moderate	Nucleates hydroxyapatite
BSP	SIBLING	Minor	Nucleates hydroxyapatite
OPN	SIBLING	Minor	Inhibits and regulates mineralization
MEPE	SIBLING	Minor	Inhibits and regulates mineralization
DSP-PG¶	Proteoglycan	Major	Unclear

DMP1-PG#	Proteoglycan	Major	Inhibits mineralization
Biglycan	Proteoglycan	Minor	Unclear
Decorin	Proteoglycan	Minor	Involved in collagen fibrinogenesis
Osteocalcin	NCPs	Major	Unclear
Osteonectin	NCPs	Moderate	Unclear

* Noncollagenous components are classified into the SIBLING family and the proteoglycan category. Those that cannot be classified into either of the two groups (such as osteocalcin and osteonectin) are listed as noncollagenous proteins (NCPs).
† The quantity described reflects the amount of an individual component relative to other members in the same category. For example, DSP-PG is the most abundant proteoglycan, so it is listed as a major component, although it is relatively much less abundant than DPP.
‡When the functions of a component are not well defined, they are labeled unclear.
§DSP and DPP are the cleaved products of DSPP.
||DMP1 37-kDa and DMP1 57-kDa fragments are the cleaved products of DMP1.
¶DSP-PG is the proteoglycan form of DSP.
#DMP1-PG is the proteoglycan form of DMP1 37-kDa fragment.

DSPP

Gene mutation studies in humans[104–106] and investigations involving *Dspp* gene knockout mice[107] have demonstrated that DSPP is crucial for dentinogenesis. Dentin in *Dspp*-null mice is hypomineralized and the predentin is widened, giving rise to a phenotype similar to the manifestations of DI types II and III in humans.

The large precursor protein, DSPP, predicted from cDNA,[112–114] gives rise to two proteins: dentin sialoprotein (DSP) with coding sequences in the 5’ end and dentin phosphoprotein (DPP) representing the 3’ end of the DSPP messenger RNA. The occurrence of one gene transcribing a single messenger RNA that encodes both DSP and DPP indicates that the translated product, DSPP, must be cleaved by a proteinase, giving rise to the individual proteins DSP and DPP. Consistent with this conclusion, DSP and DPP are found in abundance in dentin ECM (Fig 1-21a), but DSPP (the protein representing the entire sequence) is not.

DPP, discovered about four decades ago,[116] is the most abundant NCP in dentin ECM. The unusual feature of DPP is the occurrence of large amounts of acidic residues such as aspartic acid and phosphoserine. Data obtained through in vitro

mineralization studies indicate that DPP is an important initiator and modulator for the formation and growth of hydroxyapatite crystals.[117–119] Following its synthesis and secretion by odontoblasts, DPP is transported to the mineralization front, where it binds to collagen fibrils and assumes a conformation that promotes the formation of initial hydroxyapatite. As the mineralization process proceeds and predentin is converted to dentin, these mineral crystals grow in an oriented fashion under the modulation of DPP and other NCPs that bind to the growing hydroxyapatite faces.[120,121]

DSP, discovered about 25 years ago,[122] is another abundant NCP in dentin ECM. The functions of DSP are presently ill defined because little or no effect on in vitro mineralization has been shown.[123] Recently, a proteoglycan form of DSP (see Fig 1-21a) has been isolated and characterized by several research groups.[124–126]

Thus, the ECM of dentin contains three variants derived from the DSPP amino acid sequence: DSP, DPP, and a proteoglycan form of DSP (referred to as *DSP-PG*). It is likely that these variants, which vary greatly in biochemical structure, play different roles during dentinogenesis. DPP is known to be an initiator and modulator for the formation and growth of hydroxyapatite, but information regarding the biologic roles of DSP and DSP-PG is lacking. Based on the presence of DSP and DPP and the absence of DSPP in dentin ECM, along with the observed roles of DPP in the nucleation and modulation of apatite crystal formation, it is likely that the conversion of DSPP to DSP and DPP is an activation event, converting an inactive precursor to active fragments (Fig 1-21b), as in the case of zymogen activation. This activation step would represent one of the controlling mechanisms in dentin formation.[99]

The correct structure for DSP (within DSPP) may be required for proper folding or transport of DSPP, reducing the level of DPP that is delivered to the site. The folding and/or structure of this large precursor DSPP may prevent formation of the three-dimensional structure that would allow DPP to bind to calcium or collagen, in a manner prescribed for it to initiate and control mineralization.

For about two decades, the expression of DSPP was thought to be tooth specific.[127] Recent studies have demonstrated that DSPP is also expressed in bone and osteoblasts at a level much lower than that in dentin and odontoblasts.[128–130] In tooth, DSPP and/or its cleaved products (ie, DSP and DPP) are expressed in preameloblasts, odontoblasts, predentin, and dentin (Fig 1-21c).

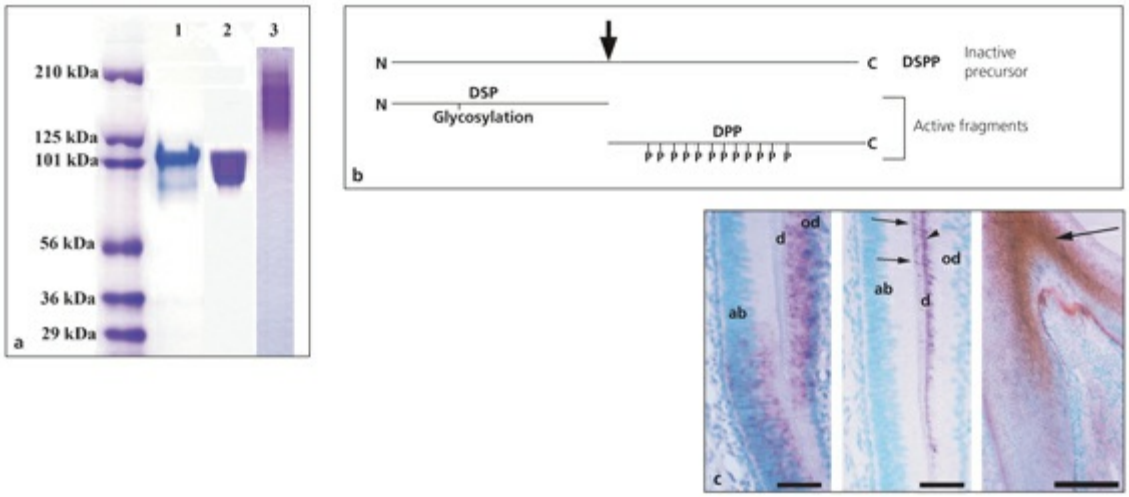

Fig 1-21 *(a)* Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). All staining (Sigma Aldrich) of DSP, DPP, and DSP-PG isolated from rat dentin ECM. DSP (lane 1, at ~95 to 100 kDa) and DPP (lane 2, at ~90 to 95 kDa) are abundant, but their precursor, DSPP (theoretical migration rate, ~200 kDa), is absent in the ECM of dentin. DSP-PG purified from dentin ECM appears as a very broad band migrating between 125 kDa and over 200 kDa on SDS-PAGE (lane 3). *(b)* Hypothetical proteolytic activation of DSPP. Full-length DSPP may be an inactive precursor that is proteolytically processed into DSP and DPP originating from the NH2-terminal and COOH-terminal regions of DSPP, respectively. DSP is rich in carbohydrates (highly glycosylated) but contains fewer phosphates (P), whereas DPP is devoid of glycosylation but contains an unusually large number of phosphates. As in the case of zymogen activation, DSPP proteolysis may be necessary to liberate the active forms of DPP and/or DSP at a site and time that is appropriate for their functions during dentin formation. *(c)* Expression of DSPP in tooth. *(left)* DSPP messenger RNA *(purple)* is detected in the odontoblasts (od) and preameloblasts (ab) in the mandibular first molar of 1-day-old rat. d, dentin (bar = 50 μm). *(center)* DSP protein *(purple)* is visualized in predentin *(arrowhead)* and dentin (d, *arrows)* of the mandibular first molar of a 1-day-old rat by immunohistochemical staining using an anti-DSP antibody (bar = 50 μm). *(right)* Dentin of the first molar from an 8-week-old rat shows a strong immunoreactivity *(arrow, brown area)* to the anti-DSP antibody (bar = 200 μm). (Reprinted from Baba et al[115] with permission.)

DMP1

DMP1, discovered by cDNA cloning,[131] is an acidic phosphoprotein. Originally postulated to be dentin specific, the expression of DMP1 was later observed in bone.[132–134] The distinctive feature of DMP1 (predicted from cDNA) is a large number of acidic domains, a property that implicates it as a possible participant in regulating matrix mineralization. The importance of DMP1 for dentin mineralization has been demonstrated by knockout experiments in mice: *Dmp1*-null mice show profound dental defects, including widening of predentin and hypomineralization of

dentin.[135]

Like DSPP, DMP1 is present in the ECM of bone and dentin as an NH_2-terminal (37-kDa) fragment, a COOH-terminal (57-kDa) fragment,[136] and a proteoglycan form (DMP1-PG) of the NH_2-terminal fragment.[137] These three forms (Fig 1-22a), differing dramatically in biochemical structure, may have different functions in dentinogenesis and osteogenesis. Several in vitro mineralization studies have indicated that the 57-kDa fragment, like DPP, promotes mineralization by acting as a nucleator for hydroxyapatite formation.[138–140] DMP1-PG appears to inhibit biomineralization.[141] In fact, DMP1 is more highly expressed in bone than tooth. In the tooth, DMP1 is primarily present in predentin and dentin. In mineralized dentin, DMP1 is predominantly localized in the peritubular region.[115]

Based on the existence of DMP1 as processed fragments in the ECM of dentin and bone, the absence of a significant quantity of full-length DMP1 and the observed roles of the 57-kDa fragment in mineralization, it is reasonable to believe that the proteolytic conversion of DMP1 to 37-kDa and 57-kDa fragments may be an activation step, releasing active fragments at the correct time and site to control the mineralization process of dentin and bone (Fig 1-22b).

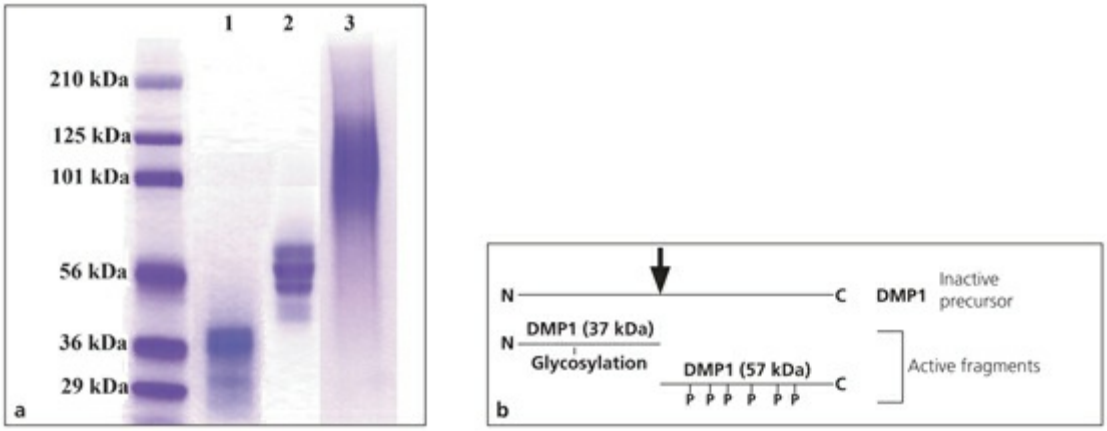

Fig 1-22 *(a)* SDS-PAGE. All staining of purified DMP1 variants. Lane 1: The processed NH_2-terminal fragment of rat DMP1 migrates at approximately 37 kDa on SDS-PAGE. Lane 2: The COOH-terminal fragment of rat DMP1 runs around 57 kDa. Lane 3: DMP1-PG migrates between 80 and 160 kDa on SDS-PAGE. *(b)* Hypothetical proteolytic activation of DMP1. Full-length DMP1 may be an inactive precursor that is proteolytically processed into 37- and 57-kDa fragments originating from the NH_2-terminal and COOH-terminal regions of the DMP1 amino acid sequence, respectively. The 37-kDa fragment is rich in carbohydrates (highly glycosylated) but contains fewer phosphates (P), whereas the 57-kDa fragment is devoid of glycosylation but contains a large number of phosphates. The cleavage of DMP1 may be an activation step employed to liberate the active forms (ie, the 57-kDa and 37-kDa fragments). (Reprinted from Qin et al[136] with permission.)

BSP

The primary sequence of BSP was first deduced from a rat cDNA sequence.[142] BSP consists of many fewer amino acid residues than DSPP or DMP1. The biologic functions of BSP in mineralized tissues are largely unknown, although some data suggest that BSP acts as a nucleator for the formation of initial apatite crystals; then, as this mineral grows on the collagen matrix, BSP acts as an inhibitor in directing the growth of the crystals.[143]

The tissue distribution of BSP is relatively restricted to the mineralized tissues, primarily in bone. In the tooth, BSP can be observed in cementum and predentin, and under physiologic conditions (primary and secondary dentinogenesis), mineralized dentin contains little or no BSP.[141,144] In tertiary dentin, the level of BSP is remarkably elevated (Fig 1-23).

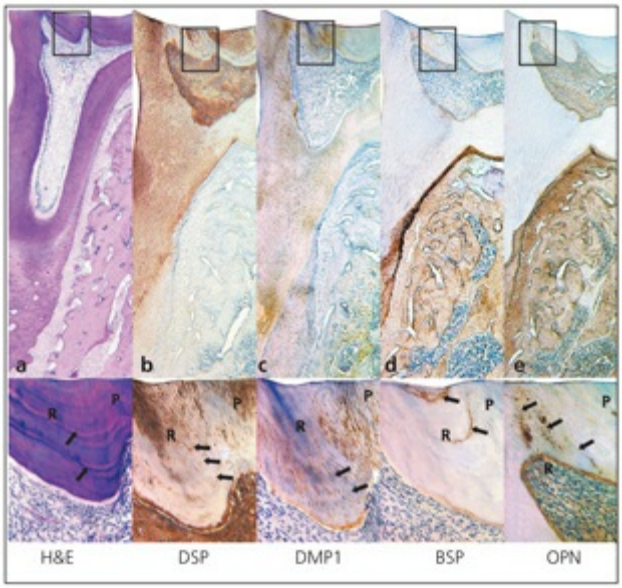

Fig 1-23 Expression of DSP, DMP1, BSP, and OPN in tertiary (reactionary) dentin (R) versus primary dentin (P). Photomicrographs are from the mesiodistal sections of the first molar of a 36-week-old rat. In ×400 photographs, the reactionary dentin fills nearly all of the field of view. The boxes in the upper section denote the locations of the images in the lower section. *(a)* ×40 magnification with H&E staining; *(inset)* ×400 magnification with H&E staining. *arrows*, incremental staining pattern. *(b)* ×40 magnification for DSP immunostaining *(brown)*; *(inset)* ×400 magnification for DSP immunostaining. *arrows*, incremental lines in reactionary dentin stained for DSP. *(c)* ×40 magnification for DMP1 immunostaining *(brown)*; *(inset)* ×400 magnification for DMP1 immunostaining. *arrows*, incremental staining pattern in the hematoxylin background. *(d)* ×40 magnification for BSP immu-nostaining *(brown)*; *(inset)* ×400 magnification for BSP immunostaining. Note the absence of BSP in primary dentin and the elevated expression of this protein in reactionary dentin. *arrows*, reactionary dentin. *(e)* ×40 magnification for OPN immunostaining *(brown)*; *(inset)* ×400 magnification for OPN immunostaining. *arrows*, incremental lines stained for OPN. Note the absence of OPN in primary

dentin and the elevated expression of this protein in reactionary dentin. (Reprinted from Moses et al[144] with permission.)

OPN

The name *osteopontin* was introduced to reflect the potential of this protein in bone to serve as a bridge between cells and hydroxyapatite through arginine-glycine-aspartate tripeptide and polyaspartic acid motifs in its primary amino acid sequence, which was discovered by cDNA cloning.[145] OPN contains a similar number of amino acids as BSP.

Although OPN is present in bone in relatively large quantities, it is also expressed in a variety of other tissues and cells[146,147]; the broad expression of OPN indicates a multiplicity of functions in diverse biologic events. Under physiologic conditions, only minor amounts of OPN exist in the ECM of dentin.[148] In tertiary dentin formation, the level of OPN is remarkably elevated (see Fig 1-23). In mineralized tissues, OPN is believed to be an effective inhibitor of apatite formation and growth.[149–151]

Dentinogenesis is a dynamic process, involving an interplay among a number of molecules, including type I collagen, NCPs, and proteoglycans. Collectively, these molecules work to precisely control the site and rate of apatite formation. Despite the efforts and progress made in the past several decades, the precise process of dentin mineralization is not well understood. The available data indicate that dentin matrix proteins, in particular the SIBLING family members, play important roles in the mineralization of this tissue, although the exact mechanisms by which each individual molecule participates in biomineralization are unclear. More information about dentin matrix proteins is warranted for a better understanding of the roles of these molecules; such information will not only help to clarify the fundamental mechanisms involved in the formation of mineralized tissues but will also enhance understanding concerning the pathogeneses of dentin defects that occur in systemic diseases such as DI and dentin dysplasia. A better elucidation of the pathogeneses of these dental defects is essential for establishing scientifically based treatment modalities for such diseases.

Future Directions

Following the completion of the initial mapping of the human genome (Human Genome Project), it is anticipated that nearly every human disease gene will be identified and isolated. The current postgenomic era will stimulate new research on the nature of proteins and their defects. Gene discoveries will lead to genetic screening and prevention strategies, and it is highly likely that the genetic code for the human dentition will be unraveled through the use of reverse genetics.

For researchers studying pulpal biology, this era will undoubtedly provide challenging and exciting opportunities to explore several basic biologic issues that are not well understood. With the help of commercially available DNA microarrays (also referred to as *DNA chips* and *oligonucleotide arrays*) that allow screening of the entire human and mouse genomes, it will soon be possible to catalog the complete genetic and biochemical profile of odontoblasts (Fig 1-24). Through this method, odontoblast-specific and dental pulp–specific determinants during health and disease can be identified. Such knowledge can quickly be extrapolated to studies directed at understanding the nature of cells within the subodontoblastic layer as well as other pulp cell populations. Furthermore, the underlying mechanisms of pulpitis and the molecular predictors of reversible versus irreversible pulpitis will be explored in depth.

Such new molecular data will be applied to tissue engineering and biomimetic approaches that are geared toward dentin regeneration after injury from caries and operative dental procedures. Basic science approaches directed toward understanding how key dentin matrix genes are regulated will lead to further studies on the molecules that control the terminal phases of odontoblast differentiation. Identification and isolation of growth factors and transcription factors will encourage the use of a multipronged approach for the treatment of injuries to the pulpodentin complex. This may require the use of genetically engineered mouse models prior to translational studies performed on human teeth. Knowledge of these genes will generate a candidate list of genes whose role in inherited disorders of dentin, in particular the dentin dysplasias, can be analyzed in depth.

Finally, the discovery that dental pulp houses cell populations that retain the capacity to differentiate into multiple cell types is exciting and offers new directions for regenerative therapies aimed at the pulpodentin complex and supporting tooth structures. Applications of these therapies will require greater interactions between endodontists, pulpal biologists, and basic science researchers.

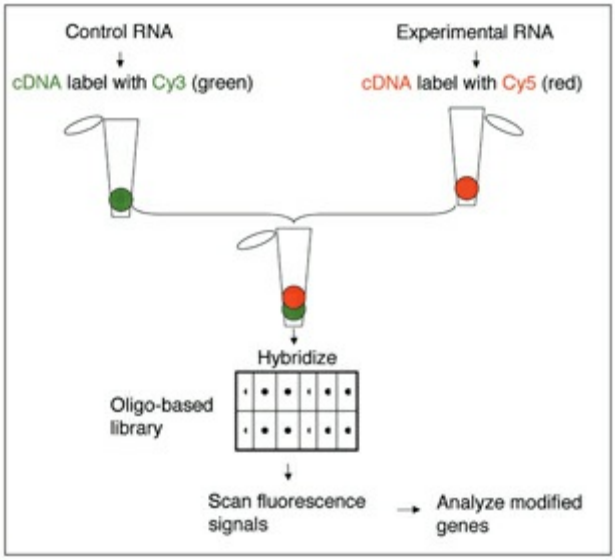

Fig 1-24 Experimental use of microarray technology. This technique is useful for studying different levels of gene expression during development, disease, and repair. The identification of genes that are either upregulated or downregulated helps researchers understand the underlying mechanisms of gene expression.

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Formation and Repair of Dentin in the Adult

Anthony J. Smith, BSc, PhD

The structure and responses of the pulpodentin complex throughout life are intimately related to the behavior of the odontoblasts and other cells of the pulp. Classification of the orthodentin of mammals as primary, secondary, or tertiary[1] has provided a basis for understanding how dentin forms over the course of a lifetime (Fig 2-1). *Primary dentin* is the regular tubular dentin formed prior to eruption and completion of the apical region of the tooth, including the first-formed mantle dentin. *Secondary dentin* is the regular circumpulpal orthodentin formed (in tubular continuity with the primary dentin) at a slower rate throughout the remaining life of the tooth. *Tertiary dentin* represents the more or less irregular dentin formed focally in response to noxious stimuli such as tooth wear, dental caries, cavity preparation, and restorative procedures. This category has been proposed to encompass a range of sometimes confusing terms, including *irregular secondary dentin, irritation*

dentin, reparative dentin, irregular dentin, reaction dentin, replacement dentin, and *defense dentin*.

Primary and secondary dentin, including mantle dentin, are the exclusive secretory products of the tightly packed layer of primary odontoblasts found on the formative surface of the tissue. Barring injury, these postmitotic cells generally survive for the life of the tooth and provide both vitality to the tissue and the ability to respond to a wide variety of environmental stimuli. An appreciation of odontoblast behavior throughout life is critical to our understanding of dentin formation and the development of regenerative endodontic procedures (see chapter 5). This is especially important in the context of pulpodentin tissue regeneration and engineering. Recent reports on whole-tooth engineering[2–7] highlight the importance of understanding how to replicate developmental cell and molecular events.

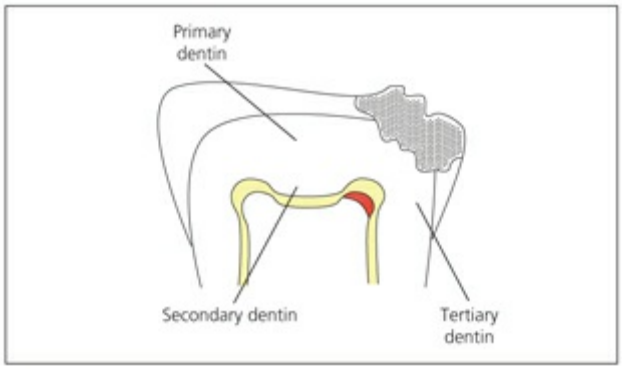

Fig 2-1 Locations of primary, secondary, and tertiary dentin in the human tooth.

Odontoblasts and Dentinogenesis

Odontoblasts and their relationship to the pulp

The traditional understanding of the morphology of the odontoblast is that of a tall, columnar secretory cell with a polarized, basal nucleus and a single cytoplasmic process. Although this concept holds true during active dentinogenesis, it is now clear that an odontoblast varies throughout its life cycle both in size and in content of cytoplasmic organelles and that these changes are closely related to its functional activity[8–11] (Fig 2-2).The relationship between size and secretory activity of the cells is borne out by the differences in size between odontoblasts in the crown and

those in the root of the tooth,[12] which may be related to the varying rate of dentinogenesis in these two areas of the tooth.

The phenotype of the odontoblast is defined both by its morphology and by its polarized secretion of a characteristic set of molecules,[13,14] leading to deposition of a mineralizable matrix that has a regular tubular structure within which the odontoblast processes lie. These features may be important to the specificity of any repair responses after injury to the tooth.

However, the odontoblast requires the presence of other pulpal elements to survive and function. Attempts to culture odontoblasts in isolation have met with little success; organ cultures involving the entire pulpodentin complex have been required for maintenance of their growth in vitro,[15,16] although immortalized pulp cell lines with odontoblast-like cell characteristics have been established.[17–19] Several reports have suggested that stimulation of pulp cell cultures with mineralization-promoting agents such as dexamethasone and β-glycerophosphate results in development of an odontoblast-like cell phenotype based on the morphologies and molecular expression profiles of these cells.[20–22] However, more robust characterization of these cells is required before they can be considered to resemble the primary odontoblasts responsible for physiologic dentinogenesis.

The cell-rich layer of Höhl underlying the odontoblast layer shows some unique phenotypic characteristics in terms of cellular morphology[23] and may function to support odontoblast activity, most conspicuously in the crown of the tooth during active dentinogenesis. During the last cell division of the preodontoblast prior to terminal differentiation, one of the daughter cells is positioned adjacent to the dental basement membrane and receives the inductive signal to differentiate into an odontoblast while the other does not and may contribute to the cell-rich layer of Höhl. These cells may contribute to the progenitor cell population for odontoblast-like cell differentiation during tertiary dentinogenesis.

Associated with this cell-rich layer is a rich capillary plexus that probably plays a key role in the transport of nutrients for secretion of the mineralized organic matrix during active dentinogenesis. Correlation of the blood supply of the developing tooth with the extent of mineralization[24] has demonstrated the intimate relationship between angiogenesis and dentinogenesis (see chapter 6). The extensive vascular network in the coronal part of the pulp has been elegantly demonstrated in resin casts[25] (Fig 2-3). The importance of an adequate vascular supply to the odontoblasts for dentinogenesis is also highlighted during tertiary dentinogenesis, when successful outcomes of the repair process generally require angiogenic activity at the injury

site. Molecular signaling of these angiogenic processes by growth factors, especially vascular endothelial growth factor (VEGF),[26–28] is likely central to tissue events.

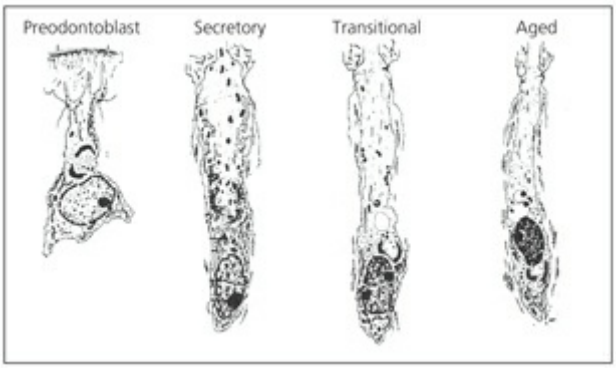

Fig 2-2 Ultrastructural appearance of the human odontoblast throughout its life cycle. (Reprinted from Couve[10] with permission.)

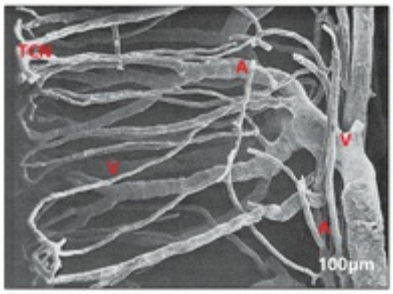

Fig 2-3 Resin cast of the subodontoblastic capillary plexus in a dog pulp. TCN, terminal capillary network beneath the dentin; A, terminal arteriole; V, venule. (Reprinted from Takahashi et al[25] with permission.)

Secretory behavior of odontoblasts

The cytologic features of the active odontoblast have been well described[29] and reflect those of a unidirectional secretory cell. Such features include a basal nucleus with parallel stacks of rough endoplasmic reticulum aligned parallel to the length of the cell, both on the apical side of the nucleus and at the apical end of the cell on either side of the prominent Golgi apparatus. The saccules are more distended on the mature aspect of the apparatus, and secretory granules, which are also found in apical areas of the cell and in the odontoblast process, are seen in the nearby cytoplasm. These sites are probably associated with exocytosis of the secretory granules. The terminal web, comprising transverse microfibrils, morphologically separates the cell body from the process, which has fewer cytologic features,

reflecting its secretory role.[30]

Use of radiolabeled proline and autoradiography has shown that the pathway of collagen synthesis and secretion is typical of most connective tissue cells. The radiolabel appeared first in the rough endoplasmic reticulum, then in the Golgi apparatus, and finally in the presecretory and secretory granules.[31,32] The label appeared in the predentin within 4 hours in the rat, presumably by exocytosis, but was not observed in the dentin until nearly a day after pulse labeling. Similar pathways are responsible for secretion of the other matrix components of dentin, including phosphoproteins, glycoproteins, and proteoglycans, although these demonstrate much more rapid incorporation—of the order of minutes rather than days.[33,34] This highlights possible differences in the control of secretion of the various components of dentin matrix, although much more needs to be learned about the control of odontoblast secretion.

The concept of two levels of secretion from the odontoblast has been proposed by Linde.[35] The major level of secretion is envisioned as being at the proximal end of the odontoblast cell body to form a matrix comprising collagen and proteoglycans, which reaches the advancing mineralization front after approximately 24 hours. The second, distal level of secretion is anticipated as being close to the mineralization front, where various tissue-specific noncollagenous matrix components, including phosphoproteins, are secreted (Fig 2-4). The latter components have been implicated in the mineralization process as nucleators for hydroxyapatite crystal formation,[36] and their secretion at this site could explain the mineralization of the collagenous predentin after a certain interval of time. Furthermore, this model might also explain the formation of peritubular dentin at this site, with its collagen-poor and noncollagenous protein–rich matrix. Although plausible, this model of dentin secretion is merely hypothetical at this time.

Complex matrix remodeling occurs during the transition of the matrix from predentin to dentin, particularly in the proteoglycans. The matrix metalloproteinases are a complex family of matrix-degrading enzymes, and their expression by odontoblasts[37] ,[38] and presence near the mineralization front is likely to be associated with the matrix remodeling taking place there. The involvement of this family of enzymes (especially matrix metalloproteinases 2, 9, and 20) in dentinogenesis,[39] which is just starting to be unraveled, should clarify the maturation changes in the matrix during secretion and the mechanisms of mineralization. These enzymes appear to be involved in the onset of dentin mineralization,[40] regulation of mineralization in mantle dentin,[41] and the processing of dentin sialophosphoprotein

during dentinogenesis.[42]

Dentin has traditionally been regarded as a relatively inert tissue that does not undergo tissue remodeling to the same degree as bone. However, there is some ultrastructural evidence to indicate limited endocytosis by the odontoblast,[43] although the functional significance of this event is still unclear. Furthermore, recently it has been reported that odontoblasts develop a conspicuous autophagic-lysosomal system for turnover and degradation of cellular components to maintain their functionality.[11] Nevertheless, what is clear is that the odontoblast maintains communication with deeper areas of the matrix through its process, which lies in the dentinal tubule. Numerous lateral branches from the processes permeate the dentin matrix (Fig 2-5), and these may connect with lateral branches of other odontoblasts. Interestingly, the three-dimensional canalicular network of odontoblasts shows striking similarities to that of osteocytes at the ultrastructural level[44] (Fig 2-6). This level of communication between the cell and its matrix suggests that dentin may not be as inert as traditionally believed.

The S-shaped primary curvature of the dentinal tubules in the crown is an effect of the crowding of odontoblasts as they move toward the center of the pulp (Fig 2-7). The result is a greater tubular density nearer to the pulp, but it also has consequences for the pulpal region itself, which directly communicates with the outer surface of the dentin.

The mineralization of dentin requires the transfer of considerable quantities of mineral ions from the serum to the extracellular sites, where they are deposited as hydroxyapatite crystals. The subodontoblastic capillary plexus is well located for this transfer, although the transport of ions and the necessary regulation have to be clarified. While the odontoblastic layer represents a relatively impermeable barrier, Nagai and Frank[45] found some evidence to suggest that calcium passes through the interodontoblastic space as well as through the odontoblast, accumulating in the Golgi apparatus and mitochondria but not in secretory vacuoles. High concentrations of calcium at the distal secretory pole of odontoblasts have been demonstrated by electron probe analysis,[46] supporting the concept of the intraodontoblastic route of calcium transport. A calcium transport system in which ions become associated with matrix components as they are synthesized and secreted could have energetic advantages for the cell as well as provide a means of regulation. However, a central role for the odontoblasts, in which calcium ions are transported through the cells themselves by different transmembranous ion–transporting mechanisms, has become apparent.[47,48]

The possible nucleation of hydroxyapatite crystals on components of the organic matrix of dentin has long been suggested. The anionic nature of many of the matrix components has led to their implication in such a role,[36] but in vivo data implicating any single component are lacking. It is generally accepted that heterogenous nucleation on organic matrix components is responsible for mineralization of circumpulpal dentin after mantle dentin formation and that the globular appearance of the mineralization front arises from the fusion of calcospherites (Fig 2-8). During mantle dentin formation at the initiation of dentinogenesis, mineralization is achieved through the mediation of matrix vesicles.[49] These are small, membrane-bound vesicles rich in adenosine triphosphatase and phosphohydrolytic enzymes that arise by budding off from the odontoblast. The matrix vesicles are capable of concentrating mineral ions to overcome the solubility product to allow calcium phosphate crystal precipitation. These vesicles are present in the earliest mantle dentin matrix, adjacent to the large coarse fibrils of collagen lying perpendicular to the site of the dental basement membrane (Fig 2-9), but they are absent from the matrix subsequent to mantle dentin formation.

The need for an alternative mechanism of mineralization during mantle dentin formation may relate to the fact that odontoblasts are still completing their terminal differentiation at this stage and may not be able to fully exhibit the odontoblast phenotype in terms of expression of dentin-specific matrix components. However, as soon as mantle dentin formation is completed and the odontoblasts are visible as a discrete, tightly packed layer of cells, mineralization proceeds in association with the extracellular matrix, and matrix vesicles can no longer be observed.

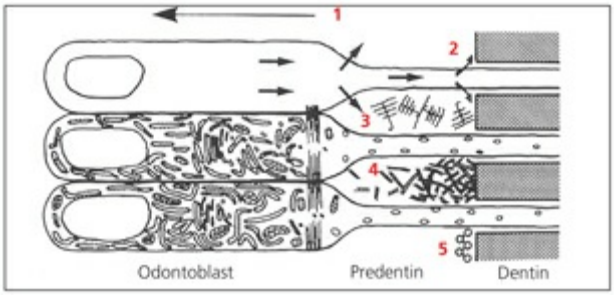

Fig 2-4 Dentinogenically active odontoblasts with two proposed levels of secretion. Secretion of collagen (1) and proteoglycans (3) occurs at the proximal level and accumulates in the predentin (4), while secretion of noncollagenous components, including phosphoproteins, γ-carboxyglutamate–containing proteins, and proteoglycans (5), occurs at the distal level (2) just prior to the mineralization front. (Reprinted from Linde[35] with permission.)

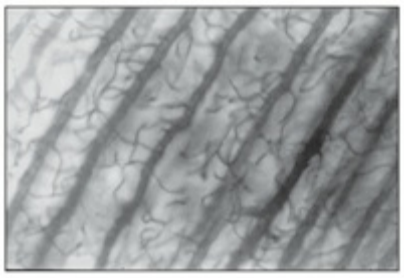

Fig 2-5 Odontoblast processes in human dentin with numerous lateral branches (original magnification ×2,000).

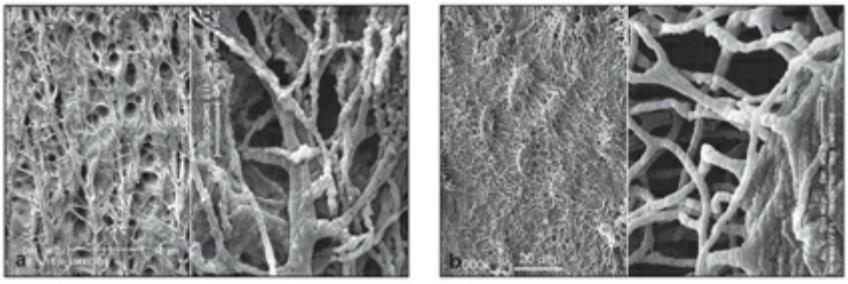

Fig 2-6 Low- *(left)* and high-power *(right)* scanning electron microscopic images of the odontoblast tubular network and osteocyte lacunocanicular network in samples of dentin *(a)* and mandibular bone *(b)*, illustrating the similarities in these networks. (Reprinted from Lu et al[44] with permission.)

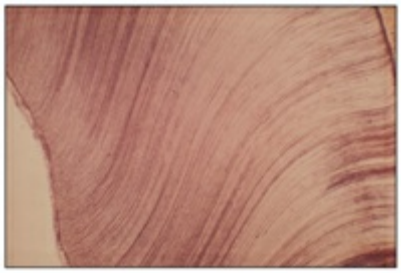

Fig 2-7 S-shaped primary curvature of the dentinal tubules in human crown dentin (hematoxylin-eosin [H&E] stain; original magnification ×80).

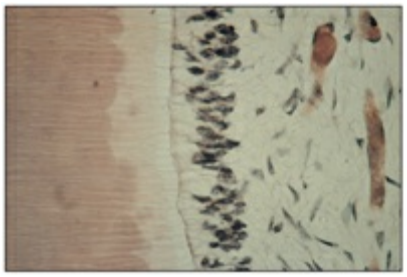

Fig 2-8 Globular appearance of the mineralization front in dentin arising from fusion of globules of hydroxyapatite or calcospherites (H&E stain; original magnification ×400).

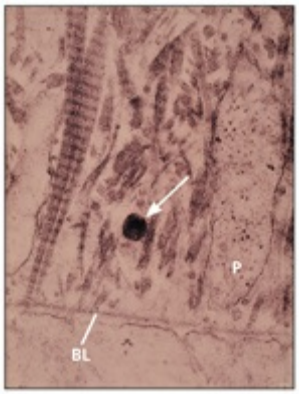

Fig 2-9 Matrix vesicle *(arrow)* adjacent to an odontoblast process (P) and beside the dental basement membrane (BL) in the early mantle dentin matrix. Large, coarse fibrils of collagen showing a typical striated appearance can be observed perpendicular to the basement membrane. (Reprinted from Eisenmann and Glick[49] with permission.)

Primary Dentinogenesis

When mantle dentin formation is completed and the odontoblasts eliminate the extracellular compartment between them to form a tightly packed layer of cells, the matrix of dentin is produced exclusively by the odontoblasts. Elaboration of this matrix involves secretion of collagen fibrils that are smaller than are those in mantle dentin and associated non-collagenous organic matrix or ground substance. The odontoblasts lie on the formative surface of this matrix and move pulpally as the matrix is secreted, leaving a single cytoplasmic process embedded within a dentinal tubule in the matrix.

These tubules, which increase in density nearer to the pulp, confer the property of permeability on the dentin. The gradient of tubular density as the dentin is traversed has clinical implications related to the depth of cavity preparation and tissue permeability[50] (Fig 2-10). This intertubular dentin matrix comprises the bulk of the circumpulpal dentin.

As this matrix forms, a matrix with a rather different composition, known as the *peritubular dentin matrix*, is secreted around the tubule perimeter. Peritubular dentin is more highly mineralized than the intertubular dentin matrix, contains few collagen fibrils, and is rich in noncollagenous matrix components (Fig 2-11). Its continued deposition throughout primary dentinogenesis leads to regional differences in its thickness through the dentin matrix.

The dentinal tubules are tapered structures because of peritubular dentin

formation, and they vary in diameter from approximately 2.5 mm near the pulp to 0.9 mm near the dentinoenamel junction. Complete occlusion of dentinal tubules may occur. The translucent appearance of areas of matrix containing such tubules has been described as *sclerotic dentin*, and its presence appears to be age related. Sclerotic dentin shows a preferential distribution in the apical third of the root, the crown midway between the pulpal and outer surfaces of the tooth, and on the pulp surface of the dentin (Fig 2-12; see also chapter 18).

The derivation of this sclerotic dentin may be varied. Its presence in root dentin of adolescent premolars in the absence of any external influence is suggestive of a physiologic response involving continued secretion of peritubular dentin. However, it might also arise from deposition of mineral within the tubule in the absence of peritubular dentin formation, from diffuse calcification within a viable process, or from calcification of both the process and the tubular contents.[12] Whatever the derivation, the presence of sclerosis will reduce the permeability of dentin and has obvious clinical significance in terms of dentinal sensitivity and the potential transport of irritants along the tubules. The apparently differing rates of peritubular and intertubular dentin secretion after completion of crown and root formation suggest that the secretory processes for these two matrix compartments may be under separate regulatory control.

The secretion of dentin occurs rhythmically, showing alternate phases of activity and quiescence, leading to formation of incremental growth lines in dentin perpendicular to the dentinal tubules. Both daily and 5-day rhythmic patterns of incremental lines, the latter showing a 20-µm periodicity, can be observed in dentin, although there has been confusion over the nomenclature associated with these lines.

Control of this rhythmic dentin deposition has been suggested to be associated with the circadian rhythmic activity of peripheral adrenergic neurons that produce variations in blood flow to the odontoblasts.[53] However, this explanation does not correspond with the different periodicity observed for these lines in the crown and root of the tooth. The rate of dentin deposition is slower in the root than in the crown, and yet circadian rhythms are likely to influence cell secretion similarly in both areas.

This raises a critical question: What mechanisms control odontoblast secretion? Odontoblast secretion proceeds rapidly throughout primary dentin formation (with differences in rate between the crown and the root of the tooth) with a clear blueprint for both the crown and the root. Once these parts of the tooth have been completed, the rate of secretion abruptly decreases. Does the odontoblast have to be continually stimulated for secretion of the primary dentin matrix, or is it in its normal

state as an actively secreting cell whose activity has to be downregulated by some signaling block to arrest secretion as secondary dentinogenesis is initiated? Clarification of the nature of these control mechanisms is also fundamental to an understanding of the factors controlling tertiary dentin secretion during repair after injury to the pulpodentin complex.

The control mechanisms for physiologic dentin secretion remain elusive, but various growth factors, hormones, and transcription factors have been implicated in the regulation of odontoblast secretory activity (reviewed by Smith and Lesot[54]). Identification of various growth factors, particularly the transforming growth factor β (TGF-β) family, and signal transduction pathways provides some clues as to how odontoblast secretion may be regulated. Both paracrine and autocrine control of expression of these growth factors may have a strong regulatory effect on odontoblast secretion.

Although such signaling molecules may be capable of regulating odontoblast secretion, it is unclear what determines their control to upregulate and downregulate specific phases of dentinogenesis. Modulation of their activity by extracellular matrix molecules[55] perhaps involves a "chicken and egg" situation, whereby growth factors can influence extracellular matrix secretion, and extracellular matrix molecules in turn modulate growth factor activity. Transcriptional control of growth factor expression may also provide a key mode of regulation. Over 500 genes are differentially regulated in odontoblasts involved in primary and secondary dentinogenesis, and these changes in the odontoblast transcriptome are associated with activation of the p38 MAP kinase pathway.[56] Upregulation of p38 expression and activation of p38 protein are seen when odontoblast-like cells are stimulated, such as during tertiary dentinogenesis.[57] Regulation of the MAPK pathway by TGF-β1[58,59] provides some clues as to how odontoblast secretion may be regulated.

Some form of preprogramming of odontoblasts to regulate their secretory activity cannot be excluded. Programming is a feature of cell death or apoptosis, and it appears to occur to some extent in odontoblasts,[60] although to a lesser degree than in most tissues. An immortalized odontoblast-like cell line was observed to undergo apoptosis in a dose-dependent manner in response to TGF-β1,[61] highlighting the delicate balance between odontoblast behavior and interaction with molecules of its extracellular matrix. However, the ability to upregulate odontoblast secretion during tertiary dentinogenesis suggests that, for the majority of the primary odontoblast population, local cellular signaling mechanisms can override any preprogramming of cellular secretory activity. Clearly, the physiologic regulation of odontoblast

secretion will provide many challenges to researchers and will represent a key topic of study for the future.

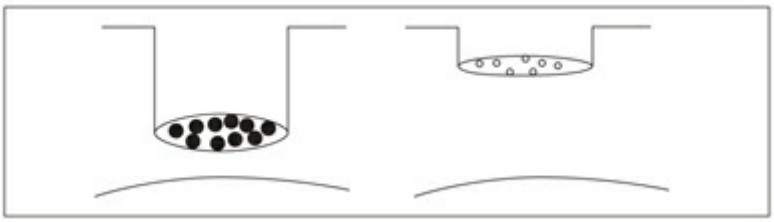

Fig 2-10 Differences in tubular density of the floor of deep *(left)* and shallow *(right)* cavities prepared in dentin.

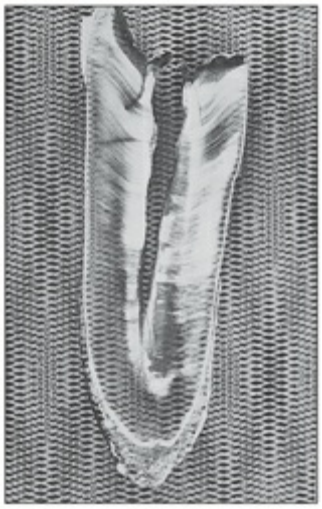

Fig 2-11 Scanning electron microscopic appearance of dentin showing the dentinal tubules cut in cross section. Each tubule has a surrounding collar of peritubular dentin matrix, which has a homogenous nonfibrillar appearance. The fibrillar, collagenous matrix of the intertubular dentin contrasts in appearance and composition with that of the peritubular dentin. (Reprinted from Scott et al[51] with permission.)

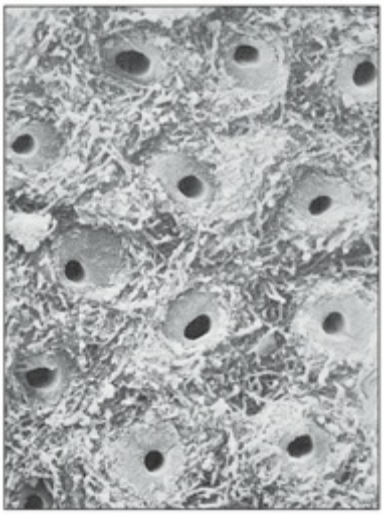

Fig 2-12 Apical sclerotic dentin in a ground section of an old tooth. The translucent appearance of the

dentin allows visualization of the mesh pattern underlying it. (Reprinted from Ten Cate[52] with permission.)

Physiologic Secondary Dentinogenesis

Physiologic secondary dentinogenesis represents the slower-paced deposition of dentin matrix that continues after completion of the crown and root and spans a lifetime. While secondary dentin is deposited all around the periphery of the tooth, its distribution is asymmetric, with greater amounts on the floor and roof of the pulp chamber. This leads to pulpal recession, the extent of which will depend on the age of the individual. Thus, secondary dentinogenesis may potentially increase the difficulty of endodontic procedures.

Historically, there has been considerable confusion over what constitutes *physiologic secondary dentin* and over use of the term *secondary dentin* to describe tertiary dentin formed in response to an external influence. Differences between the composition of glycosaminoglycans and that of other glyco-conjugates for secondary dentin have been reported on the basis of histochemical stains,[62] although it is unclear whether these differences can be ascribed to true physiologic secondary dentin.

The tubules of the secondary dentin matrix are largely continuous with those of the primary dentin, suggesting that the same odontoblasts are responsible for primary and secondary dentin secretion. However, downregulation of the secretory activity of these cells means that secondary dentin is deposited relatively slowly.

The wide-ranging rates of secondary dentin deposition (from less than 1 to 16 μm per day) that have been reported perhaps reflect in part the unclear identification of secondary and tertiary dentins. However, early studies from Hoffman and Schour[63] in the rat molar provide insight into both the dynamics and the gradients of secondary dentin secretion. In the pulp horns, a daily rate of 16 μm observed at 35 to 45 days had declined to 1.25 μm per day at 500 days. On the roof and floor of the pulp cavity, the rate was 2.5 μm per day at 125 to 135 days and declined to 0.69 μm at 500 days. Thus, the rate of formation varies in different areas of the tooth and appears to slow with age to a rate of only about 8% to 25% of that observed at a younger age. However, if the greater rate of secretion in the pulp horns reflects a response to attrition, at least in part, then some of this secretion represents strictly tertiary dentinogenesis. Because of this ambiguity, terminology describing the

different phases of dentinogenesis should be used only as a means of understanding the processes taking place and not as an inflexible system of classification.

Tertiary Dentinogenesis

To overcome the plethora of confusing terms used to describe the focal secretion of dentin in response to external influences—including dental caries, tooth wear, trauma, and other tissue injury—Kuttler1 proposed the concept of tertiary dentin formation. Tertiary dentin encompasses a broad spectrum of responses, ranging from secretion of a regular, tubular matrix that differs little from primary and secondary dentin to secretion of a very dysplastic matrix that may even be atubular. The cellular and molecular processes responsible for this spectrum of responses also may show a number of differences.

Tertiary dentin has been subclassified as either reactionary or reparative[64,65] as a means of distinguishing the different sequences of biologic events taking place in situations of milder and stronger external stimuli responsible for initiation of the response (Fig 2-13) . *Reactionary dentin* is defined as a tertiary dentin matrix secreted by surviving postmitotic odontoblast cells in response to an appropriate stimulus. Typically, such a response will be made to milder stimuli and represents upregulation of the secretory activity of the existing odontoblast responsible for primary dentin secretion.

In contrast, *reparative dentin* is defined as a tertiary dentin matrix secreted by a new generation of odontoblast-like cells in response to an appropriate stimulus after the death of the original postmitotic odontoblasts responsible for primary and physiologic secondary dentin secretion. Such a response will normally be made to stronger stimuli and represents a much more complex sequence of biologic processes. Reparative dentin actually encompasses a broad range of responses, some of which appear to be relatively specific while others are classified as tertiary dentinogenesis only because they occur in the pulpodentin complex.

It is appropriate to consider these two variants of tertiary dentinogenesis individually in view of the diversity of the biologic processes taking place (Fig 2-14), although it must be recognized that reparative dentinogenesis will often be a sequel to reactionary dentinogenesis and that both variants may be observed within the same lesion.

Regardless of the subclassification, tertiary dentinogenesis represents a regenerative or wound-healing response, and, as in many tissues in the body, this response will be influenced by the local tissue environment. Pulpal inflammation represents a defense response to an injury, which in the case of caries is often invoked by bacteria.[66] The inflammatory processes are considered elsewhere in this book (see chapters 4, 10, and 11), but their influence on tissue regeneration should not be underestimated.

A transient inflammatory response is beneficial in terms of clearing foreign antigens and creating a conducive tissue environment in which regeneration can take place. However, a sustained inflammatory response can act to block regeneration. Bone morphogenetic protein 7–induced dental regeneration was completely inhibited when inflammatory reactions were invoked with lipopolysaccharide.[67] It is therefore crucial to eliminate bacteria and inflammation in order to create a tissue environment that is conducive to regeneration. The topic of regenerative endodontics is covered in detail in chapter 5.

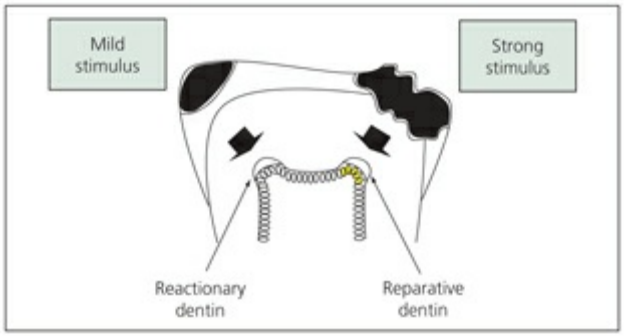

Fig 2-13 Reactionary (secreted by surviving postmitotic primary odontoblasts) and reparative (secreted by a new generation of odontoblast-like cells after death of the primary odontoblasts) variants of tertiary dentinogenesis. (Reprinted from Smith et al[65] with permission.)

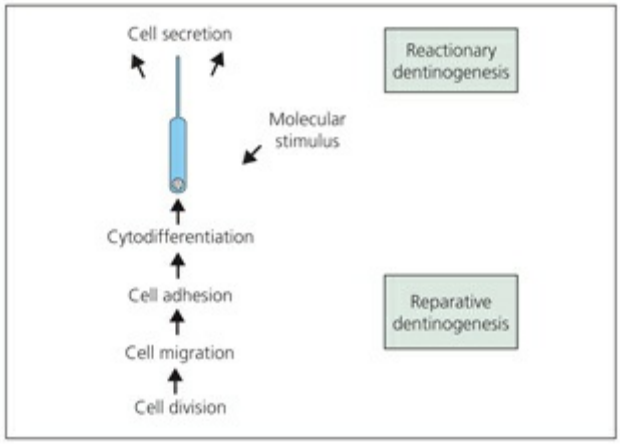

Fig 2-14 Biologic processes taking place during reactionary and reparative dentinogenesis. (Reprinted from Smith et al[65] with permission.)

Reactionary dentinogenesis

Reactionary dentin is, by definition, secreted by surviving primary odontoblasts. Therefore, to determine cell survival, its identification requires chronologic information on the postinjury events within the pulpodentin complex. While such information is often lacking in histologic studies of pulpal responses following dental injury, the presence of tubular continuity between physiologic secondary and tertiary dentin matrices has been suggested as characteristic of this response.[68]

Upregulation of matrix secretion

The biologic processes responsible for reactionary dentinogenesis represent focal upregulation of the secretory activity of the surviving odontoblasts. As such, this response might be considered an extension of the physiologic behavior of these odontoblasts, and the rationale for identification of a tertiary dentinogenic response would be the nature of the initiating stimulus (ie, injury to the tissues). This distinction is probably important in that control of upregulation of secretion is determined by the stimulus and may show differences to physiologic regulation of cell secretory behavior. The intensity of the response will reflect both the degree and the duration of the stimulus, although the extent of the response is limited to those cells in direct tubular communication with the initiating stimulus. Thus, beneath a cavity preparation, the reactionary dentinogenic response is generally limited to those areas where the dentinal tubules communicate with the cavity. In unetched preparations, variable plugging of the tubules may lead to differential stimulation of individual odontoblasts beneath the preparation and an irregular interface between the reactionary dentin and odontoblasts, possibly with fingerlike projections of matrix (Fig 2-15).

The molecular basis of odontoblast upregulation during reactionary dentinogenesis has only recently received much attention. Traditionally, it has been suggested that “irritation” from bacterial products of plaque during caries or leaching of components from restorative materials beneath preparations may be responsible for the stimulus. However, these hypotheses have never really identified the molecular signaling processes responsible for cellular upregulation. An in vivo study in which isolated dentin matrix components were implanted in the base of unexposed cavities that had been carefully prepared in ferret teeth to ensure primary odontoblast survival has shown that bioactive molecules in these isolated matrix

preparations are capable of stimulating reactionary dentinogenesis.[69] These findings indicate that the signaling molecules for reactionary dentinogenesis may be derived from and released by diffusion of the injurious agent through the dentin matrix.

Partial purification of the isolated matrix preparations to enrich their growth factor content, particularly of the TGF-β family, has implicated these molecules in the cellular signaling responsible for reactionary dentinogenesis.[65] Direct application of the TGF-β1 and TGF-β3 isoforms to the odontoblastic layer on agarose beads in cultured tooth slices has demonstrated their ability to upregulate odontoblast secretion.[70] Similar findings were made after application of a solution of TGF-β1 to cultured tooth slices using small polymethyl methacrylate tubes glued to the dentin matrix that allowed diffusion of the growth factor through the dentinal tubules.[71]

TGF-β1, TGF-β2, and TGF-β3 isoforms are expressed by odontoblasts,[72] and TGF-β1 becomes sequestered within the dentin matrix.[73] Thus, considerable endogenous tissue pools of this growth factor are found within the dentin matrix, and these are available for release if the matrix is solubilized or degraded. Other bioactive molecules within the extracellular matrix of dentin, including adrenomedullin[74] ,[75] and matrix molecules,[14,76–79] may also be released after tissue injury and contribute to cell signaling for regenerative events.

In the presence of caries, bacterial acids from plaque that diffuse through and demineralize the dentin matrix might be expected to solubilize some of this tissue pool of growth factors and other bioactive molecules. During cavity preparation, the use of etchants or cavity conditioning agents may also release these molecules. A number of commonly used etchants have been found to solubilize TGF-β1 and various noncollagenous matrix components from dentin, the most effective of these being ethylenediaminetetraacetic acid (EDTA) (Smith and Smith, unpublished data, 1998).

Restorative materials may also stimulate reactionary dentinogenesis through similar mechanisms. Calcium hydroxide can solubilize TGF-β1 and non-collagenous matrix components from dentin, and these solubilized molecules have been demonstrated to modulate gene expression in odontoblast-like cells.[80] This provides new insight into the mechanisms of action of this widely used material and offers a rational explanation for its effects on dentin regeneration. Mineral trioxide aggregate appears to have similar properties,[74] and subtle differences in the profiles of dentin matrix components released by these two pulp capping agents may contribute to their respective clinical effects. Thus, a variety of factors associated with both the injury

process to the tissue and its subsequent restoration may contribute to defense reactions of repair.

A role for the TGF-βs in tertiary dentinogenesis is supported by studies with TGF-β1 (–/–) mice, in which there appears to be decreased "secondary" (tertiary) dentin formation.[81] Although release of TGF-β1 from the dentin matrix may provide an explanation for the cellular signaling of reactionary dentinogenesis, other growth factors are also sequestrated within the dentin matrix. Insulin-like growth factors I and II,[82] bone morphogenetic proteins,[83] and a number of angiogenic growth factors[84] have been reported in dentin matrix. This diverse group of growth factors provides a powerful cocktail of bioactive molecules that may be released and participate in cellular signaling during injury and repair to the pulpodentin complex. The presence of angiogenic growth factors in dentin derived from the odontoblasts, their expression by pulp fibroblasts,[26] and the proangiogenic effects of dentin matrix preparations[85] may explain the stimulation of angiogenesis at sites of tertiary dentin formation and after ectopic transplantation,[28] but the range of cellular effects arising from release of some of the other growth factors remains to be elucidated. The concept of dentin as an inert tissue must therefore be questioned, and a variety of cellular effects arising from solubilization of its matrix remain to be identified.

Fig 2-15 Reactionary dentinogenic response beneath an unetched and unexposed cavity *(top)* prepared in a ferret canine tooth in which a lyophilized preparation of isolated dentin matrix proteins have been implanted. Reactionary dentin secretion is restricted to that area in which the dentinal tubules are in direct communication with the cavity. Note the fingerlike projections of reactionary dentin matrix *(arrows)* arising from differential stimulation of individual odontoblasts beneath the preparation.

Factors influencing reactionary dentinogenesis

A tertiary dentinogenic response beneath a caries lesion is easily recognized.[86] A reactionary response is often associated with small, slowly progressing lesions,[87] whereas in more active lesions death of the primary odontoblasts is more likely to

occur, and reparative dentinogenesis will occur if the prevailing tissue conditions allow. However, even in more slowly progressing lesions, the response may be a mixture of reactionary and reparative dentinogenesis.[87] Thus, the activity of a caries lesion will have a strong influence on the nature of the tertiary dentinogenic response.

Various factors associated with cavity preparation and restoration can influence the tertiary dentinogenic response: the method of cavity preparation, dimensions of the cavity, remaining dentinal thickness (RDT) of the cavity, etching of the cavity, and the nature of the dental materials used and method of their application for the restoration. Many studies have described the pulpal changes in response to these various factors, and the consensus is that events during cavity restoration can influence the underlying pulp cell populations to a degree that is proportionally greater than the differences in cytotoxicity of cavity restorative materials themselves[88–91] (see chapter 14).

This finding highlights the need for careful control of cavity preparation conditions in studies of pulpal responses. Use of animal models[89] for assessment of pulpal responses to restorative factors can allow more reproducible control of these factors, while in vitro organ culture approaches offer opportunities to examine some of these factors in the absence of inflammation and bacteria.[92] Nevertheless, it is still important to assess human dental responses to these factors to overcome possible species variations and to attempt to quantify their relative importance. The complex interplay between the various factors makes it difficult to unravel their involvement during different phases of the injury and repair responses. However, the interplay between inflammation and regeneration may be critical to our understanding of the interactions between injury and repair events.[93]

A histomorphometric evaluation of reactionary dentinogenesis beneath standardized cavity preparations was performed in a relatively young population of human teeth, and subsequent statistical analysis showed a strong correlation between reactionary dentin secretion and RDT, patient age, cavity floor surface area, and restoration width.[90] RDT was apparently the most significant factor determining the secretion of reactionary dentin, which is increased in area by 1.187 mm^2 for every 1-mm decrease in the RDT beneath the cavity. Increases in reactionary dentin area were also correlated with increases in the dimensions of other cavity preparation variables. A weaker correlation was observed between choice of restorative material and reactionary dentinogenesis; however, zinc oxide–eugenol appeared to have no effect on reactionary dentinogenesis compared with calcium hydroxide and

amalgam restorations.

Similar observations on the quantitative relationship between RDT and reactionary dentinogenesis were made in a larger study of 217 human teeth.[90] Reactionary dentin secretion was observed beneath cavities with an RDT greater than 0.50 mm or less than 0.25 mm; however, maximum reactionary dentinogenesis (approximately fourfold greater) was observed beneath cavities with an RDT between 0.50 and 0.25 mm. Reduced reactionary dentin secretion beneath cavities with an RDT of less than 0.25 mm appeared to be associated with reduced odontoblast survival, presumably as a result of irreversible cell damage during cavity preparation.

The choice of restorative material influenced reactionary dentin secretion, as well as odontoblast survival, to a significant degree but to a lesser extent than did RDT. In terms of their influence on reactionary dentinogenesis, calcium hydroxide had the greatest influence, followed by composite resin, resin-modified glass ionomer, and zinc oxide–eugenol. This ranking reflected a combination of the effects of the materials on odontoblast cell survival and stimulation of reactionary dentinogenesis.[91]

These findings indicate how the various restorative factors influence reactionary dentinogenesis on a mechanistic basis. RDT after cavity cutting has the potential to influence odontoblast cell survival: In deep cavities (RDT less than 0.25 mm), little more than 50% odontoblast survival may be seen,[91] whereas in shallower cavities odontoblast survival is about 85% or greater, and, despite the likely cutting of the odontoblast process, the cells respond by secretion of reactionary dentin. Although little is known about how the cell responds to cutting of its process, it is generally assumed that such breaks in the membrane integrity are soon restored. Receptors to TGF-βs have been demonstrated on odontoblasts.[94] Receptors to other growth factors may also be present, and therefore localization studies are required. In shallower cavities, the amount of reactionary dentin that is secreted can be correlated with the RDT, which suggests that the distance of diffusion of cell-signaling molecules is a determining factor.

Cavity etching can positively influence the secretion of reactionary dentin. Treatment with EDTA for 0 seconds, 60 seconds, and 120 seconds led to a ranking of 60 seconds > 120 seconds > 0 seconds for reactionary dentin secretion.[95] Reduced reactionary dentinogenesis after 0 or 120 seconds of treatment was associated with decreased odontoblast survival. The stimulatory effect of EDTA treatment on reactionary dentinogenesis might be ascribed to the ability of this

chemical to release growth factors from the dentin matrix during its solubilizing action; these growth factors could then diffuse down the dentinal tubules and bind to receptors on the odontoblasts for signaling of a reactionary dentinogenic response.

Other etchants such as phosphoric acid have a less stimulatory effect on reactionary dentinogenesis, perhaps reflecting their less effective action in solubilizing matrix-bound growth factors. Such a sequence for cellular signaling of reactionary dentinogenesis would be expected to be dependent on the distance of diffusion of the growth factor molecules, and this would be in accord with the observations on the importance of RDT to the amount of reactionary dentin secreted. Restorative materials that are capable of stimulating reactionary dentinogenesis, such as calcium hydroxide, may have similar actions on the dentin matrix–releasing growth factors, which then diffuse to the odontoblasts and prompt their upregulation.

Clearly, complex events take place during cavity preparation and restoration, involving the interplay of many factors, but odontoblast cell survival and release of endogenous matrix-bound pools of growth factors and other bioactive molecules in the tooth may be critical to the signaling of reactionary dentinogenesis.

Reparative dentinogenesis

In unexposed pulps, reparative dentinogenesis may be a sequel to reactionary dentinogenesis (Fig 2-16a), or it may occur independently in the absence of reactionary dentin if the injury is of sufficient intensity (eg, an active caries lesion). The reparative response of tertiary dentinogenesis will always take place at sites of pulpal exposure because of the loss of odontoblasts and the need for dentin bridge formation. Reparative dentinogenesis involves a much more complex sequence of biologic events than reactionary dentinogenesis in that progenitor cells from the pulp must be recruited and induced to differentiate into odontoblast-like cells before their secretion may be upregulated to form the reparative dentin matrix (see Fig 2-14).

The matrices secreted during reparative dentinogenesis show a broad spectrum of appearances, ranging from a regular, tubular matrix to a very dysplastic, atubular matrix sometimes with cellular inclusions present (Fig 2-16b). This heterogeneity in matrix morphology is often paralleled in the morphology and secretory behavior of the odontoblast-like cells responsible for its secretion, leading to considerable variations in the structure and composition of matrices (Fig 2-16c).

Although all of these responses may generally be categorized as reparative dentinogenesis, they nonetheless show considerable heterogeneity. This heterogeneity may reflect the specificity of the dentinogenic processes taking place. These processes may resemble physiologic dentinogenesis or, instead, may represent nonspecific matrix secretion. Nonspecific secretion can occur from cells with few phenotypic characteristics of odontoblast-like cells and is believed to represent part of a more generalized wound-healing response.

The differences in tubularity observed in a reparative dentin matrix will have consequences for the permeability of the matrix and thus its capacity to provide pulpal protection from the possible effects of bacteria and restorative materials (Fig 2-16d). Maintenance of the tubular physiologic structure of dentin may be a sensible goal in tissue regeneration generally, but it must be considered in relation to the tissue environment created by a restoration. Where there is a need to provide pulpal protection from the effects of bacterial microleakage and components of restorative materials, the presence of a tubular, reparative matrix will increase permeability and therefore may be disadvantageous. Thus, as novel clinical regenerative therapies emerge, these could exploit the current understanding of cell behavior to direct secretion of tubular or atubular dentin matrices according to the desired permeability properties for a particular clinical application.

Reparative dentinogenesis is often preceded by secretion of a fibrodentin matrix[23] that is atubular and associated with rather cuboidal cells with poorly developed organelles on its formative surface. Deposition of tubular matrix by polarized cells is then observed later on the surface of this fibrodentin. Whether fibrodentin deposition represents a specific dentinogenic response or a nonspecific connective tissue wound-healing response is uncertain. Data on the molecular phenotype of these cells are not available, but the morphology of the cells responsible for fibrodentin synthesis and secretion point to a relatively nonspecific response. Nevertheless, this fibrodentin may play an important role in the signaling of true reparative dentinogenesis by providing a substrate on which signaling molecules for odontoblast-like cell differentiation may become immobilized. In this way, it could mimic the role of the dental basement membrane for odontoblast differentiation during tooth development, where it has been suggested that growth factors derived from the inner dental epithelium become temporospatially immobilized on the basement membrane for presentation to the preodontoblasts to signal their terminal differentiation.[96]

It has been suggested that tertiary dentinogenesis replicates embryonic events leading to tooth development; indeed, the two processes have many common

features.[54] The need for progenitor cell recruitment, induction of differentiation, and upregulation of secretory activity are common to both processes (Fig 2-17), although the absence of physiologic regulation of the biologic events during repair may lead to more diversity in the cellular secretions. The three critical steps for reparative dentinogenesis are *(1)* recruitment of progenitor cells, *(2)* signaling of odontoblast-like cell differentiation, and *(3)* subsequent upregulation of matrix secretion by these cells. While upregulation of matrix secretion is common to reactionary dentinogenesis, the first two steps distinguish the process of reparative dentinogenesis.

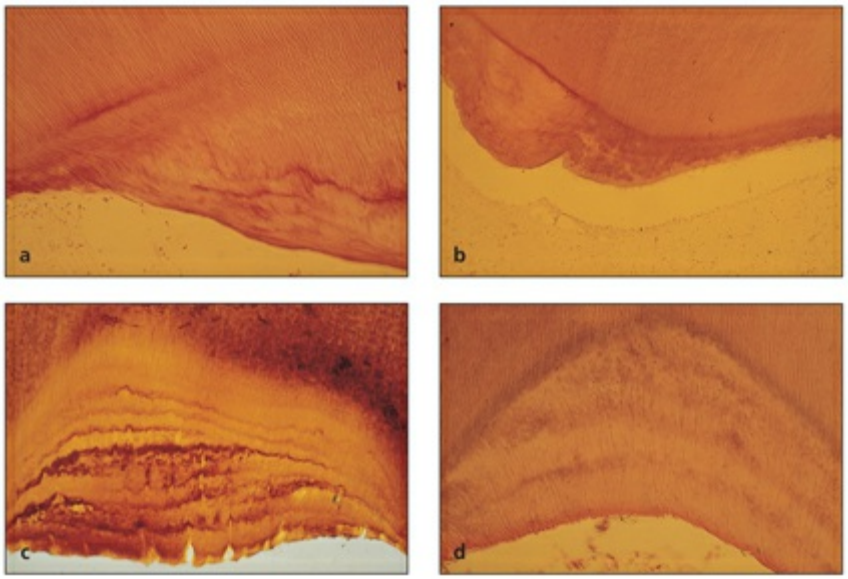

Fig 2-16 Tertiary dentinogenic responses beneath caries lesions in human teeth. *(a)* An initial reactionary dentinogenic response adjacent to the physiologic dentin with a subsequent reparative dentinogenic response, the matrix of which shows heterogeneity in its tubularity (periodic acid–Schiff stain; original magnification ×250). *(b)* Reparative dentin secreted beneath and demarcated from physiologic dentin by a darker-staining calciotraumatic line underlying a caries lesion in a human tooth. Note the considerable heterogeneity in the reparative dentin ranging from a tubular *(right)* to an atubular *(left)* matrix (periodic acid–Schiff stain; original magnification ×100). *(c)* Reparative dentin matrix secreted beneath a caries lesion in a section of a human tooth stained with silver colloid to demonstrate phosphoproteins. Note the considerable heterogeneity in the staining intensity of these matrix components, reflecting variations in odontoblast-like cell behavior during secretion (original magnification ×250). *(d)* Reparative dentin matrix secreted beneath a caries lesion in a human tooth, showing a less tubular structure than the adjacent physiologic dentin, which will reduce the permeability of this tissue (H&E stain; original magnification ×250).

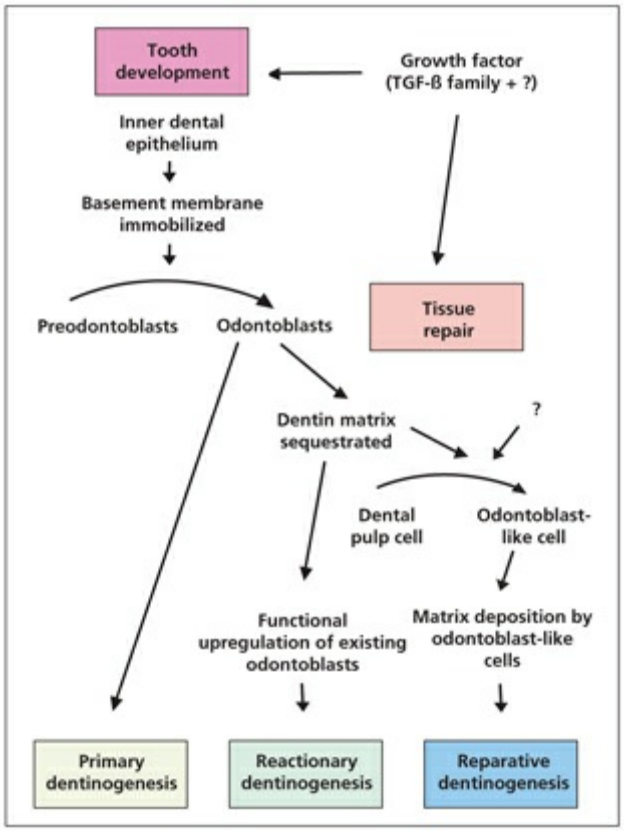

Fig 2-17 Comparison of events during tooth development and dental tissue repair, highlighting the many similarities among the processes taking place. (Reprinted from Smith and Lesot[54] with permission.)

Recruitment of progenitor cells

The derivation of the progenitor cells recruited for odontoblast-like cell differentiation during reparative dentinogenesis is still unclear.[97] However, the neural crest origin of the ectomesenchymal cells of the dental papilla giving rise to the odontoblasts is likely to be important to their phenotype. In the context of regeneration in the adult tooth, however, not all of the cells of the pulp share this embryonic derivation. During embryogenesis, neural crest cells migrate to the first branchial arch, where they associate with the local mesenchyme. With advancing development of the pulpodentin complex in the tooth, some of the cells of the central core of the pulp do not appear to be derived from the neural crest.[98] Thus, the mature pulp comprises cell populations of varied derivation.

Isolated pulp cells from adult human teeth show many similarities in their molecular phenotype to bone marrow stem cells but appear to show different behavior both in vitro and after transplantation to immunocompromised mice in vivo.[99] Establishment of dental pulp stem cell (DPSC) populations indicated the heterogenous nature of these cells with respect to their rate of odontogenesis.[100]

Importantly, these DPSCs have been reported to generate reparative dentin on the surface of human dentin after transplantation in vivo and differed in behavior from bone marrow stromal stem cells.[101] Thus, the pulpal derivation of these cells and/or the pulp tissue environment appears to confer specificity on their developmental potential.

The ability of isolated dentin matrix–derived protein extracts to induce in vitro differentiation of odontoblast-like cells from these DPSCs[102] and the modulation of their gene-expression profiles following differentiation[103] emphasize the importance of the pulpodentin tissue environment during regeneration. The perivascular niche of these DPSCs[104] raises intriguing questions as to whether these DPSCs derive from the pulp cell populations arising during development or if they migrate to the pulp through the vasculature postdevelopmentally and what their relationship is with mesenchymal stem cells from other sources. This may have implications for identification of potential cells for use in development of regenerative therapies. Interestingly, cells with dentinogenic potential have been isolated from exfoliated human primary teeth (SHED cells) and represent a readily accessible source.[105] Interestingly, these SHED cells appear to have the ability to differentiate into odontoblasts and endothelial cells when seeded on a PLA scaffold in tooth slices and implanted subcutaneously.[106]

One of the challenges facing attempts to engineer tissue or regenerate the pulpodentin complex will be selection of the appropriate stem and progenitor cell populations within pulp. Identification of suitable markers for these cells will facilitate their selection using approaches such as fluorescent-activated cell sorting and magnetic-activated cell sorting; low-affinity nerve growth factor receptor, a neural crest cell marker, has shown promise in this respect.[107–109] Cell selection may confer greater specificity on the regenerative process and allow optimization of the dentinogenic potential.

Traditionally, a variety of cell niches have been implicated in the differentiation of odontoblast-like cells during reparative dentinogenesis. The undifferentiated mesenchymal cells in the cell-rich zone of Höhl adjacent to the odontoblastic layer have been suggested as progenitors,[110] and they are attractive candidates because they have experienced a developmental history similar to that of the primary odontoblasts. During tooth development, preodontoblasts align themselves perpendicular to the dental basement membrane during the final mitotic division. One daughter cell is exposed to the epithelium-derived epigenetic signal for induction of odontoblast differentiation and the other is not[93] and is generally

assumed to join the cells in the cell-rich zone of Höhl instead. However, cells from elsewhere in the pulp have also been implicated as progenitors for the odontoblast-like cell. These include perivascular cells, undifferentiated mesenchymal cells, and fibroblasts.[111]

The relative contribution of any of these cell populations to progenitor recruitment for odontoblast-like cell differentiation is unclear, but the possibility suggests opportunities for heterogeneity in the nature of the response. Aging may influence the survival of some of these possible progenitor cell populations, and their different phenotypic characteristics may contribute to the specificity of the process if they are involved in reparative dentinogenesis. Thus, depending on the age of the patient and the survival of cells following injury to the pulpodentin complex, considerable heterogeneity may be seen in the cellular response.

Stem cells are pluripotent, and their presence in tissues can offer opportunities for regeneration in response to growth and differentiation signals. There are many genes that control the behavior and expression potential of stem cells, including the highly conserved Notch signaling pathway, which can enable equivalent precursor cells to adopt different cell potentials.[112] Such genetic control may be important in determining progenitor cell recruitment during reparative dentinogenesis. Reappearance of Notch in subodontoblastic cells during reparative responses to injury and in association with vascular structures in apical areas of the tooth root[113] may indicate that cells in these areas can contribute to reparative dentinogenesis. If this is the case, then both undifferentiated mesenchymal cells and pericytes may be progenitors of odontoblast-like cells. What has become clear is that no single stem or progenitor cell population is responsible for reparative dentinogenesis and that the heterogenous nature of such responses reflects in part the involvement of a variety of different cell populations in different circumstances.

Migration of progenitor cells in the pulp to the site of injury for reparative dentinogenesis requires an appropriate chemotactic attractant. Isolated human dentin matrix components have been found to be chemotactic in vitro to pulp cells showing characteristics of pericytes (Murray et al, unpublished data, 2001), and dissolution of dentin matrix at sites of injury could provide such a stimulus. Although the specific components in the matrix that are responsible for these effects remain to be identified, TGF-β1 is known to be chemotactic for fibroblasts, macrophages, neutrophils, and monocytes during dermal wound healing.[114] Attraction of inflammatory cells to the site of injury may further enhance the chemotaxis of other cells, including pulpal progenitor cells, because many will also produce TGF-βs and other growth factors. TGF-β1 has also been reported to be mitogenic for cells in

the subodontoblastic layer[71] and thus may stimulate both the migration of progenitor cells and their proliferation to expand the available population of progenitor cells.

Signaling of odontoblast-like cell differentiation

Following recruitment of progenitor cells to the site of injury, signaling of odontoblast-like cell differentiation has to be achieved before reparative dentin secretion can commence. While an epithelium-derived, temporospatially regulated epigenetic signal is responsible for induction of odontoblast differentiation during tooth development,[96] the absence of epithelium in the mature tooth requires an alternative derivation for this signal.

Dentin matrix can be autoinductive, and the various reports of its ability to induce odontoblast-like cell differentiation[115] concur with the histopathologic reports of matrix growth from dentin chips pushed into the pulp during cavity preparation. Implantation of isolated dentin matrix components, including fractions derived from both the soluble and the insoluble matrix compartments, in exposed cavities prepared in ferret teeth gave rise to a reparative dentinogenic response, with secretion of a regular tubular matrix by polarized, columnar odontoblast-like cells in the absence of a fibrodentin precursor matrix.[116] Culture of dissociated embryonic dental papillae with the same dentin matrix preparations induced a physiologic gradient of odontoblast differentiation, which could be inhibited by inclusion of antibodies to TGF-β1.117 Similar effects were observed when the dentin matrix components were substituted with recombinant TGF-β1 immobilized with heparin, and the odontoblast-like cells showed most of the molecular phenotypic characteristics of physiologic odontoblasts.[117,118] Thus, the epigenetic signaling of odontoblast differentiation by growth factors may be recapitulated during repair in adult tissues.

Experimental application of recombinant TGF-β1 to adult pulp tissue in dogs has confirmed the ability of this growth factor to signal odontoblast-like cell differentiation.[119] TGF-β3 may also exert similar effects because application of agarose beads soaked in this growth factor at sites of needle-punch injury in cultured tooth slices led to alignment of columnar odontoblast-like cells on the bead surface in a number of cultures.[70] Other members of the TGF-β family of growth factors, including the bone morphogenetic proteins, have also been implicated in signaling of odontoblast-like cell differentiation during reparative dentinogenesis.[120–123]

The aforementioned findings provide new opportunities to exploit endogenous pools of growth factors sequestered in dentin matrix (for example, with calcium

hydroxide and mineral trioxide aggregate[74,80]) and to develop novel biomaterials containing growth factors for use in pulp capping. Development of an alginate-based hydrogel containing TGF-β1 allowed initiation of de novo dentinogenesis with secretion of a tubular dentin matrix after application to a cut pulp surface.[124] Such biomimetic materials offer a novel and radically different approach to clinical dentistry in which biologically based therapies will emerge.

The presence of growth factors, particularly the TGF-βs, in both the soluble and insoluble tissue compartments of dentin matrix[73,84] provides opportunities for cellular interaction with these molecules in several ways. Release of growth factors from the soluble tissue compartment may arise from tissue demineralization by bacterial acids during caries. Further release of these molecules during cavity preparation and restoration may also arise from the action of cavity-etching agents and diffusion of components leached from restorative materials. Diffusion of these soluble growth factors to the pulp may provide a chemotactic attraction for progenitor cells to the injury site and a mitogenic stimulus to expand this cell population.

It is unclear whether the signaling of odontoblast-like cell differentiation may involve the soluble or insoluble tissue pools of these growth factors. However, if developmental events are mimicked, it seems likely that immobilized matrix-bound growth factors may provide the signal for differentiation. This tissue pool of growth factors appears to be masked by mineral and other matrix components and requires exposure before they can participate in signaling processes. Ultrastructural immunolabeling of untreated cut dentin surfaces for TGF-β showed absence of reactivity.[125] However, treatment of the cut surface with cavity etchants unmasked the TGF-β in the matrix to varying degrees, and different etchants demonstrated variable ability to expose these molecules (Fig 2-18). It is therefore apparent that conventional restorative procedures can act on the dental tissues in ways not previously appreciated and may contribute significantly to cellular events involved in repair after injury.

It is also important to recognize that a variety of cell-signaling molecules may be present in the extracellular milieu at sites of injury in the pulp. Although the greatest focus recently has been on TGF-βs derived from the dentin matrix, a cocktail of growth factors is present within this matrix as well as the plethora of matrix proteins with potential bioactive properties alluded to earlier. The effects of some of the individual molecules on pulp cells remain to be determined, and little attention has been paid to any synergistic effects that combinations of these molecules may have. Thus, it is possible that a variety of biologic effects arising from dentin matrix

dissolution may yet be identified.

Clearly, pulpal injury, cavity preparation, and restoration procedures may all influence these biologic effects through differential solubilization of various matrix-bound bioactive molecules. Injury events within the pulp may also directly give rise to bioactive molecules from cells, which may influence subsequent cellular events. Death of odontoblasts and other pulp cells may release intracellular contents at the site of injury. Inflammatory cells attracted to the site of injury will also give rise to cytokines and growth factors, which will modulate cellular events both directly and indirectly.[93] Thus, a complex interplay among signaling molecules in the pulp can be anticipated after injury, which may result in a range of responses (Fig 2-19).

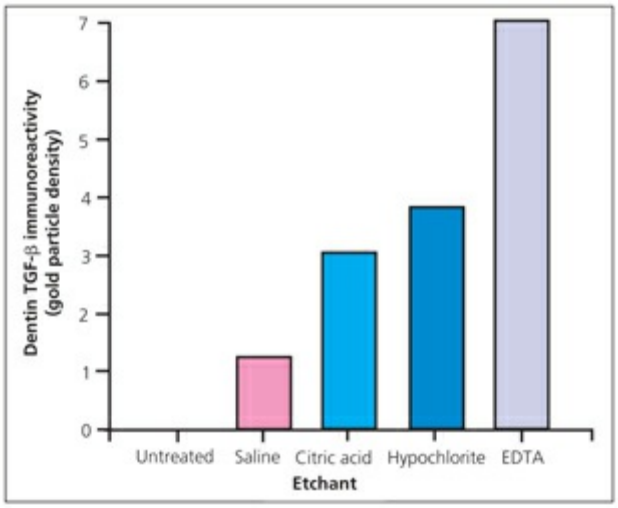

Fig 2-18 Comparison of immunoreactivity for TGF-β1 in cut human dentin matrix after treatment with various etchants. EDTA, ethylenediaminetetraacetic acid. (Data from Zhao et al.[125])

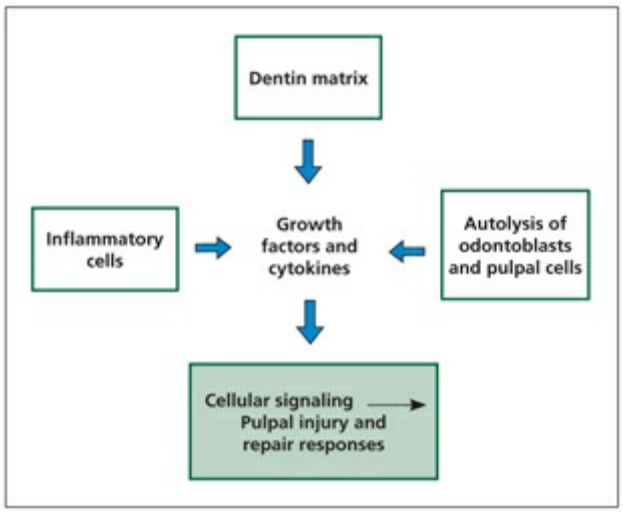

Fig 2-19 Possible derivations of signaling molecules contributing to pulpal injury and repair responses.

Formation of dentin bridges

At sites of pulpal exposure, dentinal continuity may be restored through formation of a dentin bridge across the exposure. There have been reports of bridging after pulp capping with a variety of agents, the most common being calcium hydroxide. Dentin bridge formation is not distinct from reparative dentinogenesis but rather represents a particular situation under which reparative dentin is formed. However, the extent of the injury and the reparative processes required may influence the quality or structure of the new matrix secreted within the bridge. Divergence from the normal, regular tubular structure of dentin may be common. This calls into question the specificity of the dentinogenic response in some situations. Such diversity of dentin structure may also reflect the different regulatory environment of the injured pulp on odontoblast behavior.

New dentin bridge formation is often regarded as an indication of successful pulp capping treatment, although this is probably true only where the bridge provides an effective bacterial seal. Mjör[126] cautioned that the presence of a dentin bridge may not be a suitable criterion for assessment of successful pulpal healing, especially during capping of young healthy pulps. Many bridges are permeated by pulp tissue and operative debris.

The concept of dentin bridges has been questioned because of the presence of imperfections in many bridges.[127] These imperfections, called *tunnel defects*, involve multiple perforations that allow communication between the pulp and capping material. Multiple tunnel defects were found in 89% of dentin bridges after calcium hydroxide pulp capping,[66] and 41% of these bridges were associated with recurring pulpal inflammation or necrosis and with the presence of inflammatory cells and stained bacterial profiles. The patency of these tunnel defects prevents a hermetic seal that would protect the pulp against recurring infection from bacterial microleakage.

These findings highlight the need to use materials capable of providing a long-term bacterial seal over capped pulps and the advantages of developing new capping materials that stimulate more specific dentinogenic responses during pulpal healing. Newer capping materials such as mineral trioxide aggregate have already shown clinical merit, and this may, in part at least, be a consequence of their ability to harness the bioactive signaling molecules within dentin.[74] As new capping materials emerge, it is probable that the emphasis will be increasingly on biomimetic approaches, which will provide important stepping stones toward tissue regenerative and engineering strategies for the pulpodentin complex.

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Pulpodentin Complex

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Previous chapters have discussed the developmental aspects of dentin and the pulp, the various types of dentinogenesis, and mineralization. This chapter describes the structure of dentin, its chemical and physical properties, and some of its mechanical properties. This topic is clinically significant because the permeability of dentin regulates the rate of inward diffusion of irritants that initiate pulpal inflammation and the outward diffusion of dentinal fluids that contain immunoglobulins. Because of this interaction, the pulp and dentin are often discussed together as a functional unit, the *pulpodentin complex*.

Structure of the Pulpodentin Complex

There is a great deal of evidence that dentin and the pulp are functionally coupled and hence integrated as a tissue. For example, when normal intact teeth are

stimulated thermally, dentinal fluid expands or contracts faster than the volume of the tubules that contain the fluid, which causes hydrodynamic activation of pulpal nerves. If the external tissues that seal dentin (eg, enamel and cementum) are lost for any reason, the normal compartmentalization of the two tissues is lost and they become functionally continuous.

Under these pathologic conditions, a fluid-filled continuum develops from the dentin surface to the pulp. It is through this fluid medium that bacterial substances and noxious materials may diffuse across dentin to produce pulpal reactions.[1–8] The pulp responds to these chemical stimuli in the short term by mounting an acute inflammatory response, which produces an outward movement of both fluid[9–12] and macromolecules.[13–15] In the long term, pulp tissues produce tertiary dentin as a biologic response in an attempt to reduce the permeability of the pulpodentin complex and to restore it to its original sequestration[16] (see chapter 2). In vivo, radioactive tracer experiments demonstrated the continuity of the dentinal fluid–pulpal fluid–pulpal circulation in exposed dentin and the importance of pulpal blood flow in clearing pulpal interstitial fluids of exogenous material.[17] Thus, the pulpodentin complex functions as an integrated unit.[18]

Odontoblasts are highly differentiated cells that form the tubular dentin matrix (see chapters 2 and 4). Their cell bodies reside in the pulp chamber, but their processes extend various distances through the unmineralized, freshly secreted predentin into the mineralized matrix[19] (Fig 3-1). Detailed information about the structure and function of odontoblasts can be found in other sources.[20,21]

If the odontoblastic layer is removed during endodontic therapy, the odontoblast processesare sometimes removed from the unmineralized tubules of predentin as well[22] (Fig 3-2). This dentin matrix is not yet mineralized and contains tubular openings 3 µm in diameter where no peritubular dentin matrix has yet been formed. Treatment with 5% sodium hypochlorite (NaOCl) removes the predentin and reveals the underlying mineralization front, which exhibits characteristic hemispherical structures known as *calcospherites*.[22] Mineralized dentinal tubules that are almost 3 µm in diameter are present within these calcospherites (Fig 3-3).

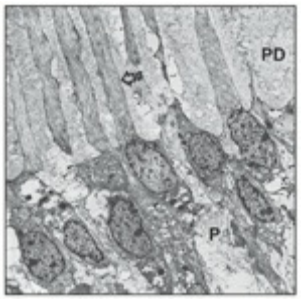

Fig 3-1 Transmission electron micrograph of the human odontoblastic layer at the junction of the pulp (P) and predentin (PD). Each cell sends an odontoblast process *(arrow)* through the predentin toward the periphery. (Reprinted from Jean et al[19] with permission.)

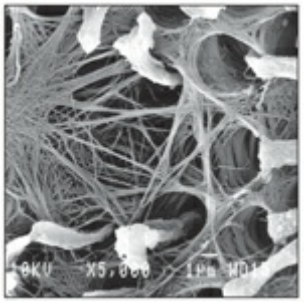

Fig 3-2 Scanning electron micrograph of predentin after removal of odontoblasts. Note the woven network of collagen fibrils approximately 100 nm in diameter. These fibrils polymerize perpendicular to the cell process, forcing the network to form circumferentially around the process shown in the tubules. (Reprinted from Jean et al[19] with permission.)

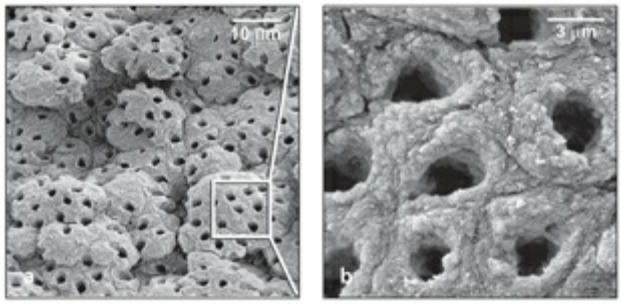

Fig 3-3 *(a)* When the predentin is removed by NaOCl treatment, the underlying mineralization front can be observed, organized in hemispherical units called *calcospherites*. *(b)* High magnification of the boxed area in *(a)*. Each calcosphe-rite contains many dentinal tubules, which are 2 to 3 μm in diameter.

Characteristics of Dentin

Morphology

Dentin is a porous biologic composite made up of apatite crystal filler particles in a collagen matrix (Fig 3-4a). This mineralized matrix was formed developmentally by odontoblasts, which began secreting collagen at the dentinoenamel junction (DEJ) and then grew centripetally while trailing odontoblast processes. Acid etching or ethylenediaminetetraacetic acid (EDTA) chelation removes the peritubular dentin

matrix, thereby enlarging the tubule orifice, and removes the mineral crystallites from around the collagen fibrils, exposing the fibrillar nature of the intertubular dentin matrix (Figs 3-4b and 3-5).

There are three types of dentin: primary, secondary, and tertiary (Fig 3-6). Primary dentin is the original tubular dentin largely formed prior to eruption of a tooth. The outer layer of primary dentin, called *mantle dentin*,[21] is slightly less mineralized (about 4%) in young dentin but is similar to that of primary dentin found in aged teeth.[23] Mantle dentin is about 150 μm wide and comprises the first dentin laid down by newly differentiated odontoblasts.[24] These cells may not be completely differentiated, or they may have had relatively short odontoblast processes providing slightly less-than-ideal mineralization.

Secondary dentin is the same circumpulpal dentin as primary dentin, but secondary dentin is formed after completion of root formation. The major difference between primary and secondary dentin is that the latter is secreted more slowly than primary dentin. Because the same odontoblasts form both types of dentin, the tubules remain continuous (Fig 3-6d). Each dentinal tubule has many submicron diameter branches that anastomose with microbranches from adjacent tubules.[25]

Over decades, a large amount of secondary dentin is formed on the roof and floor of the pulp chamber, causing the chamber to become shallower. Similarly, secondary dentin formation causes the dimensions of the root canal to become increasingly smaller with age. The presence of cellular processes on odontoblasts makes primary and secondary dentin tubular in nature.

The third type of dentin, tertiary dentin (also known as *irritation dentin*, *irregular secondary dentin*, *reactionary dentin*, or *reparative dentin*16), is found only in dentin that has been subjected to trauma or irritation, such as cervical exposure, caries, or traumatic cavity preparation (Fig 3-6).

Because the circumference of the most peripheral part of the crown or root of a tooth is much larger than the circumference of the final pulp chamber or root canal space, the odontoblasts are forced closer together as they continue to lay down dentin, developing a pseudostratified columnar layer in parts of the coronal pulp, especially over pulp horns.[26,27] Odontoblasts are cuboidal in the root canal[22] and become flat near the apex.[21] The convergence of dentinal tubules toward the pulp creates a unique structural organization in dentin that has profound functional consequences, as discussed later in the chapter. This convergence in the density of tubules has been estimated to be 5:1 in coronal dentin.[28,29] It is less in root dentin but still more than 2:1.

Each individual dentinal tubule is an inverted cone with the smallest dimensions at the DEJ and the largest dimensions at the pulp (Fig 3-7). Originally, each tubule has a diameter of nearly 3 μm. However, within each tubule is a collagen-poor, hypermineralized cuff of intertubular dentin, often called *peritubular dentin* (see Fig 3-5). It is actually *periluminal dentin* or, more accurately, *intratubular dentin*.[21,30] Its formation narrows the lumen of the tubule from its original 3 μm to as little as 0.6 to 0.8 μm in superficial dentin.[31,32] This large amount of peritubular dentin in superficial dentin near the DEJ is due in part to the fact that it is "older" than middle or deep dentin. Thus, the width of intratubular (peritubular) dentin decreases as tubules are followed inward toward the pulp, with the exception that there is no peritubular dentin in intraglobular dentin.[33,34] Very close to the pulp, where there is no intratubular or peritubular dentin, the tubule (luminal) diameter is almost 3 μm.[32] Thus, most of the narrowing of the tubule lumen at the periphery of dentin is due to deposition of peritubular dentin.[20,33,34]

Although there have been reports of giant tubules 5 to 40 μm in diameter, in human permanent and primary teeth[35] they number fewer than 30 giant tubules per tooth. They extend from the pulp chamber to the incisal DEJ, but there is some question as to their patency. Similar developmental defects in incisal regions have been reported.[36]

The composition of collagen-poor[31] and mineral-rich peritubular dentin is different from that of intertubular dentin (see Fig 3-5). The mineral is in the form of small, calcium-deficient, carbonate-rich apatite crystals, which have a higher crystallinity and are almost five times harder than intertubular dentin.[37] Little is known about the biologic control of peritubular dentin apposition. Although it is a very slow process, it can be accelerated by occlusal abrasion[33,38] and other forms of pulpal irritation such as caries and may be more rapid in primary[39] than in permanent teeth.

Although the complete composition of dentinal fluid is unknown, it presumably contains an ion product of calcium and phosphate near or above the solubility product constants for a number of forms of calcium phosphate.[40,41] This fluid tends to form mineral deposits in dentinal tubules, which may take many forms (Fig 3-8), because the outward movement of dentinal fluid presents a larger amount of mineral ions to the walls of the tubules than could occur by diffusion in sealed tubules. This principle has been used experimentally to slow the depth of demineralization of dentin in vivo under simulated caries-forming conditions.[42]

The permeability properties of dentinal tubules indicate that they have functionally

much smaller dimensions than their actual microscopic dimensions.[43,44] Although the microscopic diameter of dentinal tubules at the DEJ has been reported to be 0.5 to 0.9 μm, they function as though they are 0.1 μm in diameter. Dentin can remove 99.8% of a bacterial suspension of streptococci that are approximately 0.5 μm in diameter when pressure is applied to the solution,[45] which tends to prevent infection of the pulp even when patients masticate on infected carious dentin. This phenomenon explains why there are no bacteria in the tubules at the extreme front of the carious attack.[46]

Although bacteria can invade dentinal tubules,[46] they do not invade as fast or as far in vital dentin[47,48] (Fig 3-9), presumably because dentinal fluid moving outward contains immunoglobulins.[49] Immunoglobulin G1 was found to be the predominant immunoglobulin subclass in uninfected dentinal tubules beneath shallow and deep caries.[50] Fluid shifts across dentin may occur, but the fluid is virtually sterile because intratubular deposits of mineral and collagen fibrils form multiple constrictions within the tubule that reduce the dimensions to less than those of most microorganisms. In one study, 65% of the dentinal tubules in occlusal coronal dentin contained large collagen fibrils.[51] These intraluminal collagen fibrils would tend to trap any suspended bacteria as fluid flows through the tubules (Fig 3-10). The long-term effects of having bacteria trapped in tubules depend on the bacteria's source of nutrition and the effects of immunoglobulins from the pulp.

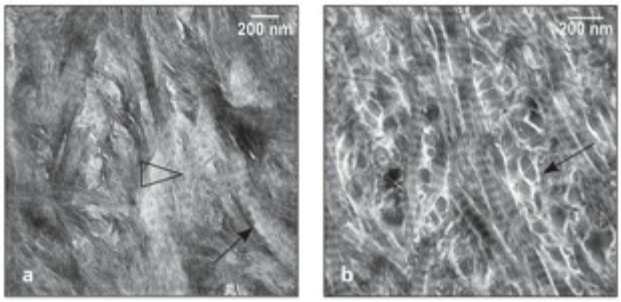

Fig 3-4 *(a)* Transmission electron micrograph of unstained, mineralized dentin showing the deposition of apatite crystallite striae within the collagen fibrils (intrafi-brillar mineralization; *arrowhead*) and between the collagen fibrils (interfibrillar min-eralization; *arrow*). *(b)* Transmission electron micrograph of stained, demineralized dentin showing the same striations or banding characteristics of the stained collagen fibrils. Dissolution of the interfibrillar apatite minerals results in empty interfibrillar spaces *(arrow)*.

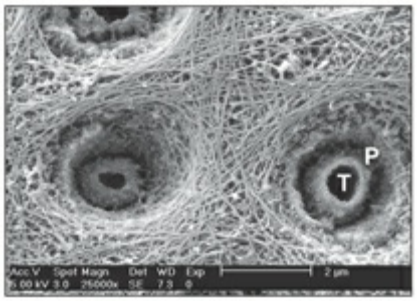

Fig 3-5 Acid etching of dentin removes a few microns of the peritubular dentin matrix (P) and strips the crystallites off the collagen fibrils, permitting the true fibrillar nature of the intertubular dentin matrix to be seen. T, dentinal tubule.

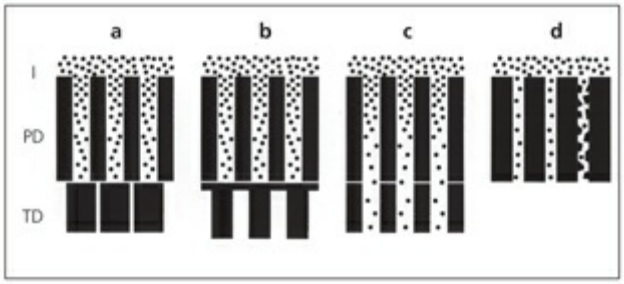

Fig 3-6 Tubular nature of various types of dentin. *(a)* Primary dentin (PD) is shown above less tubular tertiary dentin (TD). The small dots indicate the concentration of a potentially noxious substance diffusing across dentin. The original odontoblasts were destroyed, but the newly differentiated odontoblasts in the reparative dentin did not line up with the original primary dentin, greatly lowering its permeability. *(b)* Sometimes the newly formed odontoblasts lack a process and form a layer of atubular dentin that can reduce the permeability to near zero. *(c)* Injured odontoblasts sometimes do not die but simply make more dentin at a fast rate, thereby increasing dentinal thickness and reducing its permeability. *(d)* Intraluminal crystalline deposits (I) in tubules may lower dentin permeability. (Modified from Tziafas et al[16] with permission.)

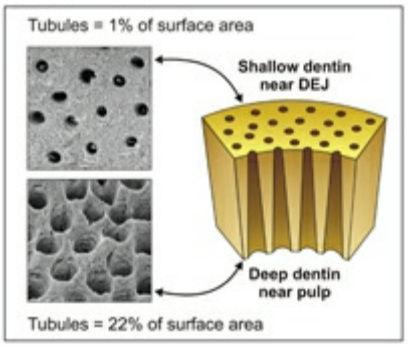

Fig 3-7 Each dentinal tubule is an inverted cone with the largest diameter at the pulp chamber or root canal and the smallest diameter at the DEJ due to the progressive formation of more peritubular dentin. Only 1% of the surface area of superficial dentin near the DEJ contains tubules, whereas 22% of deep dentin contains tubules. Hence, deep dentin is more permeable than superficial dentin. (Courtesy of Parkell, Biomaterials Division.)

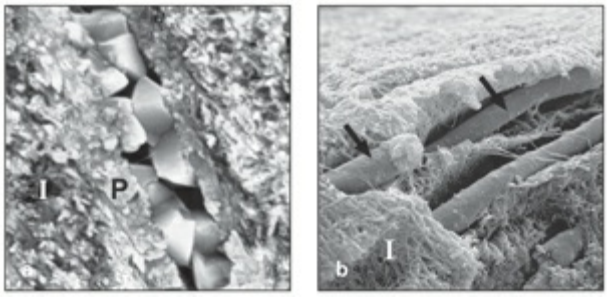

Fig 3-8 Examples of intraluminal crystalline deposits that lower dentin permeability. *(a)* Large whitlockite crystals that are composed of magnesium-substituted β-tricalcium phosphate. I, intertubular dentin; P, peritubular dentin. *(b)* Cores of fine crystalline deposits *(arrows)* resembling peritubular dentin that completely occlude the dentinal tubules. They are resistant to acid etching and are separated from the adjacent demineralized, acid-etched intertubular dentin (I) because of dehydration shrinkage. The presence of intraluminal deposits makes sclerotic dentin almost impermeable.

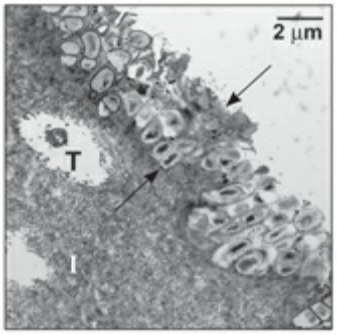

Fig 3-9 Although bacteria *(area between arrows)* can invade dentinal tubules, they are seldom found in the tubules (T) at the extreme front of the carious attack, probably because of the outward movement of dentinal fluid that contains immunoglobulins. I, intertubular dentin.

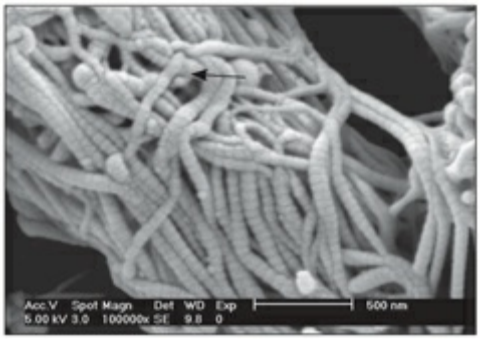

Fig 3-10 The presence of large bundles of intraluminal collagen reduces the functional diameter of the dentinal tubules and hence the permeability of dentin. These collagen bundles may also trap any suspended bacteria *(arrow)* as fluid flows through the tubules.

Changes in structure with depth

The area occupied by the lumina of dentinal tubules may be calculated as the product of the cross-sectional area of a single tubule, πr^2 , and N, the number of tubules per square centimeter. The unit r is the radius of the tubule. Because both the radius of

dentinal tubules and their number per unit area increase from the DEJ to the pulp,[29,52] the area occupied by tubule lumina also increases.

Garberoglio and Brännström[32] measured the tubule radius by carefully correcting for shrinkage artifact. Table 3-1 uses their data and provides calculations for the area occupied by tubule lumina at the DEJ and near the pulp.[53,54] Because this area is occupied by dentinal fluid, which is 95% water, these areas are also approximately equal to their tubular water content. That is, the water content of dentin near the DEJ is about 1% (volume percent), whereas that of dentin near the pulp is about 22%, a 20-fold variation. Textbooks list the water content of dentin at approximately 10% by weight or 20% by volume,[55] but that is an average value (Table 3-2).

The difficulty in bonding to deep dentin is caused in part by its high water content, which competes with resin monomers for the surfaces of collagen fibrils.[58,59] Transudation of water or dentinal fluid from dentinal tubules during bonding to vital dentin may also result in the entrapment of water blisters within the resin-dentin interface (Fig 3-11). These blisters act as stress raisers that affect the integrity of the bonded interfaces.[60,61]

Table 3-1 **Density and diameter of dentinal tubules at various distances from the pulp and the calculated areas of fluid-filled tubules, peritubular dentin, and intertubular dentin***

				Area†	
Distance from pulp (mm)	**Number of tubules × $10^6/cm^2$**	**Radius of tubules (μm)**	**Fluid-filled tubules (A_t)**	**Peritubular dentin (A_p)**	**Intertubular dentin (A_i)**
0.0	4.5	1.25	22.10	66.25‡	11.65
0.1•0.5	4.3	0.95	12.19	36.58	51.23
0.6•1.0	3.8	0.80	7.64	22.92	69.44
1.1•1.5	3.5	0.60	3.96	11.89	84.15
1.6•2.0	3.0	0.55	2.85	8.55	88.60
2.1•2.5	2.3	0.45	1.46	4.39	94.15
2.6•3.0	2.0	0.40	1.01	3.01	95.98
3.1•3.5	1.9	0.40	0.96	2.86	96.18

* Data calculated from Garberoglio and Brännström.[32]

† $At = \Pi r^2 N(100)$, where N = number of tubules per square centimeter. A_t, although an area, is also the percent of surface area occupied by water.

$A_p = \Pi N(R^2 \bullet r^2)(100)$, where $R = 2r$ and r = tubular radius.

$A_i = 100 \bullet (A_p - A_t)$.

‡ There is no peritubular dentin at the pulp surface, but it begins close to the pulp surface.

Table 3-2 Chemical composition of dentin

Source	Mineral wt% (vol%)	Organic wt% (vol%)	Water wt% (vol%)
LeGeros[55]	70 (47)	20 (30)	10 (21)
Kinney et al[56]	65 (45)	35 (48)	NA (7)
Frank and Voegel[57]	70 (47)	20 (32)	10 (21)

wt, weight; vol, volume; NA, not available.

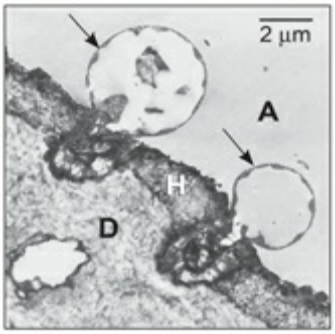

Fig 3-11 Transmission electron micrograph depicting the transudation of dentinal fluid and tubular contents *(arrows)* from the tubules during dentin bonding to acid-etched vital dentin. The fluid droplets are trapped within the dentin adhesive (A) and act as stress raisers that adversely affect the integrity of resin-dentin bonds. H, hybrid layer; D, intertubular dentin.

Fortunately, the water content can be controlled in nonvital dentin, which facilitates bonding to endodontically treated dentin. This phenomenon may become more important in endodontics as the use of adhesive resins for secondary seals increases in popularity. It is well known that loss or leakage of the access-opening provisional restorative material can lead to bacterial contamination of root canal fillings.[62,63] To avoid such contamination, several investigations have recommended the use of secondary seals on the floor of the pulp chamber that extend over the

orifice of the filled canal.[64,65] This technique not only protects the root canal filling from leakage but also seals off any accessory canals that might exist on the pulpal floor. The use of transparent, unfilled resins is preferred because they permit observation of the underlying gutta-percha and are soft enough to remove easily should retreatment be necessary.

Chemical composition

The composition of bulk dentin by weight has been reported to be 70% mineral, 20% organic, and 10% water[20] (see Table 3-2). Because of the high density of dentin (ranging between 2.05 and 2.30 g/cm^3), the weight percentage is much higher than volume percentage.

The mineral phase of dentin is a calcium-deficient, carbonate-rich apatite with platelike crystals in intertubular dentin 50 to 60 nm long, 36.4 ± 1.5 nm wide, and 10.3 ± 0.3 nm thick. This small size, relative to large apatite crystals in enamel, is thought to be responsible for the high critical pH of dentin (pH 6.7). This crystal size is clinically significant because root dentin demineralizes at less than 10% of the hydrogenion concentration required for enamel demineralization (pH 6.7 versus 5.5), making dentin more susceptible to caries than enamel once root surfaces become exposed.[66] Trace elements are also found in dentin.[55,67] About 90% of the organic portion of the dentin matrix is made up of type I collagen; noncollagenous protein growth factors, proteoglycans, and matrix metalloproteinases[68] make up the remainder.[69–71]

The water content of dentin varies with location, but bulk dentin has been reported to be between 8% and 16% water, most of which is unbound water that can be removed by heating to 120°C. A small fraction of the water (probably less than 1%) is associated with apatite crystals and collagen.

Physical characteristics

Dentin is a heterogenous composite material that contains micrometer-diameter tubules surrounded by highly mineralized (about 95% mineral phase by volume) peritubular dentin embedded within a partially mineralized (about 30% mineral phase by volume) collagen matrix (intertubular dentin).[72] The bulk of tooth structure is made up of dentin, which is the vital part of the tooth (Table 3-3).

Dentin is much softer than enamel (Knoop hardness of 68 versus 343 kg/mm^2).[73] The softness of dentin allows it to wear much faster than enamel[72] and permits

efficient endodontic instrumentation with hand or rotary files.[74] Dentin is also more elastic than enamel (modulus of elasticity of about 11 to 20 versus 86 GPa). This greater elasticity makes dentin tougher than enamel, and it also accounts for dentin's stress-breaking or shock-absorbing function for the overlying enamel.

Modifications to the atomic force microscope permit measurement of both nanohardness and the modulus of elasticity of intertubular and peritubular dentin.[37] Peritubular dentin has a greater nanohardness than intertubular dentin (2.5 versus 0.52 GPa). In addition, deep dentin tends to be more elastic than superficial dentin (modulus of elasticity of about 17 versus 21 GPa). The shear strength of dentin also varies by region; superficial dentin has greater shear strength than deep dentin (132 versus 45 MPa).[75] The shear strength of middle dentin has been reported to be about 72.4 to 86.9 MPa.[76]

The ultimate tensile strength of human mineralized root dentin has been reported to range from 63 to 96 MPa, whereas that of demineralized root dentin varies from 16 to 29 MPa; the greater values are achieved when the root dentin is stressed perpendicular to the tubules.[77,78] The bulk modulus of elasticity of human dentin has been reported to range from 10 to 19 GPa, with a value of 14 GPa derived from studies using smaller specimens.

Because of the wide variation in the microscopic structure of dentin, the mechanical properties of dentin across regions would be expected to vary. Indeed, the values given for the bulk mechanical properties of dentin are generally listed as a range because they are not uniform. For instance, microhardness values in the crown decrease from 350 kg/mm^2 in enamel to 50 kg/mm^2 in dentin just beneath the DEJ, increase to 70 kg/mm^2 within 1 mm from the DEJ, and then decline slowly toward the pulp (40 to 50 kg/mm^2 near the pulp).[79] Similar distributions are found for the modulus of elasticity.[80] Mantle dentin and the zone of interglobular dentin about 200 µm from the DEJ are less mineralized and less stiff than the inner core of dentin. The microhardness of root dentin is even lower, about 30 kg/mm^2 at the cementoenamel junction (CEJ), increasing to 52 kg/mm^2 about halfway across, and then decreasing to 40 to 45 kg/mm^2 near the pulp.[79] This variation in mechanical properties of dentin plays a critical role in the distribution of mechanical forces through a tooth.

Table 3-3 **Mechanical properties of dentin**

	Dentin*		
Mechanical properties	Bulk	Peritubular	Intertubular

Compressive strength (MPa)	217–300	NA	NA
Young modulus of elasticity (GPa)	10–19	29–30	16–21
Shear strength (MPa)	45–132	NA	NA
Tensile strength (MPa)	31–106	NA	NA
Microhardness (kg/mm^2)	40–70	NA	NA
Nanohardness (GPa)	NA	2.2–2.5	0.12–0.52

*Because dentin is not homogenous, values are given in ranges.
NA, not available.

Mechanics of root dentin

Endodontic treatment can weaken the rigidity of teeth. In a recent study using speckle pattern interferometry, preparations for access and for endodontic posts significantly increased root deformation under a 3.75-N load applied to the lingual incisal third of maxillary incisors at an angle of 135 degrees with respect to the long access of the tooth.[81] NaOCl is also known to reduce the mechanical properties of dentin: Sims et al[82] reported 15% reductions in dentin stiffness and a 39% reduction in flexural strength after exposure of beams of dentin to 5.25% NaOCl for 2 hours. Moreover, long-term use of calcium hydroxide for more than 30 days as an intracanal dressing or in apexification procedures has been shown to reduce the fracture resistance of root dentin, particularly for immature teeth with thin layers of root dentin.[83]

Old teeth are more susceptible to fracture than young teeth during cyclic stressing.[84] Fatigue cracks develop at lower stresses and propagate at a faster rate in old dentin than in young dentin.[85] It is thought that this is due in part to occlusion of tubules with mineral crystals that results in less hydration.[86]

What have been needed in dental mechanics are techniques that can show the distribution of stresses and strains in dental hard tissues under load. Modeling techniques such as the use of model teeth constructed of photoelastic materials or the use of finite element stress analysis have been questioned because they assume

uniform mechanical properties. Alternative techniques measure the distribution of stress or strain applied to whole teeth.[87] For example, grids used to support tiny tissue sections for transmission electron microscopy have been used to transfer microscopic patterns to tooth surfaces. This permits multiple measurements of regional microstrains by measuring distortions in the grid.[88]

A more sophisticated approach involves the application of Moiré gratings (200 lines/mm) to longitudinal sections of teeth, permitting measurement of two-dimensional strain fields.[89] Under these conditions, most of the strain was found in the low-modulus, 200-µm-thick region just below the DEJ and at the CEJ, suggesting that these low-modulus zones act as stress buffers or cushions.

Because of the regional differences in mineral distribution, hardness, and elastic modulus within root dentin,[90] two-dimensional studies in the x, y, and z planes are needed. For example, multiple rosette strain gauges were placed on the buccal plate of bone over a maxillary central incisor at the cervical, middle, and apical thirds of the root in a patient undergoing dental surgery.[91] The tooth was then loaded, and strains induced in the overlying bone were measured. Maximum strain was found in the bone over the cervical region, intermediate strain in the bone over the middle third, and no strain in the bone over the apical third. In another patient who had lost buccal bone to periodontal disease, the same procedures were repeated with rosette strain gauges fixed to the cervical, middle, and apical thirds of root dentin. The strains in root dentin were much lower than those in bone, and the distribution of shear strain on the bone surface was nearly constant. In these studies, most of the axial bite forces were distributed along the cervical and middle thirds of the root and of the supporting bone, thereby relieving the apical region of stress and strain. This distribution of forces may protect blood vessels entering the root apex from occlusal forces that might transiently cut off venous outflow.[91]

The ideal approach to evaluating the mechanical properties of teeth under function is use of three-dimensional quantitative microscopic x-ray tomography. Such studies will permit quantitative three-dimensional microstrains in teeth under functional loads, such as before and after endodontic therapy and the cementation of metal or fiber posts.

Permeability

Coronal dentin

The tubular structure of dentin provides channels for the passage of solutes and solvents across dentin. The *tubular density*, or number of dentinal tubules per square millimeter, varies from 15,000 at the DEJ to 65,000 at the pulp.[25,27,32,52] Because both the density and diameter of the tubules increase with dentin depth from the DEJ, the permeability of dentin is lowest at the DEJ and highest at the pulp (Fig 3-12; see also Table 3-1). However, at any depth, the permeability of dentin in vitro is far below what would be predicted by the tubular density and diameters because of the presence of intratubular material such as collagen fibrils, mineralized constrictions of the tubules, and so on. The permeability of dentin is highest for small molecules such as water, lower for larger molecules such as albumin and immunoglobulins, and still lower for molecules with molecular weight greater than 10^6 , such as endotoxins.[92]

Dentin permeability is divided into two broad categories (Fig 3-13) : *(1)* transdentinal movement of substances through dentinal tubules, such as fluid shifts in response to hydrodynamic stimuli, and *(2)* intradentinal movement of exogenous substances into intertubular dentin, as occurs with infiltration of hydrophilic adhesive resins into demineralized dentin surfaces during resin bonding or demineralization of intertubular dentin by acids.[56,93]

Dentin may be regarded both as a barrier and as a permeable structure, depending on its thickness, age, and other variables.[94] Its tubular structure makes dentin very porous. The minimum porosity of normal peripheral coronal dentin is about 15,000 tubules per square millimeter. Once uncovered by trauma or tooth preparation, these tubules become diffusion channels from the surface to the pulp. The rate of diffusional flux of exogenous material across the dentin to the pulp is highly dependent on dentinal thickness and the hydraulic conductance of dentin.[44,95] Thin dentin permits much more diffusional flux than thick dentin. However, in exposed vital dentin with open tubules, the inward diffusional flux of materials competes with the rinsing action of outward convective fluid transport.[96,97] This competition may serve a protective function in mitigating the inward flux of potentially irritating bacterial products into exposed, sensitive dentin.

The permeability of dentin is not uniform but varies widely, especially on occlusal surfaces, where perhaps only 30% of the tubules are in free communication with the pulp[98] (Figs 3-14 and 3-15). Scanning electron microscopic examination of acid-etched occlusal dentin reveals that all the tubules are exposed, but functional studies of the distribution of fluid movement across the occlusal dentin reveal that

the tubules that communicate with the pulp are located over pulp horns and that the central region is relatively impermeable. Apparently, intratubular materials such as collagen fibrils[51] and mineralized deposits restrict fluid movement even though the peripheral and central ends of the tubules are patent. Even microscopically, within any 100 × 100–μm field, only a few tubules are patent from the periphery to the pulp.[99]

Axial dentin is much more permeable than occlusal dentin.[100,101] The gingival floor, proximal boxes, or gingival extension of finish lines in crown preparations often ends in regions of high dentin permeability.[102] As many as 4 million exposed dentinal tubules are found in a surface area of approximately 1 cm^2 in complete-crown preparations on posterior teeth.[54] Although the tubules are occluded by smear layers and/or cement following placement of castings, both cement and smear layers have finite solubility and may permit some tubules to become exposed over time, most likely at the most peripheral extensions of restorations, where diffusion distances to the pulp are shortest.

The permeability of sclerotic dentin is very low,[103] regardless of whether the sclerosis was due to physiologic or pathologic processes, because the tubules become filled with mineral deposits. Indeed, this reaction is fortuitous in that it slows the caries process and tends to protect the pulp. Most pulpal reactions to cavity preparations or restorative materials used on carious dentin are due to changes that occur across adjacent normal dentin rather than the almost impermeable caries-affected dentin.

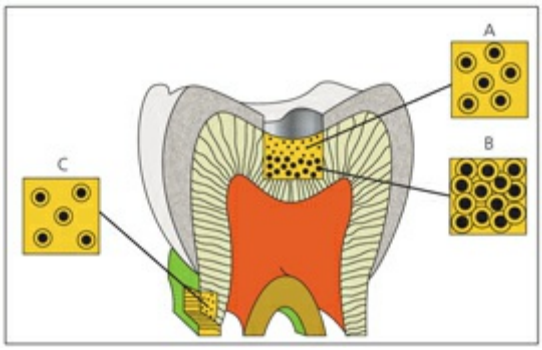

Fig 3-12 Cavities prepared on the occlusal surface of coronal dentin (A and B) and at the cervical region of the root surface (C). There are fewer tubules in superficial dentin (A) than in deep dentin (B); the tubules are not only closer together in deep dentin but larger in diameter. This combination is responsible for the exponential increase in dentin permeability with depth. (Reprinted from Pashley[18] with permission.)

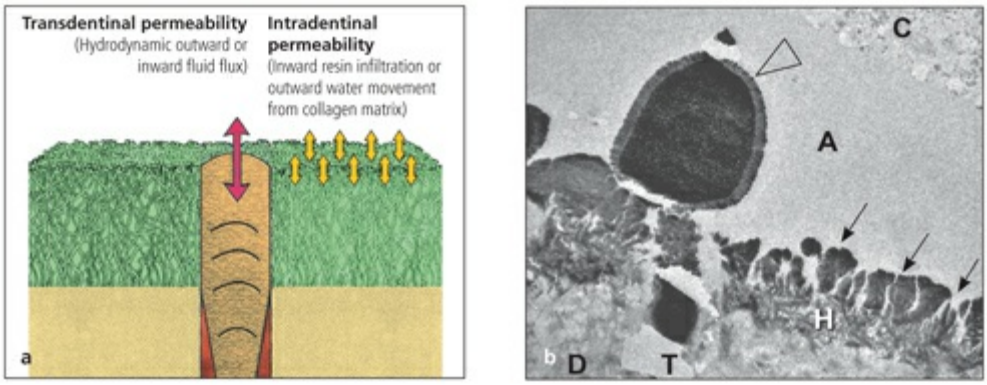

Fig 3-13 *(a)* Comparison of transdentinal permeability, arising from inward or outward movement of dentinal fluid or materials within tubules, and intradentinal permeability, in which materials or water diffuse in or out of porosities created between tubules by demineralization. *(b)* Transmission electron micrograph of a silver-impregnated interface of coronal dentin bonded with a permeable, one-step, self-etch adhesive under physiologic pulpal pressure of 15 cm H_2O. This micrograph illustrates both outward transdentinal permeation of a water droplet *(arrowhead)* into the adhesive layer (A) and outward intradentinal permeation of water *(arrows)* from the demineralized collagen matrix that is partially infiltrated by the adhesive (ie, hybrid layer [H]). The latter type of water movement has been described as *water trees*. C, composite; T, dentinal tubule; D, dentin.

Fig 3-14 Movement of dye across dentin from the underlying pulp chamber in vitro under a simulated pulpal pressure of 20 cm H_2O. The dentin is most permeable over the pulp horns, where the tubules have their largest diameters and densities.

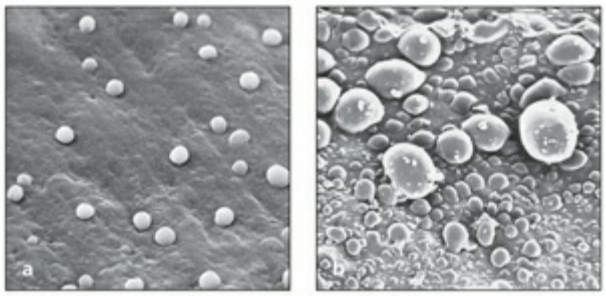

Fig 3-15 Epoxy resin replicas of dentin surfaces showing the transudation of dentinal fluid from vital, instrumented human dentin. *(a)* Dentin permeability is low when vital dentin is covered with a smear layer. *(b)* Higher permeability is observed after removal of the smear layer.

Root dentin

As in coronal dentin, the permeability of root dentin depends on dentinal thickness, the tubular density, and the diameter and patency of the tubules.[44,54,95] There are more tubules per square millimeter in deep dentin near the canal than in peripheral dentin because the outer circumference of the root is larger than the circumference of the root canal (Fig 3-16). The number of tubules (and odontoblasts) remains constant from the outer root dentin to the root canal, but the tubules become crowded together at the canal.[104]

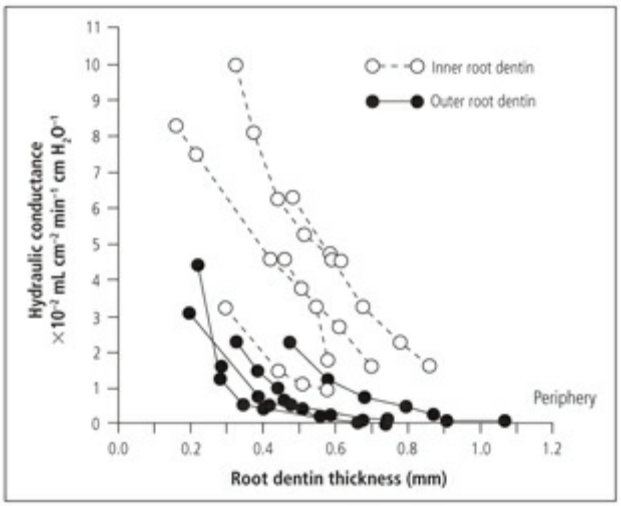

Fig 3-16 Permeability of inner and outer root dentin as a function of dentinal thickness. Flat root surfaces were split longitudinally into outer and inner halves, each about 1.1 mm thick. The permeability of the outer half *(solid circles)* was low even after removal of cementum (from 1.1 to 0.9 mm) and then only slowly increased as the dentin was made thinner by grinding the periphery. The rate of increase of root dentin permeability was much higher in inner dentin *(open circles)*. (Modified from Fogel et al[104] with permission.)

Although a number of authors have reported the reduction of tubular densities from the coronal third to the apical third of root canals[105] (Fig 3-17), few have been specific about the exact locations at which these tubular densities were observed. For example, the greater concavity of buccal or lingual walls, compared with mesial or distal walls, increases tubular density. In premolars, tubular densities of the pulpal wall at the level of the CEJ are higher on the lingual side than the mesial or distal side (72,000 versus 44,000).[27] There is less concavity in third molars (ie, the crown is more square in outline), and the corresponding dentinal tubular densities are more similar (66,000 versus 61,000).

Given these values for tubular density (72,000) and known values for the radius of each tubule (2.5 μm), the percentage of dentin surface area occupied by tubule lumina can be calculated.[29,52,54] By this calculation, 35% of the area of the buccal wall of the coronal pulp chamber of premolars is occupied by tubules.

Although the permeability of dentin should be proportional to the area occupied by dentinal tubules, quantitative comparisons of the theoretic or calculated permeability and the actual measured permeability revealed the latter to be less than 3% of the theoretic value. This finding is explained by the fact that dentinal tubules are not smooth-bore tubes but contain a good deal of intratubular material such as mineralized inclusions and collagen fibrils. Tubular sclerosis, a physiologic phenomenon that commences in the third decade of life in the apical root region and advances coronally with age, is the main factor reducing the permeability of apical root dentin[106] (Fig 3-18).

Dentin permeability has numerous clinical implications. For example, NaOCl is a commonly used irrigant in endodontic procedures. Soaking dentin disks in 5% NaOCl for 1 hour produces a 105% increase in the hydraulic conductance of human cervical dentin.[107] By contrast, soaking dentin disks with even 35% hydrogen peroxide for 1 hour only produces a 16% decrease in permeability.

During endodontic instrumentation of root canals, the softest inner dentin is removed.[74] The increase in the diameter of the root canal leads to a decrease in the tubular density (the exact opposite of what occurs in operative dentistry when the tooth is prepared from the enamel toward the pulp). In addition, the root dentin is made slightly thinner.[108] These two phenomena tend to influence root dentin permeability oppositely, but the reduction in thickness is dominant. However, the shaping of the canal also creates long smear plugs[109] and thick smear layers[104] on the instrumented surface that decrease the permeability of dentin.

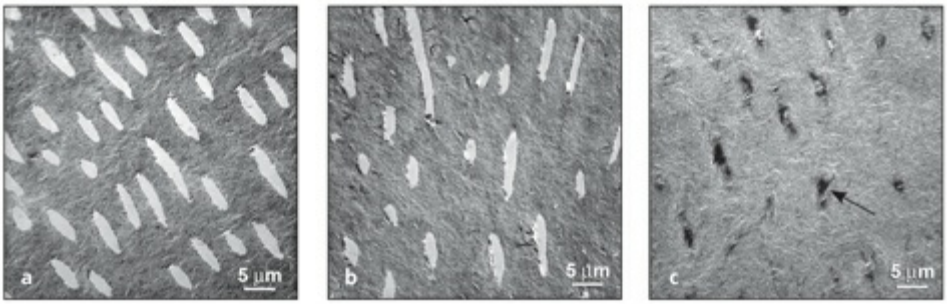

Fig 3-17 Transmission electron micrographs comparing the tubular density in the coronal third *(a)*, the middle third *(b)*, and the apical third *(c)* of intraradicular dentin. In the apical third, dentinal tubules are more irregular and are occluded by electron-dense mineral deposits *(arrow)*.

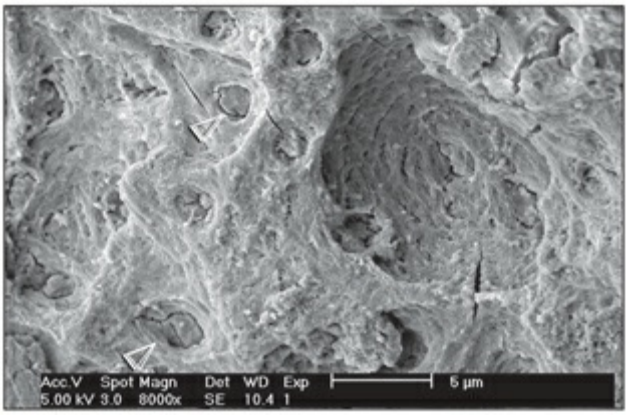

Fig 3-18 Apical third of an instrumented and NaOCl-irrigated root canal, showing completely obliterated dentinal tubules *(arrowheads)*. These occluded tubules are not amenable to penetration by bacteria. The cracks in the dentin are drying artifacts. The large circular structure is a mineralized lateral canal.

Smear layers and smear plugs

Smear plugs are composed of grinding debris whose particle sizes are smaller than tubule orifices[110] (Fig 3-19). Although most smear plugs in coronal or radicular dentinal tubules are only 1 to 3 µm long, smear plugs in instrumented root canals are up to 40 times longer (40 µm) because the tubule diameters are so much larger at the pulp chamber.[103] The presence of grinding debris in the tubule orifices and on the dentin surface also lowers the permeability of dentin.[53,111,112] Treatment of smear layers with NaOCl does not remove the smear layer[113] because most of the organic component is masked by mineral. Only after removal of the mineral with EDTA or acids can NaOCl remove the organic portion.[114] This restriction is the rationale for the sequential use of EDTA and NaOCl as endodontic irrigants for removal of the smear layer.

Any instrument placed in contact with the root canal or pulp chamber will create a smear layer (Fig 3-20). For example, instrumentation with K files decreases the permeability of root dentin by 49%.[115] The presence of the smear layer and smear plugs prevents the entry of sealer[116] or thermoplasticized gutta-percha into dentinal tubules. However, the critical seal is in the apical third, a region where it is difficult to remove the smear layer. It is important to understand that the mere presence of dental materials (eg, sealer or gutta-percha "tags") in tubules does not ensure a perfect seal.

Moreover, even the absence of a smear layer on the dentin surface may not necessarily correlate with increased dentin permeability.[117] To evaluate this possibility, the canals of single-rooted teeth were endodontically instrumented and

filled with radioactive water, and the diffusional flux of the water was measured before and after removal of the smear layer. Removal of the smear layer with 0.5 mol/L EDTA and 5% NaOCl resulted in a 34% decrease in permeability. When the measurements were repeated after the specimens were soaked in water for 2 months, the permeability of roots to tritiated water had increased 81% above the previously measured values. Apparently, during the treatment of root canals with EDTA, the concentration of ionized calcium and phosphate in tubular fluid increased to high levels, allowing the formation of precipitates in the tubules that actually lowered the permeability of dentin, even though scanning electron microscopy (SEM) studies of these surfaces indicated that the smear layer had been removed. Over the next 2 months, these precipitates slowly dissolved, leading to an increase in permeability. Thus, scanning electron microscopy (SEM) studies demonstrating smear layer removal may not indicate an increase in dentin permeability because they fail to show subsurface changes that can have a profound effect on dentin permeability.

The use of BioPure MTAD (Dentsply Tulsa Dental) as an endodontic irrigant results in the creation of a 5- to 8-µm deep demineralized zone along the root canal[118] (Fig 3-21a). The loss of mineral lowers the stiffness of that demineralized root dentin from 18,000 to 2 MPa, allowing compaction forces to collapse this layer. Current endodontic sealers are too hydrophobic to penetrate the exposed collagen fibrils by intradentin permeation (Fig 3-21b). This partially denuded collagen matrix contains matrix metalloproteinase activity[119] that can dissolve the collagen, leaving a 10-µm gap between the root filling material and the mineralized dentin wall. Recent interest in the use of self-etching primers and adhesives in endodontics[120] indicates that endodontists must review the problems associated with these techniques in the unique environment of root canals.[121]

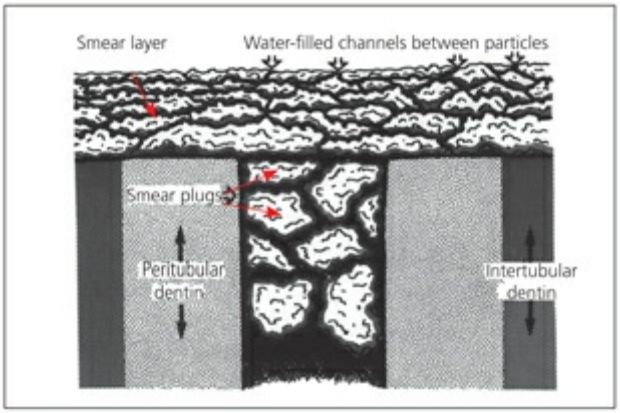

Fig 3-19 Smear layers and smear plugs, made up of submicron pieces of grinding or cutting debris burnished together and to the underlying dentin. Isotope tracer studies have shown the debris to be permeable to water-soluble materials via water-filled channels around constituent particles. (Reprinted from Pashley et al[110] with permission.)

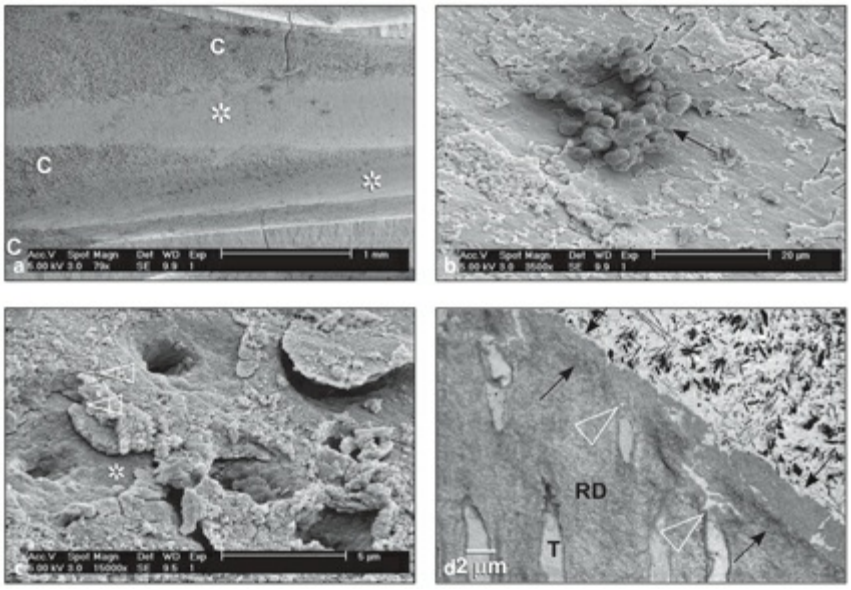

Fig 3-20 Scanning and transmission electron micrographs of endodontic smear layers. *(a)* A smear layer *(asterisks)* is created only when a rotary endodontic instrument is in contact with the root canal wall. Thus, areas untouched by the instrument leave behind granular-appearing calcospherites (C) after the instrumented canal is irrigated with NaOCl. *(b)* Higher-magnification view of the morphology of the smear layer shown in *(a)*. A bacterial biofilm cluster *(arrow)* is visible on the surface of the smear layer–covered root dentin. *(c)* Still higher magnification, showing the thickness of the smear layer *(area between arrowheads)* from a location where the smear layer was partially dislodged because of specimen dehydration, exposing the underlying mineralized root dentin *(asterisk)*. *(d)* Transmission electron micrograph of a similarly instrumented canal after restoration with a root canal sealer. Vertical sectioning through the smear layer *(area between black arrows)* reveals the presence of channels *(lower white arrowhead)* between the smear layer components. Smear plugs *(upper white arrowhead)* occlude the dentinal tubular orifices. T, dentinal tubule; RD, radicular dentin.

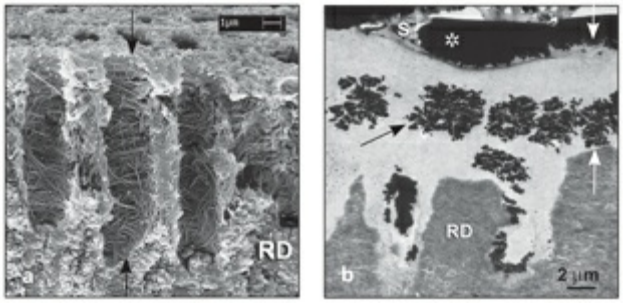

Fig 3-21 *(a)* Scanning electron micrograph showing the creation of a 6- to 8-mm-thick fibrillar demineralized collagen matrix *(area between arrows)* when root dentin (RD) is irrigated with BioPure MTAD. *(b)* Silver-impregnated transmission electron micrograph of the sealer–root dentin interface after BioPure MTAD–irrigated root dentin (RD) is filled with a root filling material and a sealer (S). Regions within the demineralized collagen matrix *(area between white arrows)* that are not infiltrated by the sealer are impregnated by silver deposits, that is, nanoleakage *(black arrow)*. A 2- to 3-mm-wide silver-filled gap *(asterisk)* is evident between the sealer and the root dentin.

Variations in permeability

The permeability of root dentin and coronal dentin is not uniform.[117] When measured for hydraulic conductance, root dentin has a permeability that is only about 3% to 8% as great as that of coronal dentin[122] (Table 3-4). It is likely that the relative impermeability of root dentin protects the periodontal tissues from the wide variety of potentially cytotoxic compounds that historically have been placed in root canals to sterilize them.[104,123,124] Similarly, the low permeability of root dentin prevents sulcular endotoxins[125] from diffusing into the pulp. Part of the barrier properties of the root are the result of the low permeability of cementum,[126] indicating that with the loss of cementum in periodontal therapy, the permeability of root dentin may increase in periodontally involved teeth. Cementum serves as a "surface resister" in conjunction with root dentin to reduce permeability. For example, the outer half of root dentin slabs, covered peripherally by cementum, has a very low permeability compared with that of the inner half of slabs[104] (see Fig 3-16). Even after removal of all cementum, the permeability remains low until more than 200 μm of outer dentin is removed because there is no sharp demarcation between cementum and root dentin,[127] and multiple branches in the tubules of root dentin[25] often modify the hydraulic conductance of dentin.[128] After removal of the most external root dentin, the permeability of the outer half of the root dentin slabs increases rapidly (see Fig 3-16).

Other studies have confirmed the importance of cementum in reducing the permeability of root dentin. In one study,[108] two groups of single-rooted teeth underwent endodontic instrumentation. One group had all of the cementum and some external root dentin removed (about 0.5 mm) along with the smear layer on the instrumented external surface, and the second group had intact cementum layers sealing the external surfaces. The group without cementum showed significant increases in permeability during extirpation of the pulp, cleaning and shaping with NaOCl, and removal of the smear layer with EDTA.

On closer examination, the permeability of root dentin itself is not uniform but displays regional differences along its axial length. Cervical and middle root dentin have higher permeability than apical dentin,[108,123,129,130] consistent with their higher tubular density as compared with apical dentin.[105] The permeability of the floor of the pulp chamber in the region of the furcation has been reported to be high because of the presence of accessory canals.[131–134] However, subsequent studies examining the floor of the pulp chamber in 100 unerupted third molars found only one tooth with a patent accessory canal.[135] The higher incidence of accessory canals reported in earlier studies may have occurred because specimens were soaked in 5% NaOCl,

which may have removed organic material in accessory canals, thereby exaggerating their incidence. Although it is possible that the use of third molars may have underestimated the incidence of accessory canals, other studies using SEM to examine the pulpal floors of human molars revealed only two accessory canals in 87 human molars.[22] Thus, the current findings clearly demonstrate that coronal and middle root dentin have greater permeability than apical dentin and that the permeability of the pulpal floor is largely influenced by the presence of patent accessory canals.

Endodontists must be especially aware of the tubular density of apical dentin because this region may be beveled during apical surgery. During such surgery, the resection of the root at an oblique angle may be favored to facilitate visibility and the placement of a reverse filling. However, this angled resection may expose dentinal tubules beneath the retrofilling material, thereby introducing the possibility of tubule leakage (Fig 3-22). The greater the angle of the root resection, the greater the potential for microleakage.[136,137] Thus, minimally angled resections have been advocated along with placement of the reverse filling below the edge of the bevel to prevent leakage through dentinal tubules exposed by the bevel (see Fig 3-22). The steeper the bevel, the deeper the reverse filling must be to prevent this leakage. In addition to apical root resection with near-zero-degree bevels, others have proposed sealing the resected surface with resin, eliminating the need for a reverse filling.[105,138–142] As described previously, the tubular density is much lower in apical dentin than in coronal dentin, and the density of apical dentinal tubules can be further reduced with age, becoming nearly translucent over time because of increased deposition of mineral crystals in the dentinal tubules.[106]

Table 3-4 **Comparison of coronal and radicular hydraulic conductance (Lp) of dentin**

	Lp ($\mu L\ cm^{-2}\ min^{-1}\ cm\ H_2O^{-1}$)	
Thickness (mm)	Coronal	Radicular
0.9	0.1210	0.0005
0.7	0.1850	0.0204
0.6	0.3900	0.0304
0.2	NA	0.0701
0.1	1.5000	0.0801

NA, not available. (Reprinted from Pashley[122] with permission.)

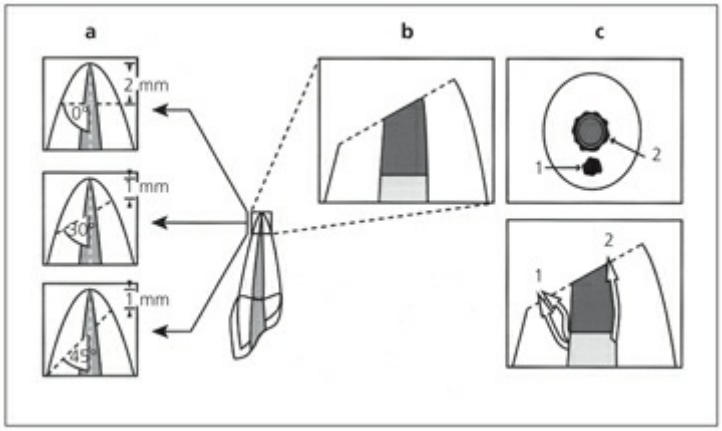

Fig 3-22 Influence of the apical bevel angle on the depth of the reverse filling required to prevent leakage of substances through exposed dentinal tubules. *(a)* Possible angles of resection. *(b)* Reverse filling. *(c)* Routes of leakage via gaps around the filling (2) or via tubules that were not sealed by filling (1). (Modified from Gilheany et al[137] with permission.)

Balance between permeation of noxious substances across dentin and clearance

There is a balance between the rate at which materials permeate exposed dentin to reach the pulp and the rate at which they are cleared from interstitial fluid by the pulpal microcirculation and lymphatic vessels.[17,18,94,95] To illustrate this principle, conical chambers were placed on the buccal and lingual surfaces of a dog molar in vivo[17] (Fig 3-23). Radioactive iodide (131I) was placed in the buccal chamber. The lingual chamber was perfused with samples collected by a fraction collector. Blood samples, drawn every 10 minutes, revealed that the pulpal circulation cleared almost all of the iodide that permeated the buccal dentin. Little iodide showed up in the rinsing fluid of the lingual chamber until epinephrine was added to the buccal chamber, causing cessation of pulpal blood flow and accumulation of iodide in the pulp, as indicated by the large increase in iodide diffusion across the lingual dentin (Fig 3-24). Procedures that reduce pulpal blood flow (eg, activation of pulpal sympathetic nerves or administration of vasoconstrictor agents with local anesthetics) upset this balance, permitting higher interstitial fluid concentrations of extrinsic material to exist than would occur at normal levels of capillary flow.[94] The control of pulpal blood flow is covered in detail in chapter 6.

Interventions that stimulate pulpal blood flow can lead to reduced interstitial levels of materials because of reduced permeability of dentinal tubules and

increased clearance by the pulpal microvasculature. For example, electrical stimulation of the inferior alveolar nerve in cats causes an increase in pulpal blood flow and an increase in outward dentinal fluid movement.[11,143–145] This increase shifts the inflow-outflow balance in the opposite direction, leading to a reduction in pulpal levels of materials. This response is believed to be due to axon reflex activity. That is, antidromic stimulation of the inferior alveolar nerve causes simultaneous depolarization of all of the nerve terminals providing sensory innervation of the teeth. This release of neuropeptides from the terminals causes both increased pulpal blood flow and extravasation of plasma proteins from the microcirculation.[13,144]

Alterations in pulpal blood flow may alter diffusion of substances through dentinal tubules by varying the rate of dentinal fluid outflow. Matthews and Vongsavan[143] speculated that hydrodynamic stimulation of exposed dentin evoked the release of neuropeptides from nerve terminals, thereby increasing local blood flow, tissue pressure, and stimulation of outward flow of fluid in dentinal tubules.[145] This outward fluid flow might rinse the tubules free of inward-diffusing noxious substances by "solvent drag." Indeed, Vongsavan and Matthews[9–12] demonstrated that Evans blue dye would not diffuse into exposed dentin in vivo but would do so in vitro. They speculated that the outward fluid flow in vivo blocked inward diffusion of blue dye.

This notion was tested in vitro by quantitation of the decrease in the inward flux of radioactive iodide when a simulated outwardly directed 15-cm H_2O pulpal pressure was applied to dentin disks. This outward fluid movement produced a 50% to 60% reduction in the inward diffusion of radioactive iodide across acid-etched dentin in vitro.[96] Thus, stimulated dentin with open tubules (ie, hypersensitive dentin) should produce higher rates of outward fluid flow and less inward diffusion of bacterial toxins than nonstimulated dentin. This protective effect is diminished in the presence of a smear layer.[96] Under these conditions, the inward flux of noxious substances may increase and permit higher interstitial fluid concentrations[17,95] to be achieved than would occur if there were more outward fluid movement. However, such partial occlusion may provide a therapeutic advantage by increasing pulpal concentrations of topically applied agents.[146]

In exposed dentin, the outward fluid flow through tubules is a first line of defense against the inward diffusion of noxious substances. Dentinal fluid also contains plasma proteins (eg, albumin and globulins) that bind or agglutinate some materials and may play a protective role.[49] A second defensive reaction occurs in freshly

exposed dentin that causes the permeability of dentin to decrease after cavity preparation in vital dog teeth but not in nonvital teeth.[95] Further, in experiments in which there was a continuous inward-directed bulk fluid movement, the rate of decrease in dentin permeability was related to the slow outward movement of some unidentified substance. These experiments were repeated on dogs treated with an enzyme that depleted fibrinogen from their blood. Under these conditions, cavity preparation produced a much smaller reduction in dentin permeability.[146]

Apparently, the outward movement of fluid is associated with an outward flux of plasma proteins,[14] including fibrinogen.[146] Although attempts to recover fibrinogen in dentinal fluid have been unsuccessful, intratubular fibrinogen has been detected in the dentin of rat molars using specific antibodies,[147] possibly because the dentinal thickness in rat molars following cavity preparation is only about 100 μm. Bergenholtz et al[4,148] found fibrinogen only in very deep cavities prepared in monkey or human teeth. Pashley et al[146] reported that dentin removed 61% of fibrinogen as it passed through a 1.0-mm disk. Presumably, even larger amounts of fibrinogen would be removed in vivo because the tubules contain odontoblast processes at their terminations, which further reduces the size of the channels through which this high–molecular weight molecule must move. This molecule may be responsible for reducing dentin permeability, especially at the pulpal terminations of the tubules, where it may polymerize into fibrin. Thus, multiple physiologic mechanisms regulate the inward diffusion of substances through dentinal tubules.

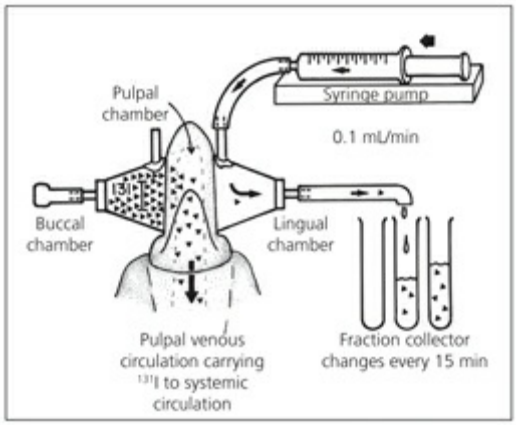

Fig 3-23 Placement of dual chambers on both the buccal and lingual surfaces of dog molars to measure both systemic absorption of substances applied to dentin and changes in pulpal interstitial fluid concentration of substances. Triangles indicate radioactive iodide. (Reprinted from Pashley[17] with permission.)

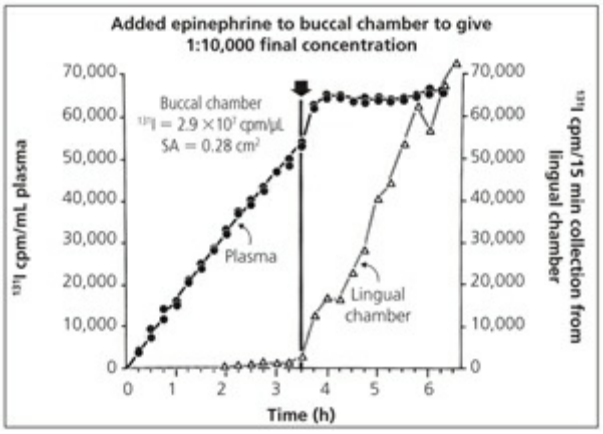

Fig 3-24 Effects of topical epinephrine on pulpal interstitial fluid iodide concentration. Over the first 3.5 hours, the plasma iodide concentration *(solid circles)* rose linearly as iodide permeated the buccal dentin into the pulp, where the microcirculation cleared it so efficiently that little iodide ever showed up in rinsings from the lingual chamber *(open triangles)*. However, as soon as epinephrine was added to the buccal chamber, the pulpal clearance stopped, and the interstitial fluid concentration rose and began diffusing across the lingual dentin into the lingual chamber. (Reprinted from Pashley[17] with permission.)

Dynamics of the Pulpodentin Complex

The pulpodentin complex is an important concept in understanding the pathobiology of dentin and pulp. Developmentally, pulpal cells produce dentin, nerves, and blood vessels. Although dentin and pulp have different structures and compositions, once formed they react to stimuli as a functional unit. Exposure of dentin through attrition, trauma, or caries produces profound pulpal reactions that tend to reduce dentin permeability and stimulate formation of additional dentin. These reactions are brought about by changes in fibroblasts, nerves, blood vessels, odontoblasts, leukocytes, and the immune system. Recent discoveries about the effects of nerves on pulpal blood vessels and vice versa have produced a new appreciation for the interaction of these two systems in response to stimuli applied to dentin. Too often, for technical or experimental reasons, the individual components of the pulpodentin complex are studied independently. However, it is becoming clear that the individual components are very interactive and that each modifies the activity of the other.

Dentin is a living tissue that can and does react to changes in its environment.[18,111] It is the only innervated hard tissue of the tooth.[15,149] Normally, dentin is covered coronally by enamel and on its root surfaces by cementum. Dentin exposed to the oral environment is subjected to considerable chemical, mechanical, and thermal

stimuli. The exposed, fluid-filled tubules permit minute fluid shifts across dentin whenever dentin is exposed to tactile, thermal, osmotic, or evaporative stimuli, which in turn activate mechanoreceptors in the pulp. These fluid shifts can directly stimulate odontoblasts, pulpal nerves, and subodontoblastic blood vessels by applying large shear forces on their surfaces as the fluid streams through narrow spaces. Moreover, as detailed in chapters 7 and 8, the sensory transduction mechanism for painful sensation to hydrodynamic stimuli is more sensitive to provocation by outward fluid flow than inner fluid flow.[97] In rats, exposure of dentin irritates the pulp and causes the release of neuropeptides such as calcitonin gene–related peptide or substance P from the pulpal nerves to create a local neurogenic inflammatory condition.[15,150,151] These stimuli also produce changes in pulpal blood vessels, leading to increased flow of plasma fluid and plasma proteins from vessels into pulp tissue spaces and out into dentinal tubules.[9–11,13,15] This extravasation of plasma can also cause increases in local pulp tissue pressure,[145,152] which tends to increase firing of sensitized neurons.[15,153] The outward fluid flow may have a protective, flushing action that may reduce the inward diffusion of noxious bacterial products in both exposed cervical dentin and perhaps even in leaking restorations[10,12,94,96] (Fig 3-25). Additional information on this topic is found in chapters 6, 7, and 8.

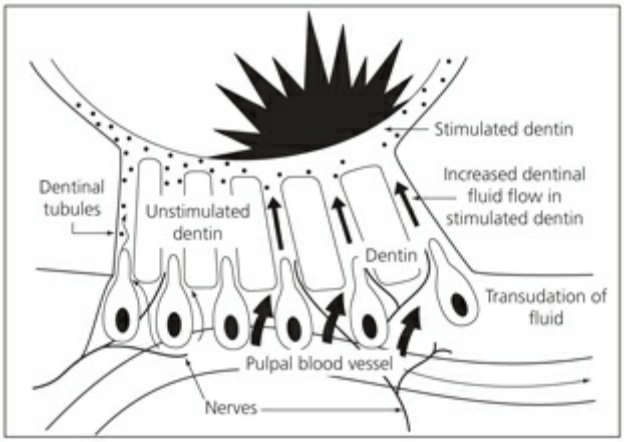

Fig 3-25 Inward diffusion of noxious material *(black dots)*, opposed by the outward movement of dentinal fluid in response to axon reflex–induced release of neuropeptides that cause vasodilation, transudation, and increased capillary permeability. (Reprinted from Pashley[153] with permission.)

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Pulp as a Connective Tissue

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Dental pulp is a connective tissue uniquely situated within the rigid encasement of mineralized dentin. Although dental pulp shares many properties with other connective tissues of the body, the unique location of dental pulp imposes several special constraints on its development, maintenance, and response to injury. It is apparent that the composition and structure of the pulp are quite different from those of the dentin. However, the two tissues exist in intimate embryologic and functional relation, which is why the dentin and pulp are usually considered together as an inseparable functional unit termed the *pulpodentin complex*.

General Properties of Connective Tissue

Connective tissue is the supporting tissue widely distributed throughout the body. The major constituent of connective tissue is its extracellular matrix, which is mainly composed of fibrillar proteins and ground substance. Connective tissue cells are

scattered within the extracellular matrix. Fibrillar proteins form an extensively meshed network of long, slender polymers that are arranged in an amorphous hydrated gel of ground substance. Collagen is the most abundant fibrillar protein and is the main component of collagen fibers, which confer strength of the tissue. Ground substance is responsible primarily for the viscoelasticity and filtration function of connective tissue. It is mainly composed of macromolecules called *proteoglycans*, which consist of a protein core and a varying number of large, unbranched polysaccharide side chains called *glycosaminoglycans*. Extracellular matrix also contains adhesive glycoproteins such as fibronectin, which primarily function to mediate cell-matrix interactions.

Fibroblasts are the principal cells in connective tissue. They form a network within extracellular matrix and produce a wide range of extracellular matrix components. They are also responsible for degrading the extracellular elements and thus are essential in the remodeling of connective tissue. Other cellular elements include blood-derived defense cells, such as macrophages, whose primary function is to cope with infection or the introduction of foreign substances.

The major function of connective tissue is to provide a matrix that binds cells and organs and ultimately gives support to the body. Connective tissue is also responsible for various activities that initiate and orchestrate reactions to pathogenic invasion, and thus it serves as an essential site of host defense. Connective tissue also has a remarkable capacity to repair damaged tissue in the form of scarring.

Structural Organization of the Pulp

In the central core of the pulp, the basic components already described are arranged in a manner similar to that found in other loose connective tissues. However, a characteristic cellular arrangement can be seen in the peripheral portion of the pulp (Fig 4-1).

As described in chapter 2, a layer of odontoblasts, the specialized cells that elaborate dentin, circumscribes the outermost part of the pulp. They form a single layer lining the most peripheral portion of the pulp, with cell bodies in the pulp and long cytoplasmic processes, the *odontoblast processes*, extending into the dentinal tubules (Fig 4-2). The shape of the cell body of odontoblasts is not uniform; rather, these cells are tall and columnar in the coronal pulp, short and columnar in the midportion of the tooth, and cuboidal to flat in the root portion.

A network of capillaries called the *terminal capillary network* exists within the odontoblastic layer.[1] Nerve fibers (terminal axons that exit from the plexus of Raschkow) pass between the odontoblasts as free nerve endings[2] (Fig 4-3). Moreover, this layer is populated by a substantial number of Class II major histocompatibility complex (MHC) molecule–expressing dendritic cells that may be responsible for detecting transdentinal antigenic stimuli.[3] Collagen fibrils,[4–6] proteoglycans,[7–9] and fibronectin[6,10] are identified between odontoblasts. They seem to be part of the interodontoblastic fibrous structure, the *Korff fibers*, which may be demonstrated by means of silver impregnation.

Subjacent to the odontoblastic layer is an area relatively free of cells. This area is known as the *cell-free zone* or *zone of Weil*. Major constituents of this zone include the rich network of mostly unmyelinated nerve fibers (see Fig 4-3), blood capillaries, and processes of fibroblasts. This zone is often inconspicuous when the odontoblasts are actively forming dentin.

More deeply situated pulpward is the cell-rich zone, which has a relatively high density of cells. The constituents of this zone are basically the same as those in the pulp proper, ie, fibroblasts, undifferentiated mesenchymal cells, defense cells (macrophages and lymphocytes), blood capillaries, and nerves. This zone is discernible because it has a higher density of fibroblasts than does the pulp proper and is much more prominent in the coronal pulp than in the root pulp. It has been suggested that this zone is a source of cells that differentiate into secondary (replacement) odontoblasts on injury to primary odontoblasts (see chapter 2).

From the cell-rich zone inward is the central connective tissue mass known as *pulp proper* or *pulp core*. This zone contains fibroblasts, the most abundant cell type; larger blood vessels; and nerves. Undifferentiated mesenchymal cells and defense cells such as macrophages are frequently located in the perivascular area. Collagen fiber bundles are much more numerous in the root pulp than in the coronal pulp. The clinical implication of this higher density of collagen fiber bundles in the apical region is the use of a barbed broach during pulpectomies. The most efficient removal of pulp tissue is achieved when the broach is passively placed apically to engage these large collagen bundles.

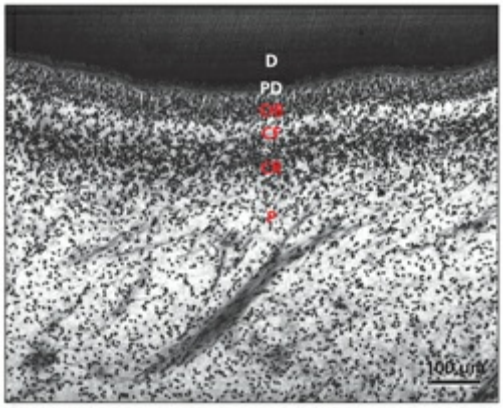


Fig 4-1 Light micrograph of mature coronal pulp (human third molar). D, dentin; PD, predentin; OB, odontoblastic layer; CF, cell-free zone; CR, cell-rich zone; P, pulp proper.

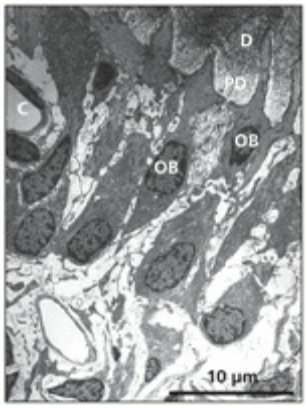


Fig 4-2 Electron micrograph of the odontoblastic layer of a rat molar. D, dentin; PD, predentin; OB, odontoblast; C, capillary.

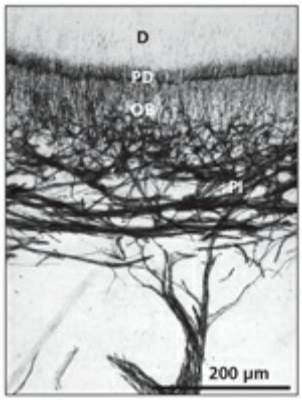


Fig 4-3 Distribution of neural elements (nerve fibers and Schwann cells) in a human third molar. Immunoperoxidase staining using a monoclonal antibody directed against nerve growth factor receptor. D, dentin; PD, predentin; OB, odontoblastic layer; Pl, plexus of Raschkow.

Pulp as the Soft Tissue Component of the Pulpodentin

Complex

As already stated, the pulp is usually considered together with the dentin as the pulpodentin complex because of their anatomical, developmental, and functional relationships. Structurally, pulpal elements such as odontoblast processes and neuronal terminals extend into the dentin. Functional coupling between pulp and dentin is exemplified in several aspects: *(1)* Encapsulation in dentin creates a low-compliance environment that influences the defense potential of the pulp; *(2)* pulpal connective tissue is able to respond to dentinal injuries, even when it is not directly stimulated; *(3)* pulp carries nerves that give dentin its sensitivity; and *(4)* pulp is capable of elaborating dentin both physiologically and in response to external stimuli.

Low-compliance environment

The most restrictive anatomical feature characteristic of the connective tissue of pulp is that it is encased in rigid mineralized tissue. This provides the pulp a low-compliance environment in which nutrition for the tissue is almost entirely supplied via vessels traversing the narrow apical foramen. Recognition of this physiologic restriction was the historical basis for the so-called self-strangulation theory that stigmatized the pulp as a connective tissue with a low capacity for defense or repair. According to the theory, increased tissue pressure, resulting from even modest increases in vasodilation and plasma exudation during inflammation, caused blood vessel compression and resultant ischemia and pulpal necrosis.

Some studies indeed showed a dramatic and sustained decrease of pulpal blood flow following application of inflammatory mediators.[11] However, later studies indicated that the pulp has physiologic feedback mechanisms that act to oppose increases in tissue pressure (ie, increased lymph flow and absorption of interstitial fluid into capillaries in non-inflamed areas).[12] This may be why inflammation of the pulp is usually long-standing within a confined area but heals following appropriate treatment measures (see chapter 6).

Dentin permeability

As described extensively in chapter 3, dentin is not a barrier that completely prevents the invasion of external noxious substances (eg, bacteria and their by-products) in the underlying pulp. This is due to its tubular structure, through which irritants may diffuse and affect the pulp in a number of clinical situations. A clear example is the effect of Class V cavity preparations in monkey teeth. Neutrophil infiltration was evident in the area of the pulp below the cut dentin when bacterial products were sealed within the cavities, whereas little or no inflammation was observed when the bacterial products were not applied.[13] Responses of the connective tissue of the pulp to several types of injury, including caries and operative procedures, are thus highly dependent on the degree of dentinal damage and resultant status of dentin permeability.[14,15]

Dentin permeability is influenced by pathophysiologic conditions of the neural and vascular systems of the underlying pulp. Microcirculatory changes of the pulp under the low-compliance environment can determine the status of pulpal blood flow, plasma exudation, and intradental tissue pressure and thus may influence the amount and direction of dentinal fluid movement.[11,12,16] As described in the next section, excitation of intradental nerves causes the release of vasoactive neuropeptides that potentially influence pulpal circulation.[15–17]

Sensory innervation

The extremely rich sensory innervation (see Fig 4-3) likely influences the defense reactions in the connective tissue of the pulp (see chapter 7). Many of the sensory fibers contain neuropeptides, such as substance P, calcitonin gene–related peptide (CGRP), and neurokinin A,[2,18,19] which are stored in the nerve terminals and may be released on depolarization. These neuropeptides are known to modulate vasodilation and increase vascular permeability (neurogenic inflammation). Several researchers have actually demonstrated that stimulation of pulpal sensory nerves induces blood flow increases and vascular leakage of proteins, which are most likely mediated by the release of neuropeptides.[20,21] Immunomodulatory effects of these neuropeptides have also been suggested.[22]

Substance P and CGRP exert trophic effects on the growth of pulp fibroblasts in vitro.[23,24] Moreover, inferior alveolar nerve sectioning and capsaicin treatment, both of which caused a decrease in the number of nerves containing substance P and CGRP, resulted in reduced secondary dentin deposition in rat molars.[25] These findings suggest that sensory nerves play a role in the modulation of extracellular matrix production and secondary dentinogenesis. Thus, pulpal sensory neurons have afferent (ie, pain-detecting) and efferent (ie, neurogenic inflammation, immunomodulatory, and healing) functions.

Extracellular Matrix of the Pulp

Collagen

Collagen is an extracellular structural protein that represents the major constituent of all connective tissues. Its structure is characterized by the presence of the triple-helical domain, which is formed by an assembly of three polypeptide chains (α chains) bound by hydrogen bonds and hydrophobic interactions. Chemically, collagen contains two characteristic amino acids: hydroxyproline and hydroxylysine. Glycine, proline, and hydroxyproline are the three main amino acid components, but there are at least 28 collagen types that differ in chemical composition, morphology, distribution, and function. Cells responsible for the synthesis of collagen include fibroblasts, chondroblasts, osteoblasts, cementoblasts, and odontoblasts.

Type I collagen is the most common form and occurs in a variety of tissues, including skin, tendon, bone, dentin, and dental pulp. Type I collagen is the major component of macromolecular structures, designated as collagen fibers. The collagen fibers are made up of fibrils in which the basic collagen molecules are aggregated in a highly organized structure. The fibrils display characteristic striations at intervals of 67 nm, determined by the stepwise overlapping arrangement of the molecules, and are a hallmark for identification of collagen fibrils under the electron microscope (Figs 4-4 and 4-5). The common molecular species contains two genetically distinct α chains that differ in their amino acid composition and sequence in a ratio of 2 to 1. This molecular arrangement is designated as $[\alpha 1(I)]_2\alpha 2(I)$. Small amounts of homotrimers ($[\alpha 1(I)]_3$) occur in some circumstances.

Differences in the combinations and linkages of the polypeptide chains making up collagen molecules are responsible for the different types of collagen. Based on their supramolecular structures, the collagens are divided into two main classes: fibrillar collagens (eg, types I to III, V, and XI) and nonfibrillar collagens. Collagen type II ($[\alpha1(II)]_3$) mainly occurs in cartilage and forms only thin fibrils that do not aggregate into fibers. This type does not occur in the pulp. Type III ($[\alpha1(III)]_3$) usually codistributes with type I collagen in a variety of unmineralized tissues and is a main component of reticular fibers, which form a loose mesh of extremely fine fibers particularly rich during embryogenesis, inflammatory processes, and wound healing. Type IV (occurs as $[\alpha1(VI)]_2\alpha2(IV)$, $[\alpha1(IV)]_3$, or $[\alpha2(IV)]_3$) and type VII ($[\alpha1(VII)]_3$) are present in the basement membranes. Type V occurs as $[\alpha1(V)]_2\alpha2(V)$, $[\alpha1(V)]_3$, or $\alpha1(V)\alpha2(V)\alpha3(V)$ and is widely distributed in a variety of unmineralized tissues. Type VI is a heterotrimer of three distinct chains—α1(VI), α2(VI), and α3(VI)—and is widely distributed in the body as interfibrillar filaments.

Collagen is a major organic component in the pulp, although the pulp appears to contain relatively lower concentrations of collagen than other collagenous connective tissues. The amount of collagen in dried human pulp is 25.7% in premolars and 31.9% in third molars.[26] These percentages are much higher than those reported for pulp in other species, such as 10.3% of the total protein in rabbit incisor pulp.[27] The content is higher in the radicular part of the pulp than in the coronal part.

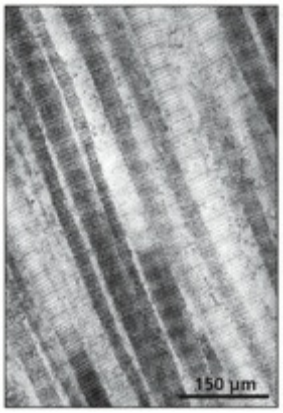


Fig 4-4 Transmission electron micrograph (TEM) of collagen fibrils in the central portion of the coronal pulp of a rat molar. Characteristic striations are visible.

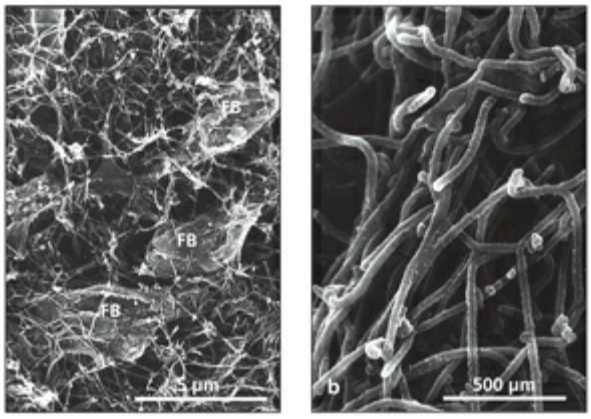


Fig 4-5 Scanning electron micrograph (SEM) of the central portion of the coronal pulp of a rat molar. *(a)* Low-power view showing network of collagen fibrils. Fibroblasts (FB) are embedded. *(b)* High-power view showing striations of the fibrils.

Of the collagen molecules occurring in the pulp, types I and III represent the bulk of the tissue collagen.[26,28,29] Type I is the predominant type and may contribute to the establishment of the architecture of the pulp. It is found mainly in thick, striated fibrils distributed in varying numbers and density throughout the connective tissue of the pulp (see Figs 4-4 and 4-5).

The relative proportion of type III collagen in the pulp is also high.[28–31] It has been reported that type III collagen constitutes 42.6% of total collagen in human pulp[26] and over 40% in bovine pulp.[28] This high level may provide the pulp a certain measure of elasticity.[32] Type III collagen usually forms thinner fibrils than type I. In the pulp proper, type III collagen appears as fine-branched filaments whose distribution is similar to that of reticular fibers.[29] In both cell-free and cell-rich zones, type III collagen is richly distributed.[33]

The composition of collagen types in dentin and predentin differs considerably from that of pulp. Dentin and predentin collagens are almost exclusively composed of type I,[34] although some investigators detected the presence of type III[33] and type V[35] collagens in predentin. Pulp fibroblasts can produce both type I and type III collagens, whereas the majority of collagen molecules produced by odontoblasts are type I.[36] This finding supports the idea that dentin collagen originates from odontoblasts and is not a combined product of odontoblasts and pulp fibroblasts.

Collagen fibers between odontoblasts were originally described as coarse argyrophilic fibers, the so-called Korff fibers, arising from the pulp, passing spirally between the odontoblasts, and entering the predentin.[4–6] These fibers are particularly evident during early dentinogenesis, suggesting that they are involved in odontoblast differentiation and formation of the mantle dentin. These fibers are mainly composed

of collagen types I and III.[6,37,38]

Type V[30,31,35] and type VI[31,35] collagen have been observed in the pulp, forming a dense meshwork of thin microfibrils throughout the stroma of the connective tissue of the pulp. Corkscrew fibers of collagen type VI have been found between fully differentiated odontoblasts toward the predentin, suggesting that these fibers are a component of Korff fibers.[31] In addition, collagen type IV is identified as a component of basement membranes of pulpal blood vessels.[31,39]

Collagen synthesis in the pulp is accelerated during the reparative process, exemplified in the process of dentin bridge formation following application of calcium hydroxide to exposed vital pulps. Calcium hydroxide initially induces the formation of a superficial necrotic zone because of its high pH. Following infiltration of inflammatory cells, fibroblast-like cells (including progenitors of secondary odontoblasts) proliferate and migrate to the injury site. This action is followed by the formation of new collagen that is arranged in contact with the superficial necrotic zone and contains cellular inclusions.[40] Thus, the application of calcium hydroxide results in accelerated collagen formation. During the early phase of reparative dentinogenesis, collagen fibrils show an interodontoblastic arrangement comparable to that of Korff fibers. However, these fibers become thinner and fewer following establishment of a firm layer of secondary odontoblasts.[6] It has thus been postulated that these fibers give support to the precursors of secondary odontoblasts before the formation of the regular odontoblastic layer.

Glycosaminoglycans and proteoglycans

Glycosaminoglycans are long, unbranched polymers of repeating disaccharide units (70 to 200 residues). The disaccharides usually consist of a hexosamine (glucosamine or galactosamine), which may contain ester sulfate groups, and a uronic acid (D-glucuronic acid or L-iduronic acid) with a carboxyl group. There are four main types of glycosaminoglycans that differ in the composition of the disaccharides and in tissue distribution: chondroitin sulfate/dermatan sulfate; heparan sulfate/heparin; keratan sulfate; and hyaluronic acid.

Hyaluronic acid is not sulfated and exists as free chains. The other glycosaminoglycans are present as constituents of proteoglycans, which consist of a

central protein core to which side chains of glycosaminoglycans are covalently linked. The structure of proteoglycans is heterogenous in terms of the size of the core protein and the size and number of glycosaminoglycan chains. In general, the three-dimensional structure of proteoglycans can be portrayed as an interdental brush, with the wire stem representing the protein core and the bristles representing the glycosaminoglycans.

Because of the abundance of carboxyl groups, sulfated hexosamines, and hydroxyl groups in glycosaminoglycan chains, proteoglycans are intensely hydrophilic and act as polyanions. The long glycosaminoglycan chains form relatively rigid coils constituting a network in which a large amount of water is held. Thus, proteoglycans are present as a characteristic gel that occupies a large space relative to their weight and provides protection against compression of connective tissue.

Given their spatial organization and high negative charge, proteoglycans prevent diffusion of larger molecules but attract cationic material. Importantly, most extracellular matrix proteins and many growth factors such as transforming growth factor β (TGF-β) have binding sites for glycosaminoglycans. Thus, proteoglycans regulate tissue organization by linking together several extracellular matrix components (which serve as cellular binding sites) and may act as a reservoir for bioactive molecules. Some proteoglycans, such as syndecan, are located on the cell membrane. These feature an extracellular domain that is able to bind to extracellular glycoproteins (collagen, fibronectin, tenascin, etc) and a cytoplasmic domain that links with the cytoskeleton. Thus, they are cell-surface receptors that connect extracellular matrix molecules to the cytoskeleton of the cell and control cell functions.

The pulp contains several types of glycosaminoglycans that normally occur in other connective tissues.[41] In human pulp, chondroitin sulfate, dermatan sulfate, and hyaluronic acid are consistently found.[42,43] A variety of proteoglycans are also identified in the connective tissue of the pulp by means of immunohistochemistry.[7–9] Predentin possesses various types of glycosaminoglycans and proteoglycans, whereas dentin mainly contains chondroitin sulfate.[7,44]

Glycosaminoglycans and proteoglycans are thought to play an important role during dentinogenesis. They show an affinity for collagen and thus influence its fibrinogenesis, which takes place before the period of mineralization. Chondroitin sulfate, the major glycosaminoglycan present in teeth with active dentinogenesis, has a strong capacity to bind calcium[45] and may be involved in maintaining calcium phosphate during mineralization.[46] An in vitro study has demonstrated that

glycosaminoglycans may also be involved in the maintenance of the polarized state of cultured odontoblasts.[47]

In another study employing human teeth, decorin, a small chondroitin–dermatan sulfate proteoglycan consisting of a core protein and a single glycosaminoglycan chain, was immunolocalized in odontoblast cell bodies and its processes were located within predentin and along the calcification front and dentinal tubules[8] (Fig 4-6). This finding suggests that decorin is synthesized by odontoblasts and transported through the odontoblast processes, where an accumulation along the calcification front may be involved in mineral nucleation.

During infection and inflammation, the high viscosity of proteoglycans may present a mechanical barrier to bacteria. However, several bacterial species, such as some strains of streptococci, produce hyaluronidase as a spreading factor. This enzyme reduces the viscosity of the barrier by hydrolyzing glycosaminoglycans and thus contributes to bacterial penetration of connective tissues. By virtue of this ability, hyaluronidase is presumed to be a factor promoting the destruction of periodontal tissues.[48] The enzyme activity has been detected in bacterial isolates from infected root canals,[49] although its relevance to pulpal pathosis is not completely understood.

Fig 4-6 Distribution of the small proteoglycan decorin in the pulpodentin complex of a human tooth (immunofluorescence staining using an antiserum against decorin). Intense immunoreactivity extends along the calcification front *(arrows)* and dentinal tubules. Odontoblast cell bodies (OB) and their processes *(arrowhead)* in predentin (PD) also express specific antibody reaction. D, dentin; P, pulp. (Reprinted from Yoshiba et al[8] with permission.)

Noncollagenous proteins

Fibronectin is a multifunctional stromal glycoprotein that exists as *(1)* a circulating plasma protein, *(2)* a protein that attaches on the surface of cells, and *(3)* insoluble

fibrils forming part of the extracellular matrix. This molecule, with a molecular weight of 440,000, features sites that bind collagen, glycosaminoglycans, and several cell-adhesion molecules such as integrins. By virtue of this ability, fibronectin acts as a mediator for cell-cell and cell-matrix adhesion and thus has a major effect on the proliferation, differentiation, and organization of cells.

Fibronectin is ubiquitously distributed in the extracellular matrix of the pulp. In the pulp proper, it forms a reticular network of fibrils, with an increased concentration around the blood vessels.[33,50,51] Fibronectin is also immunolocalized in the odontoblastic layer, where it forms corkscrew fibers that pass from the pulp into predentin parallel to the long axis of odontoblasts[10,50] (Fig 4-7), which suggests that fibronectin is a constituent of Korff fibers. Fibronectin at this position is believed to mediate the interaction between fully differentiated odontoblasts and extracellular fibers and may contribute to the maintenance of the specific morphology of these cells.[10] Immunoreactivity of fibronectin is also seen at the border between odontoblast cell bodies and predentin, suggesting that fibronectin may contribute to the maintenance of a tight seal at this site.[10]

Fibronectin is implicated in the terminal differentiation and polarization of primary odontoblasts.[52] Preodontoblasts in the dental papilla are initially surrounded by fibronectin associated with the dental basement membrane. During terminal differentiation of odontoblasts, however, fibronectin is restricted to distribution around the apical pole of polarizing odontoblasts. Consequently, a nonintegrin, 165-kDa fibronectin-binding protein is transiently expressed at the apical pole. It has been postulated that an interaction between extracellular fibronectin and this protein modulates the shape and polarity of the odontoblasts through reorganization of the cytoskeleton.

As in other connective tissues, fibronectin may be involved in cell migration and anchorage in the wound-healing process of the connective tissue of the pulp. Moreover, it is implicated in reparative dentinogenesis as well. In exposed human pulps capped with calcium hydroxide, fibronectin is immunolocalized in the zone of initial dystrophic calcification located just beneath the superficial necrotic layer. Progression of odontoblast differentiation has been observed beneath this zone.[53,54] In another study in which cavity preparations were made in rat molars, fibronectin was shown to accumulate first in the exudative lesion below the cavity and then be distributed in newly formed predentin.[55] These findings suggest that fibronectin regulates the migration and differentiation of secondary odontoblasts.

The extracellular matrix of the pulp contains some noncollagenous glycoproteins

that are predominantly distributed in the matrix of mineralized tissues such as dentin and bone. Osteonectin, a 43-kDa glycoprotein, has been immunolocalized in rat[56] and human[57] pulp tissue as well as cultured human pulp fibroblasts.[57,58] This protein has strong affinity for calcium and hydroxyapatite and promotes the deposition of calcium phosphate mineral onto collagen. Bone sialoprotein, a major sialic acid–rich phosphoglycoprotein in bone, has also been immunolocalized in the extracellular matrix of the human pulp.[57] Cultured pulp fibroblasts are able to synthesize osteopontin, a highly phosphorylated sialoprotein localized in dentin, bone, and cementum, as well as in a wide range of nonmineralized tissues.[59]

The presence of noncollagenous proteins associated with tissue mineralization suggests a probable role of these proteins in the mediation of mineralized tissue formation of the pulp, However, the pulp matrix lacks several noncollagenous proteins that are identified in dentin, such as dentin sialoprotein, dentin phosphoprotein, dentin matrix protein 1, and osteocalcin.[60] The absence of these dentin-specific proteins could partly explain why the pulp does not mineralize under physiologic conditions.

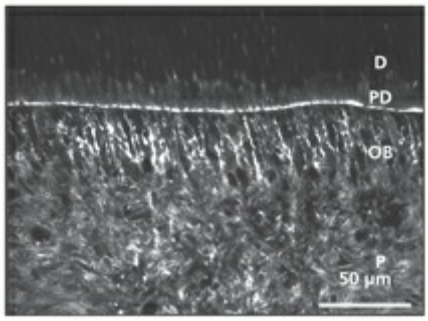


Fig 4-7 Distribution of fibronectin in the pulpodentin complex of a human tooth (immunofluorescence staining using an antifibronectin antibody). Between odontoblasts (OB), fibrous structures, some of which extend into predentin (PD), show specific antibody reaction. A fluorescent line is visible at the border between odontoblast cell bodies and predentin. D, dentin; P, pulp. (Reprinted from Yoshiba et al[10] with permission.)

Cells of the Pulp

Odontoblasts

Structure

Odontoblasts, the most highly differentiated cells of the pulp, are postmitotic neural crest–derived cells whose primary function is to elaborate dentin. The cytoplasmic feature of the odontoblast cell body varies according to the cell's functional activity.[61,62] Actively synthesizing odontoblasts exhibit all the characteristics of matrix-synthesizing cells. They display prominent organelles consisting of an extensive rough endoplasmic reticulum, a well-developed Golgi complex, numerous mitochondria, and numerous vesicles (Fig 4-8). A large, oval nucleus is eccentrically located in the basal part of the cell body. This nucleus contains up to four nucleoli and is surrounded by a nuclear envelope. A particularly well-developed rough endoplasmic reticulum is found throughout the entire cell body, closely associated with numerous mitochondria. The rough endoplasmic reticulum consists of closely stacked cisternae that are usually aligned parallel to the long axis of the cell body. Numerous ribosomes are associated with the membranes of the cisternae. A well-developed Golgi complex composed of several stacks of saccules is centrally located in the supranuclear region. Numerous transport vesicles are accumulated at the immature face of the Golgi complex, and secretory granules of various sizes are found at the mature face.

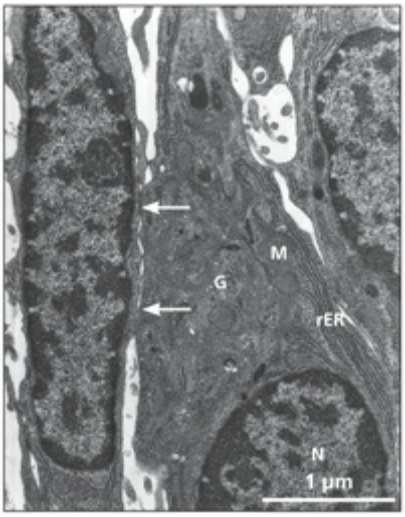


Fig 4-8 Ultrastructure of an odontoblast cell body (rat molar). N, nucleus; G, Golgi complex; M, mitochondria; rER, rough endoplasmic reticulum; *arrows,* cell-cell junctions.

Autoradiographic studies using a radioactive collagen precursor (^{3}H-proline) as a tracer revealed that the synthesis, migration, and release of collagen precursors in odontoblasts follow the classic pathway for secretion of extracellular proteins. The isotope was rapidly incorporated in the rough endoplasmic reticulum and transported to the Golgi apparatus, where it was packed into secretory vesicles. The

vesicles then migrated to the base of the cellular process, where the labeled vesicles fused with the cell membrane and the contents were released into the predentin matrix.[63]

Numerous mitochondria are evenly distributed throughout the cell body. Besides being the major site of adenosine triphosphate production, mitochondria in odontoblasts may also serve as sites for intracellular storage and regulation of calcium.[63] Numerous filaments and microtubules are located among the organelles described above. This cytoskeleton contributes to cell shape and polarity.

Quiescent odontoblasts are shorter and less polarized than actively synthesizing cells and show a reduction in number and size of the endoplasmic reticulum, Golgi complex, and mitochondria.[61,62] When the cells are in the transitional stage between active synthesis and quiescence, these organelles tend to show perinuclear distribution. Autophagic vacuoles can be seen within the cytoplasm, and thus an autophagic process may mediate the reduction of organelles. At the final stage of the cell's life cycle, these organelles are located only within the infranuclear region; the supranuclear region is devoid of organelles except for large, lipid-filled vacuoles.[61]

Odontoblast process

This is a direct extension of the cell body and occupies most of the space within the dentinal tubules (Fig 4-9). Its diameter is 3 to 4 μm at the pulp-predentin border and gradually narrows as it passes within the dentinal tubules. The process has numerous side branches that may contact the branches of other odontoblasts. In contrast to the main cell body, the process is virtually devoid of major organelles for synthetic activity. A few cisternae of the endoplasmic reticulum, sparsely occurring mitochondria, and occasional ribosome-like granules are seen, mostly at the level of the predentin. However, the process displays a well-developed cytoskeleton as its principal component, filled with numerous microfilaments and microtubules oriented parallel to its long axis.[62,64,65] The process also contains numerous secretory vesicles and coated vesicles of various sizes and shapes.

Cavity or crown preparation may disturb odontoblast processes, leading to irreversibly damaged odontoblasts. Thus, information on the extent or length of odontoblast processes is important to clinicians because it allows for better estimation of the impact of tooth preparation on the pulpodentin complex. There has been a long-standing controversy regarding the extent of the odontoblast process, ie, whether the process extends the full length[66,67] or whether it is present only in the inner portion of the tubules.[68,69] However, a study employing fluorescent

carbocyanine dye labeling of process membranes and confocal laser scanning microscopy convincingly demonstrated that the processes in rat molars do not extend to the outer dentin except during the early stage of tooth development.[70] Similar findings have been obtained in a study in which fluorescence labeling and transmission electron microscopy were employed in human teeth.[71]

Odontoblast junctions

Several types of cell-cell junctions occur between adjacent odontoblasts. Desmosome-like junctions, which do not contain the intercellular disks found in typical epithelial desmosomes, occur along the lateral surfaces of the odontoblasts.[72,73] This type of junctional contact may promote cell-cell adhesion and play a role in maintaining the polarity of the odontoblasts.

Gap junctions have also been described between the lateral surfaces of the odontoblasts.[72,73] These specialized junctions may provide pathways for intercellular transfer of ions and small water-soluble metabolites and thus may play a role in controlling cytodifferentiation of the odontoblasts and mineralization of dentin. From this perspective, gap junctions help to coordinate intercellular responses. Studies have demonstrated that the expression of connex-in 43, a gap junction structural protein, shows an increase in differentiating odontoblasts.[74,75] This finding suggests that gap junctional intercellular communication may contribute to odontoblast differentiation. Gap junctions may also be present between odontoblasts and subjacent pulp cells, as demonstrated by electron microscopy[72] and dye coupling analysis.[76] This suggests that information on external stimuli detected by odontoblasts may be conducted to pulp fibroblasts.

The most prominent contact between odontoblasts is in the border region between the cell body and the process. This region contains the connecting apparatus, terminal bar–terminal web structures, which consists mostly of small-gap and tight junctions.[72,73] The tight junctions are seen exclusively in this region and may prevent passage of material between the odontoblasts. It is still unclear whether they completely encircle the odontoblasts (ie, as zonular tight junctions) or whether they are macular or “leaky.” It is known that some small nerve fibers[2] and collagen fibers[4,5] pass through the interodontoblastic space and reach the predentin. This observation may indicate that the junctional complex in odontoblasts does not completely encircle the cell body.

A study using immunohistochemistry to ZO-1, a membrane protein present at the cytoplasmic surface of tight junctions, has also demonstrated that the junctions are

macular.[75] When the permeability of the junctions was tested by perfusion of intercellular tracers, no penetration of the tracers occurred beyond the tight junctions,[77,78] which may mean that the odontoblastic layer acts as a physiologic barrier. However, another study demonstrated that the barrier was leaky when a higher dose of the tracer was used.[79] Experiments also demonstrated that the tracer penetrated predentin and dentin following cavity preparation.[77,79] Such perturbation of the barrier suggests an increased outward flow of dentinal fluid. This phenomenon may contribute to reparative processes in that it may transfer reparative compounds and ions to the site of injury and prevent inward diffusion of external noxious substances.

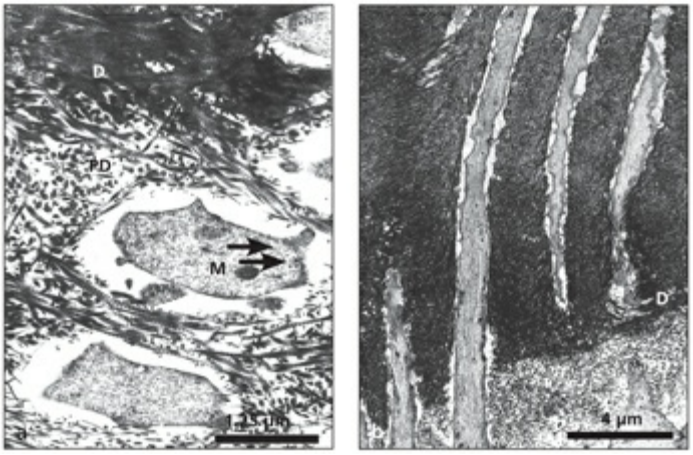


Fig 4-9 Ultrastructure of an odontoblast process (rat molar). D, dentin; PD, predentin. *(a)* Cross section of processes in predentin showing mitochondria (M) and vesicles *(arrows)*. *(b)* Longitudinal section of processes situated in predentin and dentin showing parallel alignment of microtubules and filaments.

Functions

Secretion and synthesis

Odontoblasts are secreting cells whose primary function is to synthesize many kinds of organic matrix components of the dentin, mainly type I collagen. Moreover, odontoblasts also produce several bioactive substances (eg, chemokines, growth factors such as TGF-β, matrix metalloproteinases [MMPs]) and express molecules that are involved in defense-and-repair reactions (eg, toll-like receptors [TLRs]). With this property, odontoblasts may be regarded as defense cells that act as the first cell in the pulpodentin complex to be encountered by transdentinal bacterial challenges.

Collagens, mainly type I, are the main organic components of the predentin and dentin that odontoblasts secrete. Proteoglycans are also secreted by these cells.

Moreover, odontoblasts also synthesize various noncollagenous proteins associated with mineralization, including bone sialoprotein, dentin sialoprotein, phosphophoryn (dentin phosphoprotein), dentin matrix protein 1, osteocalcin, osteonectin, and osteopontin.[60] Dentin sialoprotein and phosphophoryn have been considered to be dentin specific,[80] although one study has demonstrated that bone cells synthesize much smaller amounts of these proteins.[81] These molecules are secreted at the apical end of the odontoblasts' cell bodies as well as along the cytoplasmic processes within the tubules of the predentin. Moreover, odontoblasts may intracellularly transport calcium ions to the mineralization front.[82]

In addition to synthetic activity, odontoblasts are involved in the degradation of organic components via production of a group of zinc enzymes called *matrix metalloproteinases* (eg, collagenase, gelatinase, stromelysin), which catalyze the degradation of matrix macromolecules such as collagens and proteoglycans. MMPs known to be synthesized by odontoblasts in fully developed human teeth include MMP-8 (collagenase 2), MMP-2 and -9 (gelatinases), membrane-bound MMP-14 (MT1-MMP), and MMP-20 (enamelysin).[83] They may be incorporated in dentin and play some role in its physiologic processes, although precise functions remain unclear. It has also been proposed that these dentinal MMPs are involved in the progression of dentinal caries.[84] The dentinal MMPs may also cause hydrolysis of incompletely resin-infiltrated collagen fibers underlying adhesive resin composites and thus may adversely affect the longevity of the restorations.[85]

Growth factors are proteins that induce cellular differentiation and/or proliferation following binding to receptors on target cells. Among these, members of the TGF-β/bone morphogenetic protein (BMP) superfamily are considered important for the differentiation and matrix secretion of odontoblasts because these factors are known to participate in the modulation of cell growth, differentiation, immune responses, and wound healing. In mature teeth, odontoblasts are reported to be the main source of TGF-β in the pulpodentin complex.[86,87] Some isoforms of TGF-β may be secreted and sequestered within the dentin matrix and could later act to stimulate repair following dentinal injury.[60]

Odontoblasts express several integrins, which are a group of heterodimeric cell-surface glycoproteins that promote cell-cell and cell–extracellular matrix adhesion. Integrins are composed of two noncovalently linked polypeptide chains, α and β. At least 18 α subunits and 8 β subunits have been identified. The various combinations of α and β subunits constitute about 30 different integrin molecules that differ in their ligand-binding specificity and expression on different types of cells. Integrins

identified on human mature odontoblasts include αvβ3, αvβ5, and perhaps αvβ1.[88] Fibroblasts from human pulp also express α1, α3, α5, α6, αv, and β1 integrin subunits.[89] The specific roles of integrins in the fully matured pulpodentin complex are not well understood, although these molecules may contribute to the maintenance of the integrity of the pulpodentin complex through interodontoblast and odontoblast–extracellular matrix bindings. On injury, integrins could exert some signaling functions that regulate repair processes.

Innate immunity

There is growing evidence to show that odontoblasts are involved in innate immunity as the first cells that are exposed to transdentinally derived bacterial challenges. Findings that support this notion include the following: *(1)* Odontoblasts express the pattern-recognizing TLRs[90–93]; *(2)* odontoblasts are able to produce several chemokines and express chemokine receptors[90,93–95]; and *(3)* odontoblasts are able to produce antimicrobial peptides such as β-defensins.[93,96,97]

TLRs are a type of pattern-recognition receptor that enables the cells to differentially recognize pathogen-associated molecular patterns. Ten TLRs have been identified in humans, and each TLR shows ligand-recognition specificity; TLR-2 recognizes products of gram-positive bacteria such as peptidoglycans and lipoteichoic acid, whereas TLR-4 recognizes lipopolysaccharides produced by gram-negative bacteria.

Following TLR activation by ligands of microbial origin, several types of host defense responses, including production of cytokines, chemokines, and antimicrobial peptides such as defensins, are induced. In a study in which TLR and chemokine expression was examined in vitro in differentiated human odontoblasts, it was demonstrated that odontoblasts challenged by lipoteichoic acid—a wall component of gram-positive bacteria—upregulated TLR-2 (lipoteichoic acid receptor) and produced various chemokines.[90] The study also revealed that supernatants from lipoteichoic acid–stimulated odontoblasts augmented migration of immature dendritic cells, suggesting that odontoblasts, on stimulation by lipo-teichoic acid, initiate innate immune responses via generation of chemokines that induce dendritic cell migration.[90]

In another study utilizing organotypic tooth crown cultures in which human odontoblasts were maintained, messenger RNA (mRNA) expression of several molecules involved in innate immunity, such as chemokines, chemokine receptors, TLRs (TLR-2 and TLR-4), cytokines, and β-defensins, was detected in

odontoblasts.[93] Stimulation of these cells with TLR-2 and TLR-4 agonists differently altered the expression pattern of the aforementioned molecules, suggesting that odontoblasts differentially respond to gram-positive and gram-negative bacteria. Together, these findings support the view that odontoblasts play a role in the innate immune system against caries and other bacterial challenges via the dentinal tubules.

Responses of odontoblasts to injury

The pulpodentin complex has a unique defense-and-repair reaction not seen in most other connective tissues. This reaction involves the formation of new mineralized tissue in response to injury (see chapter 2). Either the odontoblasts already exist in the pulp (primary odontoblasts) or newly generated mineralized tissue–forming cells may elaborate the new mineralized tissue.

Under physiologic conditions, primary odontoblasts in the adult tooth produce new dentin (secondary dentin) at a very slow rate. Once the primary odontoblasts are injured, the dentin production may be accelerated as a defense-and-repair reaction. Depending on the nature, magnitude, and duration of the injury, the primary odontoblasts may be reversibly damaged or may actually die. In the latter situation, the dead cells may be replaced by secondary (replacement) odontoblasts that produce the matrix of new dentin (tertiary dentin). Some researchers define *reactionary dentin* as the new dentin secreted by surviving primary odontoblasts, in contrast to *reparative dentin*, which is produced by newly recruited secondary odontoblasts[98] (see chapter 2).

Although controversy still surrounds the origin of secondary odontoblasts, undifferentiated mesenchymal cells in the pulp proper and/or differentiated pulp cells that dedifferentiate into undifferentiated cells and then redifferentiate into odontoblasts have been implicated as likely sources.[99–101] Stem-like cells that possess self-renewing capacity, high proliferative potential, and the ability to undergo multilineage differentiation have been isolated from pulp tissues.[102–105] These cells may represent a population of less-differentiated progenitors of secondary odontoblasts among a hierarchy of more committed progenitors[103] (see chapters 2 and 5).

Although variable in morphology, secondary odontoblasts may be less columnar and more sparsely arranged than primary odontoblasts. However, secondary odontoblasts seem to share several phenotypic and functional properties with primary odontoblasts. A study of reparative dentinogenesis in rats demonstrated that secondary odontoblasts express mRNA for type I but not type III collagen and that

they are immunopositive for dentin sialoprotein, a dentin-specific protein that marks the odontoblast phenotype.[36]

Fibroblasts

Fibroblasts are the most numerous connective tissue cells with the capacity to synthesize and maintain connective tissue matrix (Fig 4-10; see also Fig 4-5a). They are widely distributed throughout the connective tissue of the pulp and are found in high densities in the cell-rich zone of the coronal pulp.

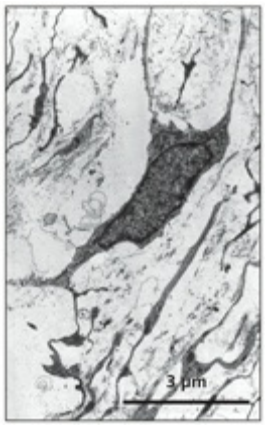


Fig 4-10 TEM of a fibroblast in the coronal pulp of a rat molar. This cell has several long cytoplasmic processes and relatively well-developed organelles.

The morphology of pulp fibroblasts varies according to their functional state, which is in common with fibroblasts in other parts of the body. Intensely synthetic cells have several irregularly branched cytoplasmic processes with a nucleus located at one end of the cell. They are rich in rough endoplasmic reticulum, and the Golgi complex is well developed. This type of cell is particularly common in the young pulp. However, quiescent cells, frequently found in older pulp, are smaller than the active cells and tend to be spindle shaped with fewer processes. The amount of rough endoplasmic reticulum in these cells is also smaller. When the quiescent cells are adequately stimulated, their synthetic activity may be reactivated. The mitotic activity of fibroblasts is quite low in adult connective tissues, but active cell division occurs when the tissue is damaged.

Synthesis of type I and type III collagens is a main function of fibroblasts in the pulp, as in fibroblasts elsewhere in the body. They are also responsible for the synthesis and secretion of a wide range of noncollagenous extracellular matrix

components, such as proteoglycans and fibronectin. Pulp fibroblasts also produce some noncollagenous proteins responsible for hard tissue mineralization, such as osteonectin, bone sialoprotein,[57,58] and osteopontin.[59] Moreover, these cells express growth factors, such as members of the TGF-β/BMP superfamily.[87,106,107] TGF-β1, BMP-2, and BMP-4 either enhanced or suppressed TGF-the alkaline phosphatase activity and matrix protein expression of pulp cells,[106] suggesting that the behavior of pulp cells is controlled by these molecules, especially with respect to the extracellular matrix protein synthesis and mineralizing potential of these cells.

Fibroblasts are implicated in the degradation of extracellular matrix components and thus are essential in the remodeling of connective tissues. Fibroblasts are able to phagocytose collagen fibrils and digest them intracellularly by lysosomal enzymes. Moreover, these cells are a source of MMPs (eg, collagenase, gelatinase, stromelysin) that degrade matrix macromolecules such as collagens and proteoglycans. Expression of various classes of MMPs has been detected in human pulp tissue.[108,109] The expression is characterized by a high level of MMP-13 (collagenase 3), which has very limited expression in other normal adult tissues.[109] In vitro studies demonstrated that MMP production from cultured pulp fibroblasts showed an increase following stimulation with cytokines and/or bacterial components.[110,111] These findings suggest that fibroblasts stimulated by inflammatory cytokines and bacterial by-products play a role in the degradation of pulpal connective tissue during pulpal inflammation.

Undifferentiated mesenchymal cells and stem cells

Undifferentiated mesenchymal cells are distributed throughout the cell-rich zone and the pulp core, frequently occupying the perivascular area. These cells appear as stellate-shaped cells with a relatively high nuclear-to-cytoplasmic ratio. However, they are usually difficult to distinguish from fibroblasts under light microscopy. After receiving appropriate stimuli, they may undergo terminal differentiation and give rise to either fibroblasts or odontoblasts. At least some of these cells may represent multipotent stem cells and their descendants.

Multipotent stem cells are found throughout the body and divide to replenish dying cells and repair injured tissues. Recently, much attention has been given to the potential utility of stem cells for regenerative therapy of injured tissues and organs,

including the pulpodentin complex[112] (see chapter 5). Thus, attempts have been made to isolate pulp cell populations that possess self-renewal capacity, high proliferation potential, and the ability to undergo multilineage differentiation.[102–105] These stem-like cells may be a less differentiated progenitor of secondary odontoblasts among a hierarchy of more committed progenitors.[103]

Stem cells reside in niches, a specialized cellular environment that provides stem cells with the support needed for self-renewal. Although the location of niches in the pulp is not yet fully understood, largely due to the lack of clear markers for the stem-like cells of the pulp, a considerable part of these cells may reside within perivascular niches of the pulp.[113] The stem-like cells may proliferate in the pulp and migrate to the injury site following pulpal exposure.[114]

Immunocompetent cells

The ability of connective tissue to generate and support local inflammatory and immune reactions makes it an active participant in host defense. A considerable part of this capacity depends on immunocompetent cells residing in the tissue. These cells are recruited from the bloodstream, where they reside as somewhat transient inhabitants. Once foreign antigens gain entry to connective tissue, these cells interact to create mechanisms that help defend the tissue from antigenic invasion.

Lymphocytes

The specificity of immune responses is due to lymphocytes because they are the only cells in the body capable of specifically recognizing different antigens. They are broadly divided into B lymphocytes and T lymphocytes, which are quite different in phenotype and function. B lymphocytes differentiate into antibody-secreting cells (ie, plasma cells) and thus play a major role in humoral immunity. They carry membrane-bound forms of antibodies by which they can recognize antigens. T lymphocytes play a central role in specific immune responses to protein antigens.

T lymphocytes are subdivided into helper ($CD4^+$) and cytotoxic ($CD8^+$) types. The main function of cytotoxic T lymphocytes is to cause lysis of other cells that carry foreign antigens, such as cells infected by intracellular microbes (eg, viruses). Thus, cytotoxic T lymphocytes are predominantly involved in cell-mediated

immunity. Helper T lymphocytes play a crucial role in orchestrating both humoral and cell-mediated immune responses through production of *cytokines*, bioactive molecules that regulate the intensity and/or duration of the immune response by either stimulating or inhibiting the action of various target cells.

Following activation, helper T lymphocytes secrete several cytokines. Depending on the profile of cytokine production, these cells are further classified into T_H1 and T_H2 cells. T_H1 cells predominantly produce interleukin 2 and interferon γ and are primarily involved in activation of macrophages. T_H2 cells predominantly produce interleukins 4, 5, and 6 and stimulate proliferation and differentiation of B lymphocytes.

Mechanism of activation

The T-lymphocyte response to protein antigens requires the participation of antigen-presenting cells. These cells uptake protein antigens, convert them into peptide fragments, assemble the peptides with proteins encoded for the MHC, and then express the assembly on their surface. T lymphocytes are able to recognize not the antigens themselves but the assembly.

Class I and Class II MHC molecules bind to CD8 and CD4 molecules, respectively, on T lymphocytes and are involved in the T-lymphocyte response. Class I MHC molecules, designated as *human leukocyte antigen (HLA) -A*, *-B*, and *-C* in humans, are expressed on almost all cells of the body and are involved in the activation of $CD8^+$ T lymphocytes. Class II MHC molecules (HLA-DR, -DP, and -DQ in humans) are expressed on limited types of cells; dendritic cells and B lymphocytes constitutively express these molecules, whereas macrophages, endothelial cells, and some other types of cells can be induced to express them.

The interaction between antigen-presenting cells and $CD4^+$ T lymphocytes involves contact between the Class II MHC molecule–associated peptide and the T-cell receptor, the first signal required for T lymphocytes to become activated. Binding of several costimulatory molecules on antigen-presenting cells to their ligands on T lymphocytes is also necessary for the activation. Throughout this chapter, the term *antigen-presenting cells* is used to denote cells that are involved in the Class II–restricted antigen recognition, although it seems appropriate that target cells of $CD8^+$ T lymphocytes should be included among the antigen-presenting cells as well.

Lymphocytes of the pulp

The composition of lymphocytes in the pulp resembles that of other connective tissues, such as the dermis of the skin. T lymphocytes are recognized as normal residents of human and rat dental pulp (Fig 4-11). These cells are scattered predominantly along the blood vessels in the pulp proper, although numerically fewer among pulp cellular elements. Several immunohistochemical studies have demonstrated that $CD8^+$ T lymphocytes outnumber $CD4^+$ T lymphocytes.[115–119]

In normal human pulp, T lymphocytes usually express CD45RO, a marker for memory T lymphocytes.[116,117,119] Thus, they are predominantly composed of memory T lymphocytes. However, cells expressing phenotypic markers for activated T lymphocytes, such as CD25 (interleukin 2 receptor), are rarely found.[119] As described later, $CD4^+$ T lymphocytes may be involved in the initial immunodefense of the pulp following interaction with Class II MHC molecule–expressing cells. The roles of memory $CD8^+$ T lymphocytes in the normal pulp remain unclear, although it has been proposed that this type of cell patrols nonlymphoid tissues to achieve effective immunosurveillance.[120]

B lymphocytes and plasma cells, the terminally differentiated B lymphocytes with a specialized capacity for antibody synthesis, are rarely encountered in normal human pulp.[115–118] In studies of rat molar pulp, a few plasma cells were occasionally detected in the coronal pulp.[121,122] At present, it seems difficult to identify a significant role for B lymphocytes in the normal pulp.

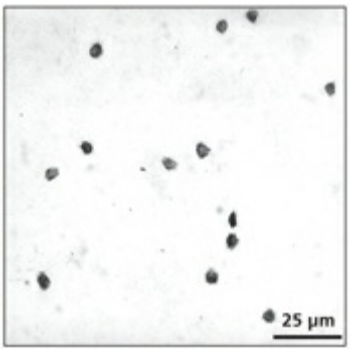


Fig 4-11 T lymphocytes in the coronal pulp of a human third molar visualized by immunoperoxidase staining using a monoclonal CD3 antibody (reactive to all T lymphocytes). Small, round cells are scattered in the connective tissue of the pulp.

A recent study has demonstrated that normal rat pulp contains natural killer cells, a subset of lymphocytes that are deeply involved in innate immunity.[123] These cells are known to recognize and kill cells that express no or low levels of MHC Class I molecules, such as virally infected cells, without any need for immunization. The roles these cells play in the pulpal immune responses remain unclear, although it has

been reported that certain natural killer cell populations have immunoregulatory functions to promote maturation of dendritic cells,[124] a major component of the pulpal immunodefense system.

Macrophages

Macrophages are constituents of the mononuclear phagocyte system, which consists of heterogenous populations of bone marrow–derived cells whose primary function is phagocytosis. They primarily act as scavenger cells that phagocytose and digest foreign particles (eg, microbes) as well as self-tissues and cells that are injured or dead. Macrophages are activated by a variety of stimuli and acquire several properties that contribute to the defense and repair of connective tissues. For example, activated macrophages show an elevated production of various bioactive substances such as bactericidal enzymes, reactive oxygen species, cytokines, and growth factors. On expression of Class II MHC molecules on their cell surface, macrophages acquire the capacity of antigen presentation and thus play a role in T-lymphocyte activation.

Macrophages show diversity in terms of morphology, phenotype, and function. This heterogeneity mostly reflects local microenvironmental conditions and the resulting difference in the state of differentiation and activation.[125] The morphology of macrophages varies according to the state of activation and differentiation, but it is generally characterized by an irregular surface with protrusions and indentations, a well-developed Golgi complex, many lysosomes, and a prominent rough endoplasmic reticulum (Fig 4-12).

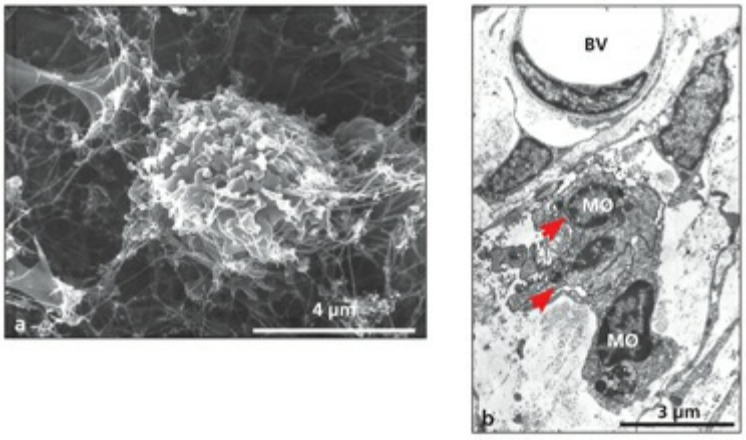
Macrophages of the pulp

Macrophages are classically described as histiocytes predominantly located in the vicinity of blood vessels. Immunohistochemical studies have demonstrated that a remarkably high number of cells throughout the pulpal connective tissue express macrophage-associated antigens[121,122,126] (Fig 4-13). These cells are particularly rich in the perivascular area of the inner pulp. The morphologic appearance of these cells is diverse, but cells with long, slender, branching processes are predominant. Typically, the ultrastructural appearance of these cells is characterized by an irregularly indented cell surface and the presence of relatively well-developed lysosomal structures within the cytoplasm.

Macrophages of the pulp have several phenotypes. They express varying combinations of several macrophage-associated antigens, such as CD14, CD68,

coagulation factor XIIIa, and HLA-DR in humans.[126] In the rat, the majority of cells immunoreactive to the monoclonal antibody ED1 (a general macrophage marker that recognizes an intracytoplasmic CD68-like antigen) coexpress immunoreactivity to ED2 (a monoclonal antibody exclusively reactive with tissue-resident macrophages [see Fig 4-13]). This expression indicates that macrophages of the pulp are predominantly composed of typical resident-type cells, as are macrophages in most other connective tissues.

Moreover, some of these macrophages coexpress Class II MHC molecules (see Fig 4-12b) and thus may have a capacity for antigen presentation to T lymphocytes. In rat molars, approximately 30% of the $ED1^+$ cells in the coronal pulp and 15% of cells in the root pulp coexpress Class II MHC molecules.[121] This ratio may be higher in human pulp because it has been reported that 86.9% of $CD68^+$ cells coexpress HLA-DR.[126] As described later, a certain proportion of these Class II MHC molecule–expressing cells may represent dendritic cells, although precise discrimination of dendritic cells from macrophages is difficult at present because of a lack of dendritic cell–specific markers.



4-12 Ultrastructure of macrophages (coronal pulp of rat molars). *(a)* SEM of a macrophage with **b 3 µm** numerous microvilli on its surface. *(b)* TEM of two macrophages (MØ) in the central portion of the coronal pulp. Immunoperoxidase staining using a monoclonal antibody against rat Class II MHC molecules (OX6) was used. One of the macrophages *(upper cell)* shows OX6-immunoreactivity on its cytoplasmic membrane. *arrowheads,* phagosomes; BV, blood vessel. (Courtesy of Dr T. Kaneko, Tokyo Medical and Dental University.)

Fig 4-13 Macrophages in the coronal pulp of a rat molar visualized by immunoperoxidase staining using a monoclonal antibody against tissue-resident macrophages (ED2). Cells with diverse profiles are distributed throughout the connective tissue of the pulp.

Dendritic cells

Dendritic cells are discrete populations of hematopoietically derived leukocytes sparsely distributed in almost all tissues and organs of the body. They are characterized by *(1)* peculiar dendritic morphology, *(2)* constitutive expression of a high amount of Class II MHC molecules, *(3)* high motility, *(4)* limited phagocytic activity, and *(5)* a potent capacity for antigen presentation to T lymphocytes.[127] Dendritic cells generally express several cell-surface molecules, including Class II MHC molecules and various adhesion and costimulatory molecules. Expression of myeloid-associated antigens is generally weak or lacking. However, the profile of the expression of those markers varies, mostly because of the difference in the state of maturation and local microenvironmental conditions.

Following maturation in the bone marrow and circulation in the bloodstream, dendritic cells populate peripheral nonlymphoid tissues, where they monitor the invasion of antigens and thus act as an immunosurveillance component. During primary immune responses, they are the only cell type able to stimulate naïve T lymphocytes (cells that have not previously been exposed to any antigen). Dendritic cells capture invaded antigens and then migrate through afferent lymphatics to lymphoid tissues, where they fully mature and present antigens to resting T lymphocytes. During secondary immune responses, both dendritic cells and Class II MHC molecule–expressing macrophages may present antigens to locally recruited memory T lymphocytes.

Dendritic cells of the pulp

In human pulp, Class II MHC molecule–expressing ($HLA\text{-}DR^+$) cells form a

continuous reticular network throughout the entire pulp.[115,116,126,128] The majority of these cells coexpress coagulation factor XIIIa, a marker for antigen-presenting cells of the dermis[126] (Fig 4-14). The HLA-DR$^+$ cells have three or more branched, 50-µm-long cytoplasmic processes. They are particularly rich in the periphery of the pulp (in and just subjacent to the odontoblastic layer), where they compete for space with the odontoblasts and sometimes extend their processes into the dentinal tubules.[116,128] The cells are also rich in the perivascular area, where they are arranged with their longitudinal axes parallel to the endothelial cells.[126] Rat pulp tissue contains similar types of cells.[121,129]

Class II MHC molecule–expressing cells of the pulp are most likely composed of macrophages and "true" dendritic cells, although it is often difficult to make a clear-cut discrimination solely by means of light microscopic appearances. Transmission electron microscopy has thus been employed, and cells with ultrastructural characteristics comparable to those of dendritic cells in other tissues (true dendritic cells) have been identified (Fig 4-15). The true dendritic cells are reported to exhibit narrow, tortuous cytoplasmic processes and to contain fine tubulovesicular structures, a moderately developed Golgi apparatus, and poorly developed lysosomal structures within the cytoplasm.[115,128–132] These cells are predominantly located in and just beneath the odontoblastic layer of the coronal pulp.

Other studies employing double-labeling immunohistochemistry have shown that the pulp contains Class II MHC molecule–expressing cells that lack expression of macrophage markers and thus may belong to the dendritic cell lineage.[121,125] In human pulp, these cells account for about 13% of HLA-DR$^+$ nonendothelial cells.[126] In the rat, cells immunoreactive to OX62 (a marker for rat dendritic cell subpopulations, ie, veiled cells), account for approximately 30% of Class II MHC molecule–expressing cells in the coronal pulp, although OX62 may also recognize certain cells other than dendritic cells.[133] Taken together, it seems reasonable to assume that Class II MHC molecule–expressing cells in the pulp are composed of macrophages as the major subpopulation and true dendritic cells as a minor but distinct subpopulation.

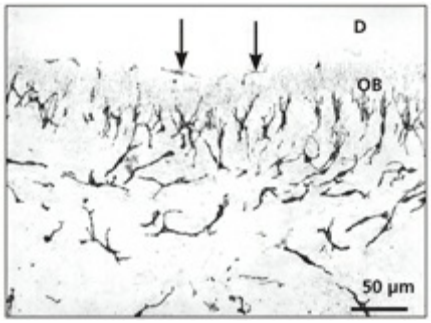


Fig 4-14 Distribution of cells expressing coagulation factor XIIIa (a marker for antigen-presenting cells of the dermis) in the peripheral portion of the coronal pulp (human third molar). Immunoperoxidase staining using an antiserum against factor XIIIa was used. Positively stained cells with a dendritic profile are arranged in and just subjacent to the odontoblastic layer (OB). Cells located in the vicinity of the pulp-predentin border *(arrows)* extend their processes into dentinal tubules. D, dentin.

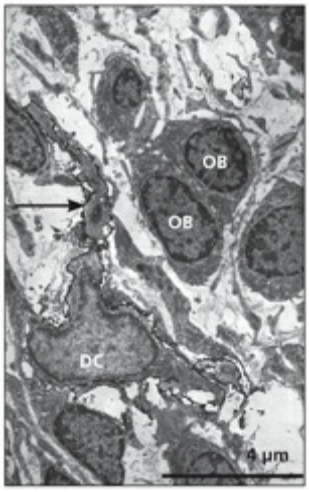


Fig 4-15 TEM showing a dendritic cell (DC) in the odontoblastic layer of a rat molar. Immunoperoxidase staining using a monoclonal antibody against rat class II MHC molecules (OX6) was used. Immunoreaction is visible on the cytoplasmic membrane. The positively stained cell has a long cytoplasmic process *(arrow)*. OB, odontoblasts. (Courtesy of Dr T. Kaneko, Tokyo Medical and Dental University.)

In a study in which phenotypic characterization of dendritic cells was performed in the mouse molar pulp, existence of two subpopulations, ie, $CD11c^+$ and $F4/80^+$ dendritic cells, has been demonstrated.[134] The former type of cells was localized at the pulp-dentin border and coexpressed CD205 (a dendritic cell–specific marker), TLR-2, and TLR-4 and thus may play a crucial role in sensing microbial challenges via dentinal tubules.

The functions of Class II MHC molecule–expressing cells have been investigated in vitro using enzymatically released pulp cells from rat incisors. The results indicated that *(1)* dendritic cells may correspond to a minor subpopulation of Class II MHC molecule–expressing cells with weak phagocytic capacity[129] and that *(2)* dendritic cells may have a stronger capacity for providing signals to cause proliferation of mitogenstimulated T lymphocytes than macrophages.[135] The T-lymphocyte proliferation was influenced by neuropeptides (substance P and CGRP), suggesting that dendritic cell–T-lymphocyte interaction in the pulp may be modulated by these neuropeptides.[22]

In summary, the pulp is equipped with dendritic cells as a minor but distinct

subpopulation of Class II MHC molecule–expressing cells. Their primary function may be to monitor invasion of antigens. Following ingestion of invading antigens, they may act in either of two ways (Fig 4-16): *(1)* migrate to regional lymph nodes, where they present antigens to antigen-specific naïve T lymphocytes in order to initiate primary immune responses, or *(2)* locally present antigens to patrolling memory T lymphocytes when the antigens again challenge the pulp (secondary immune responses).[3] Class II MHC molecule–expressing macrophages may interact only with memory T lymphocytes and thus may be involved in the initiation of secondary immune responses.

Dendritic cells are particularly rich in and just subjacent to the odontoblastic layer. This characteristic distribution suggests that dendritic cells are strategically positioned in the area where the opportunity for these cells to encounter exogenous antigens is the greatest.

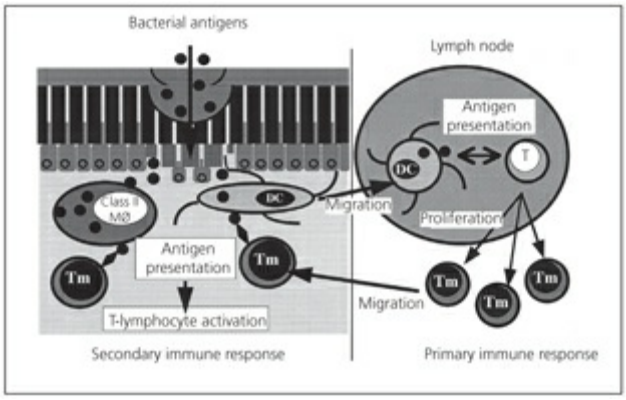


Fig 4-16 Role of Class II MHC molecule–expressing cells in the pulp. Den-dritic cells (DC) capture antigens in the pulp and then migrate to regional lymph nodes, where the dendritic cells present antigens to antigen-specific naïve $CD4^+$ T lymphocytes (T) (primary immune responses). The antigen presentation causes clonal expansion and development of antigen-specific memory T lymphocytes (Tm), which leave the lymph nodes and scan peripheral tissues such as the pulp. When the same antigens again challenge the pulp, both dendritic cells and macrophages (MØ) are able to present the antigens directly in the pulp to the memory T lymphocytes (secondary immune responses). The resultant activation of the memory T lymphocytes triggers the effector phase of immune responses.

Effects of Transdentinal Bacterial Challenge on Pulpal Immunocompetent Cells

Immunocompetent cells residing in the connective tissue of the pulp may respond to a number of clinical situations that cause loss of hard tissue integrity, including caries, tooth fracture, and cavity preparation. Bacteria and their by-products

invading from the oral cavity are the key elements associated with such a response. However, the response may be initiated even when the pulp is not directly exposed to the oral environment[119] (see chapters 3 and 10).

Studies have revealed that Class II MHC molecule–expressing cells respond promptly and actively to dentinal injuries, presumably by detecting incoming antigens and subsequently initiating immune responses by acting as antigen-presenting cells.[119] The Class II MHC molecule–expressing cells are most likely composed of true dendritic cells and macrophages. These two types of cell are collectively termed *pulpal dendritic cells* because complete discrimination of these cells is not always possible.

Rat molars lack an enamel covering at the tip of the cusps, and this anatomy may normally result in transdentinal oral bacterial challenges. Thus, the paraodontoblastic accumulation of pulpal dendritic cells may be, at least in part, a consequence of an invasion of bacterial antigens. This view is supported by the observation that an active increase of $OX6^+$ cells occurred coincidentally with the period of active tooth eruption[122] and that the densities of Class II MHC molecule–expressing cells and $OX62^+$ cells in germ-free rats were significantly lower than those in conventional rats.[119] These findings may further support the notion that bacterial challenges are necessary for the establishment of normal paraodontoblastic accumulation of pulpal dendritic cells in rat molars. Moreover, reimplantation of rat molars causes a marked accumulation of Class II MHC molecule–expressing cells along the pulp-dentin border, corresponding to the exposed dentin at the tip of the cusps.[136,137] This peculiar accumulation may in-dicate that these cells are responding actively to an influx of bacterial elements via the naturally exposed dentin because a rapid decrease of pulp tissue fluid pressure owing to the severance of the vasculature may cause a rapid increase in dentin permeability.[138]

Several studies have addressed molecular mechanisms underlying the dendritic cell migration to the site of dentinal injuries. It has been postulated that TGF-β1 originating from dentin contributes to the migration of dendritic cells because accumulation of these cells was observed after transdentinal TGF-β1 challenge in vitro.[139] Chemokines generated from odontoblasts may also be involved in the recruitment of dendritic cells to the odontoblastic layer.[90,93]

Response to cavity preparation and restoration

Cavity preparation in rat molars causes a rapid and intense accumulation of pulpal dendritic cells under the freshly exposed dentinal tubules. The accumulation is transient and gradually subsides following initiation of reparative dentinogenesis.[132,140,141] In a study using mouse molars, it was observed that a subpopulation of pulpal dendritic cells expressing CD11c, CD205 (a mouse dendritic cell marker), TLR-2, and TLR-4 showed an accumulation 2 hours after cusp trimming and acid etching. This may represent the sentinel role of this subpopulation of pulpal dendritic cells.[134]

In another study using rat molars, responses of pulpal dendritic cells to deep cavity preparation and immediate restoration with 4-META/MMA-TBB resin were studied.[142] Results demonstrated that the restoration reduced the accumulation of these cells and subsequently sound reparative dentin was formed; unrestored teeth, in contrast, initially developed an intrapulpal abscess and then exhibited partial pulpal necrosis.

After deep cavity preparation and immediate restoration with resin composites in human teeth, dendritic cells remained accumulated after 1 month; the accumulation was replaced with newly developed odontoblast-like cells at 2 months.[143] When cavities were prepared by laser abrasion in rat molars, however, a transient accumulation of pulpal dendritic cells followed by abscess formation was observed.[144] This may be due to the lack of smear layer after laser abrasion, which may have allowed continuous bacterial infections via dentinal tubules.

Together, findings from these studies[132,134,140–144] indicate that pulpal dendritic cells are able to respond to a transdentinal bacterial challenge resulting from acute dentinal exposure. Restoration most likely reduces the bacterial challenge and thereby diminishes the response of pulpal dendritic cells. This phenomenon may strongly support the key concept that reduction of transdentinal bacterial challenge by the use of dentin-adhesive materials is of utmost importance for vital pulp therapy (see chapters 3 and 13).

Response to dentinal caries

The kinetics of pulpal dendritic cells was investigated following experimental caries induction in rat molars.[145] The initial pulpal response was characterized by a localized accumulation of these cells beneath the pulpal ends of dentinal tubules

communicating with early caries lesions. The accumulation in this position may indicate that these cells respond promptly and actively to incoming bacterial antigens diffusing through the dentinal tubules. On the other hand, the accumulation was not evident following reparative dentin formation, suggesting that, in such cases, the influx of bacterial antigens is reduced or inhibited. However, these cells again accumulated markedly when the reparative dentin was invaded by caries. These findings support the view that the intensity of inflammatory and immunologic responses beneath dentinal caries does not necessarily correspond to the depth of the lesion but may be associated with the status and quality of reactive and reparative processes of dentin that influence dentin permeability.

A similar pattern is observed in human teeth that have dentinal caries without pulpal exposure: There is a marked localized accumulation of HLA-DR$^+$ pulpal dendritic cells in the paraodontoblast region immediately subjacent to the pulpal end of the carious dentinal tubules (Fig 4-17). Here, numerous dendritic cells extend their cytoplasmic processes into the affected dentinal tubules, probably representing the high motility of these cells.[116,128] This localized accumulation is more evident in teeth without reparative dentin.[116]

Studies have also demonstrated that T lymphocytes increase under these conditions (Fig 4-18). This increase is evident even in teeth with relatively shallow caries lesions, whereas an increase of B lymphocytes is noticeable only in teeth with deep lesions.[116,117] Thus, T lymphocytes may be more deeply involved in the initial immunologic reactions that take place following caries attack than are B lymphocytes. The majority of T lymphocytes may be memory T lymphocytes expressing CD45RO.[116,119]

Taken together, these findings suggest an early critical role of local interaction between pulpal dendritic cells and memory T lymphocytes in the initial immunodefense of the pulp against dentinal tubule–derived carious antigenic stimuli. It is postulated that the interaction results in the activation of both T lymphocytes and pulpal dendritic cells, which in turn may facilitate the recruitment and activation of several types of effector cells and thus may trigger a cascade of immunopathologic events involved in the process of pulpal pathosis associated with dental caries.

It seems reasonable to expect that accumulation of pulpal dendritic cells beneath dentinal caries lesions subsides after caries removal and restoration. However, Yoshiba et al[143] have demonstrated that the accumulation remains evident even 6 months after caries removal with the aid of a caries detector dye and restoration with adhesive resin composite (Fig 4-19). This suggests that immunologic responses

may continue even after proper and meticulous caries treatment, probably in response to bacteria left in the deeper parts of affected dentinal tubules.

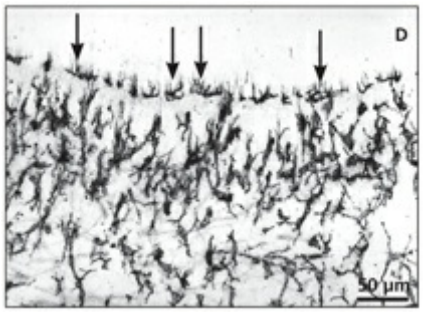


Fig 4-17 Distribution of pulpal dendritic cells in a human third molar with dentinal caries, with the peripheral portion of the pulp corresponding to carious dentinal tubules. Immunoperoxidase staining with an anti–HLA-DR monoclonal antibody was used. Note the accumulation of immunopositive cells. Many cells located in the vicinity of the pulp-predentin border extend their processes *(arrows)* into dentinal tubules. D, dentin. (Courtesy of Dr K. Sakurai.)

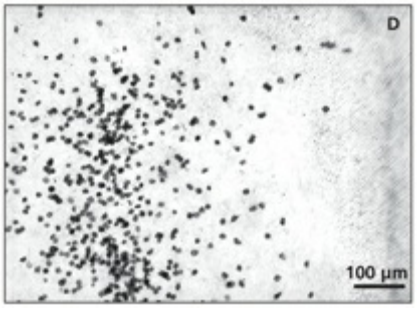


Fig 4-18 Accumulation of T lymphocytes in the peripheral portion of the coronal pulp in a human third molar with dentinal caries. Immunoperoxidase staining with a monoclonal CD3 antibody (reactive to all T lymphocytes) was used. D, dentin. (Courtesy of Dr K. Sakurai.)

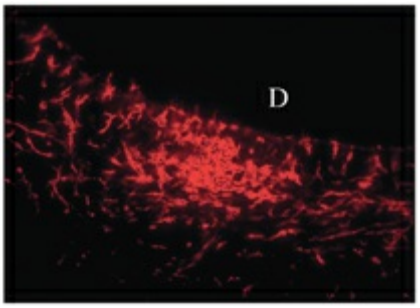


Fig 4-19 Accumulation of pulpal dendritic cells in a human third molar 3 months after removal of deep dentinal caries and immediate restoration with bonded composite resin, with the peripheral portion of the pulp corresponding to the restored region. Immunofluorescence staining with an anti–HLA-DR monoclonal antibody was used. D, dentin. (Courtesy of Dr K. Yoshiba.)

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Stem Cells and Regeneration of the Pulpodentin Complex

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Survival of everyday environmental conditions and entropic processes requires a mechanism that can maintain the functional integrity of tissues. That mechanism is *regeneration*. Generally, regeneration maintains or restores the original structures and function of tissues by replicating part of their embryonic development. Some tissues, such as blood and epithelia, undergo continual turnover, a process called *maintenance* or *homeostatic regeneration*. These tissues, as well as most others, also regenerate on a larger scale when damaged, a process called *injury-induced regeneration*.

The pulpodentin complex regulates and maintains dentinogenesis and tooth vitality throughout life. The human pulpodentin complex has the capacity to mineralize and to increase the rate of dentinogenesis in response to caries and trauma. The success rate of direct pulp capping to restore the structure of a tooth with dental materials, especially following a carious pulpal exposure, is extremely low. The weak regenerative capacity of the human pulpodentin complex is the main reason for the

need for pulpotomy or tooth extraction following symptoms of chronic pulpitis.

Several animals, such as salamanders, sharks, and some types of crocodiles, continually regenerate teeth throughout their lives to replace teeth that are lost. Several other types of animals, such as rodents and cows, have teeth that continually grow. Research efforts are underway to investigate the biochemical differences between human teeth and the aforementioned animal teeth to explain their superior regenerative capacity and specifically to identify and isolate the role of stem cells and signaling molecules. The end product of these investigations is expected to be the development of new dental treatments that translate the superior regenerative ability of some animal teeth to humans.

Over the long term, it may even be possible to harvest newly formed human teeth from "tooth farms." The potential to stimulate the regeneration of the human pulpodentin complex and to create human replacement teeth has generated excitement and high expectations for dental pulp research.

Many aspects of regenerative dentistry and endodontics are thought to be recent inventions, but the long history of research in this field is surprising. Regenerative dental procedures originated in 1952, when B. W. Hermann[1] reported on the application of calcium hydroxide in a case report of vital pulp amputation. Subsequent regenerative dental procedures include the development of Emdogain (Straumann) for periodontal tissue regeneration,[2] recombinant human bone morphogenetic proteins (rhBMPs) for bone augmentation,[3] and preclinical trials on the use of fibroblast growth factor 2 for periodontal tissue regeneration.[4] Surgical regeneration therapies include guided tissue or bone regeneration procedures, distraction osteogenesis,[5] and the application of platelet-rich plasma for bone augmentation.[6] Despite the success of these dental regenerative therapies, as well as regenerative medical procedures such as bone marrow transplants and skin and cornea grafts, there has not been significant translation of any of these applications into clinical endodontic practice.

Regenerative endodontic procedures are biologically based procedures designed to replace damaged structures, including dentin and root structures as well as cells of the pulpodentin complex. The objectives of regenerative endodontic procedures are to *(1)* regenerate pulplike tissue, ideally the pulpodentin complex; *(2)* regenerate damaged coronal dentin, such as following a carious exposure; and *(3)* regenerate resorbed root, cervical, or apical dentin. The importance of the endodontic aspect of tissue engineering has been highlighted by the formation of a standing committee on regenerative endodontics by the American Association of Endodontists in 2007.

Foundations of Regenerative Medicine

Regenerative medicine can perhaps be best defined as the use of a combination of stem cells and three-dimensional scaffold materials, in the presence of suitable biochemical molecules and absence of inhibitory molecules, to improve or replace biologic functions in an effort to effect the advancement of medicine. Regenerative medicine is based on tissue engineering therapies.

Probably the first definition of the term *tissue engineering* was suggested by Langer and Vacanti,[7] who stated that it is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function." MacArthur and Oreffo[8] defined tissue engineering as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." Our own description expands on this idea: Tissue engineering is the employment of biologic therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function.

The changes in the definition of *tissue engineering* over the years have been driven by scientific progress. While there can be many differing definitions of *regenerative medicine*, in practice the term has come to represent applications that repair or replace structural and functional tissues, including bone, cartilage, and blood vessels, among other organs and tissues.[9] The principles of regenerative medicine can be applied to endodontic tissue engineering. The steps followed to create dental pulp constructs as part of regenerative endodontic procedures are shown in Fig 5-1.

The counterargument to the development of regenerative endodontic medicine is that the pulp in a fully developed tooth plays no major role in form, function, or esthetics, and thus its replacement by a filling material in conventional root canal treatment is sufficient management. In terms of esthetics, this view does not take into consideration that certain endodontic filling materials and sealers or medicaments have the potential to discolor the tooth crown[10] or weaken the tooth.[11] A retrospective study of tooth survival times found that, although root canal treatment prolongs tooth survival, these teeth are still compromised compared to control teeth not receiving any treatment.[12] Moreover, dental pulp contains proprioceptors and therefore provides important sensory feedback on masticatory forces during chewing,[13] a function that may reduce the potential for tooth fracture. Thus,

regeneration of the pulpodentin complex offers the potential to increase tooth survival.

Although there are several potential benefits for regeneration of dental pulp, this tissue may also be susceptible to new infections and may conceivably require retreatment. However, the potential benefits outweigh these concerns, and research in this field is worth pursuing. In addition, this treatment approach would give patients a clear alternative to the synthetic tooth implants that are currently available.[14] Thus, regenerative therapies have the potential to revolutionize future dental treatments.

Progenitor and stem cells

The term *progenitor cell* is used in cell biology and developmental biology to refer to immature or undifferentiated cells, typically found in postnatal animals. While progenitor cells share many common features with stem cells, the former term is far less restrictive. A *stem cell* is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells and tissues.[15] All tissues originate from stem cells.

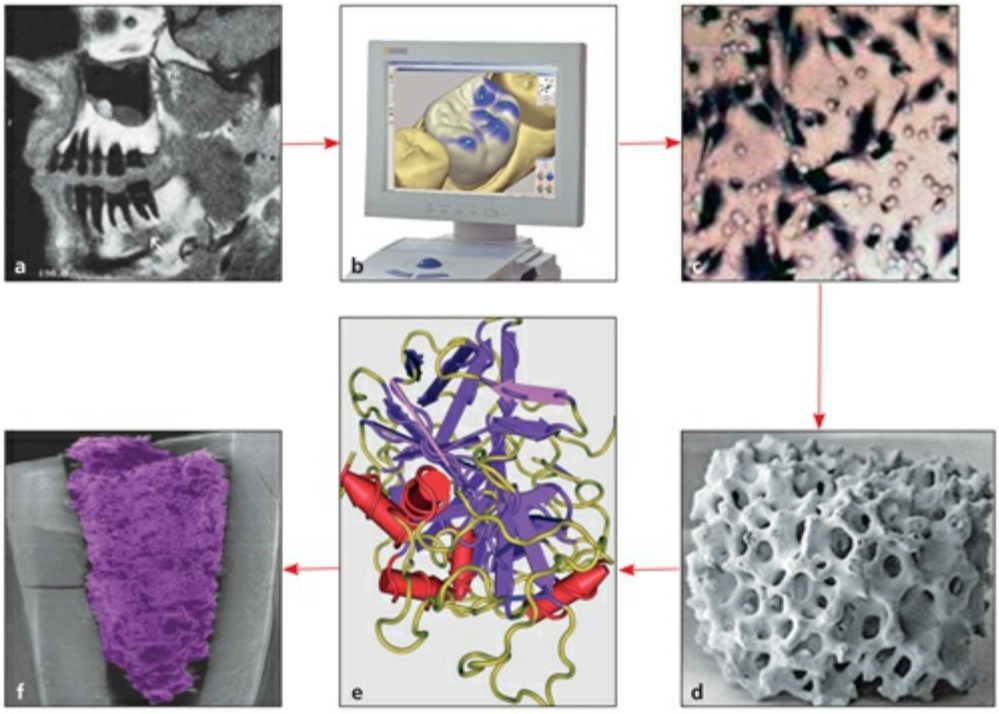

Fig 5-1 Several steps are required to create dental pulp constructs as part of regenerative endodontic procedures. Computer-aided biomodeling may be needed to define the anatomical dimensions of the

tissue to be regenerated *(a)*. Software may be needed to design the biomimetic structure of the replacement tissue *(b)*. Stem cells are needed to tissue engineer a dental pulp construct *(c)*. The cells are seeded on three-dimensional scaffolds *(d)* and maintained in culture. Growth factors may be used to promote tissue regeneration *(e)*. The dental pulp construct must then be transplanted into cleaned and shaped root canals *(f)*.

Stem cell populations are established in *niches*— specific anatomical locations that regulate how they participate in tissue generation, maintenance, and repair.[16] The niche saves stem cells from depletion while protecting the host from overexuberant stem cell proliferation. It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues. The simple presence of stem cells is not sufficient to define a niche. The niche must have both anatomical and functional dimensions and is an entity of action.[17]

Stem cells are commonly distinguished as either embryonic/fetal or adult/postnatal.[18] We prefer the term *embryonic* rather than *fetal* because the majority of these cells are embryonic. We also prefer the term *postnatal* rather than *adult* because these same cells are present in babies, infants, and children. It is important to distinguish between embryonic and postnatal stem cells because these cells have a different potential for developing into various specialized cells (ie, plasticity). Researchers have traditionally found that the plasticity of embryonic stem cells is much greater than that of postnatal stem cells, but recent studies indicate that postnatal stem cells are more plastic than first imagined.[19] The plasticity of the stem cell defines its ability to produce cells of different tissues.[20] Stem cells are also commonly subdivided into totipotent, pluripotent, and multipotent categories, according to their plasticity (Fig 5-2).

Their greater plasticity makes embryonic stem cells more valuable among researchers for developing new therapies.[21] However, the source of embryonic stem cells is controversial and surrounded by ethical and legal issues, which reduces the attractiveness of these cells for developing new therapies. This explains why many researchers are now focusing attention on developing stem cell therapies from postnatal stem cells donated by the patient or a close relative.

The application of postnatal stem cell therapy was launched in 1968, when the first allogeneic bone marrow transplant was successfully used in treatment of severely compromised immunodeficiency.[22] Since the 1970s, bone marrow transplants have been used to successfully treat leukemia, lymphoma, various

anemias, and genetic disorders.[23] Postnatal stem cells have been recovered from umbilical cord blood, umbilical cord, bone marrow, peripheral blood, body fat, and almost all body tissues,[24] including the pulp tissue of teeth.[25]

One of the first stem cell researchers was John Enders, who received the 1954 Nobel Prize in Medicine for growing polio virus in human embryonic kidney cells.[26] In 1998, James Thomson isolated cells from the inner cell mass of the early embryo and developed the first human embryonic stem cell lines.[27] Also, in 1998, John Gearhart derived human embryonic germ cells from cells in fetal gonadal tissue (primordial germ cells).[28] Pluripotent stem cell lines were developed from both donated embryonic cells.

However, it is now evident that the legal limitations and the ethical debate related to the use of embryonic stem cells must be resolved before the great potential of donated embryonic stem cells can be realized to regenerate diseased, damaged, and missing tissues as part of future medical treatments.[29] Accordingly, there is increased interest in autogenous (autologous) postnatal stem cells as an alternative source for clinical applications because they are readily available and have no immunogenicity issues, even though these cells may have reduced plasticity.

Stem cells are often categorized by their source. The most practical clinical application of a stem cell therapy would be to use a patient's own donor cells. Autologous stem cells are obtained from the same individual in whom they will be reimplanted. Bone marrow harvesting of a patient's own stem cells and their transplantation back to the same patient represents one clinical application of autogenous postnatal stem cells. Stem cells could be taken from the bone marrow,[30] peripheral blood,[31] fat removed by liposuction,[32] the periodontal ligament,[33] oral mucosa, or skin. An example of an autologous cell bank is one that stores umbilical cord stem cells.[34] From a medical perspective, the most valuable stem cells are those capable of neuronal differentiation[35] because these cells have the potential to be transformed into different cell morphologies in vitro using lineage-specific induction factors, including neuronal, adipogenic, chondrogenic, myogenic, and osteogenic cells.[36] It may be possible to use stem cells derived from adipose tissue[36] instead of bone marrow cells.

Autologous stem cells have the fewest problems with immune rejection and pathogen transmission.[37] The patient's own cells are the least expensive to harvest, and their use avoids legal and ethical concerns.[38] However, in some patients, such as very ill or elderly persons, suitable donor cells may not be available. The most promising cells for endodontic regeneration are autologous postnatal stem cells[39]

because they appear to have the fewest disadvantages that would prevent them from being used clinically.

Autologous cells originate from a donor of the same species.[40] Examples of allogeneic donor cells include blood cells used for a blood transfusion,[41] bone marrow cells used for a bone marrow transplant,[42] and donated egg cells used for in vitro transplantation.[43] These donated cells are often stored in a cell bank, to be used by patients requiring them. An alternative to donated allogeneic cells is the creation of autogeneic cell lines. The use of preexisting cell lines and cell organ cultures removes the problems of harvesting cells from the patient and waiting weeks for replacement tissues to form in cell organ tissue cultures.[44] However, there are some ethical and legal constraints to the use of human cell lines to accomplish regenerative medicine.[45] The most serious disadvantages are the risks of immune rejection and pathogen transmission.[38]

Xenogeneic cells are those isolated from individuals of another species. Pig tooth pulp cells have been transplanted into mice to produce tooth crown structures.[46] This suggests that it is feasible to accomplish regenerative therapy wherein donated animal pulp stem cells are eventually used to create tooth tissues in humans. In particular, animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular replacement tissues.[47] The harvesting of cells from donor animals removes most of the legal and ethical issues associated with obtaining cells from other humans. However, many problems remain, such as the high potential for immune rejection and possible pathogen transmission from the donor animal to the human recipient.[38] The future use of xenogeneic stem cells is uncertain and largely depends on the success of the other available stem cell therapies. If the results of allogeneic and autologous pulp stem cell tissue regeneration are disappointing, then the use of xenogeneic endodontic cells may remain a viable option for developing an endodontic, or even a whole tooth, regeneration therapy.[48]

Regenerative medicine solves medical problems by using cells as engineering materials. Examples of these uses could be cartilage repaired with living chondrocytes, artificial skin that includes living fibroblasts,[49] or other types of cells used in other ways. Stem cells hold great promise in regenerative medicine, but many unanswered questions, especially with regard to the safety of the procedures, have to be addressed before these cells can be routinely used in patients. The potential for pulp tissue regeneration from implanted stem cells has yet to be tested in animals and clinical trials. The US Food and Drug Administration has established a Center for Biologics Evaluation and Research to regulate the use of human cells or

tissues intended for implantation, transplantation, infusion, or transfer into a human recipient.

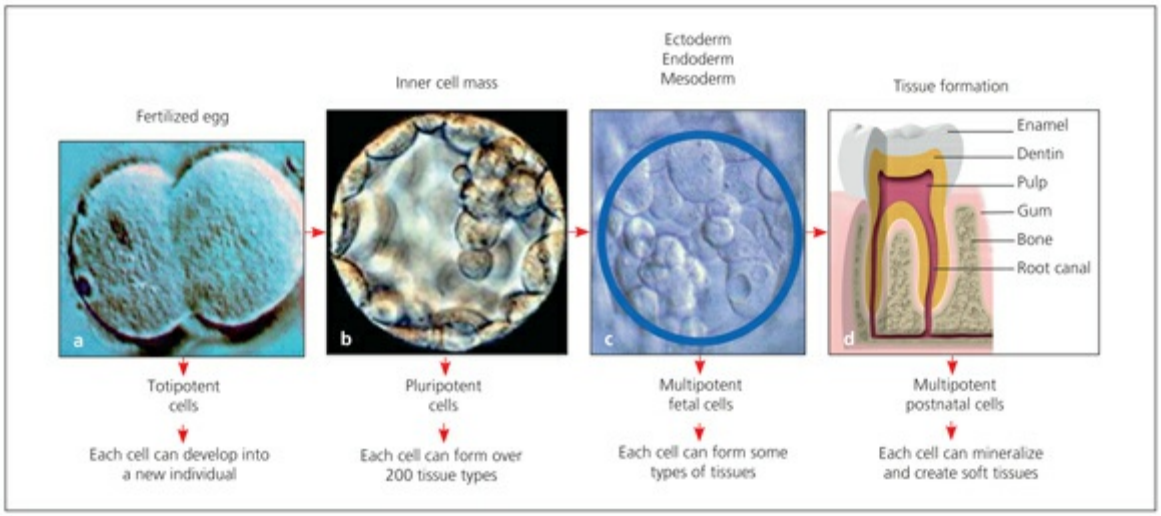

Fig 5-2 Stem cells and tissue development can be divided into three stages. *(a)* Cells from the early embryo (1 to 3 days) are totipotent and can develop into all of the tissue types in a new individual. *(b)* Cells from the blastocyst (5 to 14 days) are pluripotent and can form more than 200 tissue types. *(c)* Cells from the ectoderm, endoderm, and mesoderm can be obtained from embryonic tissues, and these can form some tissue types. *(d)* Cells from postnatal sources, including cord blood, fat, and teeth, are multipotent and have the potential to mineralize and create soft tissues.

Dental pulp stem cells

One of the most significant prerequisites for the creation of replacement pulp tissue for use in regenerative endodontics is the ability to obtain progenitor cells that will continually divide and produce cells or pulp tissues that can be implanted in root canal systems. One possibility is the development of human dental pulp stem cell lines that are disease and pathogen free. Several human dental pulp stem cell lines would likely need to be developed to match the antigenic markers of groups of patients, similar to those used for bone marrow and blood donations, to avoid causing potentially harmful immunologic reactions. With the use of a human pulp stem cell line, patients do not need to provide their own cells through a biopsy, and pulp tissue constructs can be premade for quick implantation when they are needed. The sourcing of stem cells and the difficulty in proving that they will provide a reliable and effective therapy are significant factors limiting the development of new endodontic, dental, and medical therapies.

The dental pulp contains stem cells that are called *pulp stem cells*[50] or, in the case

of immature teeth, *stem cells from human exfoliated deciduous teeth (SHED)*.[51] Sometimes pulp stem cells are called *odontoblastoid cells* because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells they replace.[52] Other similar types of cells include dental pulp stem cells from the apical papilla (SCAP)[53] and dental follicle progenitor cells (DFPCs).[54]

The SHED, SCAP, and DFPC cell populations have a potent capacity to differentiate into odontogenic cells. These cells also share the ability to give rise to osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells, similar to mesenchymal stem cells derived from bone marrow.[54] Following severe pulpal damage or mechanical or caries exposure, the odontoblasts are often irreversibly injured beneath the wound site.[55] Odontoblasts are postmitotic terminally differentiated cells and cannot proliferate to replace subjacent irreversibly injured odontoblasts.[55]

The source of the odontoblastoid cells that replace the odontoblasts and secrete reparative dentin bridges has proven to be controversial. Initially, it was suggested that irreversibly injured odontoblasts were replaced by predetermined odontoblastoid cells that do not replicate their DNA after induction. It was proposed that the cells within the subodontoblastic cell-rich layer, or *zone of Höhl,* adjacent to the odontoblasts[56] differentiate into odontoblastoid cells. However, the purpose of these cells appears to be limited to an odontoblast-supporting role because the survival of these cells was linked to the survival of the odontoblasts, and no proliferative or regenerative activity was observed.[57]

The use of tritiated thymidine and autoradiography to study cellular division in the pulp following damage revealed a peak in fibroblastic activity close to the exposure site about 4 days after successful pulp capping of monkey teeth.[58] An additional autoradiographic study of dentin bridge formation in monkey teeth, after calcium hydroxide direct pulp capping for up to 12 days,[59] revealed differences in the cellular labeling depending on the location of the wound site. Labeling of specific cells among the fibroblasts and perivascular cells shifted from low to high over time if the exposure was limited to the odontoblastic layer and the cell-free zone, while labeling changed from high to low if the exposure was deep into the pulp tissue. More cells were labeled close to the reparative dentin bridge than in the pulp core. The autoradiographic findings did not show any labeling in the existing odontoblastic layer or in a specific pulp location. This provided support for the theory that the progenitor stem cells for the odontoblastoid cells are resident undifferentiated mesenchymal cells.[60] The origins of these cells may be related to the

primary odontoblasts during tooth development,[61] or they may migrate to the dental pulp through the vasculature postdevelopmentally (see chapter 2).

The ability of both young and old teeth to respond to injury by induction of reparative dentinogenesis suggests that a small population of competent progenitor pulp stem cells may exist within the dental pulp throughout life. However, the debate on the nature of the precursor pulp stem cells giving rise to the odontoblastoid cells as well as questions concerning the heterogeneity of the dental pulp population in adult teeth remain to be resolved.[62]

Stem cells can be identified and isolated from mixed cell populations by four commonly used techniques: *(1)* staining of the cells with specific antibody markers and use of a flow cytometer in a process called *fluorescent antibody cell sorting (FACS)*; *(2)* immunomagnetic bead selection; *(3)* immunohistochemical staining; and *(4)* physiologic and histologic criteria, including phenotype (appearance), chemotaxis, proliferation, differentiation, and mineralizing activity. FACS, together with the protein marker CD34, is widely used to separate human stem cells expressing CD34 from peripheral blood, umbilical cord blood, and cell cultures.[63] Different types of stem cells often express different proteins on their membranes and are therefore not identified by the same stem cell protein marker. The most studied dental stem cells are those of the dental pulp. Human pulp stem cells express von Willebrand factor CD146, α–smooth muscle actin, and 3G5 proteins.[64] Human pulp stem cells also have a fibroblast phenoptype, with specific proliferation, differentiation, and mineralizing activity patterns.[65]

Growth factors and odontogenic induction agents

If the field of regenerative endodontics is to have a significant impact on clinical practice, it must primarily focus on providing effective therapies for regenerating functioning pulp tissue and ideally restoring lost dentin structure.[66] Toward this aim, increased understanding of the biologic processes mediating tissue repair has allowed some investigators to mimic or supplement tooth-reparative responses.[67] Dentin contains many proteins capable of stimulating tissue responses. Demineralization of the dental tissues can lead to the release of growth factors following the application of cavity etching agents or restorative materials and even caries.[68] Indeed, it is likely that much of the therapeutic effect of calcium hydroxide

and mineral trioxide aggregate may be due to their extraction of growth factors from the dentin matrix.[69] Once released, these growth factors may play key roles in signaling many of the events of tertiary dentinogenesis, a response of pulpodentin repair.[70]

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/ or differentiation.[71] Many growth factors are quite versatile, stimulating cellular division in numerous different cell types, whereas others are more cell specific.[72] The names of individual growth factors often have little to do with their most important functions and exist because of the historical circumstances under which they arose. For example, fibroblast growth factor was found in a cow brain extract by Gospodarowicz[73] and tested in a bioassay, which caused fibroblasts to proliferate.

Currently, a variety of growth factors with specific functions that can be used as part of stem cell and tissue engineering therapies have been identified.[74] Many growth factors can be used to control stem cell activity by means such as increasing the rate of proliferation, causing differentiation of the cells into another tissue type, or mediating stem cells to synthesize and secrete mineralized matrix.[75] Odontogenic differentiation of progenitor cells may be induced by culturing them for 3 weeks in odontogenic/ osteogenic induction medium consisting of 10 mmol/L β-glycerophosphate, 0.2 mmol/L ascorbate-2-phosphate, and 100 nmol/L dexamethasone in Dulbecco's modified Eagle medium with 15% fetal bovine serum.[76] A summary of the function, source, and activity of some common growth factors and odontogenic induction agents is shown in Table 5-1.

Growth factors, especially those of the transforming growth factor-β (TGF-β) family, are important in cellular signaling for odontoblast differentiation and stimulation of dentin matrix secretion (see chapters 1 and 2). These growth factors are secreted by odontoblasts and deposited within the dentin matrix,[77] where they remain protected in an active form through interaction with other components of the dentin matrix.[78] The addition of purified dentin protein fractions has stimulated an increase in tertiary dentin matrix secretion.[79]

Another important family of growth factors in tooth development[80] and regeneration[81] is the bone morphogenetic proteins (BMPs). Iohara et al[82] reported that rhBMP-2 stimulates differentiation of postnatal pulp stem cells into an odontoblastoid morphology in culture. The similar effects of TGF-β1, TGF-β2, TGF-β3, and BMP-7 have been demonstrated in cultured tooth slices.[83,84] Furthermore, rhBMP-2, -4, and -7 induce formation of reparative dentin in vivo.[85,86]

The application of recombinant human insulin-like growth factor 1 together with collagen has been found to induce complete dentin bridging and tubular dentin formation.[87] This indicates the potential for adding growth factors prior to pulp capping or incorporating them into restorative and endodontic materials to stimulate dentinal and pulpal regeneration.[88] In the long term, growth factors will likely be used in conjunction with postnatal stem cells to accomplish the tissue engineering replacement of diseased tooth pulp.

Table 5-1 **Function, source, and activity of common growth factors**

Growth factor	Function	Source	Activity
Bone morphogenetic protein (BMP)	Promotes cell differentiation and tissue mineralization	Bone matrix	Promotes differentiation of dental pulp stem cells into odontoblastoid cells
Transforming growth factor β (TGF-β)	Promotes cell differentiation and tissue mineralization	Dentin matrix, activated T-helper (TH1) cells, and natural killer cells	Is anti-inflammatory, promotes wound healing, and inhibits macrophage and lymphocyte proliferation
Colony-stimulating factor (CSF)	Promotes cell proliferation	Wide range of cells	Stimulates the proliferation of specific pluripotent bone stem cells
Epidermal growth factor (EGF)	Promotes cell proliferation	Submaxillary gland and Brunner gland	Promotes proliferation of mesenchymal, glial, and epithelial cells
Fibroblast growth factor (FGF)	Promotes cell proliferation	Wide range of cells	Promotes proliferation of many cells
Platelet-derived growth factor (PDGF)	Promotes cell proliferation	Platelets, endothelial cells, and placenta	Promotes proliferation of connective tissue, glial, and smooth muscle cells
Transforming growth factor α (TGF-α)	Promotes cell differentiation into a specific tissue type	Macrophages, brain cells, and keratinocytes	Induces epithelia and tissue structure development
Nerve growth factor (NGF)	Promotes cell differentiation into a specific tissue type	Protein secreted by a neuron's target	Is critical for the survival and maintenance of sympathetic and sensory neurons

Potential Technologies for Regenerative Endodontics

Three major areas of research may have application in the development of regenerative endodontic techniques: *(1)* postnatal stem cell therapy, *(2)* dental pulp constructs, and *(3)* root canal revascularization via blood clotting. These regenerative endodontic techniques are based on the basic tissue engineering principles already described and include specific consideration of cells, growth factors, and tissue engineering scaffolds.

Postnatal stem cell therapy

The simplest method to administer cells of appropriate regenerative potential is to inject postnatal stem cells into disinfected root canal systems after the apex is opened widely enough to permit revascularization. Postnatal stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone.[89] A major research obstacle is identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp (eg, fibroblasts, endothelial cells, and odontoblasts). Technical obstacles include development of methods for harvesting and any necessary ex vivo methods required to purify and/or expand cell numbers sufficient for regenerative endodontic applications.

Possible approaches would be to use dental pulp stem cells derived from *(1)* a patient's (own) cells that have been retrieved from umbilical cord stem cells that have been cryogenically stored since birth, *(2)* an allogeneic purified pulp stem cell line that is disease and pathogen free, or *(3)* xenogeneic (animal) pulp stem cells that have been grown in the laboratory. Presently, no purified dental pulp stem cell lines are available. Although umbilical cord stem cell collection is advertised primarily to be used as part of a future medical therapy, these stem cells have yet to be used to engineer any tissue constructs for regenerative medical therapies.

There are several advantages to an approach using postnatal stem cells. First, autologous stem cells are relatively easy to harvest, easy to deliver by syringe, and have the potential to induce new pulpal regeneration. Second, this approach is already used in regenerative medical applications, including bone marrow replacement, and several endodontic implications have recently been reviewed.[90]

However, a delivery method that involves the injection of cells has at least two disadvantages. First, the cells may have low cell survival. Second, the cells may migrate to different locations within the body,[91] possibly leading to aberrant patterns of mineralization. A solution for this issue may be to apply the cells together with a fibrin clot or other scaffold material. This would help to position and maintain cell location. In general, scaffolds, cells, and bioactive signaling molecules are needed to induce stem cell differentiation into a dental tissue type.[92] Therefore, the probability that injecting only stem cells in a saline solution into the pulp chamber will produce new, functioning pulp tissue may be very low. Instead, pulpal regeneration must consider all three elements (cells, growth factors, and scaffold) to maximize potential for success.

Dental pulp constructs

To create a practical endodontic tissue engineering therapy, dental pulp stem cells must be organized into a three-dimensional scaffold that can support cell organization and vascularization. This may be accomplished using a porous tissue engineering scaffold seeded with pulp stem cells to create a dental pulp construct[93] that can be implanted within endodontically treated teeth.[94] The scaffold may contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development.[95] The scaffold may also contain nutrients that promote cell survival and growth[96] and possibly antibiotics to prevent any bacterial ingrowth in the canal systems. The engineering of nanoscaffolds may be useful in the delivery of pharmaceutical drugs to specific tissues.[97] In addition, the scaffold may exert essential mechanical and biologic functions needed by a replacement tissue.[98]

In teeth with pulpal exposures, dentin chips have been found to stimulate reparative dentin bridge formation.[99] Dentin chips may provide a matrix for pulp stem cell attachment[100] and act as a reservoir of growth factors.[101] The natural reparative activity of pulp stem cells in response to dentin chips provides some support for the use of scaffolds to regenerate the pulpodentin complex. A dental pulp construct implanted in vitro in a human root canal is shown in Fig 5-3.

To achieve the goal of pulp tissue reconstruction, scaffolds must meet specific requirements. Biodegradability is essential because scaffolds must be absorbed by the surrounding tissues to eliminate the need for surgical removal.[102] A high porosity

and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients.[103] The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation. This means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually break down, leaving the newly formed tissue, which will take over the mechanical load.[104]

Most of the scaffold materials used in tissue engineering have had a long history of use in medicine as bioresorbable sutures and as meshes used in wound dressings.[105] Scaffold materials are either natural or synthetic and biodegradable or permanent. Synthetic materials include polylactic acid, polyglycolic acid, and polycaprolactone, which are common polyester materials that degrade within the human body.[106] These scaffolds have all been successfully used for tissue engineering applications because they are degradable fibrous structures with the capability to support the growth of various stem cell types. The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size. This has led researchers to concentrate efforts to engineering scaffolds at the nanostructural level in order to modify cellular interactions with the scaffold.[107]

Scaffolds may also be constructed from natural materials. In particular, different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth.[108] Several proteic materials, such as collagen or fibrin, and polysac-charidic materials, such as chitosan or glycosaminoglycans, are available. Early results are promising in terms of their supporting cell survival and function,[109] although immune reactions to these types of materials may threaten their future use as part of regenerative medicine. Metabolizing cell survival within dental pulp constructs is shown in Fig 5-4.

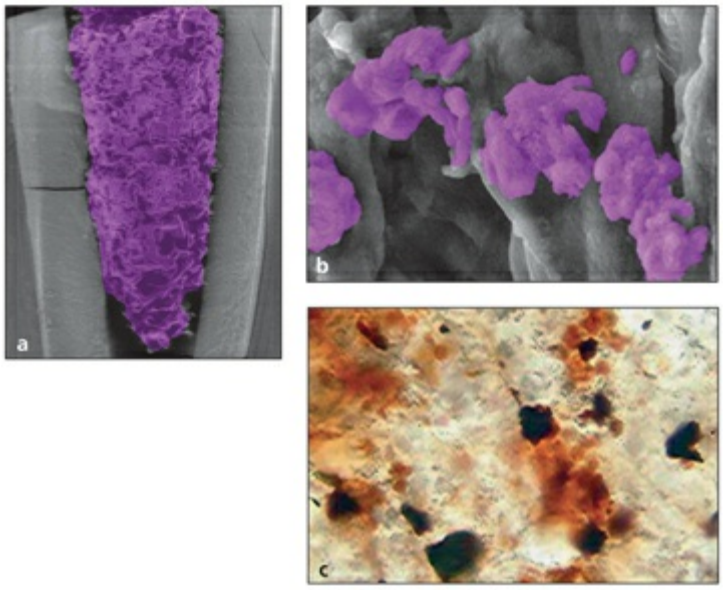

Fig 5-3 Scanning electron micrograph of a collagen scaffold seeded with dental pulp stem cells to create a dental pulp construct implanted in a cleaned and shaped root canal. *(a)* Good adherence is observed between the tissue construct and the root canal walls (original magnification ×3). *(b)* Some stem cells have attached to the scaffold, but coverage is not complete after 1 to 14 days in culture (original magnification ×400) *(c)* Histologic analysis of the pulp construct with a stain during culture demonstrates that the cells are metabolizing, suggesting that the construct is vital (neutral red metabolic stain; original magnification ×400) (Murray PE and García-Godoy F, unpublished data, 2009).

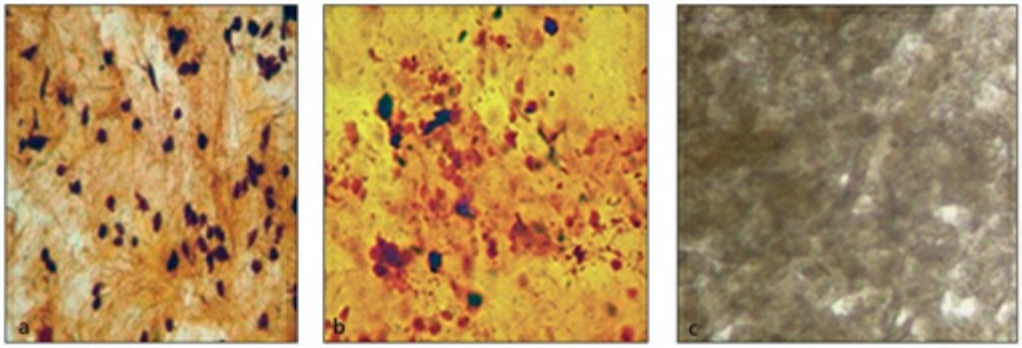

Fig 5-4 Dental pulp constructs have been created by seeding dental pulp stem cells on three types of tissue engineering scaffolds. *(a)* Good cell survival, as shown by the presence of metabolizing cells dispersed throughout the construct, is observed via vital dye staining of the open polylactic acid scaffold, a material used for resorbable surgical sutures. *(b)* Good cell survival is also observed on the collagen scaffold. *(c)* No or few vital cells are observed after seeding on the calcium phosphate scaffold. These observations suggest that the chemical and surface properties of scaffolds can influence the success of dental pulp constructs (neutral red metabolic stain; original magnification ×400) (Murray PE and García-Godoy F, unpublished data, 2009).

Rigid tissue engineering scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support.[110] However, in root canal systems, a tissue-engineered pulp is not required to provide structural support for the tooth. This will allow tissue-

engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix such as a polymer hydrogel. *Hydrogels* are injectable scaffolds that can be delivered by syringe.[111] Hydrogels have the potential to be noninvasive and easy to deliver to root canal systems. In theory, a hydrogel may promote pulpal regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.[112]

A past problem with hydrogels was the limited control over tissue formation and development, but advances in formulation have dramatically improved their ability to support cell survival.[113] Despite these advances, hydrogels are at an early stage of research, and this type of delivery system, although promising, has yet to be proven functional in vivo. To make the hydrogels more practical, research is focusing on making them photopolymerizable to form rigid structures once implanted in the tissue site.[114]

Root canal revascularization via blood clotting

Biologically based endodontic therapies can result in continued root development, increased dentin wall thickness, and apical closure when necrotic immature permanent teeth are treated.[115] Several case reports have documented revascularization of necrotic root canal systems when disinfection was followed by the establishment of bleeding into the canal system via overinstrumentation.[116,117] The revascularization method assumes that the root canal space has been disinfected and that the formation of a blood clot forms a matrix (eg, fibrin) that traps cells capable of generating new tissue. It is not clear that the regenerated tissue's phenotype resembles dental pulp; however, case reports published to date do demonstrate continued root formation and the restoration of a positive response to thermal pulp testing.[116] The potential of disinfection and blood clotting to revascularize necrotic infected immature dog teeth was demonstrated by Thibodeau et al.[118]

In addition, recent case reports indicate that an important aspect is the use of intracanal irrigants (sodium hypochlorite and chlorhexidine) and the placement of antibiotics (eg, a mixture of ciprofloxacin, metronidazole, and minocycline paste) or calcium hydroxide for several weeks. The triple antibiotic mixture, in particular, effectively disinfects root canal systems[119] and increases revascularization of

avulsed and necrotic teeth,[120] suggesting that this is a critical step in revascularization.

The selection of various irrigants and medicaments is worthy of additional research because these agents may confer several important effects for regeneration in addition to their antimicrobial properties. For example, tetracycline enhances the growth of host cells on dentin not by an antimicrobial action but via exposure of embedded collagen fibers or growth factors.[121] However, it is not yet known if minocycline shares this effect and whether these additional properties might contribute to successful revascularization.

While these case reports largely involve teeth with incomplete apical closures, it has been noted that avulsed teeth with an apical opening of approximately 1.1 mm or larger demonstrate a greater likelihood of revascularization than do teeth with smaller openings when reimplanted.[122] This finding suggests that revascularization of necrotic pulps with fully formed (closed) apices may require enlargement of the tooth apex to approximately 1 to 2 mm in apical diameter to allow systemic bleeding into the root canal systems.

Another important point is the patient's age; it is unclear if the young age of the patient in most of these case reports had a positive effect on healing. Younger adults generally have a greater capacity for healing.[123]

There are several advantages to a revascularization approach. First, this approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology.[115–122] Second, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission that arises if the pulp is replaced with a tissue-engineered construct.

However, some concerns must be addressed in prospective research. First, although the case reports that a blood clot has the capacity to regenerate pulp tissue are exciting, caution is required because the source of the regenerated tissue has not been identified. More animal and clinical studies are required to investigate the potential of this technique before it can be recommended for general use in patients. Generally, tissue engineering does not rely on blood clot formation because the concentration and composition of cells trapped in the fibrin clot are unpredictable. This is a critical limitation to a blood clot revascularization approach because tissue engineering is founded on delivery of effective concentrations and compositions of cells to restore function. It is very possible that variations in cell concentration and composition, particularly in older patients (in whom circulating stem cell

concentrations may be lower), may lead to variations in treatment outcome.

On the other hand, some aspects of this approach may be useful; plasma-derived fibrin clots are being used for development as scaffolds in several studies.[124] Platelet-rich plasma has a considerably elevated concentration of growth factors and appears to enhance revascularization.[125,126]

A second concern is that enlargement of the apical foramen is necessary to promote vascularization and to maintain initial cell viability via nutrient diffusion. Related to this point, cells must have an available supply of oxygen, and therefore it is likely that cells in the coronal portion of the root canal system would either not survive or would survive under hypoxic conditions prior to angiogenesis. Interestingly, endothelial cells release soluble factors that promote cell survival and angiogenesis under hypoxic conditions, while other cell types demonstrate similar responses to low oxygen availability.[127]

A summary of the possible approaches to accomplishing regenerative endodontic therapy is shown in Table 5-2.

Table 5-2 Approaches to accomplish regenerative endodontic therapy

Technique	Method	Advantages	Disadvantages
Root canal revascularization (see Fig 5-6)	Tooth apex is opened to 1 mm to allow bleeding into empty root canals	• Lowest risk of immune rejection • Lowest risk of pathogen transmission	• Minimal case reports published to date • Potential risk of necrosis if reinfected
Postnatal stem cell therapy (see Fig 5-5b)	Autologous or allogeneic stem cells are delivered to teeth via injectable hydrogel matrix	• Quick and easy to deliver • Least painful • Cells are easy to harvest	• Low cell survival • Cells do not produce new functioning pulp • High risk of complications
Dental pulp construct (see Fig 5-3a)	Pulp cells are seeded onto a three-dimensional scaffold made of polymers and surgically implanted	• Structure supports cell organization • More stable than injection of dissociated cells	• Low cell survival after implantation • Must be engineered to fit root canal precisely

Research Priorities for Regenerative Endodontics

The following represents an initial framework to identify major research priorities in developing regenerative endodontic techniques. They are not listed in order of priority but rather in the approximate sequence that they might be applied in a particular case.

Improvement of root canal disinfection and shaping

The simplest approach to pulp tissue regeneration would be to regrow pulp over infected or partially necrotic tissue. However, attempts to regenerate pulp tissue under these conditions have proved unsuccessful,[128] and it is generally recognized that the long-term prognosis of infected tissue after direct pulp capping is poor, and the procedure is not recommended.[129] In the presence of infection, the pulp stem cells that survive appear to be incapable of mineralization and deposition of a tertiary dentin bridge. The majority of the available evidence suggests that necrotic and infected tooth pulp does not heal. Therefore, in the foreseeable future, it will be necessary to disinfect the root canal systems and remove infected hard and soft tissues prior to using regenerative endodontic treatments.

Dental pulp stem cells will readily attach and grow on cleaned and shaped root canal systems,[130] but none, or very few, will attach to smear layer (Fig 5-5). To successfully attach and adhere to root canal dentin, the stem cells must be supported within a polymer or collagen scaffold.[93,94] Furthermore, pulp stem cells, periodontal stem cells, and fibroblasts will not adhere to or grow in infected root canal systems; the presence of infection renders the treatment unsuccessful (Murray PE and García-Godoy F, unpublished data, 2008).

Odontoblasts express toll-like receptor 4 (TLR-4), and exposure to lipopolysaccharides alters their phenotype.[131] This indicates that, for regenerative endodontics to be successful, the disinfection of necrotic root canal systems must be accomplished in a fashion that does not impede the healing and integration of tissue-engineered pulp with the root canal walls. In addition, biocompatible irrigants are needed to promote pulp stem cell attachment to root canal dentin, which is essential to accomplish some regenerative endodontic therapies.[130]

Moreover, the inclusion of a small local amount of antibiotics may need to be considered in developing these biodegradable scaffolds. Regenerative endodontics would benefit from a new generation of irrigants that are as effective as current

irrigants but are nonhazardous to patient tissues.

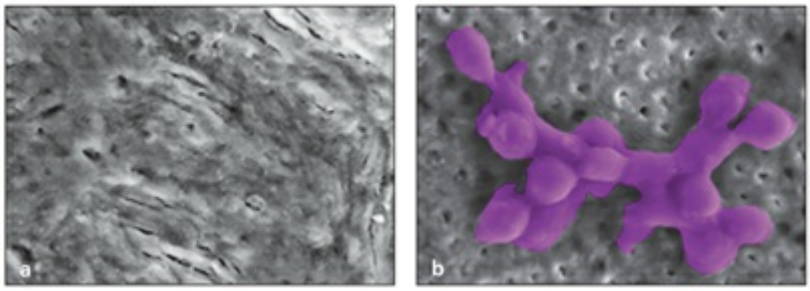

Fig 5-5 Stem cell attachment to root canals appears to be influenced by the removal of the smear layer. *(a)* No or few dental pulp stem cells have attached to a smear layer within an instrumented in vitro human root canal. *(b)* However, the cells have attached to cleaned and shaped root canal walls following irrigation with 6% sodium hypochlorite and conditioning with 17% ethylenediaminetetraacetic acid (EDTA). These observations suggest the importance of cleaning and shaping root canals to the success of regenerative endodontic therapy (original magnification ×2,000).

Improvement of smear layer removal

The presence of a smear layer on root canal walls may inhibit the adherence of implanted pulp stem cells,[130] potentially causing the regenerative endodontic treatment to fail. Improved methods to remove the smear layer from the root canal walls appear to be necessary to help promote the success of regenerative endodontics. The smear layer is a 1- to 5-µm-thick layer[132] of denatured cutting debris produced on instrumented cavity surfaces; it is composed of dentin, fragments of odontoblastic processes, nonspecific inorganic contaminants, and microorganisms.[133] The removal of the smear layer from the instrumented root canal walls is controversial. Its removal provides better sealing of the endodontic filling material to dentin and will prevent the leakage of microorganisms into oral tissues.[134]

Chemical chelating agents are used to remove the smear layer from root canal walls, most commonly a 17% solution of ethylenediaminetetraacetic acid (EDTA), which is applied as a final flush.[135] Several other solutions have been investigated for removing smear layers, including doxycycline, a tetracycline isomer,[136] citric acid,[137] and most recently MTAD (Bio-Pure MTAD, Dentsply),[138] a promising smear layer removal agent.

MTAD is an aqueous solution of 3% doxycycline, 4.25% citric acid, and 0.5% polysorbate 80 detergent.[139] This biocompatible intracanal irrigant[140] is

commercially available as a two-part set that is mixed on demand. In this product, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxy-2-naphthacenecarboxamide monohydrochloride hemiethanolate hemihydrate (doxycycline hyclate) is used instead of its free base, doxycycline monohydrate, to increase the water solubility of this broad-spectrum antibiotic.[141] MTAD has been reported to be effective in removing endodontic smear layers,[142] eliminating microbes that are resistant to conventional endodontic irrigants and dressings,[143] and providing sustained antimicrobial activity through the affinity of doxycycline to bind to dental hard tissues.[144] Adequate root canal disinfection, like cleaning, shaping, and smear layer removal, appears to be critical to accomplishing dental pulp stem cell attachment to root canal walls (see Fig 5-5).

Engineering of a functional pulp tissue

The success of regenerative endodontic therapy is dependent on the ability of researchers to create a technique that will allow clinicians to create a functional pulp tissue within cleaned and shaped root canal systems. The source of pulp tissue may come from stem cell therapy, which involves the delivery of autologous or allogeneic stem cells into root canals; dental pulp constructs, which involves the surgical implantation of synthetic pulp tissue grown in the laboratory[93,94]; or root canal revascularization, which involves enlarging the tooth apex to approximately 1 mm to allow bleeding into root canals and generation of vital tissue that appears capable of forming hard tissue under certain conditions. Each of these techniques for regenerating pulp tissue has advantages and limitations that still have to be defined through basic science and clinical research.

Improvement of delivery methods

Ideally, the delivery of regenerative endodontic procedures must be more clinically effective than current treatments. The method of delivery must also be quick and cost effective and not cause any serious health hazards to patients. The most promising delivery method for regenerative endodontic procedures is the potential use of

autologous stem cells from oral mucosa. Research is needed to evaluate these stem cells for their potential to form pulplike tissues. The oral mucosa cells are readily accessible as a source of oral cells, and in this way patients would not be required to store umbilical cord blood or save third molars immediately after extraction. The oral mucosa cells may be maintained using in vitro cell culture under sterile conditions designed to minimize infection.[145] The cells may then be seeded on the apical 1 to 3 mm of a tissue engineering scaffold; the remaining coronal 15 mm or more contains an acellular scaffold that supports cell growth and vascularization. This tissue construct may involve an injectable slurry of hydrogel, cells, and growth factors or just hydrogel and growth factors.

This two-layer method (the delivery of cells seeded on a scaffold, plus another scaffold for growth) would be fairly easy to accomplish. Moreover, because cells are seeded only in the apical region, the demand for numbers of cells derived from the host is reduced. Instead, most of the cell proliferation would occur naturally in the patient. This would reduce the need to grow large quantities of cells in the laboratory. This also reduces the need for an autologous pulp stem cell population that is not readily available to endodontists because the teeth requiring treatment are infected and necrotic. This proposed delivery method would help to avoid the potential for immune and infection issues surrounding the use of an allogeneic pulp stem cell line. Of course, alternative methods must be investigated using preclinical in vitro studies, usage studies in animals, and, eventually, clinical trials.

Measurement of appropriate clinical outcomes

Once avulsed teeth have been reimplanted or once tissue-engineered pulps have been implanted, it is not ethical to remove functioning tissues to conduct a histologic analysis. Therefore, it will not be possible to investigate mineralizing odontoblastoid cell functioning or nerve innervations. Clinicians will have to rely on the noninvasive, flawed, subjective tests in use today, involving laser Doppler blood flowmetry in teeth[146]; pulp testing involving heat, cold, and electricity[147]; and absence of signs or symptoms. Magnetic resonance imaging has shown the potential to distinguish between vital and nonvital tooth pulps,[148] but the machines are very expensive and must be greatly reduced in price before their use becomes widespread in endodontics.

The ideal clinical outcome is a nonsymptomatic tooth that never needs retreatment, but nonsubjective vitality assessment methods are essential to validate that regenerative endodontic techniques are truly effective. A summary of the challenges to the introduction of regenerative endodontics is shown in Box 5-1.

Box 5-1	Challenges to the introduction of regenerative endodontics

Creation of replacement dental pulp and oral tissues
- Stem cells from allogeneic, autologous, xenogeneic, and umbilical cord sources
- Growth factors, including rhBMP-2, rhBMP-4, rhBMP-7, TGF-β1, TGF-β2, and TGF-β3
- Tissue engineering from cell cultures, scaffolds, and hydrogels
- Pulp revascularization by apex instrumentation

Disinfection and shaping of root canals to permit regenerative endodontics
- Chemomechanical debridement, that is, cleaning and shaping of root canals
- Irrigants, including 6% sodium hypochlorite, 2% chlorhexidine gluconate, and alternatives
- Medicaments, including calcium hydroxide, triple antibiotics, MTAD, and alternatives

Delivery of replacement pulpodentin tissues
- Surgical implantation methods
- Specialist training

Measurement of appropriate clinical outcomes
- Application of biomedical imaging techniques
- Measurement of blood flow, nerve activity, and dental mineralization
- Absence of any signs or symptoms

Conclusion

The proposed therapies involving stem cells, growth factors, and tissue engineering all require pulpal revascularization, in itself an enormous challenge. One of the most challenging aspects of developing a regenerative endodontic therapy is to understand how the various component procedures can be optimized and integrated to produce the outcome of a regenerated pulpodentin complex. The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application to patients. A recent survey of endodontists found that 96% of participants thought that more regenerative therapies will be incorporated in future endodontic treatments.[149] The survey suggests that endodontic practitioners are supportive and optimistic about the future use of regenerative endodontic procedures. However, the survey also identified the

need for action from scientists, funding agencies, and the endodontic profession to pool resources to hasten the development of such procedures.[149] The unleashed potential of regenerative endodontics may benefit millions of patients each year.

The success rates of endodontic treatments can exceed 90% per year.[150] However, many teeth are not selected for endodontic treatment because of concerns about restorability and are subsequently replaced with an artificial prosthesis such as an implant. Several developmental issues that are relevant to the evolution of endodontic regeneration have been described. Each one of the regenerative techniques has advantages and disadvantages, and some of the techniques are hypothetical or at an early stage of development. The available case reports of pulp revascularization generally involve young patients, who have high stem cell populations, and teeth with open apices. In order for regenerative endodontic procedures to be widely available and predictable, endodontists will have to depend on tissue engineering therapies to regenerate pulpodentin tissue. Several innovations with the potential to be used as part of regenerative endodontic practice are under development (Fig 5-6).

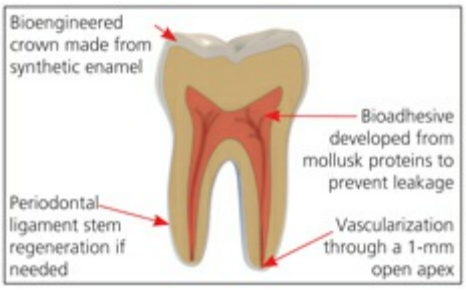

Fig 5-6 Regenerative endodontic therapy will require a new approach to clinical practice that involves several innovations. A dental pulp construct or acellular scaffold may be transplanted to the cleaned and shaped root canal. To vascularize the dental pulp construct, a blood supply will have to be created by opening the tooth apex up to 1 mm. A bioengineered crown formed from synthetic enamel may be engineered in the laboratory to restore the tooth. To restore the tooth and help prevent leakage, a new bioadhesive may be created from mollusk proteins. If the periodontal ligaments are damaged, periodontal stem cell therapy may be used to support the tooth.

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Circulation of the Pulp

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The microcirculatory system of dental pulp serves many essential roles. This system is critically important in maintaining tissue homeostasis and yet is capable of undergoing a dynamic response to injury by altering local capillary filtration rates, initiating immunologic responses to injury and inflammation via endothelial expression of adhesion molecules,[1] and even sprouting via angiogenesis. This chapter reviews the anatomy and physiology of this important system and emphasizes its response characteristics following pulpal inflammation caused by dental procedures, infection, or trauma. This information is critical for clinicians so that they can minimize injury to pulp during dental procedures and assess the status of the pulp's microcirculatory system in individual patients.

Organization of Pulpal Vasculature

The dental pulp is a microcirculatory system because it lacks true arteries and veins; the largest vessels are arterioles and venules. Its primary function is to regulate the local interstitial environment of dental pulp via the transport of nutrients, hormones, and gases and the removal of metabolic waste products. However, the pulpal microcirculation is a dynamic system that regulates blood and lymph flow in response to nearby metabolic events (including dentinogenesis). It also responds to inflammatory stimuli with a great change in circulatory properties and the endothelial expression of certain proteins, leading to the recruitment of immune cells to the site of tissue injury (see chapters 4, 7, 11, and 12). Clearly, knowledge of this system is essential to an understanding of the dental pulp in health and disease.

Blood vessels (arteriole-capillary-venule system)

The pulp has an extensive vascular supply[2] (Fig 6-1). The organizational structure of dental pulp is presented in Fig 6-2. Arterioles and venules are arranged axially in the pulp with capillary loops extending out toward the dentin.[2]

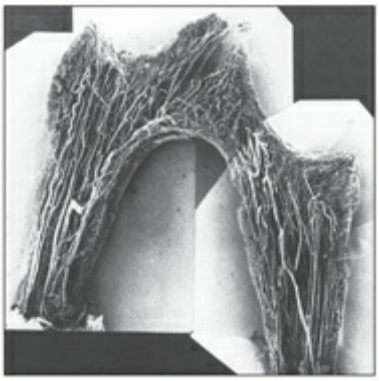

Fig 6-1 Montage of scanning electron micrographs of dental pulp illustrating the extensive vascular network in the dog mandibular first molar. The superficial capillary layer has been removed to better illustrate the organizational features of the pulpal circulatory system. (Reprinted from Kishi and Takahashi[2] with permission.)

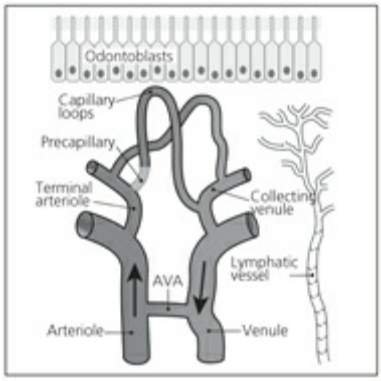

Fig 6-2 Major organizational features of the microcirculatory system of dental pulp. AVA, arteriovenous anastomosis.

Arterioles

The arterioles are resistance vessels, measuring approximately 50 μm in diameter. They have several layers of smooth muscle, which regulate vascular tone. The transitional structure between arterioles and capillaries is called the *terminal arteriole*. This segment of the arteriole has the same dimensions as a capillary but is surrounded by a few smooth muscle cells. These smooth muscle cells are organized in a spiral fashion surrounding the endothelial cells.[3] Intercellular electrical coupling exists between adjacent endothelial cells of arterioles. However, the resistance of myoendothelial couplings is appreciable, and the endothelium therefore may be important as a low-resistance path connecting many smooth muscle cells.[4]

The arterioles divide into terminal arterioles and then precapillaries. Scanning electron micrographs of resin injection casts of dental pulp vasculature illustrate the extensive arborization of capillaries from the metarterioles.[2] Metarterioles give off capillaries, which are about 8 μm in diameter (Fig 6-3). The arterioles, the capillaries, and the venules form functional units that respond to signals elaborated from the nearby tissue (discussed later in the chapter). This is an important concept because virtually every cell in the body is within 50 to 100 μm of capillaries. Thus, there is a functional coupling between cellular activity and nearby capillary blood flow.

The branch points of terminal arterioles and capillaries are characterized by the presence of clumps of smooth muscle that serve as precapillary sphincters. These sphincters are under neuronal and local cellular control (via soluble factors) and act to regulate local blood flow through a capillary bed. These functional units permit localized changes in blood flow and capillary filtration so that adjacent regions of the pulp have substantially different circulatory conditions. Thus, pulpal inflammation can elicit a localized circulatory response restricted to the area of

inflammation and does not necessarily produce pulpwide circulatory changes.[5]

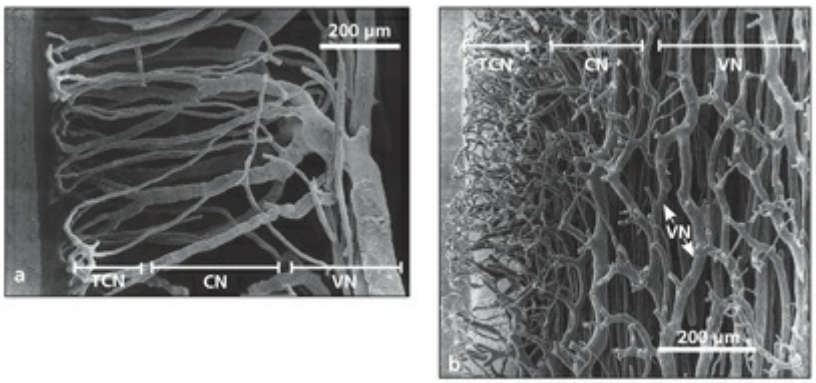

Fig 6-3 *(a)* Scanning electron micrograph of a resin injection cast of dental pulp vasculature illustrating the extensive arborization of capillaries from the metarterioles. TCN, terminal capillary network; CN, capillary network; VN, venular network. *(b)* The superficial layer of the vascular network has been removed to show the venular network (VN). The terminal capillary network (TCN) is on the left side, the capillary network (CN) is in the middle, and the venular network is on the right side. (Reprinted from Kishi and Takahashi[2] with permission.)

Capillaries

Capillaries serve as the workhorse of the circulatory system because they function as the exchange vessels regulating the transport or diffusion of substances (eg, gases, fluids, proteins) between blood and local interstitial tissue elements. At any given moment, only about 5% of the blood supply circulates in capillaries, but these are the major sites of nutrient and gas exchange with local tissues. Capillaries consist of a single layer of endothelium surrounded by a basement membrane and a loose group of reticular and collagenous fibers. In dental pulp, capillaries often form extensive loops in the subodontoblastic region[2] (see Fig 6-3). The basement membrane is composed of fine reticular filaments embedded in a mucopolysaccharide matrix.

The wall of a capillary is about 0.5 µm thick and serves as a semipermeable membrane. This semipermeable membrane restricts egress of proteins and cells from the vascular compartment under normal conditions, and it is this filtering property that generates a colloidal osmotic pressure within the vascular system. This has important implications for the regulation of capillary filtration under normal and inflamed conditions, as described in detail later in the chapter.

There are several major classes of capillaries that differ dramatically in their properties as semipermeable membranes.[6] The first class of capillary is the *fenestrated capillary*. These structures are characterized by endothelium with openings (fenestrations) in the capillary walls. These fenestrations can be open or occluded by a thin diaphragm. Fenestrated capillaries are found in dense networks in

dental pulp as well as in the renal glomerulus, the gastrointestinal mucosa, and the sulcular gingiva.[7,8]

The second class is the *continuous* (or *non-fenestrated*) *capillary*; these vascular structures are defined by endothelium devoid of fenestrations. Continuous capillaries are found in dental pulp as well as in the heart, lungs, skin, and muscle.[8] The intercellular space contains gap junctions, which are localized openings with a width of 5 to 10 nm. Continuous capillaries are found near odontoblasts during early tooth development (ie, before dentin is formed). With the active expression of primary dentin, capillaries become fenestrated and form a dense network adjacent to the odontoblastic layer. When dentin formation is nearly complete, the capillary bed switches back to a continuous capillary morphology and retreats to below the odontoblastic layer.[8] Thus, the morphology and location of pulpal capillary beds follow odontoblast activity levels during development (see chapter 1).

The third major class of capillary is the *discontinuous capillary*. These capillaries consist of discontinuous endothelium with wide intercellular spaces of approximately 5 to 10 nm. The basement membrane is also discontinuous. Discontinuous endothelium is found in the spleen, liver, and bone marrow.

The fourth major class of capillary is the *tight-junction capillary*, found in the central nervous system and the retina. The differences in the semipermeable properties of these classes of capillary play an essential role in defining the basal filtration properties of these vessels.

The microcirculatory organization of the subodontoblastic region is divided into three major layers[2] (see Fig 6-3). The terminal capillary network is located in the first layer, called the *odontoblastic layer*. The second layer, also known as the *capillary network*, contains precapillary and postcapillary vessels organized adjacent to the odontoblastic layer. The third layer consists of a *venular network* of vessels. During aging, there is a general reduction in pulpal metabolism, and the capillary organization is often simplified and becomes one single layer of capillaries terminating directly in venules[2] (see chapter 18). This change may be associated with an electrophysiologic finding that odontoblasts function as a syncytium through electrical coupling and that young odontoblasts have greater electrical conductance.[9,10] Measurement of oxygen distribution with an oxygen-sensitive electrode has shown that odontoblasts may be a major oxygen consumer in the rat incisor pulp[11] (Fig 6-4). Thus, odontoblasts, especially in the younger stage, may need more metabolic support from circulating blood during development.

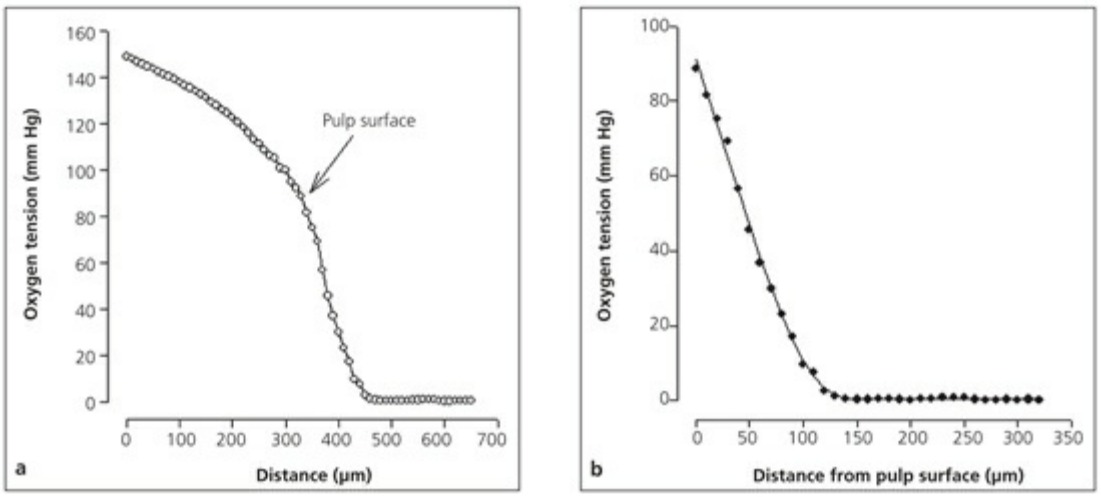

Fig 6-4 Oxygen profile of the rat incisor pulp. *(a)* Original tracing of oxygen profile recorded from nonperfused pulp. Pulpal oxygen distribution is characterized by a moderate decline in oxygen tension in the saline medium overlying the pulp and a steeper gradient in the interface before a localized oxygen-consuming region (the odontoblastic layer), followed by a plateau in oxygen tension in the middle of the pulp. *(b)* Truncated oxygen profile of that shown in *(a)* as a function of distance from the pulp surface. A fitted curve is superimposed on the data points *(filled diamonds)*. (Reprinted from Yu et al[11] with permission.)

Venules

The venular organization in dental pulp has several important characteristics. First, the collecting venules receive pulpal blood flow from the capillary bed and transfer it to the venules. As described earlier, these structures are characterized by a spiral organization of smooth muscle. Accordingly, the contractile state of these vessels plays an important role in regulating postcapillary hydrostatic pressure. Moreover, arteriovenous anastomosis (AVA) shunts and vascular loops permit regional control of pulpal blood flow via direct shunting of blood from arterioles to venules following tissue injury[2,12–14] (Fig 6-5; see also Fig 6-2).

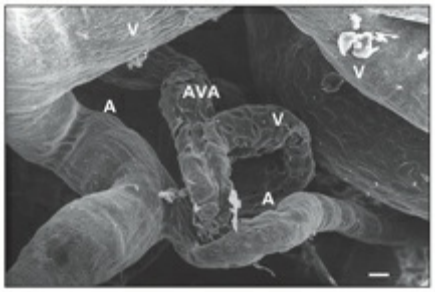

Fig 6-5 Scanning electron micrograph of a resin cast illustrating the presence of a Y-shaped arteriovenous anastomosis (AVA) between an arteriole (A) and a venule (V) using a sample from a dog tongue (bar = 10 μm). (Reprinted from Kishi and Takahashi[2] with permission.)

Lymphatic vessels

The pulp lymphatic system coexists with blood vessels and plays a critical role in tissue homeostasis and response to injury. Because of the semipermeable nature of blood capillaries, they do not absorb solutes of high molecular weight (eg, proteins such as albumin). Instead, the lymphatic system is the dominant mechanism for removal of high molecular weight solutes from interstitial fluid. This reduces the interstitial colloidal osmotic pressure and regulates the development of tissue edema or interstitial pressure. In addition, the lymphatic vessels transport lymph to regional lymph nodes prior to reentry into the vascular compartment. This provides an important immunosurveillance function by direct transport of antigens to nodal collections of immune cells.

Lymphatic vessels are formed from a fine mesh of small, thin-walled lymph capillaries. The lymphatic vessels coalesce to form larger vessels that resemble veins equipped with valves to prevent backflow (Fig 6-6). An extensive network of lymphatic vessels and ducts carries the tissue fluid back into the vascular system. Lymph from dental pulp (and nearby orofacial tissues) drains into the submaxillary and submental lymph glands and eventually into superficial and deep cervical glands that are distributed along the external and internal jugular veins.

Dental pulp contains lymphatic vessels. This statement was controversial in the older dental literature because early histologic studies (eg, hematoxylin and eosin stain preparations) had difficulty distinguishing lymphatic vessels from similar-appearing veins or capillaries. The main structural differences between the lymphatic vessels and capillaries are the lack of a basement membrane and the absence of fenestration in the endothelial cells. However, careful observation at different light microscopic levels[15–18] has provided evidence supportive of the presence of pulp lymphatic vessels. The existence of lymphatic vessels of irregular shapes with an incomplete or undeveloped basal membrane and anchoring filaments has been demonstrated in feline[15] and human dental pulp[19] at the electron microscopic level.

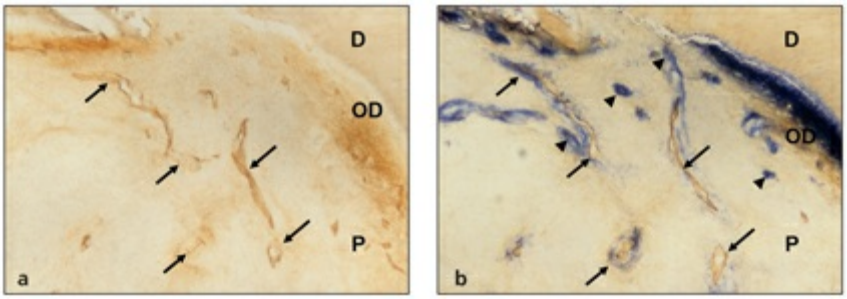

Fig 6-6 Light micrographs of cryostat sections of dental pulps (P) from human decalcified teeth with histochemical demonstration of lymphatic vessels *(arrows)* and blood vessels *(arrowheads)*. *(a)* Dark brown 5'-Nase–positive lymphatic vessels are visible near the odontoblastic layer (OD) beneath dentin (D). *(b)* 5'-Nase–ALPase double staining of a section adjacent to that in *(a)*. Note the ALPase reaction in the odontoblastic layer and around 5'-Nase–positive lymphatic vessels. (Reprinted from Matsumoto et al[22] with permission.)

Physiologic tracking studies have provided additional evidence demonstrating that lymph capillaries originate as blind openings near the zone of Weil and odontoblastic layer.[20] The collecting vessels then pass apically in the pulp, accompanying blood vessels and nerves. The large-caliber lymphatic vessels contain valves, which are not present in similar-sized veins.

More definitive studies at the light microscopic level have confirmed the presence of lymphatic vessels in dental pulp. An enzyme-histochemical submental method, which exploits the enzyme activity differences between lymphatic vessels (higher activity of 5'-nucleotidase [5'-Nase]) and blood vessels (higher activity of alkaline phosphatase [ALPase]), is useful for discriminating between the two kinds of vessels.[17,20–23] Pulp lymphatic vessels are observed mainly around the cell-free zone just underneath the odontoblastic layer, and some vessels are also found close to the odontoblastic layer[23] (see Fig 6-6).

Immunohistochemical staining methods with monoclonal antibodies to the human thoracic duct and desmoplakin also can identify lymphatic vessels specifically. Reaction products are on the endothelial cells.[24]

In human frozen sections, both immunohistochemical and enzyme-histochemical methods demonstrated that large lymphatic vessels are located in the central part of the pulp, whereas small lymphatic vessels are found in the periphery of the pulp (Fig 6-7). The pulp lymphatic vessels run through the root canal, and multiple collecting lymph vessels exit through the apical foramen to drain into large lymph vessels in the periodontal ligament.[25] Some of the lymphatic vessels in the pulp and periodontal tissues are connected to those of the periosteum in the alveolus. Collectively, lymphatic drainage of the dental pulp starts from the periphery of the pulp and

collects in the central part of the pulp[22,24] and then leaves the pulp through the apical foramen.[23]

The lymph network is critical for triggering systemic immune reactions to antigens coming through dentinal tubules. Csillag et al[26] have shown the contribution of the nervous signal to the emigration of immunocompetent cells. That is, electrical stimulation (20 to 25 seconds every 5 minutes for a total of 4 hours) of sympathetic nerves causes recruitment of $CD43^+$ polymorphonuclear cells, whereas the stimulation of sensory nerves causes the emigration of Ia antigen–expressing dendritic cells in the dental pulp.[26] Berggreen et al[27] have shown the dental lymphatic system during wound healing using the lymphatic endothelial hyaluronan receptor-1 (LYVE-1) and vascular endothelial growth factor receptor-3 (VEGFR-3).

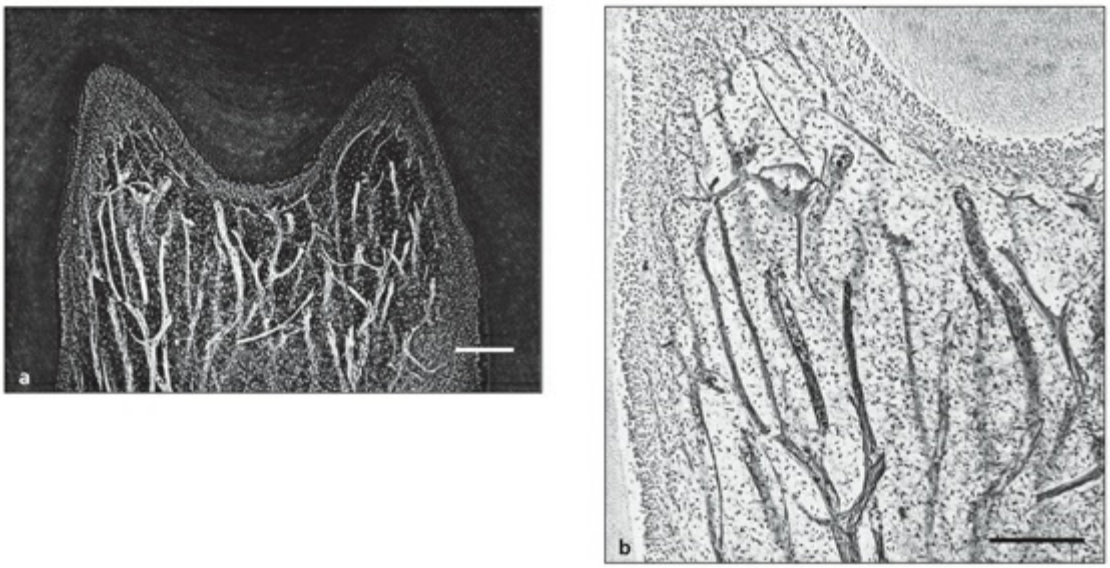

Fig 6-7 Backscattered electron micrographs of 5'Nase–positive lymphatic vessels in the monkey tooth pulp. *(a)* Longitudinal section of the pulp (bar = 200 µm). (Reprinted from Matsumoto et al[23] with permission.) *(b)* Magnified view (black-and-white reversal image) of the area near the pulp horn shown in *(a)* (bar = 200 µm). (Courtesy of Dr Y. Matsumoto.)

Effect of Microvascular Exchange on Interstitial Pressure

Microcirculation

The exchange of nutrients, hormones, metabolic wastes, and gases between capillaries and the interstitial compartment is controlled by two major determinants. The first determinant is the control of the microcirculation. This process directs capillary blood flow to local pulpal regions with the greatest metabolic need. Moreover, alterations in capillary blood flow produce changes in capillary hydrostatic pressure (P_c); this in turn regulates fluid balance between the vascular and interstitial compartments.

Not all capillaries are continuously perfused, and the proportion of perfused capillaries may range from 10% (during vasoconstriction) to nearly 100%. The terminal arterioles and precapillary sphincters play major roles in the control of capillary perfusion (see Fig 6-3). In contrast, the major sites of blood volume control and postcapillary resistance are the muscular venules. Pulpal blood flow (PBF) is determined by the following relationship:

$$PBF = (P_A - P_V)/R_T$$

where P_A is the arteriolar hydrostatic pressure, P_V is the venular hydrostatic pressure, and R_T is the total resistance. Under normal conditions (ie, no changes in systemic pressure), the major determinant of pulpal blood flow is R_T, which is determined primarily by arteriolar resistance. Thus, interventions that produce vasoconstriction of pulpal arterioles (eg, epinephrine and norepinephrine) reduce pulpal blood flow by increasing R_T.

Transcapillary exchange

The second major determinant in the exchange of nutrients, wastes, and gases between capillaries and the interstitial space is transcapillary exchange. Several factors regulate the exchange of materials between the vascular compartment and the interstitial space. First is the morphology of the capillary bed. Clearly, fenestrated capillaries possess much higher exchange rates than continuous or tight-junction capillaries.[6] The junctional openings in capillary walls permit passage of many low–molecular weight substances (eg, glucose, molecular weight = 180), while they restrict exchange of larger plasma proteins (eg, albumin, molecular weight = 69,000).[6] The semipermeable nature of the junctions helps maintain vascular

colloidal pressure. These junctional openings permit passive exchange of solutes and gases by either diffusion (ie, the net movement of molecules such as glucose, oxygen, carbon dioxide, and water down their respective concentration gradients) or osmosis (ie, the selective movement of fluid and solutes through a semipermeable membrane). The osmotic-driven exchange of fluid and solutes is termed *capillary filtration*.

A second factor regulating exchange is the composition and concentration gradient of the substance of interest; smaller or more lipophilic substances cross cell membranes relatively easily, whereas larger or more hydrophilic substances require capillary openings or transport mechanisms. A third factor is the process of active transport via pinocytosis. Of these general processes, capillary filtration is the major mechanism for the transcapillary exchange of solutes. The rate of capillary filtration is defined by the *Starling forces*, named for the English physiologist who first described them.[28] The difference between capillary hydrostatic pressure (P_C) and interstitial hydrostatic pressure (P_I), that is, $P_C - P_I$, generally favors an outward direction of fluid flow (filtration) at the arteriolar end of capillaries. The second Starling force is the difference between capillary colloidal osmotic pressure (COP_C) and interstitial colloidal osmotic pressure (COP_I), or $COP_C - COP_I$; this generally favors an inward direction of fluid flow (absorption) at the venular end of capillaries.

Thus, under normal conditions, there is a net outward flow of fluid (filtration) at the arteriolar end of capillaries because the filtration pressure gradient exceeds the colloidal osmotic pressure gradient and a net inward flow of fluid (absorption) at the venular end of capillaries because the colloidal osmotic pressure gradient exceeds the filtration pressure gradient (Fig 6-8). Overall, the amount of fluid movement across the entire capillary system of the body is enormous; it has been estimated that every minute the net capillary filtration rate equals the entire plasma volume, with an equal absorption rate back into capillaries and lymphatics. The clinical significance of the Starling forces is that they are altered during inflammation, giving rise to dramatic increases in localized interstitial pressure that may have pathophysiologic significance for injured regions of dental pulp.[12]

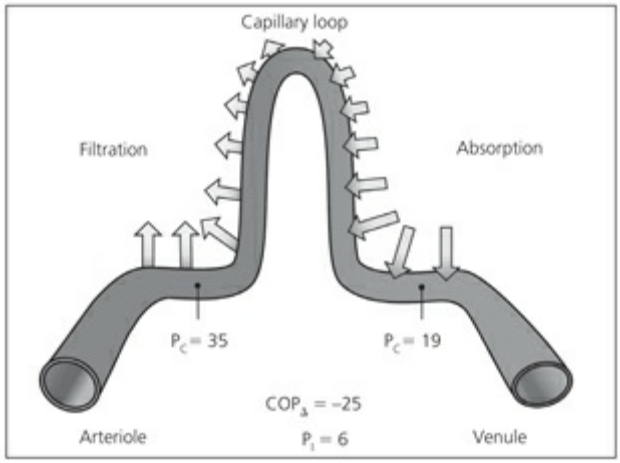

Fig 6-8 Capillary loop in dental pulp. Theoretical values (mm Hg) are given for capillary hydrostatic pressure (PC) at the arteriole and venule ends of the capillary, interstitial hydrostatic pressure (PI), and colloidal osmotic pressure gradient (COP_Δ) between the capillary and interstitial spaces. The negative value for COP_Δ indicates a net inward pressure. The size and direction of the arrows illustrate the relative magnitudes of fluid flow out of (filtration) and into (absorption) the capillaries.

Pulpal interstitial pressure

Several experimental techniques have been developed to measure pulpal interstitial pressure. Determination of this pressure in conditions of homeostasis and inflammation is critical for understanding vascular responses to pulpal injury. Methods to determine P_I include photoelectric methods,[29] pressure transducer systems,[30] tonometric measurements,[31] and micropuncture techniques.[5,12,13]

Many of the earlier testing methods in this area produced relatively large injuries to the pulp, and P_I values of about 16 to 60 mm Hg were recorded.[30,32,33] The micropuncture technique, using glass micropipettes connected to a servocontrolled counter-pressure system to measure not only P_I but also the intravascular pressure of teeth, is much less invasive; the pipettes have tip diameters of only 2 to 4 µm.

These studies recorded P_I values of about 5 to 6 mm Hg under controlled conditions.[5,13,34,35] According to these hydrostatic measurement techniques, P_A is 43 mm Hg, P_C is 35 mm Hg, P_V is 19 mm Hg, and P_I is 6 mm Hg.[34]

Studies have demonstrated that P_I increases in response to inflammation.[33] The P_I in cat dental pulp was 16.3 mm Hg at the site of pulpal inflammation and 7.0 mm Hg at a site 1 to 2 mm away. The pulpal P_I in control teeth (ie, in the contralateral arch)

was 5.5 mm Hg.

The results of the aforementioned study are important because they demonstrate that the P_I response to pulpal inflammation is restricted to the site of injury and is not generalized throughout the pulp. A generalized increase in P_I during pulpal inflammation has been theorized to occur and would lead to a generalized collapse of venules and cessation of blood flow (the pulpal strangulation theory). Studies such as the one by Tønder and Kvinnsland[5] have refuted the concept of pulpal strangulation. Instead, it appears that circulatory responses to pulpal inflammation are localized reactions to the release of inflammatory mediators or other factors.[12,13,36,37] This finding is similar to that of other studies demonstrating localized tissue responses to pulpal inflammation (eg, localized sprouting of calcitonin gene–related peptide fibers; see chapter 7).

A number of factors help restrict the increase in P_I to the site of tissue injury and prevent a generalized pulpwide increase in P_I.12 First, an increase in P_I will reduce capillary filtration by reducing the Starling hydrostatic pressure gradient (ie, an increase in P_I reduces the difference $[P_C - P_I]$). Second, an increase in P_I at the site of inflammation may lead to an increase in capillary absorption in nearby uninflamed pulp tissue. Third, increased lymph outflow will reduce both interstitial tissue fluid volume and interstitial protein concentrations (thereby reducing COP_I). This is an important function of lymphatic vessels because the lymphatic system is the predominant mechanism for removing osmotically active proteins from the interstitial space. These factors help restrict increases in P_I to the site of injury under many conditions of pulpal inflammation. However, it is still possible that major insults to the pulp (eg, involving microvascular hemorrhage or extreme capillary permeability) may lead to widespread increases in P_I.

Collectively, studies indicate that numerous factors regulate the interstitial hydrostatic pressure in dental pulp. Changes in the interstitial fluid pressure (ΔP_I) are due to changes in the volume of the interstitial fluid and tissue compliance:

$$P_I = \Delta V / C_I$$

where ΔV is the change in pulp tissue volume and C_I is the compliance of dental pulp tissue. The change in pulp tissue volume (ΔV) is regulated to a large extent by the capillary filtration rate; increases in filtration rate lead to increased fluid in the interstitial space.

The value for C_I is low because pulp is encased in an avascular hard mineralized structure: dentin. In many tissues in the body, an increase in capillary filtration rate will not necessarily lead to great changes in tissue pressure because the tissue is compliant and can expand to accommodate increased fluid volume. This is not the case in dental pulp, where localized increases in interstitial fluid volume can lead to great localized changes in P_I. In the dental pulp, the arteriolar pressure is lower and the venular pressure is higher than in other tissues.

The following factors lead to increased interstitial fluid volume (DV):

1. Arteriolar dilation (increased P_C)
2. Venular constriction (increased P_C)
3. Decreased colloidal osmotic pressure in capillaries (COP_C)
4. Increased colloidal osmotic pressure in interstitial compartment (COP_I)
5. Increased capillary permeability (eg, certain inflammatory mediators)
6. Reduced lymphatic outflow

The first four factors are merely the Starling forces described earlier. Clearly, alteration in these forces will lead to alterations in capillary filtration rate and a net outpouring of fluid (ie, increased ΔV), leading to increased interstitial fluid pressure (ΔP_I). The fifth factor, increased capillary permeability, occurs after release of certain inflammatory mediators and increases the capillary filtration rate in a dramatic fashion. Increased interstitial fluid volume compensates for the sixth factor, reduced lymphatic outflow. The rest of this chapter focuses on mechanisms regulating pulpal circulation and describes the effects of dental procedures, drugs, injury, and inflammation on pulpal hemodynamics.

Regulation of Pulpal Blood Flow

Measurement

Pulpal blood flow has been measured in animals with a variety of methods: tracer

disappearance (eg, potassium [42K], lead [86Pb], iodine [131I], hydrogen [2H], or xenon [133Xe]), electrical impedance, plethysmography, and other techniques.[37–42] Of these, the 42K, 86Pb, and 133Xe tracer disappearance and the microsphere methods yield the highest and most similar blood flow values: approximately 40 to 50 mL/min per 100 g of pulp tissue.[43] Unfortunately, one of the difficulties of studying pulp tissue is that a cavity has to be cut into the tooth; this is associated with inherent risks that may affect the parameters under study.[18]

Blood flow values have been measured by intravenous injection of 15-µm radiolabeled microspheres in young dogs maintained at a 45% hematocrit, and results for various oral and visceral tissues, such as gingiva, salivary gland, skeletal muscle, and brain, have been compared.[37] Blood flow in the pulp is the highest among oral tissues and is similar to levels found in the brain. However, the blood flow per unit weight for the kidney, spleen, and other vital organs is about 5- to 10-fold greater than pulpal blood flow. Thus, blood flow seems to reflect the functional activity of an organ.

The anatomical heterogeneity of the vascular network within the pulp is closely related to the heterogenous regional flow distribution.[37,38,43,44] The highest capillary density occurs in the peripheral layer of the coronal region. The central core of the apical region has the lowest density. Overall, about 14% of the volume of dental pulp consists of blood vessels; the mean density of capillaries in cat pulp is 1,402/mm^2.[45] The blood flow of the coronal half of the pulp is about twice that of the apical half of the pulp. The average peripheral blood flow in the coronal region is 70 mL/min per 100 g of tissue, whereas the average flow in the central core region of apical dental pulp measures 15 mL/min per 100 g.

Comparisons among flow values, measured with 8-, 9-, and 15-µm microspheres, indicate considerable shunting of 8- and 9-µm spheres in the pulp. The shunting occurs mostly in the apical half of the pulp,[38] suggesting that AVA shunts participate in the regulation of pulpal blood flow. The shunting is facilitated by U-turn loops in the apical half of the pulp as well as the numerous AVA shunts.[46]

Using intravital microscopy, Kim et al[41] found that the fastest mean intravascular flow velocity in a 42-µm arteriole was 2.1 mm/s; the slowest mean intravascular flow velocity (0.11 mm/s) was measured in an 11-µm postcapillary venule. The mean flow velocity sharply decreased with decreasing vessel diameter on the arterial side. On the venous side, a gradual increase in the velocity curve was observed in 24- to 72-µm-diameter vessels. The fastest mean velocity obtained in the venous side was 0.7 mm/s in a 61-µm venule, substantially slower than the

fastest mean velocity achieved in a comparable arteriolar vessel.

As described earlier in the chapter, pulpal blood flow is regulated by the arteriolar-venular pressure gradient as well as the total vascular resistance: PBF = $(P_A - P_V)/R_T$. During homeostasis, the major determinant of pulpal blood flow is R_T, which is determined primarily by arteriolar resistance. Therefore, factors that regulate arteriolar vasoconstriction have major effects on pulpal blood flow. These factors can be divided into three broad classes: *(1)* metabolic factors; *(2)* neuronal factors; and *(3)* paracrine, autocrine, and endocrine factors (Fig 6-9).

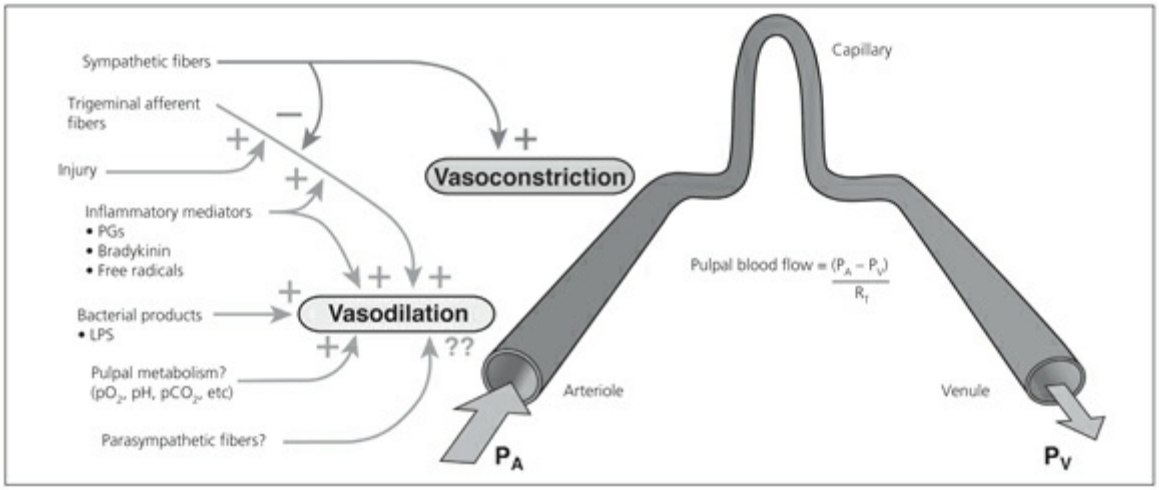

Fig 6-9 Major mechanisms regulating pulpal blood flow. PGs, prostaglandins; LPS, lipopolysaccharide; P_A, arteriolar hydrostatic pressure; P_V, venular hydrostatic pressure; R_T, total resistance.

Metabolic regulation

In most tissues of the body, the arteriolar vascular tone is regulated by locally released metabolic by-products. This serves as an efficient mechanism to couple local blood flow with increased metabolic activity. The actual by-product that evokes arteriolar vasodilation differs in different tissues. In the heart, interstitial oxygen tension regulates vascular tone. In the brain, interstitial carbon dioxide levels (or pH) regulate vascular tone. In other tissues, such as muscle, more than one by-product appears to regulate vascular tone. These effects are independent of nerves because these by-products have been shown to have direct vasodilatory effects on vascular smooth muscle.

Comparatively few studies have evaluated the effect of metabolic activity on pulpal blood flow.[42,47] This is a relatively difficult problem for investigation

because pulp tissue is not easily accessible and the pulp is thought to have a relatively low basal metabolic rate. However, studies have suggested that adenosine, low interstitial pO_2 levels, low pH, or elevated pCO_2 levels may increase pulpal blood flow via vasodilatory effects.[42,48] Graded systemic hyperoxia does not change the pulpal interstitial oxygen tension but decreases pulpal blood flow. This finding suggests that AVA shunts, through which oxygen may bypass the exchange vessels in the pulp and be carried away by the main pulpal venules, may serve as a protective mechanism against hyperoxia-induced toxicity.[48] On the other hand, there is no evidence that transient vascular blockage (20- and 60-second occlusion of the external carotid artery) produces reactive hyperemia in pulp.[45]

Collectively, these studies are consistent with the notion that localized increases in pulpal activity (eg, dentinogenesis) may lead to localized increases in pulpal blood flow. However, the relatively low overall metabolic activity of the pulp suggests that metabolic control of pulpal blood flow may not be a major regulatory mechanism for the entire dental pulp.

Neuronal regulation

In contrast to the relative dearth of studies evaluating metabolic control of pulpal blood flow, numerous investigations have characterized neuronal regulation of pulpal blood flow. Three major neuronal systems are implicated in the regulation of pulpal blood flow: sympathetic fibers, parasympathetic fibers, and afferent (sensory) fibers.

Sympathetic nerves

Andrew and Matthews[49,50] have electrophysiologically recorded the neural activity of two categories of C fibers (afferents and sympathetic fibers) innervating the same tooth, although they had been examined separately. Afferent fibers produce vasodilation and respond to a hot stimulus, suggesting that they may form part of an axon reflex or similar mechanism. Sympathetic postganglionic fibers innervate the arterioles as free nerve endings in the periphery as well as center of the pulp,[3,40,51] although other vessels also receive some innervation[3,51,52] (Figs 6-10 and 6-11). Studies conducted with human dental pulp have confirmed that norepinephrine is present in pulp, released from local terminal endings in pulp tissue, and regulated by

several presynaptic receptors.[53–57] The distribution of sympathetic fibers is highest in blood vessels in the pulp horns near the odontoblastic region and is lowest in the apical region of mouse molars.[58]

Depolarization of sympathetic nerve fibers in pulp of animals or humans leads to the local release of several neurotransmitters, including norepinephrine, neuropeptide Y (NPY), and adenosine triphosphate, which constrict vessels expressing the appropriate receptors.[59] However, the fibers do not respond to any form of drying, hot, cold, osmotic or hydrostatic pressure, or mechanical stimulation of exposed dentin other than electrical.[49] Such actions of sympathetic as well as sensory C fibers have been electrophysiologically determined with a precisely controlled, elegant experimental design that enables the threshold of each unit to electrical stimulation of the nerve filament to be determined using the collision technique.[49] At the resting stage, pulpal vessels are not under the tonic influence of sympathetic nerve discharge. However, electrical stimulation of the cervical sympathetic trunk causes a pronounced reduction of pulpal blood flow[43,60] owing to activation of α-adrenergic receptors.[37,61] Stimulation of pulpal sympathetic fibers reduces pulpal blood flow by more than 80%; this effect is blocked by pretreatment with an α-adrenergic receptor antagonist, phenoxybenzamine (see Fig 6-10).

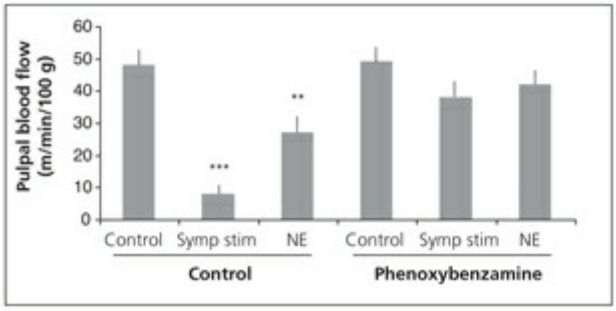

Fig 6-10 Effect of the role of α-adrenergic receptors in regulating pulpal blood flow. Pulpal blood flow was measured with 15-μm microspheres, and blood flow was altered by either electrical stimulation of pulpal sympathetic fibers (by stimulation of the cervical sympathetic nerve [Symp stim]) or administration of norepinephrine (NE). Phenoxybenzamine pretreatment was used to block α-adrenergic receptors. $^{**}P < .05$; $^{***}P < .001$. (Redrawn from Kim and Dörscher-Kim[37] with permission.)

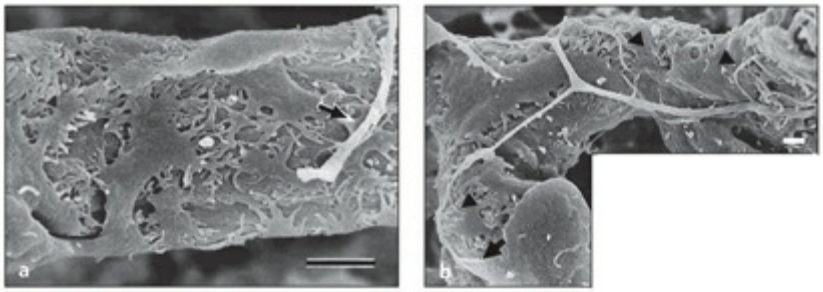

Fig 6-11 Scanning electron micrographs of sympathetic nerve innervation of blood vessels in the rat

incisor pulp. *(a)* Small arteriole in the center of the pulp. A nerve fiber, 0.8 µm in diameter, can be seen *(arrow)*. Processes of smooth muscle cells circumferentially surround endothelial cells (bar = 3 µm). *(b)* In the periphery of the pulp, nerve fibers 0.5 µm in diameter can be seen running on the pericytes or on the endothelial cells. Numerous collaterals, 0.1 to 0.2 µm in diameter, branch off from the nerve fiber and attach to the pericytes *(arrowheads)*. Terminal nerve fibers were observed on the pericytes of the capillaries *(arrow)* (bar = 1 µm). (Reprinted from Tabata et al[3] with permission.)

Reflex excitation of the entire sympathetic nervous system by experimental hypotension (nitroprusside infusion and graded hemorrhage) or a decrease in systemic oxygen transport (extreme hemodilution and hemoconcentration) also causes pulpal vasoconstriction and a reduction of pulpal blood flow.[40,44] Similar studies have been conducted in human dental pulp; results indicated that systemic alterations of the activity of the sympathetic nervous system produce alterations in pulpal blood flow.[61] Thus, activation of the sympathetic fibers innervating dental pulp by either local or reflexive activity produces a profound reduction in pulpal blood flow.

Administration of sympathetically derived neurotransmitters also reduces pulpal blood flow. The catecholamines, such as epinephrine and norepinephrine, exert their physiologic effects on α-adrenergic and β-adrenergic receptors in the blood vessels. The α-adrenergic receptors are responsible for contraction of vascular musculature and produce vasoconstriction. Norepinephrine-induced reduction in pulpal blood flow is mediated by activating α-adrenergic receptors because pretreatment with an α-adrenergic receptor antagonist blocks the effect[37] (see Fig 6-10). This general finding has been replicated in numerous studies and several species.[43,44] In addition, administration of the α_1-adrenergic agonist phenylephrine causes a sharp decrease of pulpal blood flow that is blocked by pretreatment with the α_1-adrenergic antagonist prazosin. Administration of the α_2-adrenergic agonists results in a less pronounced reduction in pulpal blood flow than does α_1-adrenergic receptor activation.[62,63]

In anesthetized dogs, the infiltration injection of 2% lidocaine with 1:100,000 epinephrine produces a significant reduction in pulpal blood flow, as measured with the microsphere injection method, whereas 3% mepivacaine without epinephrine increases it.[64] The effect of the former is due to the catecholamine component because injection of plain 2% lidocaine produces a vasodilatory effect. Thus, α_1-adrenergic receptors appear to be primarily responsible for mediating reduced pulpal blood flow by either endogenous catecholamines (eg, stimulation of pulpal sympathetic fibers) or exogenous adrenergic agonists (eg, administration of "vasoconstrictors" in local anesthetics).

NPY is another sympathetically derived neurotransmitter that is co-localized in terminals with norepinephrine. Human dental pulp contains NPY in sympathetic fibers, and pulpal blood vessels express the Y1 class of NPY receptors.[52,65,66] Figure 6-12 illustrates the perivascular distribution of pulpal nerves that express NPY immunoreactivity.[52] Administration of NPY to dental pulp reduces pulpal blood flow via vasoconstriction.[67] Administration of NPY produces a reduction in pulpal blood flow that is similar in magnitude to that produced by electrical stimulation of the sympathetic fibers that innervate the pulp (Fig 6-13).

Stimulation of β-adrenergic receptors causes a relaxation of the vascular musculature. Pulpal blood vessels respond to β-adrenergic agonists,[68] but the β-adrenergic receptors may be expressed at relatively low levels. The local administration of isoproterenol, an α-adrenergic agonist, either to exposed dentinal tubules[68] or intraluminally, produces a small vasodilatory effect,[47] as measured by dose-related increases in pulpal blood flow. In contrast, the injection of systemic (intra-arterial) or larger doses of isoproterenol causes an initial increase and subsequent decrease in pulpal blood flow in dogs.[43,68–70]

Three hypotheses have been advanced to explain this biphasic finding.[37,69] First, the reduction in pulpal blood flow following intra-arterial administration of isoproterenol may result from "stealing" of blood flow by the adjacent tissues, which have a much greater vasodilator response to isoproterenol.[36,70] Second, the effect of systemic isoproterenol may have been due to active AVA shunting, especially in the apical region of the dental pulp. Third, the biphasic effect may have been due to initial vasodilation of pulpal arterioles, leading to an increase in pulpal interstitial pressure. This increase in P_I then led to a collapse of pulpal venules and reduced pulpal blood flow.[37,43,44] There is strong evidence to support this third potential mechanism, and increased interstitial pressure may serve as an edema-preventing mechanism because it will lead to increased lymphatic outflow.[12,29]

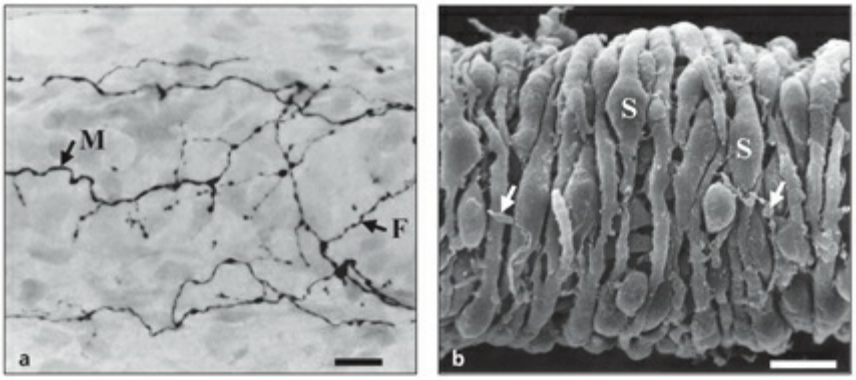

Fig 6-12 *(a)* Light micrograph showing a perivascular meshwork of NPY–immunoreactive nerves. The

meshwork consists of both fine nerve fibers (F) and medium-sized nerve fibers (M). Terminal varicosities are numerous in the fine fibers, but there are only a few in the medium-sized fibers. The vascular axis is horizontal (bar = 5 µm). *(b)* Scanning electron micrograph showing the muscle layer of terminal arterioles. Fragments of nerve fibers *(arrows)* can be seen on circular smooth muscle cells (S). The vascular axis is horizontal. (Reprinted from Zhang et al[52] with permission.)

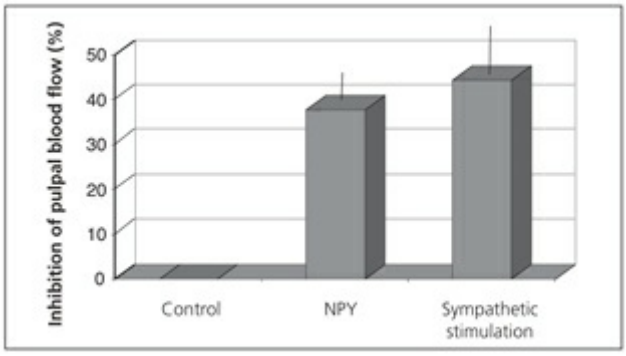

Fig 6-13 Effects of NPY administration (1.3 to 2.0 mg/kg) and electrical stimulation of the sympathetic fibers that innervate dental pulp on pulpal blood flow in anesthetized cats. (Redrawn from Kim et al[67] with permission.)

Parasympathetic nerves

The parasympathetic nervous system does not appear to have as dominant a role in regulating pulpal blood flow as the sympathetic nervous system, and some investigators have suggested that the former may be a relatively minor player in the regulation of pulpal circulation.[63]

The two major neurotransmitters derived from parasympathetic fibers are acetylcholine and vasoactive intestinal polypeptide. Histologic studies have suggested the existence of pulpal parasympathetic fibers in dental pulp.[71–73] Acetylcholinesterase is a key enzyme in the degradation of acetylcholine and has been found in dental pulp.[71] However, the presence of acetylcholinesterase is not a selective marker for parasympathetic fibers. Moreover, at least some species do not possess a parasympathetic (cholinergic) vasodilator mechanism.[74] The local administration of acetylcholine to exposed dentinal tubules had been shown to produce an increase in pulpal blood flow, and this effect is blocked by the muscarinic receptor antagonist atropine.[68,69] Yu et al[75] observed an acetylcholine-induced, dose-dependent vasodilation using an in vitro microperfusion system that enables the perfusion of pig pulpal arterioles intraluminally as well as extraluminally (Fig 6-14; see also Fig 6-17). This vascular relaxation has been abolished in the presence of atropine and by saponin treatment that leads to loss of endothelial function, suggesting that acetylcholine-induced vasodilation of pulpal

arterioles is endothelium dependent and mediated by muscarinic receptors.[75]

Immunoreactive vasoactive intestinal polypeptide is present in pulp, and the intra-arterial injection of vasoactive intestinal polypeptide also increases pulpal blood flow.[63,77] However, the existence of neurotransmitters and receptors does not necessarily indicate that they are derived from parasympathetic fibers, and the magnitude of parasympathetic contribution to pulpal blood flow appears to be low.[63]

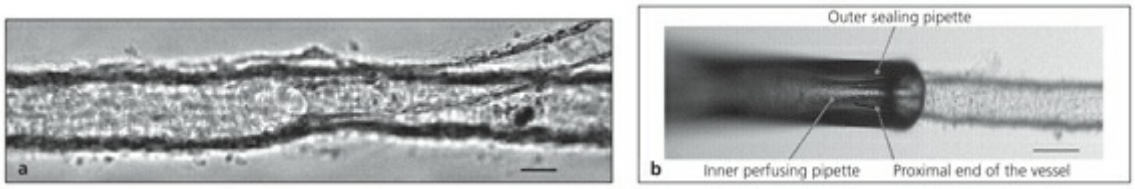

Fig 6-14 In vitro perfusion system of an isolated pulpal arteriole. *(a)* Frame-grabbed image of an isolated perfused pulpal arteriole. The vessel is perfused through the proximal end in the orthograde direction. Agonists can be delivered either intraluminally via the perfusate stream or extraluminally by adding into the incubation chamber (bar = 50 μm). *(b)* Cannulation micropipette in focus together with the proximal end of an isolated perfused pulpal arteriole (bar = 100 μm). (Reprinted from Yu et al[47] with permission.)

Peptidergic afferent fibers

Dental pulp is innervated by sensory neurons originating from the trigeminal ganglion. Although these neurons are classified as sensory, they have major efferent functions because of their release of neuropeptides from their peripheral terminals, innervating tissues such as dental pulp[63,78,79] (see chapter 7). Neuropeptides released from these fibers include substance P and calcitonin gene–related peptide (CGRP).

It has been estimated that about 80% of neuropeptides such as substance P are transported to the peripheral terminals of these afferent fibers rather than to their central terminals.[80] Thus, there is a substantial pool of these neuropeptides in peripheral terminals. This "sensory" system of afferent fibers plays a major role in modulating pulpal circulatory and immune systems via peripheral release of neuropeptides (see chapters 7 and 11). Studies conducted in dental pulp indicate that sensory-derived neuropeptides, such as immunoreactive substance P, CGRP, and neurokinin A, terminate primarily near blood vessels, although some free nerve endings are also observed.[3,73,81–83] Both substance P and CGRP are released from terminals of pulpal nociceptors consisting of certain unmyelinated C[84] and thinly myelinated Aδ[85,86] fibers. This is consistent with an idea that a relatively small number of Aδ fibers, like C fibers, evoke vasodilation in the tooth pulp. Other studies have demonstrated that the appropriate receptors for these neuropeptides are

also found in dental pulp.[87,88]

Activation of trigeminal sensory neurons has several effects on the pulpal circulatory system. Antidromic stimulation of sensory nerves induces pulpal vasodilation and increases pulpal blood flow.[45,50,79] This is a powerful system; stimulation of just a single pulpal C fiber is capable of inducing a detectable increase in pulpal blood flow.[50] In contrast, electrical stimulation of denervated pulp has no effect on pulpal blood flow (Fig 6-15). The effect of electrical stimulation is inhibited by pretreatment with antagonists to the receptors for substance P or CGRP and is greatly diminished after 4 to 5 hours of stimulation.[79,89–91] These studies suggest that finite pools of substance P and CGRP are available for regulation of pulpal blood flow, requiring replenishment via axonal transport from the neuron's cell bodies, located in the trigeminal ganglion.

Activation of capsaicin-sensitive fibers in dental pulp increases pulpal blood flow.[92–94] Capsaicin is an extract of hot chili peppers and causes pain (ie, activates polymodal nociceptors) when eaten or injected because capsaicin receptor is expressed on a major subclass of nociceptors, including unmyelinated C fibers and some lightly myelinated Aδ fibers.[85,86,95] Activation of pulpal nociceptors with capsaicin evokes the peripheral release of neuropeptides such as substance P and CGRP.[96]

Given these findings, it is not surprising that the application of capsaicin to exposed dentinal tubules increases pulpal blood flow in intact teeth but not teeth that have been previously denervated (see Fig 6-15). Repeated application of capsaicin reduces the vascular response, possibly because of neuropeptide depletion or destruction of the terminals.[86] These and other findings[63,79] (see chapter 7) support the conclusion that capsaicin-sensitive fibers represent a major source of the substance P and CGRP that regulates pulpal circulatory responses.

There is evidence that the basal rate of release of CGRP and, to a lesser extent, substance P regulates resting pulpal blood flow. In ferret dental pulp, this resting vasodilator tone is due to tonic release of CGRP, substance P, and nitric oxide (NO).[89,90] Karabucak et al[97] demonstrated that substance P induces endogenous NO production by activating NO synthase (NOS) in endothelial cells. The NOS inhibitor, L-NAME, completely blocks NO production. These results indicate that substance P–induced vascular relaxation can be mediated by inducing NOS, and subsequently NO, production in endothelial cells.[97] The intra-arterial infusion of the CGRP receptor antagonist, $CGRP_{(}8\text{-}37)$ (150 μg/kg), results in a 32% reduction in basal pulpal blood flow measured with a laser Doppler flowmeter (Fig 6-16). The

administration of neurokinin 1 receptor antagonist, SR 140.333 (300 mg/kg), produces a 17% decline in basal pulpal blood flow.[91] These results suggest that the basal tonic release of these neuropeptides in dental pulp plays an important role in regulating the basal vasodilatory state and therefore pulpal homeostasis.

Studies have indicated that the peripheral release of afferent neuropeptides such as substance P and CGRP has additional effects besides vasodilation. For example, these neuropeptides are known to produce plasma extravasation in other tissues as well as in dental pulp.[96,98] Plasma extravasation increases outflow of fluid from the vascular compartment as well as inflammatory mediators normally sequestered within the vascular compartment (eg, the kinin system). Thus, tissue injury may activate these high-threshold nociceptors, sending signals back to the brain as well as initiating neurogenic inflammatory responses[78,79] (see chapter 7). Moreover, prolonged sensory nerve stimulation leads to an accumulation of immune cells in dental pulp.[79]

The administration of neurotransmitters derived from trigeminal sensory neurons also evokes major effects on pulpal circulation. Administration of exogenous substance P and CGRP induces vasodilation in pulpal vasculature.[69,99,100] The direct application of substance P to exposed dentinal tubules leads to vasodilation, whereas the intra-arterial injection of substance P has been reported to produce biphasic effects (an initial vasodilation followed by a vasoconstriction).[69,99] It is unclear whether these contrasting effects are the results of differences in dose, route of injection, methods of measurement, physiologic responses, or species studied. However, substance P–induced vasodilation is a consistent observation.

Both CGRP and substance P exert their effects predominantly on precapillary vessels, whereas nitric oxide acts predominantly on postcapillary vessels.[90] In the mature rat molar pulp, the main targets for substance P acting through the neurokinin 1 receptors are blood vessels in the odontoblastic and subodontoblastic layer.[87]

Given the importance of trigeminal sensory neurons in regulating basal and stimulated pulpal blood flow, it is not surprising that other systems act to modulate the release of these neuropeptides. There is strong evidence that sympathetic fibers act to inhibit the release of substance P or CGRP via presynaptic actions (see Figs 6-11 and 6-12), supporting both an adrenergic and NPY mechanism for inhibition of these fibers.[98,101] Other studies have shown that bradykinin and prostaglandins enhance release of CGRP from dental pulp terminals[102] and that the ability of bradykinin to increase pulpal blood flow is significantly reduced in denervated animals[92] (see Fig 6-15). Thus, the peripheral release of substance P and CGRP can

be presynaptically modulated, resulting in either reduced or enhanced release.[96]

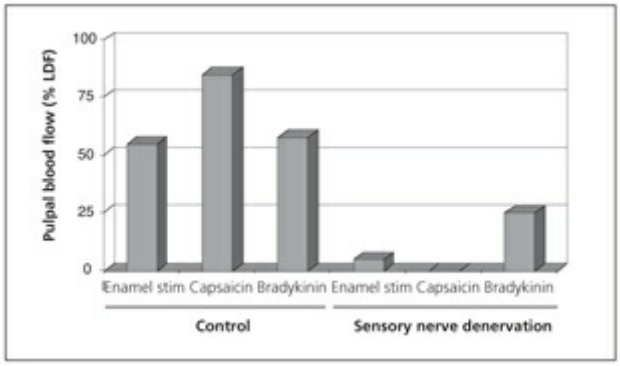

Fig 6-15 Effect of denervation of a sensory nerve on peak pulpal blood flow after electrical stimulation of the enamel (five impulses, 2 Hz at 50 ìA [Enamel stim]), application of capsaicin (1 mmol/L), or application of bradykinin (1 mmol/L) to exposed dentinal tubules. Pulpal blood flow was measured with a laser Doppler flowmeter (LDF). Denervation was accomplished by transsection of the inferior alveolar nerve 10 days prior to the experiment. The contralateral nerve was used as the control. (Data from Olgart.[92])

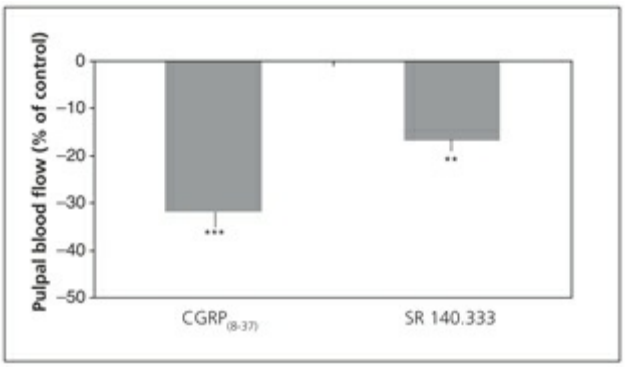

Fig 6-16 Reduction in basal pulpal blood flow by antagonists of neuropeptides. Blood flow was measured with a laser Doppler flowmeter in anesthetized ferrets before and after intra-arterial infusion of an antagonist to the CGRP receptor, $CGRP_{(8-37)}$ (150 µg/kg), or an antagonist to the neurokinin 1 receptor, SR 140.333 (300 mg/kg). $^{**}P < .05$; $^{***}P < .005$. (Data from Berggreen and Heyeraas.[91])

Paracrine, autocrine, and endocrine regulation

Another group of factors that regulates pulpal blood flow is substances of paracrine, autocrine, or endocrine origin. Paracrine and autocrine factors are locally produced or released at their site of action and do not circulate in the bloodstream. Paracrine factors affect secretory cells, and autocrine factors affect nearby cells. Endocrine factors are released from a distant gland and circulate in the bloodstream to modify the activity of the target cell. An example of hormones thought to participate in

regulation of pulpal blood flow includes circulating epinephrine and norepinephrine, although the magnitude of this effect may be relatively small.

There are several examples of paracrine factors that regulate pulpal blood flow. One factor is bradykinin. Bradykinin is locally produced at a site of inflammation via plasma extravasation of its precursor (kininogen) and releasing enzyme (kallikrein) that normally circulate in the bloodstream.[103] Bradykinin levels are elevated in irreversible pulpitis,[104] and the application of bradykinin increases pulpal blood flow.[69,92] About half of bradykinin's effect is lost in denervated pulp, suggesting that this paracrine factor acts partly by activating sensory neuron release of neuropeptides and partly by a direct action on pulpal vasculature (see Figs 6-11 and 6-15).

Bradykinin has been shown to evoke prostaglandin release from a number of tissues, and the two inflammatory mediators can produce additive or synergistic effects when coadministered to pulp.[102] The coadministration of indomethacin, a cyclooxygenase inhibitor, with bradykinin significantly reduces the effect on pulpal blood flow compared with that produced by bradykinin alone.[69] Microsphere studies have indicated that bradykinin can produce a biphasic effect on pulpal blood flow similar to that induced by other substances.[105] One interpretation of the biphasic flow response is related to the low-compliance environment of the tooth, in which active arteriolar vasodilation increases interstitial hydrostatic pressure, which in turn could increase flow resistance by compression of venules, thereby causing a reduction in pulpal blood flow.

A second example of a paracrine factor is the prostaglandins. As mentioned, prostaglandins enhance the effect of bradykinin on increasing pulpal blood flow,[69] and prostaglandin levels are elevated in samples of dental pulp with the clinical diagnosis of irreversible pulpitis.[106] Administration of prostaglandin E_2 increases pulpal blood flow by more than 60% over basal levels and induces a moderate degree of plasma extravasation.[105]

A third paracrine factor is histamine. Histamine increases pulpal blood flow to a moderate extent but produces a profound increase in plasma extavasation.[69,105] Histamine's effect on blood flow is reduced by pretreatment with diphenhydramine, a histamine receptor antagonist.[69] The effect of histamine on plasma extravasation is also reduced by pretreatment with ciproxifan or BP 2-94, antagonists to the H_3 histamine receptor.[107] Compared with administration of histamine alone, administration of the combination of histamine and prostaglandin E_2 produces

relatively little change in pulpal blood flow but evokes nearly 50% greater plasma extravasation.[105]

Fourth, adenosine is released from ischemic and hypoxic tissues and acts interstitially in an autocrine or paracrine manner in rat incisor pulp. Extraluminal adenosine causes vasodilation but intraluminal does not, suggesting a different site of action.[108]

Fifth, the action mode of endothelin 1, a vasoconstrictor peptide, has been suggested to be either local in a paracrine or autocrine manner or systemic as a circulating hormone and neuropeptide.[109] This peptide may be mainly released toward smooth muscle.[76,108] Compared with intraluminal application, extraluminal application of endothelin 1 induces a greater dose-dependent vasoconstriction in a calcium-dependent manner[76,108] (Fig 6-17). This discrepancy is likely to be caused by the tight junction of the endothelial barrier, which may prevent endothelin 1 from reaching receptors located on smooth muscle cells.[76] However, it is well known that endothelial cells have cell-to-cell communication via gap junctions.

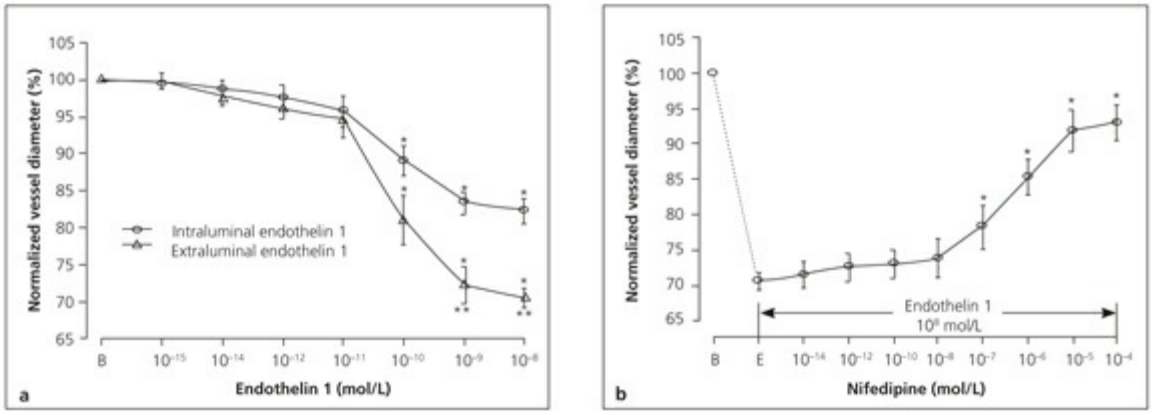

Fig 6-17 *(a)* Dose-dependent vasoconstriction induced by both intraluminal and extraluminal application of endothelin 1 in isolated porcine pulpal arterioles. There is significant constriction (*$P < .05$; **$P < .001$) compared with the diameter before endothelin 1 administration (B). *(b)* Nifedipine reverses endothelin-induced constriction. The data were normalized to the diameter before endothelin 1 administration (B). *Significant dilation ($P < .05$) compared with the diameter precontracted with endothelin 1 (E). (Reprinted from Yu et al[76] with permission.)

Yu et al[47,75,76,108] have isolated and cannulated pulpal arterioles and perfused them. This in vitro method has enabled both intraluminal and extraluminal perfusion to evaluate the vasoactive responses induced by multiple agonists (see Fig 6-14).

Circulatory Responses to Drugs, Dental Procedures,

and Inflammation

Effect of local anesthetics

Vasoconstrictors are added to local anesthetic agents to prolong the anesthetic state and obtain a deeper anesthesia. This effect is the result of arteriolar vasoconstriction, which reduces pulpal blood flow primarily by increasing vascular resistance (see Figs 6-9 and 6-10). Several studies have characterized the reduction in pulpal blood flow that occurs following local anesthetic injection with various vasoconstrictors such as epinephrine.[110] At doses of epinephrine exceeding 10^{-8} mol/L, the pulpal vessels collapse and total ischemia of the pulp results.[111]

Fortunately, dental pulp recovers from these periods of reduced pulpal blood flow. Human dental pulp may not be damaged permanently by injections of local anesthetic agents because the metabolic activity of rat pulps after 2 to 5 hours of ischemia is not significantly different from that of control pulps.[112] These investigators concluded that the anaerobic metabolic activity of the dental pulp would enable it to survive the vasoconstrictive action of the local anesthetic.

Both infiltration and intraligamentary routes of injection of local anesthetic with vasoconstrictor produce a profound reduction in pulpal blood flow because injection of lidocaine alone actually causes an increase in pulpal blood flow and has limited success in obtaining anesthesia.[64] Similar results have been found in clinical trials, in which epinephrine (1:80,000) increased the duration of 2% lidocaine anesthesia from 25 to 100 minutes after infiltration injection.[113] When both routes of injection used the same local anesthetic (2% lidocaine with 1:100,000 epinephrine), the infiltration route of injection produced about a 50% greater reduction in pulpal blood flow than did the intraligamentary route of injection.[64,110] This difference is most likely due to the difference in the injection volume (ie, dose) used in infiltration and intraligamentary techniques.

Although the vasoconstrictor is necessary for successful anesthesia through these routes of injection,[113] there is no significant difference between intraligamental injection of 2% lidocaine with 1:100,000 epinephrine and 2% lidocaine without 1:100,000 epinephrine in onset of anesthesia in the cat.[114]

Restorative or prosthetic procedures

Dental procedures alter pulpal microcirculation via two major routes: *(1)* thermal stimulation when handpieces or certain techniques are used and *(2)* the effects of dental treatment, including restorative materials. The effects of restorative materials (see chapters 4 and 14) and thermal stimulation (see chapter 15) on dental pulp are discussed elsewhere in this text. This chapter focuses on circulatory responses to dental procedures.

Drilling of outer dentin produces vasodilation in intact but not in chronically denervated teeth.[115] It is critical that clinicians use water spray to control heat buildup when performing restorative or prosthetic dental procedures. Heat generated by tooth preparation can cause major changes in the pulpal microcirculation, including extensive plasma extravasation. Figure 6-18 shows a vascular cast of a dog molar after cavity preparation without water spray.[14] The development of plasma extravasation is indicated by the extensive leakage of resin near the site of the cavity preparation.

Other studies have replicated the finding that water spray minimizes the development of pulpal inflammatory changes after restorative procedures. Figure 6-19 illustrates the effect of water spray on pulpal blood flow after crown preparation.[116] Crown preparation without water spray causes about a 95% reduction in pulpal blood flow by 1 hour after preparation. In contrast, the use of water spray virtually eradicates any alteration in pulpal blood flow. The reduction in coronal pulp blood flow is the result of an increase in blood flow through the apically positioned AVA shunts and a redistribution of blood flow from the drilled side to the opposite side of the pulp.[41,105]

What physiologic system mediates this large response in blood flow in coronal pulp? Of the several potential factors known to regulate pulpal blood flow (see Fig 6-9), the trigeminal sensory nerves appear to mediate pulpal circulatory responses to cavity preparation. This was demonstrated in an elegant study in which cavity preparations were performed in control and inferior alveolar nerve–denervated teeth[115] (see Fig 6-19). Denervation of the inferior alveolar nerve reduces the peak pulpal blood flow responses by about 75% in shallow dentinal preparations created with a low-speed handpiece (1.5-mm round diamond) that was applied three times, each time for 1 second.[115] The study indicates that water spray plays a critical role

in reducing pulpal inflammatory responses to dental preparation procedures and that the absence of water spray induces a circulatory response that is mediated primarily by activation of trigeminal sensory nerves in tooth pulp.

A systematic evaluation of changes in microcirculatory dynamics following various dental procedures and materials currently used in dentistry has obvious clinical importance in minimizing pathophysiologic pulpal responses. The placement of zinc phosphate cement at the base of a Class V preparation results in a biphasic response to pulpal blood flow; an initial 33% increase is followed by a subsequent 33% decrease in blood flow after the cement hardens.[117] Acid etching of rat incisors with 36% phosphoric acid produces a minimal effect when done over 15 to 20 seconds but produces profound vasoconstriction when continued for longer periods (eg, 60 seconds), with up to 40% of tested pulps showing stasis of blood flow.[118]

A vital microscopic study was performed to investigate the immediate vascular effect of a new self-etching adhesive, Prompt L-Pop (composite and compomer version, 3M Espe), when it was applied to dentin; the vessel diameters in rat decreased significantly during the experimental period (0 to 60 minutes).[119] Longer-term effects following restorative dental procedures can also occur. Many of these changes are mediated via diffusion of substances through exposed dentinal tubules (see chapters 4, 14, and 15). For example, the application of zinc phosphate base to shallow cavity preparations results in a 40% to 50% elevation of pulpal blood 1 week and 1 month after completion of treatment.[120] Care also must be taken to ensure that small molecules of lucifer yellow can permeate even through intact enamel and reach the tooth pulp.[121]

Mechanical, chemical, and microbiologic irritation of dentin under a provisional restoration could cause severe hyperemia when there is no adhesive lining. It is of vital importance that the freshly exposed dentin surface be covered securely. Application of adhesive resins to coat freshly exposed dentin seems to provide a new technique for minimizing pulpal irritation for indirect restoration.[122]

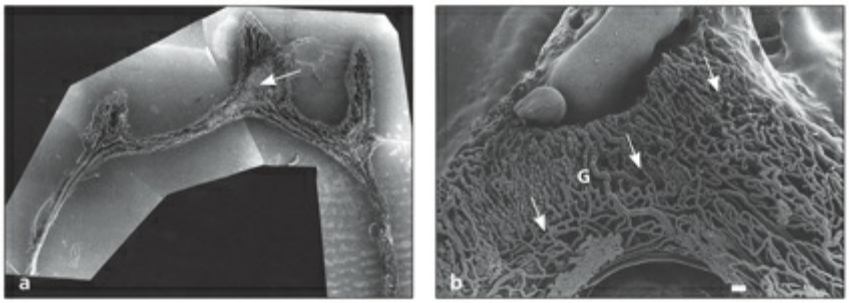

Fig 6-18 *(a)* Vascular cast of a first molar 4 hours after cavity preparation with a carbide bur in a high-speed handpiece used without water spray. There is extensive plasma extravasation *(arrow)*, as indicated by leakage of resin into the interstitial space. The terminal capillary network on the superficial

layer of pulp never showed any particular changes. *(b)* High-magnification view of a vascular cast of a mandibular premolar 1 week after cavity preparation. Furrows *(arrows)* are located between the glomerulus-like network (G) and normal network (bar = 100 μm). (Reprinted from Takahashi[14] with permission.)

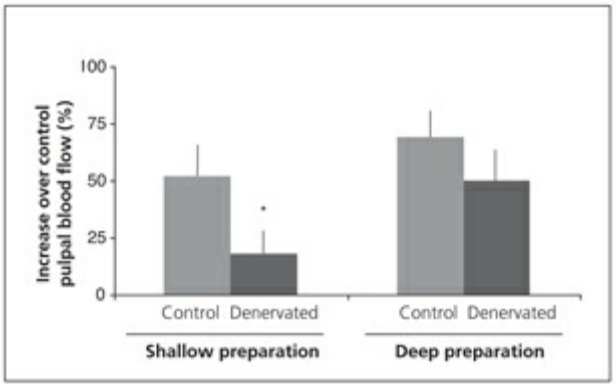

Fig 6-19 Effect of dentin grinding on pulpal blood flow in control teeth and inferior alveolar nerve–denervated teeth. Flow was measured with a laser Doppler flowmeter. Dentin was stimulated by grinding with a low-speed handpiece (1.5-mm round diamond) three times, each time for 1 second. Shallow preparations were in outer dentin. Deep preparations were in inner dentin. *$P < .05$. (Redrawn from Olgart et al[115] with permission.)

Endodontic therapy

If the pulp is partially extirpated during endodontic therapy, profuse hemorrhage may result because of the rupture of wide-diameter vessels in the central part of the pulp. There would be less hemorrhage if the pulp were extirpated closer to the apex of the tooth. Therefore, excessive bleeding during instrumentation of the canal may indicate that some pulp tissue remains in the apical portion of the root canal. The apex locator is useful for detecting the position of the apical foramen in such cases.[123]

Kishi et al[124] reported that a flat and dense vascular network is newly formed 1 week after pulpotomy in dog molars. Eight weeks after pulpotomy, the vascular network just beneath the thick dentin bridge consists of three normal layers. Unfortunately, because of the location of the remaining pulp tissue, neither electrical nor thermal testing can determine the vitality, and hence the presence of circulation, in the apical portion of the pulp.[125]

Calcium hydroxide is a commonly used intracanal medicament. Rapid and transient constriction of rat mesenteric arterioles caused by calcium hydroxide solutions has been viewed under a digital microscope[126] (Fig 6-20). It is still not known whether external $Ca2^{+}$ and/or pH, accurate concentrations of which cannot be adjusted clinically, has a direct action in eliminating persistent intracanal exudation.

Further studies are needed to clarify the detailed mechanism of this constriction.

Orthodontic therapy

Orthodontic treatment has substantial effects on the microcirculatory system of the dental pulp. Following localized orthodontic tooth movement, vasodilation and a steady and significant increase in blood flow can be observed not only in the periodontal tissues[127] but also in the pulp.[127,128] However, human pulpal blood flow is not altered during the application of a brief, 4-minute, intrusive orthodontic force.[129] At later time points, orthodontic movement stimulates a large and coordinated sprouting in pulpal nerve terminals and blood vessels.[130] This has raised the hypothesis that orthodontic movement leads to an increase in pulpal concentrations of angiogenic growth factors. Indeed, extracts of human pulp taken after orthodontic movement have higher concentrations of angiogenic factors and show increased growth of blood vessels even when cultured in vitro.[131] Other studies have shown that growth factors, including vascular endothelial growth factor, are embedded in dentin and are released from the matrix following injury[132] (see chapter 3). Thus, it is possible that dental procedures that lead to release of these growth factors from the dentin matrix may result in enhanced angiogenesis.

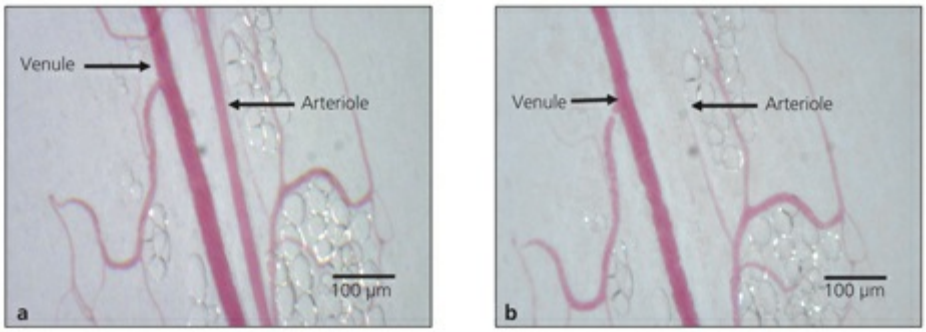

Fig 6-20 Rapid and transient constriction of a rat mesenteric arteriole caused by calcium hydroxide solution. *(a)* Before application. *(b)* Thirty seconds after application. Note the constriction of the arteriole. Five minutes after washing out, the diameter recovered to the dimension shown in *(a)*. (Reprinted from Kikuchi et al[126] with permission.)

Orthognathic surgery

Occlusal abnormalities may be corrected by maxillary or mandibular segmental osteotomies. Attempts have been made to determine the effects of such procedures on the blood flow to the oral mucosa, bone, and dental pulp. Techniques that have been used for this purpose include microangiography,[133] isotope fractionization and particle distribution,[134] hydrogen washout technique,[135] and laser Doppler

flowmetry (LDF).[136] Of all the tissues, pulpal blood flow was most severely decreased (by 82%) immediately after surgery.[135] However, blood flow has been reported to be reestablished at later time points.

Studies using a laser Doppler flowmeter have reported that, although pulpal blood flow is detectable 6 months after a Le Fort I procedure, it is still significantly lower than preoperative levels.[137] Early studies have claimed that tooth pulp responses to thermal and electrical stimuli return to normal in more than 90% of patients,[138] although recovery of pulpal sensitivity may take days or weeks.[139] Thus, preemptive endodontic therapy or extraction is not warranted in patients undergoing these types of surgeries unless there is evidence of periradicular pathoses.[140]

Effect of inflammation

A number of inflammatory mediators that are released after pulpal injury may have direct effects, or indirect effects via modulation of trigeminal sensory nerve fibers, on pulpal vasculature (see Fig 6-9). Previous sections of this chapter have already reviewed several of these mediators, including bradykinin, prostaglandins, and histamine.

The two major actions of mediators of acute inflammation are alterations in pulpal blood flow and increases in capillary permeability, leading to plasma extravasation. The increased permeability of the vessels permits the escape of plasma proteins and leukocytes from the capillaries into the inflamed area to carry out neutralization, dilution, and phagocytosis of the irritant. In the acute pulpitis stage following cavity preparation without water coolant, increased permeability of blood vessels is seen not in the superficial, terminal capillaries just beneath the cavity but in the venular and capillary networks. In the inflamed pulp, vascular loops, AVA shunts, increased blood flow, and increased lymphatic outflow may represent protective changes against inflammation.[2,12–14]

The introduction of bacterial plaque to dental pulp or the administration of bacterial lipopolysaccharide induces profound alterations in pulpal blood flow. Pulpal blood flow can increase up to 40% over control levels in dental pulp moderately inflamed with bacterial plaque.[105] As pulp becomes partially necrotic, there is a reduction in blood flow.[105] In other studies, application of bacterial lipopolysaccharide to pulp has resulted in a large increase in pulpal prostaglandins,

leukotrienes, and plasma extravasation.[5,105] Thus, bacteria and their by-products produce profound circulatory responses in dental pulp.

During chronic inflammation, pulp tissue pressure is elevated, although not as greatly as it is during acute inflammation.[5] The muscular elements in the microcirculation reestablish control over capillary pressure. Capillary permeability is gradually decreased as repair occurs.

In prolonged inflammation, the lymphatic vessels are closed, resulting in persistently increased fluid and pulpal pressure. The final result may be pulpal necrosis. Care must be taken, however, to assess the vital pulp restricted to the apical portion of the root in teeth with coronal necrosis, especially in young patients.[125] In addition, histologic and physiologic changes, including pulp tissue pressure,[34,36] could take place at the site of inflammation while the neighboring region shows no sign of inflammation.[6] Thus, total pulpal necrosis represents the gradual accumulation of local necrosis.

Detection of Pulpal Circulation as a Diagnostic Test

Given the essential role of the pulpal microcirculation in maintaining the health of this tissue, it is not surprising that clinicians have proposed that the circulatory status of dental pulp be assessed to determine the vitality of this tissue. Several general methods have been used to clinically evaluate pulpal circulation, including LDF, pulse oximetry, and transmitted-light photoplethysmography.

Laser Doppler flowmeters are based on the principle that reflected light from blood flow will demonstrate a Doppler (frequency shifting) effect, depending on the relative velocity of the blood flow compared with the probe. Several studies have suggested a reliability of greater than 80% to 90% for LDF assessment of vitality.[141–144] However, others have observed lower levels of reliability or technical difficulties, including the potential confounding factor that up to 80% of the signals measured by LDF originate from the periodontal tissue.[137,143,145,146] The use of a dual-probe LDF system has been suggested to increase the reliability of this method, as has the use of a 633-nm laser source placed 2 to 3 mm above the gingival margin.[141,147] The following must be taken into consideration when use of LDF is planned: First, the method is contraindicated in heavily restored teeth and teeth with apical viability (because LDF probes detect only coronal pulpal blood flow).[148]

Second, pulpal perfusion is significantly higher when patients are in a supine position than when they are in a standing or sitting position.

The use of a pulse oximeter is a clinically accepted technique for evaluating oxygen saturation of tissues. Pulse oximeters are often configured as finger or ear probes. Pulse oximeters have been used to assess pulpal vitality; some studies have reported greater success[143,145] than others.[146] Although the pulse oximetry approach is promising, additional developmental research should be conducted before this method is considered clinically acceptable.

Transmitted-light photoplethysmography[149] may be useful for vitality testing of young permanent teeth.[150] Validation studies have reported that 565 nm gives a peak intensity of signal when transmitted-light photoplethysmography is used on extracted teeth.[149] Measured values of plethysmography are in proportion to the pulpal volume.[151]

Although promising, these current techniques suffer from concerns about sensitivity, specificity, and ease of use. However, given the central importance of their approach (assessment of pulpal microcirculation as a measure of pulpal vitality), it is hoped that future research and development will lead to the introduction of methods that are reliable, valid, and easy to apply.

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Dental Innervation and Its Responses to Tooth Injury

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The pulpodentin complex is among the most densely innervated tissues in the body, yet we rarely perceive sensations from this structure unless a tooth is injured or inflamed. We are well acquainted with the sharp pain that is felt as soon as our teeth are injured, and much is known about acute pain mechanisms and dental anesthesia. However, there still are questions about the sensory activation mechanisms (mechanical, nociceptive, polymodal, and thermal) and the functions of the largest Aβ fibers compared to Aδ and C fibers.

Inflammatory pain mechanisms within the dental pulp differ from acute pain and are complex. Evidence is mounting that inflammation causes molecular changes in nerve fibers before pain is detected in the affected tooth. This observation leads to many questions regarding the specific role that nerve fibers play in the dental pulp. Some of the most intriguing are related to possible roles beyond those associated

with the perception of pain, such as contributions to reparative processes involved in the response to injury. What is the relationship of nerve fibers to the other cell types within the dental pulp and how do these relationships change following different insults? What are the specific genes, proteins, and receptors that are selectively activated by the dental innervation for each condition?

This chapter highlights the advances in the understanding of dental innervation and its injury responses, first from general, anatomical, and neurocytochemical perspectives in animal and human research and then concerning the basic physiology of pulpal nociceptors, dentinal sensitivity, and injury reactions, with emphasis on recent advances and reviews. Some previous seminal reviews are also helpful.[1–14] The related topics of pulpal vasoregulation (see chapter 6), pain mechanisms in the pulp (see chapter 8), pharmacologic control of tooth pain (chapter 9), and differential diagnosis of odontalgia (see chapter 19) are covered in other chapters.

Different questions concern recent demonstrations that odontoblasts express neural molecules.[15–20] Much current work is focused on testing whether that gene expression reveals direct or indirect detection of tooth stimuli by odontoblasts and/or an ability of odontoblasts to affect neural activity. Alternatively, odontoblast expression of neural genes may be part of the expanding evidence that many non-neural cells throughout the body adapt those genes for non-neural requirements.[21] Some use of neural signal systems might be required for odontoblast regulation of morphogenesis of the pulp-dentin border and its mature function as a barrier, participation in innate immunity, supervision of dentin matrix, interactions with vasculature, including regulation of dentinal fluid, and responses to injury and inflammation. Conversely (or additionally), sensory requirements of teeth may sometimes depend on initial activity of the odontoblasts[22] or other pulp cells.[23] An understanding of these issues is key to an understanding of why teeth have such dense innervation and why they have special pain problems, injury reactions, and healing mechanisms.

General Aspects of Dental Innervation

The preservation of teeth over many decades is enhanced by healthy dentin and pulp. Nerve fibers contribute to tooth survival by detecting dental stimuli, triggering dental reflexes, and interacting with other pulp cells such as odontoblasts, fibroblasts,

blood vessels, and immunocompetent cells (Fig 7-1) for protection, maintenance of healthy tooth function, and repair. Tooth nerves can be classified based on sensory perception (perceived sharp pain, dull pain, or prepain, compared to unperceived sensory activity), effective stimulus (mechanical, thermal, chemical, noxious, or polymodal), or conduction velocity (Aβ, Aδ-fast, Aδ-slow, and several types of C fiber).

Many of the nerve fibers also secrete neuropeptides into the pulp. The timing, concentration, and location of the neuropeptides provide important paracrine signals for other pulp cells about the status of the tooth. In addition to regulating normal pulpal functions, neural agents also stimulate neurogenic inflammation and contribute to pulp and dentin repair.[24] They may also mimic antimicrobial peptides,[25] thus providing an additional function for neuropeptides released from fibers that innervate the dentin and pulp.

During different phases of injury and healing, the nerve fibers (and other cells) adjust their responses, either to enhance defensive functions or to promote inflammation and tissue repair, and then return to the normal resting basal condition. The neuropulpal interactions shown in Fig 7-1 occur in preterminal and/or terminal nerve fibers within the tooth. In addition, nerve fibers extend long distances and have their control center (cell body) located far away from the tooth in the trigeminal ganglion for sensory neurons, and in the cervical sympathetic ganglia for sympathetic fibers, with further connections to the central nervous system. Dental neural functions are therefore affected by events in the brainstem, ganglion, and alveolar or sympathetic nerves as well as in the tooth. Chemical signals about those events are communicated back and forth within the neurons by fast and slow axonal transport (both anterograde [toward the cell body] and retrograde [away from the cell body]) as well as by the very rapidly conducted electrophysiology signals (Fig 7-2). Those transported and conducted signals affect neural functions at peripheral and central endings as well as regulation of neural gene expression.

The nerve fibers in pulp and dentin are components of a large somatosensory system that also includes innervation of the gingiva, junctional epithelium, periodontal ligament, tongue, lips, mastication muscles, and temporomandibular joint[4,11,12,26] (Fig 7-3). Each part of the system contributes different kinds of somatosensory information needed for integrated oral function and tissue preservation during tooth use. For example, the gingiva provides sensations of touch, pressure, and temperature via activation of special mechanoreceptors or thermoreceptors. The junctional epithelium is richly innervated by sensory fibers that release neuropeptides to regulate vasodilation, transmigration of leukocytes

across the epithelium into the oral cavity, and antimicrobial actions. The periodontal ligament contains many large Ruffini mechanoreceptors from the trigeminal ganglion or mesencephalic nucleus that give sensations of tooth touch and occlusal plane location during chewing, speech, and swallowing.[26–29] Some part of the sensory information from the periodontal mechanoreceptors may remain unconscious and serve automatic reflex responses needed for the regulation of the masticatory functions. All the orofacial tissues also have a variety of nociceptive fiber types, including polymodal nociceptive nerve fibers (eg, silent nociceptors) that initiate acute pain sensation or trigger inflammatory pain, as discussed later in this chapter in the section on human teeth. Together, the multiple nerve fiber systems of these regions provide an integrated regulatory system that acts on teeth and their supporting tissues.

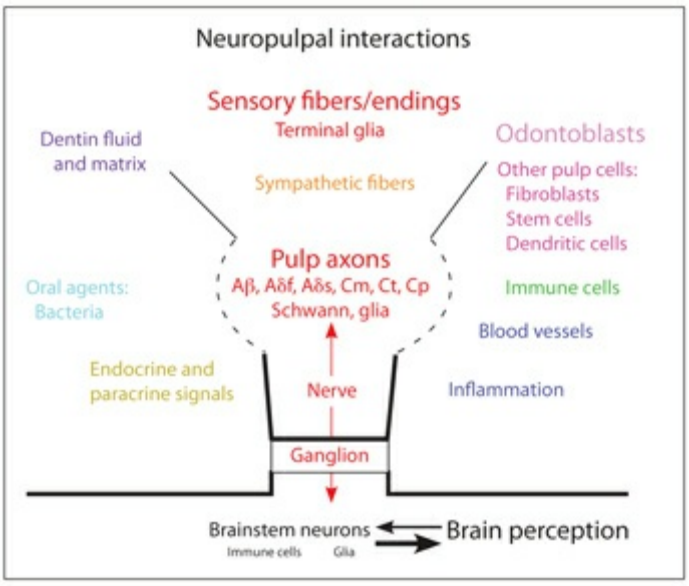

Fig 7-1 Neuropulpal interactions, all of which can affect neural activity, are represented with different colors: sensory endings and intradental axons plus their terminal glia, trigeminal nerve, and ganglion *(red)*; sympathetic fibers *(orange)*; dental pulp cells *(pink)*; immune cells *(green)*; blood vessels and inflammation *(blue)*; endocrine and paracrine signals *(yellow)*; factors from dentin matrix, interstitial fluid, or dentinal fluid *(purple)*; oral agents such as bacterial antigens *(teal)*, which can penetrate into dentin; and the central nervous system and the blood-brain or blood-nerve barriers *(thick black lines)*. All of these interactive cells and molecules can affect the sensory endings connected with the axons that carry the information from the tooth through the ganglion to brainstem to brain, where perceptions of tooth pain occur. Six types of sensory axon are listed: three types of A fiber (large, fastest conduction [Aβ]; medium fast [Aδf]; small, slow, myelinated [Aδs]) and three types of unmyelinated C fiber (mechanosensitive [Cm], thermosensitive [Ct], and polymodal [Cp]).

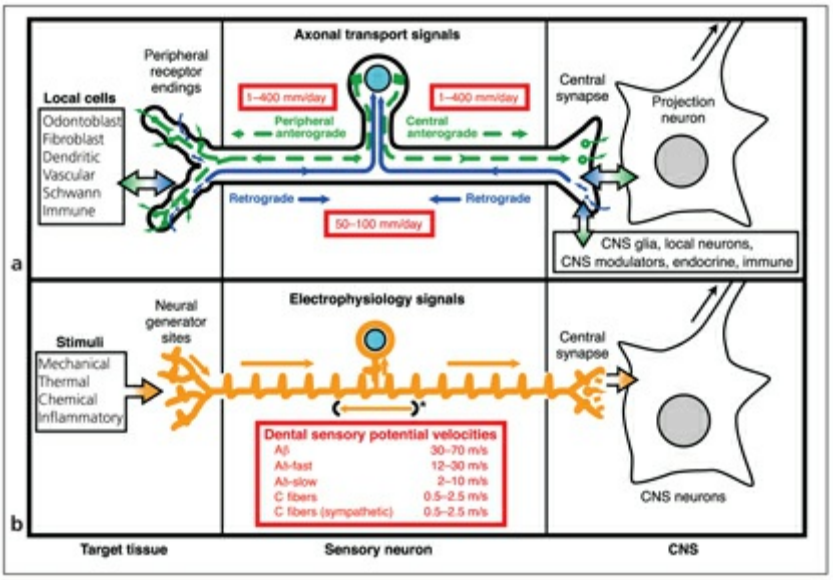

Fig 7-2 Two main information systems for neurons are shown: axonal transport *(a)* and electrophysiologically conducted action potentials *(b)*. For axonal transport, specific molecules in vesicles or cytoplasm can go in the retrograde direction from the endings (receptive or central synaptic endings) to the cell body or in the anterograde direction from the cell body out to the endings. The rates of axonal transport vary from 1 to 400 mm/day for the anterograde and from 50 to 100 mm/day for the retrograde system, both of which are much slower than the conducted action potentials. Local cells, such as odontoblasts, fibroblasts, dendritic cells, blood vessels, and immune cells, modulate the activity of sensory receptive endings. At the central endings there are modulatory interactions with glia, local neurons, axons from higher centers, and endocrine and immune signals. The receptive generator sites in the nerve endings detect tissue stimuli such as mechanical, thermal, chemical, or inflammatory signals. Their conducted action potentials usually travel from periphery to central endings, where they trigger synaptic communication with central neurons. The signals in some cases *(*)*, such as axon reflex, travel in the opposite direction out to the peripheral endings, where they stimulate the secretion of neuropeptides and other neural agents into the pulp. CNS, central nervous system.

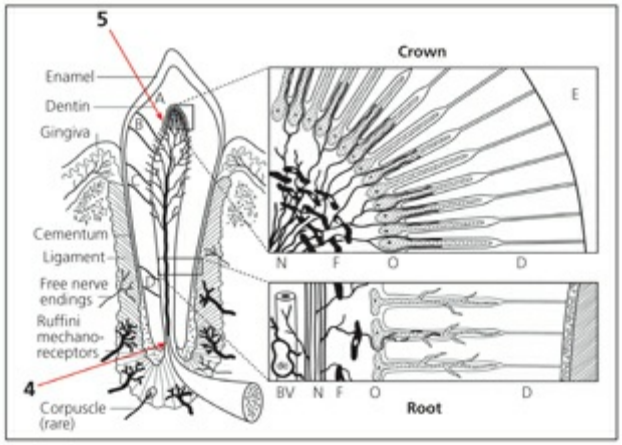

Fig 7-3 Dentinal, pulpal, and periodontal innervation of a mature erupted tooth are shown here. The labeled dentinal zones (A, B, C, and D) on the left side of the tooth have been found to have more than 40% of dentinal tubules innervated at the tip of the pulp horn; nerve density decreases progressively from midcrown to cervical dentin to the root.[3,6] The panels on the right show differences in pulpodentin organization and nerve incidence in the crown and the root. BV, blood vessels; DC, dendritic cell; N,

nerve; F, fibroblast; O, odontoblasts; D, dentin; E, enamel; 4, location of the image in Fig 7-4; 5, location of the images in Fig 7-5. (Modified from Byers and Närhi4 with permission.)

The sensory innervation of teeth includes hundreds of branches from each of the incoming myelinated A or unmyelinated C fibers (Figs 7-4 and 7-5). Most of the A fibers terminate in the coronal odontoblastic layer, predentin, and inner dentin (Figs 7-5 and 7-6), while most C fibers end in the pulp and along pulpal blood vessels. The biggest axons, the Aβ fibers, make large endings near odontoblasts along the dentin-pulp border near the pulp horn tip and lack the receptors for the low-affinity nerve growth factor (NGF) receptor.[36] They are the fibers that are most sensitive to mechanical (hydrodynamic) stimulation of dentin.[5,13] The large endings of these Aβ fibers make close appositions with odontoblasts (see Fig 7-6), and individual fibers can contact a group of neighboring odontoblasts.[8]

The majority of A fibers are small and medium myelinated fibers, many of which contain the neuropeptide calcitonin gene–related peptide (CGRP) and express receptors for NGF[36] (see Figs 7-4 to 7-6). Most of these innervate dentin, predentin, and the odontoblastic layer in the coronal regions underlying enamel.[3,4,12] The dentinal endings occur close to the odontoblast processes. No synaptic or gap junctions have been found for nerve-odontoblast associations, but a paracrine signaling mechanism would be facilitated by close association without cellular or matrix barriers between them (see Fig 7-6).

Most of the Aδ innervation is concentrated in dentin near the pulp horn tip; it is progressively less frequent toward the cervical region and least prevalent in the root dentin. Thus, there are focal regions of dense innervation of dentin oriented toward the occlusal contact zones and specific gradients of innervation. There are also some slow-conducting thin Aδ fibers (see Fig 7-4) that have capsaicin sensitivity,[37] as discussed later. Finally, at least half of the nerve fibers in human teeth are unmyelinated, slow-conducting C fibers[38] (see below).

During development, there is close coordination between the maturation of pulp and dentin and the arrival and maturation of sensory innervation that relies heavily on discrete expression of neurotrophin growth factors.[39,40] All dental nerve fibers seem to require an NGF-dependent stage,[41] but in the adult, a major component of dental innervation is regulated by glial cell line–derived neurotrophic factor (GDNF),[42] while the rest of the fibers retain their NGF dependence[43] (Fig 7-7). That organization is also found elsewhere throughout the body; the GDNF-dependent group mainly has mechanosensitive functions, and the NGF-dependent adult fibers

have polymodal or nociceptive sensitivity as well as paracrine signaling via release of neuropeptides. An additional special feature of dental innervation is that all fibers continue to express growth-associated protein (GAP-43) in the adult, unlike similar fibers in other tissues, such as skin.[46] Thus, the mature sensory innervation of teeth is continually adjusting its position in relation to the status of the pulpodentin complex so that important cytochemical changes continue to occur throughout the life of the tooth.[39,43]

Additional details about molecular subspecializations within the Aβ, Aδ, and C nerve fibers are still being discovered, especially in relation to the ion channels and membrane receptors that regulate their functions,[10] as described in the section on human teeth and in chapter 8.

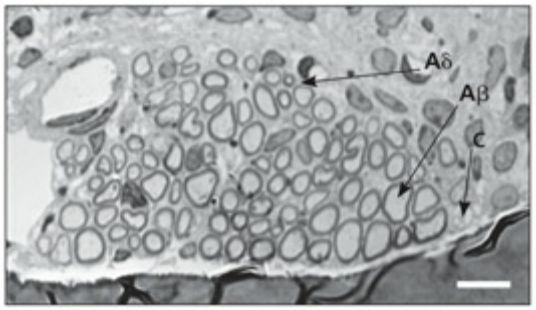

Fig 7-4 Various sizes of myelinated A-fiber axons and unmyelinated C-fiber bundles are visible in this cross section of a nerve that has just entered the root of a mouse molar. The A fibers include many large Aβ myelinated and medium or small Aδ myelinated axons (bar = 0.01 mm).

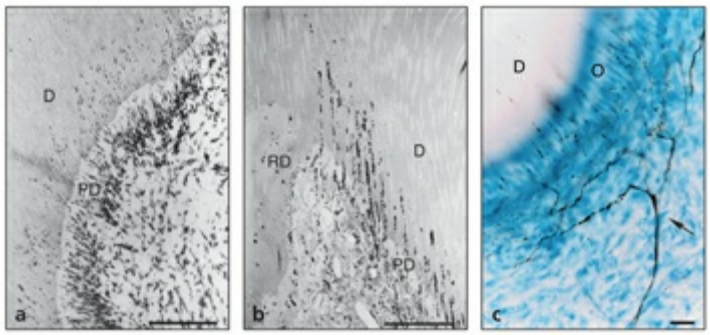

Fig 7-5 Light microscopic autoradiography of nerve endings in dentin. *(a)* About half of the tubules in the inner dentin (D) of this dog tooth have autoradiographic labeling indicating the location of trigeminal nerve endings. PD, predentin (bar = 0.1 mm). (Modified from Byers et al[30] with permission.) *(b)* Autoradiographically labeled trigeminal nerve endings in an adult rat molar are concentrated in the tubular dentin (D) while avoiding the atubular reparative dentin (RD). The predentin (PD) is wider at the innervated regions than it is for other crown dentin (bar = 0.065 mm). (Modified from Byers et al[31] with permission.) *(c)* A highly branched single Aδ ending is shown here for the crown dentin (D) and pulp of a rat molar. Its terminal branches *(arrow)* extend into the odontoblastic layer (O) and dentinal tubules (bar = 0.01 mm). (Modified from Byers[32] with permission.)

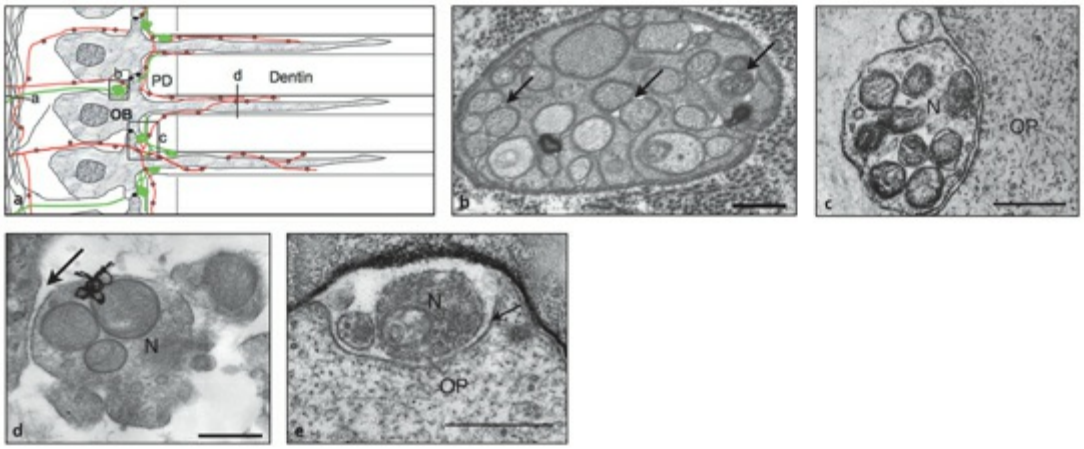

Fig 7-6 *(a)* Odontoblasts (OB) connected by gap junctions *(black dots)* that are associated with beaded peptidergic intradentinal innervation *(red fibers)* and large nerve endings that end close to odontoblasts on the dentin side of their gap junctions and in the predentin portions of dentinal tubules *(green fibers and endings)*. Approximate positions for the electron micrographs are shown *(b to e)*. *(b)* Preterminal axons in the plexus of Raschkow from a human tooth. All have lost myelin and some are close to each other *(arrows)* without a Schwann cell sheath (bar = 0.001 mm). (Modified from Byers et al[33] with permission.) *(c)* This nerve-like fiber (N) in a human tooth maintains a separation from the adjacent odontoblast process (OP) (bar = 0.005 mm). (Modified from Frank[34] with permission.) *(d)* An autoradiographically labeled trigeminal fiber (N) is separated by a small, uniform space *(arrow)* from an odontoblast, as well as from other nerve-like fibers, in a rat molar (bar = 0.005 mm). (Modified from Byers[35] with permission.) *(e)* This dentinal tubule from a human tooth contains two nerve-like processes (N) that maintain their separation *(arrow)* from the odontoblast process (OP) in that region of the terminal fiber (bar = 0.005 mm). (Modified from Byers et al[33] with permission.)

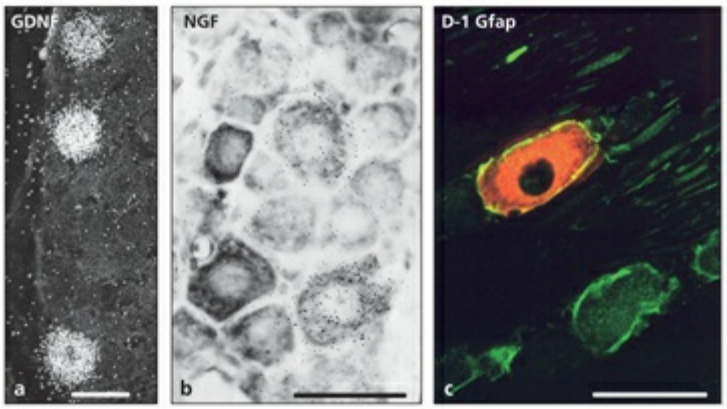

Fig 7-7 Trigeminal ganglion dental nerve cell bodies, labeled with tracers from rat molars. *(a)* Radioactive glial-derived neurotrophic factor (GDNF) *(white dots)* was transported back from rat molars to large neuronal cell bodies in the trigeminal ganglion (bar = 0.05 mm). (Reprinted from Kvinnsland et al[42] with permission.) *(b)* Radioactive nerve growth factor (NGF) molecules *(black dots)* are transported retrogradely to large and medium cell bodies in the trigeminal ganglion within 15 hours (bar = 0.05 mm). (Modified from Wheeler et al[44] with permission.) *(c)* Red dye (D-I) was transported from an injured molar to the cell body in trigeminal ganglion and is associated with a glial injury reaction *(light green)* in its satellite cell. Nearby satellite cells of uninjured neurons are also reacting by expressing glial fibrillary acidic protein (Gfap) *(dark green)* (bar = 0.05 mm). (Reprinted

from Stephenson and Byers[45] with permission.)

Sympathetic and Parasympathetic Innervation of Teeth

The vasodilatory functions of sensory innervation in teeth are opposed by the vasoconstricting activity of the sympathetic fibers[9,14,47] (see chapter 6). The sympathetic fibers are much less numerous than the sensory fibers, although there are differences among species in those relative proportions. Sympathetic fiber distribution also differs from that of sensory fibers in that the sympathetic fibers are located mainly in deeper pulp and along blood vessels. Parasympathetic activity can affect blood flow in teeth, but it is not clear whether that activity derives from intradental or periodontal sites. In any case, the relative importance of parasympathetic activity is much less than that of sympathetic activity for regulation of pulpal homeostasis and blood flow. These autonomic functions counterbalance the vasodilation produced by sensory nerve fibers, as discussed in detail in chapter 6.

Role of Odontoblasts in Tooth Sensation

Odontoblasts are complex cells that regulate dentinogenesis and its maintenance, set up barriers between dentin and pulp, help regulate blood flow and dentinal fluid, and have antimicrobial defense and immune functions.[48] In addition, odontoblasts are closely associated with nerve fibers of the pulpodentin complex, and much current work concerns their possible involvement in sensitivity of teeth. Their lack of synaptic or gap junction connections with the nerve fibers[3] suggests that they do not have primary sensory functions. Subsequent technologies have shown profuse cytochemical paracrine signaling in teeth that greatly expanded the possibilities for neuromodulating actions by odontoblasts[15] or by other pulp cells[23] as well as expanding the range of pulpal responses to neural factors (Box 7-1).

Demonstrations of neural-like ion channels[19] and TREK-1, a mechanosensitive potassium channel,[20] in odontoblasts show that they are excitable and mechanosensitive cells, as does their expression of a variety of voltage-gated ion channels.[16] In addition, they actively attract nerve fibers[49] and express neurotrophin

factors and receptors in development and after injury.[39,42,50] Pulp stem cells can even acquire neural-like functions under special conditions.[51,52] Recent work shows that keratinocytes directly affect neural activity and nociceptive sensitization in skin,[53] and odontoblasts may similarly influence events that make teeth hurt. Those mechanisms in normal teeth would change during pulpitis. Recent efforts to define neuropulpal interactions are discussed further in the section on human teeth later in this chapter.

Box 7-1	Some agents involved in neuropulpal interactions*

Neural agents that affect pulp cells and blood vessels
- Sensory neuropeptides: CGRP, substance P, neurokinin A, somatostatin, galanin
- Sensory neurotransmitters: glutamate, acetylcholine
- Autonomic factors: norepinephrine, peptide histidine isoleucine, acetylcholine, neuropeptide Y
- Schwann cell factors: NGF, GDNF, neurotrophin receptors

Dentinal, pulpal, vascular, or immune agents that affect dental nerve function
- Odontoblast-specific molecules
- Neurotrophic factors: NGF, brain-derived neurotrophic factor, GDNF
- Inflammatory mediators: serotonin, histamine, bradykinin, prostanoids, cytokines
- Cellular breakdown products: adenosine triphosphate, cyclo-oxygenase, oxidative radicals
- Altered pH and its excitation or inhibition of molecular functions
- Heat shock proteins
- Somatostatin and endocrine factors
- Antinociceptive agents (eg, opioid peptides, cannabinoids, adenosine)
- Extracellular matrix factors (eg, laminin and metalloproteinases)
- Ionic environment
- Oxygen tension and interstitial fluid pressures
- Bacterial agents

"Neural" factors expressed by pulp
- Neurotensin, nestin, protein gene product 9.5
- Tachykinin precursor and receptor
- Neurotrophin receptors: tyrosine kinase A, p75
- Neurotrophins: NGF, brain-derived neurotrophic factor, GDNF
- Nitric oxide
- Neural-like calcium, sodium, and potassium channels

* See text for references about neuropulpal interactive signaling.

Structural and Cytochemical Responses to Tooth Injury and Infection

Reactions inside teeth

Injury to the pulpodentin complex causes neuronal responses that vary for different types of fibers and along a time spectrum from milliseconds to weeks or months.[4,9,10] The nerve fibers not only send rapid electrophysiologic signals to the ganglion and central pain pathways but also release neuropeptides and other agents from their peripheral terminals that regulate vasodilation and leukocyte invasion of the injury site and affect every local cell type; this includes themselves via autocrine actions. In addition, they pick up and transport local pulp factors such as NGF that convey information about pulp status to the neuronal cell body.

The dental sensory fibers react to tooth injury by extensive anatomical and cytochemical changes in their preterminal branches and endings. In rat models of dental injury, there is an initial depletion of neuropeptides followed by an increase in neuropeptide content and sprouting of the terminal fibers within 1 day after injury. Those responses differ in intensity and duration depending on the severity of the injury.[9,10,14,54] Innocuous stimuli such as vibration may cause nerve fibers to be activated at levels sufficient to cause changes in neurally regulated pulpal homeostasis, blood flow, and interstitial fluid pressure (see chapter 6).

Stimuli that injure the pulpodentin complex have been classified at four different levels.[4] Type I injuries are least damaging. They cause a transient change in pulp, sometimes including reactive dentinogenesis (the original odontoblasts survive and are not replaced by reparative cells). There is extensive sprouting of neuropeptide-rich nerve fiber endings near the injury that return to normal within a few days to a few weeks. Those changes have been correlated with local production of NGF by the fibroblasts near the injury site.[44,55] Under these conditions, there is little or no invasion of leukocytes, and the local defense mechanisms are sufficient. Examples of this type of injury are shallow cavity preparations, shallow scaling of cervical dentin, and strong orthodontic forces.

Type II injuries have more extensive dentinal injury with some loss of pulp tissue and focal inflammation. Invasion of leukocytes and local vascular responses occur, but the pulp can repair itself and form reparative dentin. For these lesions, there is extensive sprouting of sensory fibers that have enhanced neuropeptide contents such as CGRP and substance P. Examples of this type of injury include deep dentinal cavities, small pulpal exposures, and heat stimulation of long duration and/or high intensity.

For these intermediate injuries, the sprouting and CGRP upregulation continue as long as there is active inflammation that has not been walled off by scar formation (Fig 7-8). During aging of teeth, the pulp narrows, the innervation is reduced, and the nerve fibers contain fewer neuropeptides.[57] While those changes may alter the ability of teeth to defend against pathogens or injuries, a study of dentinal cavity injury in old rats found the same ability for sprouting responses as in the younger teeth.[58]

Type III injuries cause enough pulpal damage and infection that local repair is not possible, and irreversible pulpitis ensues.[4] If a tooth has been denervated prior to injury,[59] the extent of damage is greater and the progression to necrosis proceeds more quickly (Figs 7-9a and 7-9b). Some of the conditions that lead to irreversible pulpitis are large infected pulpal exposures, bacterial invasion at failed restorations, deep infected caries, failure of pulp to make a scar barrier around an abscess, and coronal pulp destruction by heat or other excessive stimulation.

An increased intensity of sensory nerve sprouting in the surviving pulp near the lesion correlates with elevated pulp cell expression of NGF after dentinal injury. In contrast, both NGF expression and sensory sprouting are low at sites of healing (see Fig 7-8c).

Type IV injuries involve other tissues in addition to dentin and pulp. These situations occur when pulpal infections expand out of the tooth into the periradicular tissues to affect ligament and bone[56,60] (Figs 7-9c to7-9e). Periodontal tissues are also involved at the time of initial injury in a variety of dental fractures. In addition, tooth extractions damage the ligament, and pulpotomy can cause long-term nerve reactions in the periradicular tissue.[61]

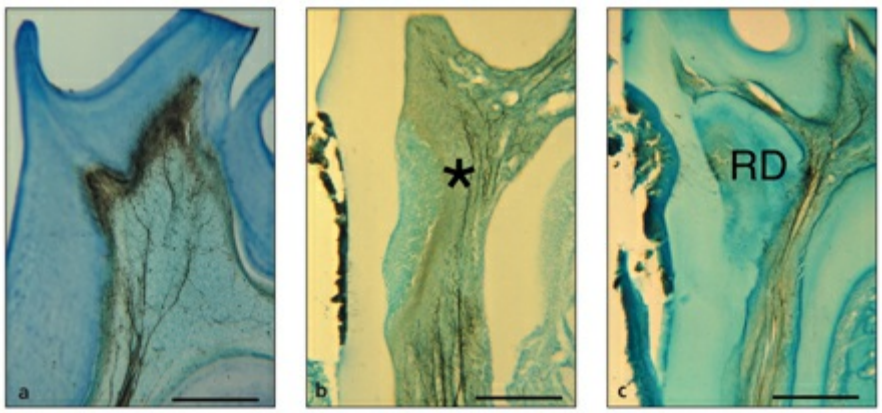

Fig 7-8 Different patterns of nerve fibers detected by immunocytochemistry for CGRP in rat molars. *(a)* Pattern in normal molars (bar = 0.2 mm). (Modified from Kimberly and Byers[56] with permission.) *(b)* Pattern 4 days after a large abscess *(asterisk)* is induced near a cervical dentinal cavity (bar = 0.2 mm). (Modified from Taylor and Byers[54] with permission.) *(c)* Pattern after 3 weeks of healing and

reparative dentin (RD) formation at an injury site that was similar to that in *(b)* (bar = 0.2 mm). (Modified from Taylor and Byers[54] with permission.)

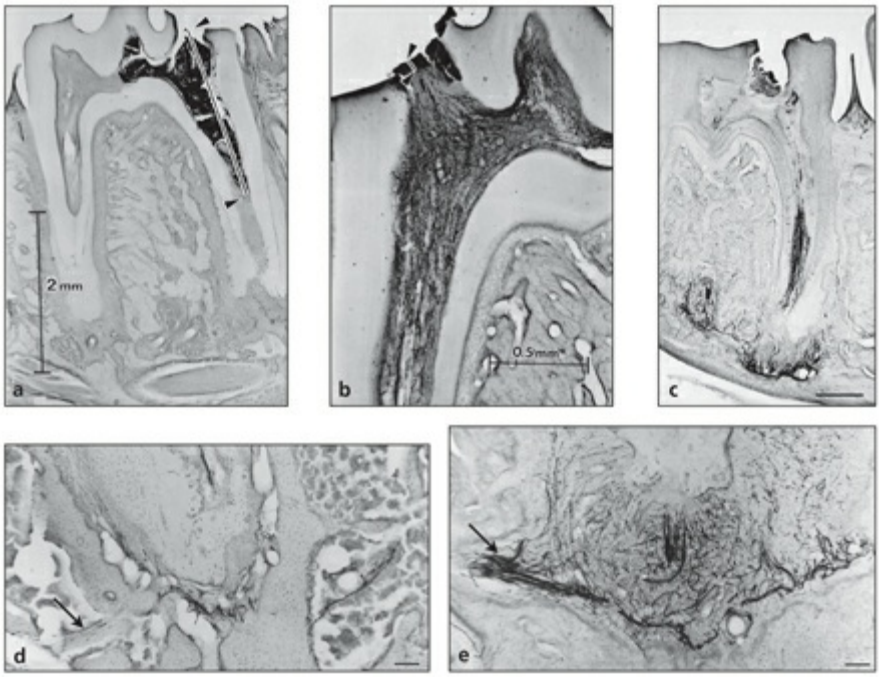

Fig 7-9 *(a)* Rat molar denervated several days before a small pulpal exposure. By 6 days later, the pulpal damage and necrosis are severe *(arrowheads and bar)*. (Reprinted from Byers and Taylor[59] with permission.) *(b)* Innervated contralateral tooth with many sprouting nerve fibers. Compared with the tooth in *(a)*, it has only a small loss of pulp *(arrowheads and bar)* after a small pulpal exposure. The sprouting nerve fibers show immunoreactivity for CGRP. (Reprinted from Byers and Taylor [59] with permission.) *(c)* Rat molar root with nerves and vital pulp retained. At 14 days after a pulpal exposure, there is already a periapical lesion with CGRP-immunoreactive sprouting fibers (bar = 0.5 mm). (Reprinted from Khayat et al[60] with permission.) *(d)* Normal periapical region of a rat molar immunoreacted for CGRP. The region shows normal, sparse innervation of the periodontal ligament. The *arrow* shows the adjoining periapical nerve (bar = 0.1 mm). (Reprinted from Kimberly and Byers[56] with permission.) *(e)* Periapical changes and sprouting nerve fibers appear 3 to 5 weeks following establishment of irreversible pulpitis subsequent to pulpal exposure lesions. Compared to that observed in *(d)*, the nerve fiber immunoreactivity for CGRP was also enhanced in the adjoining periapical nerve *(arrow)* (bar = 0.1 mm). (Reprinted from Kimberly and Byers[56] with permission.)

Distant plasticity in the trigeminal nerve, ganglion, and central endings

The discussion so far has focused on dental sensory reactions in the terminal branches within the tooth or nearby tissues. These neurons also have extensive

changes in their alveolar branches[56] (see Fig 7-9), at their cell bodies and satellite cells in the trigeminal ganglion[4,45] (see Fig 7-7), at their sensory endings in the brainstem, and in the neurons within the central nervous system. Many of the responses at the ganglion are similar to those shown for spinal nerves responding to tissue inflammation, including altered expression of neurotrophin receptors, neuropeptides, and voltage-gated ion channels by the neurons and increased expression of injury proteins by the satellite cells. Those changes can have profound effects on central pain pathways. For example, tooth injuries can cause persistent expression of the c-Fos transcription factor by central neurons, which may indicate altered central pain pathway functions.[62,63] Atypical chronic dental pain and referred pain both involve long-term shifts in central processing of peripheral inputs. Chapters 8 and 9 provide further discussion of tooth pain and the extraordinary functional and cytochemical plasticity of peripheral and central neurons responding to the input of orofacial sensory neurons.

Delayed neural reactions

Both the sensory and the sympathetic fibers can have important reactions that are not launched until days or weeks after tooth injury. For example, the alveolar nerves that carry dental axons can greatly change their neuropeptide content by several weeks after a pulpal exposure in rats[56] (see Figs 7-9d and 7-9e). The sympathetic innervation initially was not found to sprout during the early stages of neuropulpal reactions to pulpal exposure, but, by several weeks later, it too has focal responses directed toward the lesion.[9] The late sympathetic reactions have a major effect on immune cell invasion of the injured pulp and may even alter the quality of tooth pain. Thus, while the initial sensory sprouting reactions are important, subsequent reactions in those fibers, in the sympathetic neurons, and at central neural pathways must also be appreciated for their roles in tooth pain.[64–66]

Human teeth

The results of studies performed in animals have provided important information

regarding the neuroanatomical responses in the diseased or damaged dental pulp. Certainly the advantage of these studies is that responses can be evaluated at different time points following a standardized insult. Another distinct advantage is the ability to evaluate the broad effect of these injuries within the entire trigeminal neuroaxis. Even given these advantages, some limitations exist in animal studies, and most notable is the relationship of these neuroanatomical responses to pain and especially pain in humans. In this regard, knowledge gained in animal studies must be applied to the study of the human dental pulp, where pain levels and response to stimuli can be documented prior to extraction.

The human dental pulp is richly innervated—a common source of pain—and so its use is well-suited for such studies. Also, the routine extraction of both normal third molars and diseased teeth provides an abundant supply of specimens for study. Together, the results from human and basic animal studies can further the understanding of possible correlations between neuroanatomical responses and pain mechanisms in an attempt to more fully understand pulpal pain and its important relationship to the practice of endodontics. In general, the innervation of human dental pulp (Fig 7-10) is similar to that seen in experimental animals, and these similarities strengthen the use of animals as a model for understanding response to injury in the human dental pulp.

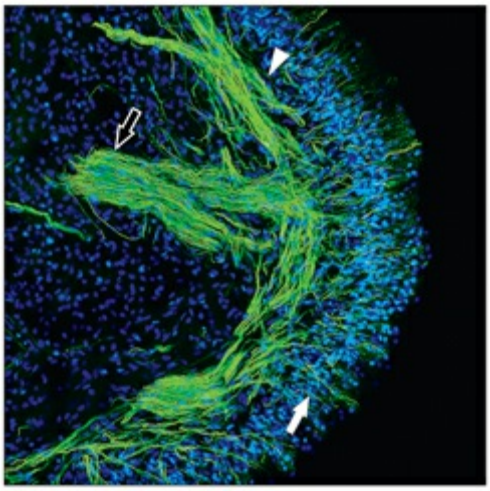

Fig 7-10 Confocal micrograph to demonstrate the overall nerve innervation pattern within the coronal region of a normal molar dental pulp. Nerve fibers are identified with both neurofilament 200-kD and GAP-43 immunoreactivities *(green)*, while nuclei are identified with TO-PRO immunoreactivity *(blue)*. Axon bundles are located within the midcoronal region *(black arrow)*, which leads to the many axons within the subodontoblastic plexus *(arrowhead)*. Some of the fibers within the subodontoblastic plexus enter and traverse the odontoblastic layer *(white arrow)*. The nuclei of the odontoblasts are more numerous and larger than are the nuclei of other cellular profiles elsewhere in the pulp.

Primary and erupting teeth

Although the innervation of permanent teeth has been extensively studied,[38] detailed descriptions of the innervation of the primary tooth are more recent. The coronal regions in both are more densely innervated than radicular areas, but, while in permanent teeth the pulp horns exhibit dense innervation, in primary teeth the cervical third of the coronal region is more densely innervated.[67,68] Dentinal tubules are innervated in both,[68] but primary teeth show lower levels of overall innervation[69] (Fig 7-11) and of some neuropeptides, including CGRP, substance P (SP), and vasoactive intestinal peptide (VIP).[70] In addition, results from animal studies show that innervation density can vary depending on tooth maturity because newly erupted teeth show fewer nerves than older teeth.[71,72]

The aforementioned data are consistent with the clinical impression that primary teeth are less sensitive than permanent teeth.[70] The special anesthesia for children's dentistry may also depend on immature central pain perception mechanisms in infants and children.[73,74] Specific studies of sensitivity during tooth eruption and root maturation show sharp increases in sensitivity once the root apices close,[75] and similar findings have been reported in animal studies.[76] That shift in sensitivity when root apices close may depend on specific maturation of the peptidergic system in rat molars at that time.[40]

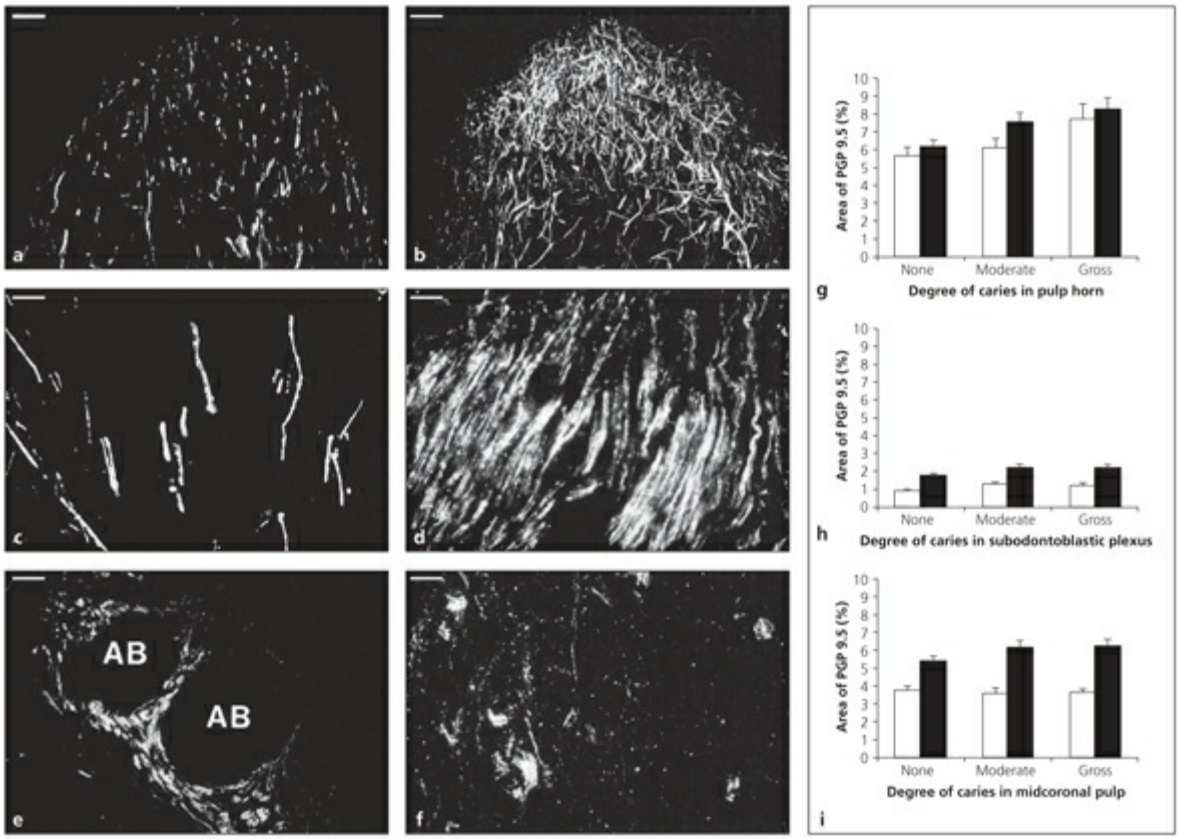

Fig 7-11 Differences in the distribution and morphology of protein gene product 9.5 (PGP 9.5)–immunoreactive nerve fibers located in intact *(a and c)* and carious *(b, d, e, and f)* human teeth. PGP 9.5–immunoreactive fibers in the pulp horn of an intact primary tooth are fewer *(a)* and thinner *(c)* than are observed in a carious primary tooth *(b and d)*. The PGP 9.5–immunoreactive fibers surround intrapulpal abscesses (AB) in the pulp horn of a carious permanent tooth *(e)*, whereas PGP 9.5–immunoreactive fibers show a fragmentation and reduction in density within the pulp horn of a grossly carious primary tooth *(f)* (bar = 13.5 µm in *c*, *d*, and *e*; bar = 27.0 µm in *a*, *b*, and *f*). Graphs show the mean percentage area of PGP 9.5 staining in the pulp horn *(g)*, the subodontoblastic plexus *(h)*, and the midcoronal pulp *(i)* regions of primary *(open bars)* and permanent *(filled bars)* teeth, according to the degree of caries present within the specimens. (Reprinted from Rodd and Boissonade[69] with permission.)

Peptidergic dental innervation

Human pulpal nerves show high levels of neuropeptides,[77] and their presence and changes in distribution within carious teeth are among the most extensively studied areas of human pulp neurobiology. Their prominent expression in pulpal axons also allows a convenient method for the visualization of pulpal axons when the anatomical response to injury is examined.

Neuropeptides that are located within human pulpal axons include the tachykinins,

neurokinin A (NKA)[78] and SP,[79] CGRP,[80] VIP,[81] neuropeptide Y (NPY),[82] cholecystokinin and somatostatin,[79] galanin,[83] and methionine- and leucine-enkephalin.[83] The importance of neuropeptides, and especially CGRP and SP, is well documented in the process of neurogenic inflammation, where the release of these peptides from sensory axons contributes to blood flow control, inflammation, and tissue repair.[14,84] Neurogenic inflammation also increases the excitability of nociceptors and thus is important as a peripheral pain mechanism.[10]

In addition to broad distributions within both myelinated and unmyelinated sensory axons throughout the pulp, some neuropeptides are intimately associated with arterioles; these include CGRP, SP, VIP, and the sympathetically derived NPY.[85] In contrast to the vasodilation produced by CGRP, SP, NKA, and VIP, NPY produces a potent vasoconstriction of pulpal blood flow,[86] so its release may counteract the effect of the vasodilatory neuropeptides. The release of sympathetically derived NPY results in an anti-inflammatory effect that may involve an inhibition of SP release,[87] and therefore the sympathetic nervous system is involved in the modulation of pulpal inflammation.[9] Because VIP is derived from the parasympathetic nervous system elsewhere,[88] its presence suggests a possible parasympathetic innervation of the pulp, although this is a point of controversy.

In contrast to SP and NKA, which have proinflammatory effects, VIP, like NPY, is considered an anti-inflammatory neuropeptide because it inhibits proinflammatory cytokines while upregulating the anti-inflammatory interleukin 10 (IL-10) cytokine.[89] The vasoactive effects of these neuropeptides are critical to the control of intrapulpal pressure, and, although involved in healing responses, the vasodilatory effect of some result in increased intrapulpal pressures that may lead to ischemia and pulp tissue degeneration.

An important contribution of neuropeptides to axonal response to injury is their effect on cytokine expression. The release of neuropeptides results in the recruitment of inflammatory cells and the release of cytokines from these cells that can dramatically increase the excitability of nociceptors.[90] Neuropeptides, and especially CGRP and SP, promote the production of cytokines such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) from inflammatory cells[91] and from fibroblasts derived from the human dental pulp.[92] Inflammatory cells that release SP include neutrophils, plasma cells, and macrophages located within acute and chronic periradicular granulomas.[93] These findings further support the emerging important role for neuroimmune interactions in both inflammatory and neuropathic pain conditions.[94]

An intensive area of research over the last decade has focused on descriptions of neuropeptide expressions in normal dental pulp and changes seen in the diseased and sometimes painful pulp. Many of these studies have evaluated SP and CGRP. Results show that tissue levels of SP are increased in teeth with irreversible pulpitis and a history of spontaneous pain when compared to normal controls,[89] and an increased SP expression in axons that is correlated with caries progression is significantly greater in painful teeth with large caries lesions when compared to both normal controls and asymptomatic teeth with caries lesions[90] (Fig 7-12). A later study found that increased axonal expressions of CGRP, SP, VIP, and NPY are correlated with caries progression in both primary and permanent dentitions, but the study did not evaluate whether these expressions were correlated with pain.[70]

Other studies have evaluated neuropeptide expression in painful teeth and found similar increases in SP, CGRP, NKA, and NPY [97] and of the receptors for CGRP[98] and SP[99] within human dental pulp. This upregulation of SP and CGRP receptors occurs as a gradient because expressions are highest in pulp tissues from teeth with irreversible pulpitis and the presence of moderate to severe spontaneous pain; intermediate expressions are found in pulp tissues from teeth where inflammation is induced, and the lowest expressions are identified within tissues from normal control teeth. In contrast, the expression of VIP was found to be stable among the three groups.[97] This last finding contrasts with the increased VIP expression mentioned earlier[70] and with another study that found greater VIP expression in dental tissues from teeth with moderate caries than in normal teeth and teeth with gross caries, although there was no indication of presence or absence of pain among the diseased specimens.[100] The dental pulp expression of NPY has also been found to vary depending on the extent of caries present: Specimens with mild to moderate caries express more than specimens with advanced caries, while normal specimens contain the least.[87] However, the presence or absence of pain was not indicated in the study. The NPY expression was also evaluated with immunocytochemistry; some NPY-positive axons show colocalization with tyrosine hydroxylase within sympathetic fibers, while others show colocalization with sensory fibers that contained SP, suggesting that this colocalization pattern may lead to decreased neurogenic inflammation. Other studies have documented increased expression of SP following deep cavity preparations[101] and within inflamed periradicular tissues,[93] increased SP and IL-1β from crevicular fluid during orthodontic tooth movement,[102] and greater levels of SP and NKA (but not CGRP) in the crevicular fluid of teeth with pulpal pain compared to that of healthy teeth.[103]

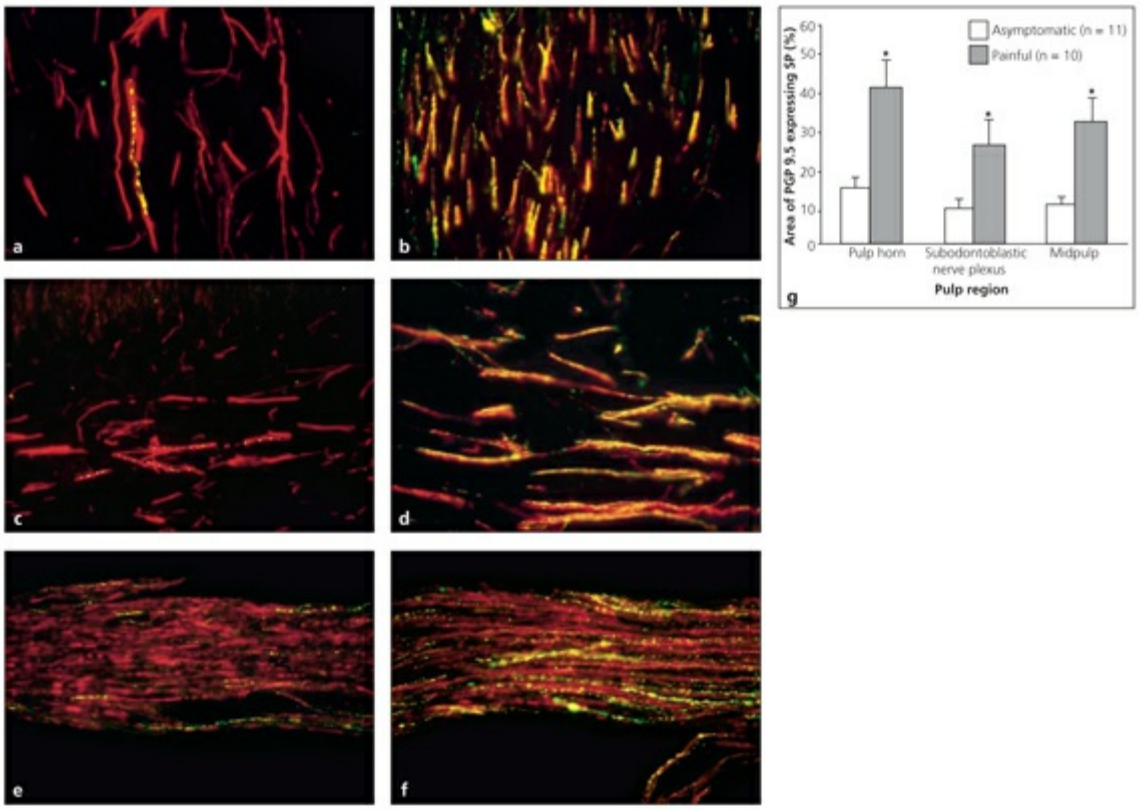

Fig 7-12 Double-exposure photomicrographs showing SP immunoreactivity *(green)* within neural tissues identified with PGP 9.5 labeling *(red)* within intact *(a, c, and e)* and carious *(b, d, and f)* teeth. Areas with SP immunoreactivity within PGP 9.5– identified nerve fibers appear yellow. Few PGP 9.5 fibers show SP immunoreactivity within the pulp horn *(a)*, the subodontoblastic region *(c)*, and the midcoronal region *(e)* of intact teeth, while the numbers of such fibers are increased within the pulp horn *(b)*, the subodontoblastic region *(d)*, and the midcoronal region *(f)* of teeth with caries. *(g)* Mean percentage area of PGP 9.5 labeling that contained SP immunoreactivity within different pulpal regions of both asymptomatic *(open bars)* and painful *(shaded bars)* specimens with gross caries. SP expression within PGP 9.5–identified nerve area was significantly greater *(asterisk)* in the painful specimens than in asymptomatic specimens. (Reprinted from Rodd and Boissonade[96] with permission.)

Together, the results of these studies document the increased expression of neuropeptides in teeth with caries and furthermore identify the involvement of neuropeptide-containing axons to the increased innervation density seen within human primary and permanent dental pulp with caries.[69] An important finding in the study by Rodd and Boissonade[69] was that increased innervation density does not correlate with reported pain experience, although their earlier report did see higher SP axonal expression in painful teeth with gross caries than in grossly carious teeth without pain. Certainly the results of these human studies and the animal studies discussed earlier identify increased neuropeptide expression as a neuroanatomical response to injury. Even so, questions remain concerning their role in pulpal pain mechanisms, given the broad effects of neuropeptides on blood flow, inflammation,

and tissue healing.

Membrane Receptors and Ion Channels

Other neuroanatomical responses to injury of the pulpodentin complex include changes in receptors and ion channels that control the excitability of pulpal nociceptors by influencing the development of generator and action potentials. Important ones include G-protein–coupled receptors (GPCRs), transient receptor potential ion channels (TRPs), voltage-gated ion channels, trk receptors, purinoceptors, and others involved in neuroimmune responses, such as IL-1, TNF-α, toll-like receptor 4, and CD14. A role for some in human pulpal pain mechanisms has been suggested by their presence in dental pulp.[104] These receptors and channels allow the peripheral terminals of nociceptors to detect and to respond to noxious signals in their environment, and changes in some of these have been seen within axons in the carious and painful dental pulp. A more detailed discussion of the role of these in peripheral odontogenic pain mechanisms is available elsewhere.[10]

The effects of most neuropeptides are mediated by receptor binding, and many of these are to due to GPCRs and subsequent activation of specific G proteins ($G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_s$) and distinct, associated signaling pathways.[105] For example, somatostatin and NPY are linked to the $G\alpha_{i/o}$ pathway, and activation of this pathway generally leads to inhibition of nerve activity, in part by decreasing cyclic adenosine monophosphate levels. Therefore, an increased expression of these neuropeptides in nociceptors would result in decreased nerve activity and an analgesic effect. Opioids also activate $G\alpha_i$ GPCRs, and this effect is thought to contribute to the analgesic effect of peripherally administered opioids.

In contrast, the $G\alpha_s$ GPCRs increase cyclic adenosine monophosphate levels and lead to excitation. The effects of prostaglandins and CGRP are linked to this pathway, and local increases in CGRP (mentioned earlier) and the prostaglandin E_2 receptor in periradicular exudates[106] and dental pulp[107] may contribute to the development of odontogenic pain. The GPCRs coupled to the $G\alpha_q$ signaling pathway include the SP neuropeptide, bradykinin, protease-activated receptors, and endothelin and leukotriene receptors and leads to activation of phospholipase C and protein kinase C and a stimulating effect on nociceptors that includes a sensitization

of the TRPV1 receptor. Increased expression of bradykinin[108] and CGRP (discussed earlier) in carious teeth may lead to increased pain levels through this mechanism.

The TRPs have a critical role in the transduction of sensory stimuli, including pain and temperature, so studies that evaluate their pulpal expressions are important. TRPV1 represents the capsaicin receptor and is the most intensely studied. TRPV1 activity is gated by temperature (≥ 43°C),[109] active at lower temperatures in the presence of inflammatory mediators,[110] and critical for the development of inflammatory hyperalgesia.[111] Given this importance in inflammatory pain, it represents a phenotypic marker for nociceptive neurons. Pulpal axons contain TRPV1,[112] and this expression is greater in both asymptomatic and symptomatic carious teeth than it is in normal teeth,[113] thus implicating TRPV1 in both pulpal inflammation and pain. Indeed, the use of capsaicin to activate TRPV1 within human dental pulp biopsies results in the activation and release of CGRP from peripheral nociceptors; use of this methodology appears as a promising tool to further evaluate the role of TRPV1 and novel pharmacologic compounds on human nociceptor sensitivity.[114]

Other putative thermoresponsive TRPs include TRPA1[115] and TRPM8,[116] which are implicated in cold transduction. Although this cold-sensing ability suggests a possible involvement in the exaggerated and prolonged pain response that is often provoked in teeth with pulpitis following the application of a cold stimulus, TRPM8 may not be involved because there is less axonal expression of TRPM8 in cold-sensitive and painful teeth than there is in normal teeth.[117] Given the importance of TRP channels to inflammatory pain mechanisms and the prominent inflammatory response in the painful dental pulp, further studies that evaluate the role of TRPs in the painful human pulp are warranted.

The activation of voltage-gated ion channels is essential to the formation and propagation of action potentials and involves calcium, potassium, and sodium channels. Much recent interest has been focused on sodium channels because changes in their expression and activation may contribute to increased neuronal excitability seen in inflammatory and neuropathic pain conditions.[118] The $Na_v1.7$, -1.8, and -1.9 sodium channel subtypes are specifically expressed within the peripheral nervous system and thus most likely involved in pulpal pain mechanisms.

The overall expression of sodium channels has been evaluated within the pulp of painful teeth with large caries lesions and normal teeth with an antibody that identifies all subtypes; the study found that sodium channel expression varies among these different specimens.[119] A common finding in the painful specimens is an

augmentation and remodeling of sodium channels within axons (Fig 7-13). Other studies have found that nerve fiber expression of the $Na_v1.7$120 and $Na_v1.8$121 isoforms is greater in teeth with pain than it is in normal teeth. The increase of $Na_v1.7$ within painful teeth varies depending on location, and the most significant increase occurs within axons located adjacent to areas with many inflammatory cells.

Some of these isoforms, such as $Na_v1.7$ and $Na_v1.8$,122 are also are located at nodes of Ranvier, where changes in their expression in disease states may contribute to spontaneous activity of myelinated fibers and the development of sharp, shooting pain that is characteristic of toothache. Indeed, a common finding in studies that have examined sodium channel expression in the painful human dental pulp is the augmentation of sodium channels at both intact and remodeling nodal sites that show a dramatic loss of myelin (Fig 7-14); this finding suggests the reorganization of ion channels at demyelinated sites as a pulpal pain mechanism.[119,120] Given the lack of a correlation between pulpal nerve fiber density and pain levels,[69] pain in teeth may involve the quality of changes within individual fibers (such as the remodeling of ion channels at localized sites), as influenced by a gradient of inflammation present within the pulp of teeth with caries lesions,[123] rather than the overall density of nerve fibers.

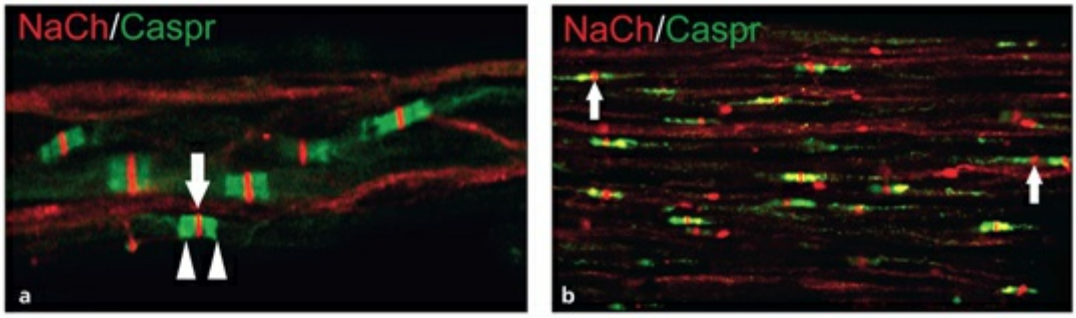

Fig 7-13 Confocal micrograph of sodium channel (NaCh) *(red)* and Caspr *(green)* immunoreactivities within pulpal axons of a normal *(a)* and painful *(b)* molar tooth pulp specimen. The NaCh antibody used in these preparations identifies all NaCh subtypes. Caspr is a paranodal protein used to identify nodes of Ranvier in myelinated fibers. The Caspr staining within the normal specimen is prominent in the paranodal region of myelinated axons *(arrowheads)*, while NaCh staining is located at a high density at the nodes of Ranvier *(arrow)* and more uniformly along axons that lack Caspr and that are most likely unmyelinated. The pattern of NaCh and Caspr immunoreactivities changes within axons in the painful specimen; the changes include an increase in the size *(arrows)* and density of NaCh accumulations, including some that show changes in Caspr relationships.

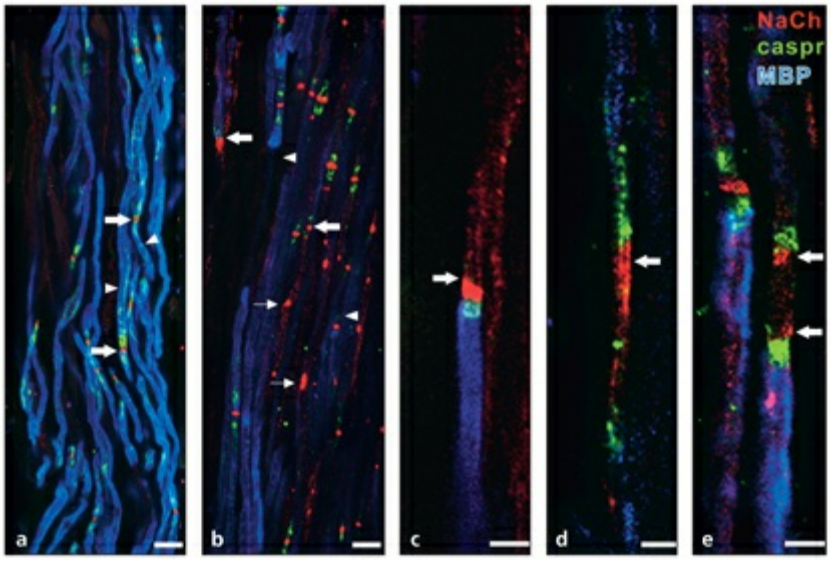

Fig 7-14 Confocal micrographs of NaCh *(red)*, Caspr *(green)*, and myelin basic protein (MBP) *(blue)* immunoreactivities within pulpal axons of a normal *(a)* and painful *(b to d)* dental pulp specimens. Myelinated fibers within the normal dental pulp *(a)* show prominent surface staining for MBP *(arrowheads)* and NaCh accumulations at Caspr-identified typical nodal sites *(arrows)*. In contrast, painful specimens *(b to d)* show generalized and focal areas of decreased MBP staining *(arrowheads)* and prominent NaCh accumulations at sites that lack Caspr *(thin arrows)* and at other sites that show alterations in Caspr relationships *(thick arrows)*. These findings identify demyelination and the remodeling of NaChs at demyelinated sites as common events within the painful human dental pulp (bar = 10 μm in *a* and *b*; bar = 5 μm in *c*, and *d*). (Reprinted from Henry et al[119] with permission.)

Dental Nerve Degeneration

Although much is known concerning the response of pulpal axons to physical and bacterial insults, other important questions remain. One of the least understood is the relationship of axon degeneration to pulpal pain states. Degeneration of pulpal axons in response to injury is a common finding when painful pulp tissues are evaluated (see Fig 7-11f). Although degenerating axons are observed in areas of pulpal necrosis, they are also commonly found to be intermixed with intact fibers within painful specimens.

The factors that influence the progression of the degenerating response are unknown but may relate to neuroimmune interactions that are prevalent in the inflamed dental pulp. The presence of degenerated fibers intermixed with intact ones also suggests a differential response to injury among various fiber types, with important implications for pain mechanisms. Although degeneration of axons may influence pain mechanisms, a more likely process involves the remodeling of axons

that occurs before or in the absence of degeneration. This change in structure in response to inflammatory influences most likely involves a remodeling of ion channels and receptors that could affect the sensitivity and activity of nociceptors.

This response is further complicated by the gradient of inflammatory changes that exist within the diseased dental pulp[123] and the effect of this inflammatory gradient on different regions of the same axon, with important implications for the development of intense spontaneous pain that may accompany the pain of toothache. These changes at individual sites suggest that pulpal pain mechanisms may relate not only to broad global changes but also to the effect of the lesion on isolated fibers. The painful human dental pulp presents a model system in which future studies can relate changes at localized sites to pain states.

Neurophysiology of Pulpal Nociceptors and Dentinal Sensitivity

Distinct groups of pulpal afferent nerve fibers can be classified, as described earlier. The classification is based on both the morphology and conduction velocities of the afferents. A number of recent studies indicate that these neuronal classes are functionally different and that their activation may mediate different types of prepain and pain sensations.[124] Generally, these studies indicate that firing of pulpal afferents in human teeth induces mostly, if not entirely, painful sensations[125,126] and that temporal summation (increase of the electrical stimulation frequency) of low-intensity electrical stimulation changes the nonpainful (prepain) sensation to a painful one.[127] However, the type of pain may vary according to the type of stimulus applied, the type of fibers activated, or the condition of the pulp. Most studies suggest that mechanosensitivity[5] or thermosensitivity[128,129] of pulpal nerve fibers does not induce mechanical or thermal perceptions in people,[1] although there is recent evidence for intradental vibration detection by humans[130] and dental Aβ-dependent brain activity.[131]

Tissue injury and inflammation can sensitize and activate pulpal afferents. In previous experimental studies on animals, pulpal inflammation has been associated with reduced thresholds to external stimulation and spontaneous discharges of pulpal nerve fibers.[124,132,133] These changes are probably due to synthesis or release of a number of different mediators, which have been shown to activate pulpal nerves and

sensitize them to external stimuli[24,132–134] (see chapter 8).

Application of a cold stimulus to hypersensitive dentin in human subjects induces pain that, in many cases, can reach a very high intensity.[133,135] Moreover, patients experiencing acute pulpitis often report moderate to severe pain.[136] However, this is not invariable: Pulpitis may proceed to a total pulpal necrosis with only minor symptoms or without any symptoms at all.[24,136] Considering the exceptionally rich nociceptive innervation of the pulp, such asymptomatic cases ("silent pulpitis") are puzzling. However, recent studies indicate that pulpal nociceptor activation may be abolished by local inhibitory mediators (eg, local opioids, cannabinoids, or somatostatin)[14,24,137] or by loss of functional terminals of these fibers (eg, via apoptosis or secondary to liquefaction necrosis). In addition to these peripheral factors, other central neural mechanisms may have a significant impact in the development of dental pain conditions[14,24,65,138,139] (see chapter 8).

Collectively, these studies indicate that there is a poor correlation between clinical pain symptoms and the histopathologic status of the pulp.[136,140] This is not surprising considering that hyperalgesia is a perceptual event mediated by peripheral and central pain mechanisms at the molecular level; these mechanisms are not necessarily discernible with microscopes evaluating biopsies of human dental pulp. In the following sections, the function of the pulpal neurons in healthy teeth and their responses to tissue injury and inflammation are described. The role of different pulpal nerve fiber groups in the mediation of pulpal and dentinal pain under normal and pathologic conditions are discussed in the next two chapters (see chapters 8 and 9).

Sensory functions of pulpal nerves under normal conditions

A major part of current knowledge regarding the function of dental nerves is based on electrophysiologic recordings performed on animals (eg, cats, dogs, and monkeys). In such experiments, single intradental nerve fibers are identified and their responses to various stimuli recorded (Figs 7-15 and 7-16). These electrophysiologic responses to various external stimuli have been compared to the perceptual responses induced by the same stimuli applied to human teeth. Such comparisons have shed light on how different pulpal nerve fiber groups contribute to different pain responses under normal and pathologic conditions. The morphologic

similarity of the intradental innervation of animals and humans serves as a good basis for such comparisons.

The classification of the pulpal primary afferents as A and C fibers is based on their conduction velocities measured in single–nerve fiber recording experiments[142–145] (see Figs 7-15 and 7-16). These two classes correspond to the myelinated and unmyelinated fibers found in morphologic studies.[3,38,146] According to the results of electrophysiologic recordings, the A and C fibers are functionally different.[1,13,37,127,128,133,147] In addition, the A-fiber group is not uniform because some slow-conducting (small) A fibers seem to be sensitive to capsaicin, whereas most of the faster-conducting fibers respond to hydrodynamic stimulation but are not activated by capsaicin.[13,37,133]

The results of electrophysiologic studies also indicate that C fibers do not respond to dentinal hydrodynamic stimulation. Instead, the sensitivity of dentin is entirely based on the function of intradental A fibers.[135,141,145] Comparison of the sensory responses from stimulated human teeth to the electrophysiologic responses from animal studies reveals functional differences between these two fiber groups in response to tissue injury.[1,13,124,133,145,148]

As already mentioned, pain and prepain are the only sensations that can be evoked by intradental nerve stimulation in human subjects, although there is recent evidence for intradental vibration perception.[130] The quality of the pain can vary depending on the type of stimuli applied and can range from sharp, stabbing pain to dull, aching, throbbing pain sensations.[1,125,134,143,148,149] The variation is caused by activation of different nerve fiber types and differences in the nerve firing patterns (temporal summation) evoked by various stimuli.[127,128,135,149]

The application of low-intensity electric stimulation of human teeth can produce a nonpainful sensation.[125–127] It has been proposed that intradental low-threshold and fast-conducting Aβ-type afferents mediate such prepain sensations.[125,127] Aβ fibers do have low electric thresholds; however, the thresholds of Aβ and Aδ fibers overlap considerably (Fig 7-17), and, accordingly, both fiber groups may be involved in the mediation of prepain sensations.[124] It is also important to note that painful sensations can be induced by increasing the stimulation frequency at prepain intensities,[127] a procedure that produces temporal summation of the nerve activity at the level of the trigeminal nuclei. Collectively, these findings suggest that prepain and pain sensations are mediated by the same afferent fibers.

On the basis of the single-fiber recordings (see Fig 7-17), it can also be concluded that activation of only a small number of pulpal afferents is needed to

evoke prepain or pain sensations.[124] This is clinically important because it suggests that pulp testing may produce a false-positive response, even in teeth with extensive pulpal necrosis, as long as at least some pulpal axons are still responsive. This could explain the clinical observation of a positive pulpal response in a tooth with a periradicular radiolucency (see also chapter 17).

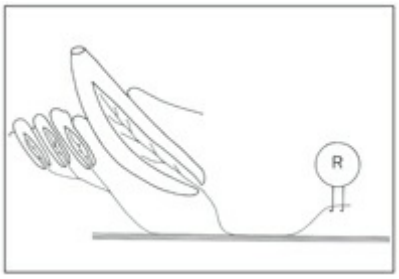

Fig 7-15 Setup for electrophysiologic recording of single intradental nerve fibers. The inferior alveolar nerve is exposed, and the nerve filaments are dissected from the nerve trunk. Single fibers innervating the canine or incisor teeth are recorded using metal wire electrodes (R). The nerve fibers are identified using electrical stimulation applied to the tooth crown. (Reprinted from Närhi and Hirvonen[141] with permission.)

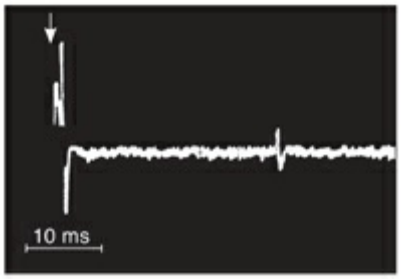

Fig 7-16 Nerve recording from a nerve filament containing one A and one C fiber. The action potential of the A fiber shows after a latency of only about 2 milliseconds (ms) after the electrical stimulus artifact on the left *(arrow)*. The C-fiber action potential on the right is delayed by about 30 ms because of slow conduction along the axon. The conduction velocity of the recorded fiber can be calculated by dividing the conduction distance (the length of the nerve fiber) by the conduction delay.

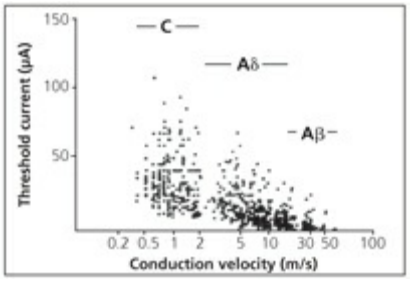

Fig 7-17 Electrical thresholds of intradental nerve fibers of the cat canine tooth plotted against their conduction velocities. Responses for C, Aδ, and Aβ fibers are shown. The Aβ and fast Aδ groups both have very low thresholds compared to the slower Aδ fibers and C-fiber groups. (Modified from Närhi et al[124] with permission.)

It has been suggested that non-noxious mechanical (tactile) stimulation of or pressure applied to the intact tooth crown activates pulpal Aβ fibers.[5,131,150] On the basis of such findings, those fibers were regarded as a discrete functional group that would be involved with the regulation of masticatory functions, the sensation of food texture between the teeth, and the control of occlusal forces. However, Aβ and Aδ fibers show similar responses to various external stimuli and to inflammatory mediators,[124,133,142–145] and the results suggest that the fibers may belong to the same functional group.

Taken together, the results of human and animal experiments indicate that a hydrodynamic mechanism mediates intradental nerve activation in response to several different stimuli[1,2,136,147,151–154] (see chapters 8 and 9) as well as release of neuropeptides.[134,155] The responding fibers consist of the Aδ and Aβ classes of neurons (Fig 7-18). Considering the tissue distortion and injury in the dentin-pulp border related to their activation,[152] the responding receptors can be classified as high-threshold mechanoreceptors or mechanical nociceptors.

The pulpal C fibers are polymodal because they respond to several different modes of stimulation and have high thresholds for activation.[124,128] They are activated only if stimuli reach their terminal endings inside the pulp. In an intact tooth, given the insulating enamel and dentinal layers, rather intense thermal stimuli are needed for their activation. The insensitivity of pulpal C fibers to dentinal (hydrodynamic) stimulation[124,142] is consistent with the location of their endings and receptive fields deep in the pulp.[3,11,142–145]

Pulpal C fibers also respond to histamine and bradykinin applied to the exposed pulp[13,124] (Fig 7-19), which indicates that this fiber group also may be activated in connection with pulpal inflammatory reactions. Thus, the dull pain induced by pulpitis may be evoked by C-fiber activation. C fibers also respond to capsaicin, which is a selective irritant of small nociceptive- and neuropeptide-containing afferents.[124,156]

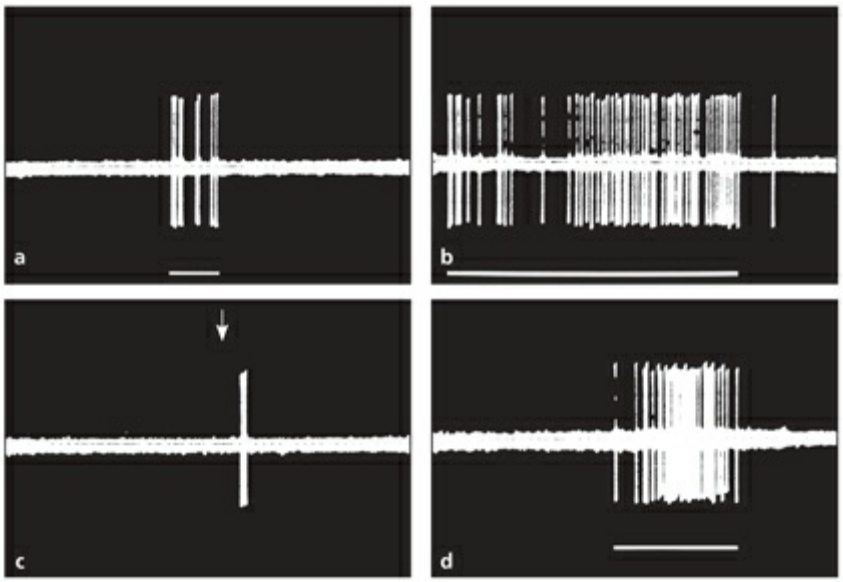

Fig 7-18 Responses of a single intradental A fiber to probing *(a)*; an air blast *(b)*; application of hypertonic, 4.9-mol/L calcium chloride to dentin *(c)*; and drilling of dentin *(d)* over a period of 1.5 seconds. The approximate timing of the stimulus application is indicated by the *horizontal lines* in *(a)*, *(b)*, and *(d)* and by the *arrow* in *(c)*. (Reprinted from Närhi et al[145] with permission.)

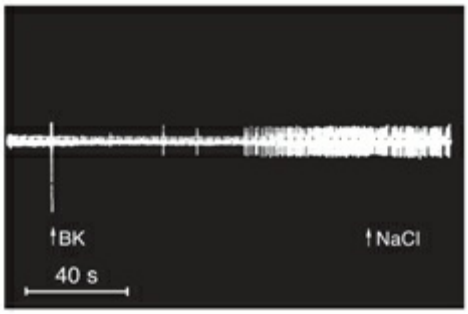

Fig 7-19 Responses of a single intradental C fiber (small action potential) in the exposed pulp of a cat canine tooth to bradykinin application (BK) and after washing with physiologic saline (NaCl). The A fiber (large action potential) in the same nerve filament only shows firing of a single action potential at the time of the bradykinin application, probably because of a mechanical effect. (Reprinted from Närhi[13] with permission.)

The application of intense heating or cooling to human teeth produces a sharp pain sensation with a short latency, typically within a few seconds. If the stimulation is continued, a dull, radiating pain response follows.[1,128] Correspondingly, biphasic responses to thermal stimuli are observed in cat teeth (Fig 7-20). The first response is an immediate or short-latency firing of intradental A fibers, followed by a delayed C-fiber activation.[124,142–145] The initial A-fiber responses are supposedly induced by the dentinal fluid flow resulting from the rapid temperature changes.[136,148,157] The delayed C-fiber activation is probably induced by a direct effect of heat and cold on the nerve endings in the pulp.[124,128,142]

The results of these thermal-stimulation studies strongly indicate that intradental A and C fibers may mediate different perceptual qualities of dental pain, ie, sharp and dull, respectively. In addition, certain other stimuli, such as air drying of exposed dentin and application of bradykinin to the exposed pulp, which are known to activate pulpal A or C fibers selectively, are also able to induce either sharp or dull pain, respectively, in human experiments.[125,157]

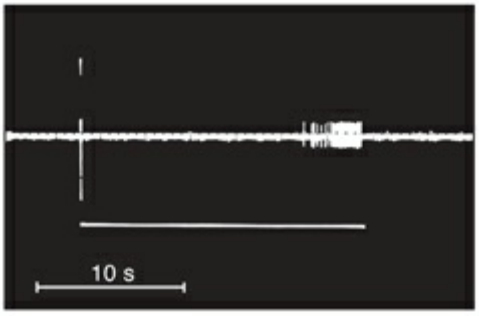

Fig 7-20 Responses of intradental nerve fibers to intense heating of an intact cat canine tooth. The timing of the stimulus application is indicated by the *horizontal line*. The A fiber (large action potential) in the filament gives an immediate response at the beginning of stimulation. In contrast, activation of the C fiber (small action potential) is much delayed. (Reprinted from Närhi[13] with permission.)

Neurophysiologic mechanisms of dentinal sensitivity

Numerous published studies indicate that the nociceptors in the dentin-pulp border area are activated by hydrodynamic fluid flow in response to dentinal stimulation (the hydrodynamic mechanism).[136,151] The fluid flow in turn stimulates the nerve endings in the dentin-pulp border area and causes their activation (Fig 7-21). Movement of dentinal fluid can also be induced in unexposed dentin, but in such cases the capillary forces are not activated and the effect of the stimulus is much weaker.

The results supporting the hydrodynamic mechanism of pulpal nerve activation are based both on in vivo studies on human subjects and experimental animals and in vitro experiments performed on extracted teeth. The results of the human experiments uniformly confirm that patency of the dentinal tubules is a prerequisite for the sensitivity of exposed dentin.[2,135,158] The relationship between the dentinal tubular condition and dentinal sensitivity was further confirmed in experiments showing a significant positive correlation between the degree of the dentinal sensitivity and the density of open dentinal tubules counted in exposed cervical dentinal surfaces in a

scanning electron microscopic replica study on human teeth.[158] In vitro measurements have also shown that opening or blocking of the tubules determines the hydraulic conductance of dentin[159] and, accordingly, the fluid flow in the dentinal tubules (see chapter 4).

Several electrophysiologic studies performed on cats and dogs have shown that acid etching of drilled dentin significantly increases the responsiveness of intradental nerves to air blasts, probing, and hyperosmotic solutions.[13,135,142–145,158,160,161] The increased sensitivity is strongly related to the patency of the dentinal tubules.[153] The sensitizing effect of acid etching can be abolished almost completely by blocking the tubules (eg, with oxalates or resin composites). Similar studies conducted in human teeth indicate that acid etching increases dentinal sensitivity.[135,136]

According to the hydrodynamic theory, rapid dentinal fluid flow serves as the final stimulus activating intradental nociceptors for many different types of stimuli. In support of this hypothesis, single intradental A fibers respond to a number of different hydrodynamic stimuli, including dentinal probing, air blasts, and hyperosmotic solutions[13,141–145] (see Fig 7-19). Studies conducted in vitro demonstrate that all of these stimuli induce fluid flow in the dentinal tubules.[137,160,161] It is the osmotic strength of solutions and not their chemical composition that elicits pain responses in human teeth, nerve responses in experimental animals, and fluid flow responses in dentinal tubules,[26,124,162] although some chemical solutions may make exceptions.[163] Also, in cold stimulation of human teeth with open or blocked dentinal tubules, the intensity of the induced pain does not seem to be related to the induced fluid flow, but some other mechanisms of the nerve activation have been suggested to play a role.[164] Much current work is examining pulp cell responses to dentinal stimulation that may modulate or contribute in some way to the neurophysiologic reactions to hydrodynamic force.[16,22]

Electrophysiologic recordings performed on cat canine teeth indicate that a direct relationship exists between dentinal fluid flow and intradental nerve activation, and a similar relationship between induced pain and fluid flow recently has been shown to exist in human teeth.[165] Accordingly, in most cases nerve activation seems to occur as a response to the fluid flow, but with certain stimuli (eg, cold and mechanosensitivity) some other mechanisms may be active.[1,128,129] When dentin is exposed, inflammation may develop, leading to sensitization of the intradental nerves.[124] Such changes may result in poor responses to treatment of hypersensitive dentin and may be significant in teeth with open dentinal tubules that have been

exposed for a long time.

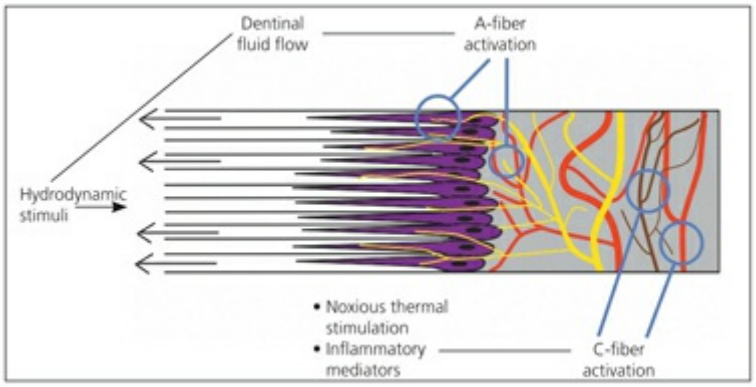

Fig 7-21 Activation mechanisms of intradental nerve fibers. A fibers in the dentin-pulp border area respond to stimulus-induced fluid flow in the dentinal tubules and consequent deformation of the peripheral pulp tissues containing the nerve endings (hydrodynamic mechanism). For C-fiber activation, the applied stimuli must reach the nerve endings, which are mostly located deeper in the pulp. C fibers also respond to certain inflammatory mediators.

Sensory functions of pulpal nerves under conditions of pulpal inflammation

As discussed in chapter 8, the two major mechanisms of pulpal pain are related to dentinal sensitivity and pulpal inflammation. Injury to the pulp can alter both of these pain mechanisms. Intense hydrodynamic stimulation may induce tissue injury in the dentin-pulp border area, including disruption of the odontoblastic layer and aspiration of the cells into the dentinal tubules.[47,136,152,166] The nerve endings may also be injured.[31,141]

The inflammation-induced elaboration of growth factors can lead to subsequent morphologic and phenotypic changes in the nociceptive nerve endings, including sprouting and increased neuropeptide expression[36,55]; these changes may contribute to long-term functional changes in the pulpal afferents.[4,133] For example, the local changes in the density of the innervation in the dentin and pulp might result in changes in the regional sensitivity of the affected tooth. However, current knowledge about the possible functional correlates of the morphologic changes is limited.

The effect of various inflammatory mediators on pulpal nerve function has been studied in cat and dog teeth. These mediators activate intradental nociceptors and/or sensitize them to subsequent stimuli (ie, they reduce the threshold for firing).[13,124] For example, serotonin activates A fibers and sensitizes them to external stimulation

(eg, hydrodynamic stimuli).[167] Intense, repeated heating sensitizes intradental nerves in cat canine teeth, and prostaglandins seem to mediate this response.[132] As stated earlier, pulpal C-fiber responses are activated by histamine and bradykinin, which may be significant for the development of pain in pulpitis.[13]

According to single-fiber recordings, the faster-conducting pulpal afferents primarily respond to hydrodynamic stimulation of dentin, although certain small-diameter myelinated afferents may also be activated.[124,135] Hydrodynamic stimulation also affects the pulpal blood flow, indicating that the nerve fibers activated by such stimulation are able to induce neurogenic vascular effects.[14,168]

Pulpal A fibers comprise functionally distinct classes of sensory neurons. Although most of the intradental A fibers are activated by hydrodynamic stimulation, there exists a rather high number of relatively slow-conducting pulpal Aδ fibers that are not sensitive to hydrodynamic stimulation of the coronal dentin of healthy teeth.[37,124,133,135] This class of "silent" A fibers can be activated only by intense heat or cold that reaches the pulp proper, and their mechanical receptive fields are located deep in the pulp.[169] However, the sensitivity of these silent Aδ fibers is enhanced in pulpal inflammation, when they significantly increase their responsiveness to dentinal stimulation,[133] and they also respond to capsaicin.[37]

Studies to date suggest that there is a functional significance to the sprouting of sensory terminals that occurs during inflammation. For example, experiments on dog teeth indicate that nerve sprouting may be reflected in the size of the receptive fields of pulpal afferents responsive to hydrodynamic stimulation of dentin.[4] In healthy teeth, gentle probing of the exposed dentinal surface revealed small receptive fields that were most often composed of a single small spot in the exposed dentin.[133,169] In contrast, gentle probing of exposed dentin in inflamed teeth revealed a dramatic change, with emergence of wide receptive fields, sometimes covering the whole dentinal surface at the crown tip in inflamed incisors.

This increase in the size of the receptive field could be caused by sprouting as well as activation of normally silent terminals of branched axons. An increase in the size of receptive fields would result in an increased overlap of receptive fields and, accordingly, would enhance spatial summation of peripheral nerve activity, increasing pain intensity in response to dentinal stimulation.[169]

Inflammation may also increase the regional sensitivity of dentin in various parts of the tooth. In normal dog teeth, the nerve fibers innervating the cervical dentin are far less responsive to hydrodynamic dentinal stimulation than are those innervating dentin in the crown tip. However, in inflamed teeth the sensitivity of cervical dentin

can increase to the same level as that of the crown tip.[169,170]

Although most inflammatory mediators activate or sensitize peripheral neurons, some mediators released in pulp after injury, including endogenous opioids and somatostatin, appear to be inhibitory. In experiments performed on inflamed dog teeth, the local application of a somatostatin antagonist increased firing of intradental nerves, suggesting that the release of endogenous somatostatin reduces firing during injury.[133] In other preliminary experiments, administration of the opioid antagonist naloxone produced a similar effect.[133] In addition, local application of morphine in deep cavities completely abolished the pulpal nerve responses to mustard oil, a substance that induces inflammation and activates nociceptive afferents.

These results suggest that in pulpal inflammation both somatostatin and endogenous opioids effectively reduce or abolish intradental nerve activity, despite the presence of other inflammatory mediators that have a stimulatory effect. These data suggest that one possible mechanism for the frequently reported lack of clinical symptoms in teeth with pulpal inflammation may be based in part on the release of local inhibitory mediators in the inflamed tissue.

Conclusion

Knowledge gained over the past two decades has greatly increased the current understanding about peripheral mechanisms of tooth sensitivity and pain, including dentinal innervation and its sensitivity, neurophysiology of pulpal nociceptors, sensory neuropeptides that affect pulp cells, vasoregulation by dental sensory and sympathetic fibers, sympathetic interactions with immune cells, responses of dental nerves to injury, the role of local factors such as growth factors or inflammatory mediators in modulating neural function, and the relationship of these different features to clinical dental pain. However, there is still more to be learned about the types of nerve fibers in teeth, their functional shifts in response to inflammation and injury, and neuropulpal and neuro-odontoblastic interactions.

Recent advances with studies of human teeth in vitro provide additional possibilities for odontoblast and neural functions, along with possible interactions of those cells in relation to tooth pain.[171,172] Animal studies of odontoblast ion channels further complicate the story by revealing at least eight different odontoblast

phenotypes, most of which do not overlap with sensory innervation terminations,[173] and those findings are consistent with other tissues in which local cells utilize "neural" genes for their own tissue responsibilities as well as for modulation of neural functions.[174,175] Many evolving paracrine communications between odontoblasts and their neighboring cells continue to be identified, including purinergic neural detection of adenosine triphosphate release from pulp cells.[176] In addition, recently evolving technology has enabled better dissection and identification of cell types and functions within the odontoblast layer of mature human teeth, with fascinating suggestions about dental mechanoreceptor mechanisms.[177]

The pace of discovery in this field suggests that new clinical insights will be developed soon concerning the peripheral mechanisms of dental pain and anesthesia, diagnostic aspects of dental pain, and the treatment of hypersensitive teeth. Some important unresolved questions concern the different mechanisms responsible for the transformation of a mild toothache to a severe one that forces the patient to seek immediate therapy. These differences may relate to important influences provided by the immune response on axons and the glia that invest them and the remodeling of ion channels and receptors within individual axons that allow them to detect noxious signals and that control axonal excitability and activity.

The pain attributes of individual toothaches vary, and this variation involves the axon response to injury and the activation of a different mix of pain mechanisms that interact with one another to form a unique fingerprint associated with each pain experience. Additional study of the human dental pulp should provide important insights into the axon response to injury and how these relate to both healing and pain. The puzzle of human pulpal pain remains, but, given that endodontic therapy is highly successful in the treatment of pulpitis, part of the answer most likely resides in the pulp, not only because infection has been arrested and removed but also because dental pain mechanisms include neuropulpal interactions that are only beginning to be understood.

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Pain Pathways and Mechanisms of the Pulpodentin Complex

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Pain conditions are common in areas innervated by the trigeminal nerve, and this especially includes odontalgia (toothaches).[1,2] It is estimated that approximately 12% of the US population suffers from odontalgia, being the primary reason patients seek oral health providers.[3,4]

Although the subject of pain is of considerable importance to all health care providers, the simple reality is that many patients consider *pain* and *dentistry* to be synonymous. This association often leads to fear and anxiety, causing patients to delay treatment, ignore their initial symptoms, and contact oral care providers only when the pain is unbearable (high intensity and chronic).[5] This association is largely based on the patient's preexisting pain and previous untoward experiences following stimulation of dental nociceptors during treatment. Other factors, such as ethnicity, age, and gender, have all been correlated with different reported pain levels.[6,7] In addition, the media sometimes portray dentists and oral care providers negatively as

professionals indifferent to their patients' pain, reinforcing anxiety toward dental treatment.[8]

In dental pain, activation of pulpal nociceptors is the initial step that ultimately results in the pain experience. However, pain is much more than activation of peripheral nociceptors; instead, pain represents an integration of nociceptive signals and emotional, cognitive, and affective components that make pain such a terrible experience.[9] Clinicians and researchers must never forget to appreciate this aspect of the pain experience.

Diagnosis and management of pain represent foundation skills necessary for the successful practice of dentistry and are especially critical when the patient with odontalgia is evaluated.[10] Accordingly, this textbook provides an extensive review of pain mechanisms that are critical to the evaluation of the patient with odontalgia, including pulpal inflammation (see chapters 4, 10, and 11), neuroanatomy, and neurophysiology of the dental pulp (see chapter 7), as well as effective pharmacologic and nonpharmacologic strategies for managing dental pain (see chapter 9). The present chapter specifically contributes to the development of this foundation skill by reviewing peripheral and central nervous system mechanisms associated with the pain response following stimulation of the pulpodentin complex. This knowledge base should help clinicians to make more accurate diagnoses and to design more effective pain-control strategies for their patients.

The awareness of pain involves three steps: *(1)* detection, *(2)* processing, and *(3)* perception (Fig 8-1). Detection is a function of the peripheral sensory (afferent) neuron; processing involves the selective activation of specific and related central nervous system pathways that is largely dependent on initial processing done within the medullary and spinal dorsal horns; and perception is the result of activity in more rostral brain regions such as the cerebral cortex. Together, the activities of these structures are responsible for the complex multidimensional aspect of pain that includes pain both as a sensation and as an emotion.[11] The clinician that diagnoses and treats odontalgia should consider the contributions provided by each of these steps because the pain experience is ultimately shaped by activity of these various structures. The neuronal activity within these different pathways can change depending on the absence or presence of disease; because most patients who seek endodontic therapy have disease, these modifying processes are active at the time of the clinical evaluation and may add to the challenges associated with the diagnostic task. This chapter reviews those peripheral and central pain mechanisms that should be considered when the clinician evaluates the symptomatic patient.

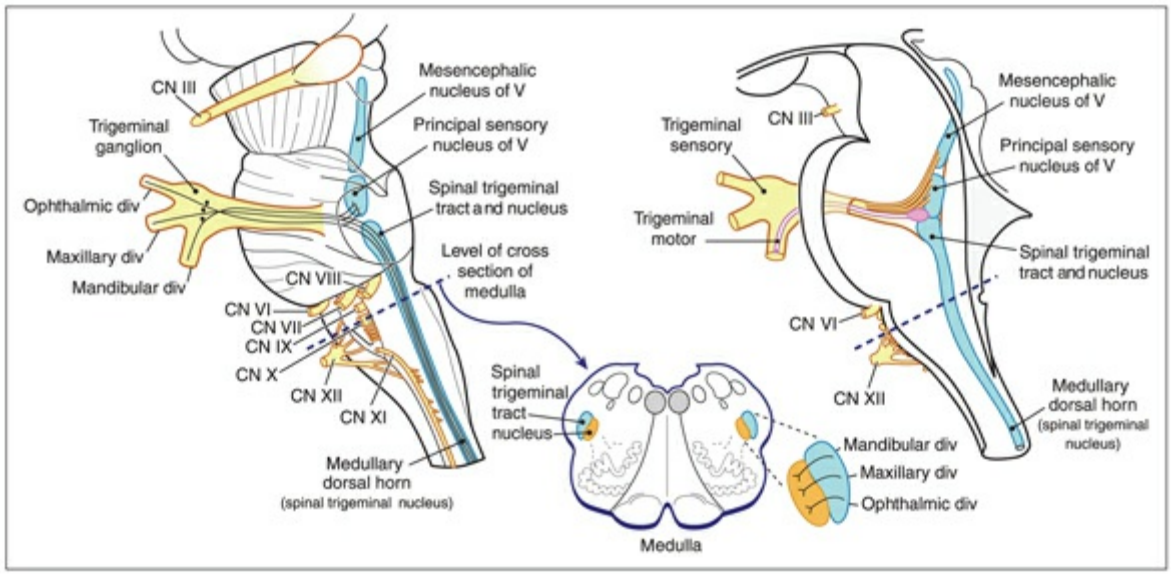

Fig 8-1 General steps in pain transmission in the orofacial region. Detection of noxious injury occurs via primary afferent nociceptors that travel in one of the three divisions (div) of the trigeminal nerve (ophthalmic, maxillary, and mandibular). (In certain chronic pain conditions, detection also may occur by other afferent fibers such as the Aβ fibers.) Processing occurs primarily in the medullary dorsal horn. Nociceptive signaling may be increased by central mechanisms of hyperalgesia or allodynia. Nociceptive signaling may also be reduced by endogenous analgesic systems. The output from the medullary dorsal horn is conveyed predominantly along the trigem-inothalamic tract. Perception occurs primarily in the cerebral cortex. Other sensory nerves are also responsible for additional craniofacial signaling (eg, cranial nerves [CN] VII, IX, and X as well as afferent fibers from the cervical spinal cord).

Pathways Responsible for Detection, Processing, and Perception of Dental Pain

Odontogenic pain is usually the result of a noxious physical stimulus or the release of inflammatory mediators that stimulate receptors located on the terminal endings of nociceptive (pain-detecting) afferent C and Aδ nerve fibers[12–16] (see chapter 7). Physical stimuli, via their effect on dentinal fluid flow, can activate the nociceptors that innervate dentinal tubules, leading to the perception of dentinal pain[16] (Fig 8-2). Inflammatory mediators, via activation of their respective receptors, can sensitize or depolarize the nociceptors that innervate pulp tissue. These topics are discussed in detail later in the chapter and elsewhere.[17] Experimental studies have shown that activation of nerves within the dental pulp by these physiologic (eg, thermal, mechanical, or chemical) stimuli results in a pure sensation of pain, although other studies using certain electrical stimuli can elicit a "prepain" sensation.[18]

The activation of the peripheral nociceptor produces a generator potential; if great enough, this depolarization will trigger a nerve impulse (action potential). The action potential is propagated along a peripheral trigeminal nerve to the primary afferent neuronal cell body located in the trigeminal ganglion and then into the central nervous system along the central process of this same neuron[14,19–21] (Fig 8-3).

The central process of the primary afferent cell body enters the brainstem at the level of the pons by way of the trigeminal root entry zone and then enters the trigeminal tract. The trigeminal tract carries the primary afferent fiber to the trigeminal sensory nucleus located in the pons and medulla, where it then terminates. The most rostral portion of the trigeminal sensory nucleus is the main sensory nucleus, while the caudal portion is represented by the spinal trigeminal nucleus. The spinal trigeminal nucleus is further subdivided into the following subnuclei: pars oralis (most rostral), pars interpolaris, and pars caudalis (most caudal).[22,23]

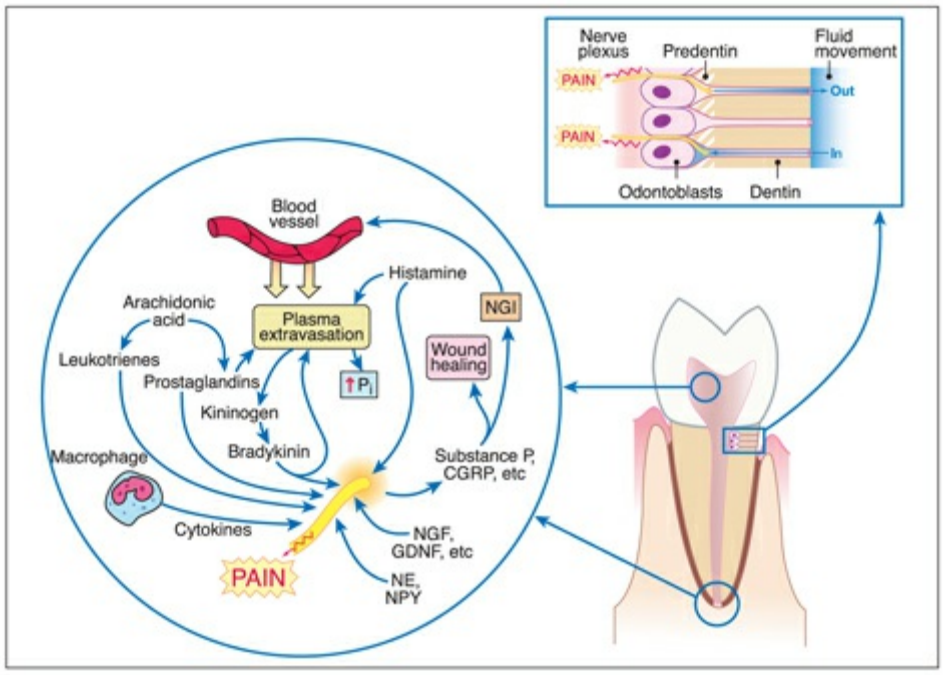

Fig 8-2 Two mechanisms for the peripheral stimulation of nociceptive nerve fibers in tooth pulp. *Acute dentinal pain:* According to the hydrodynamic theory, stimuli that cause fluid movement in exposed dentinal tubules result in the stimulation of nociceptive nerve fibers. *Pain with inflammation:* Inflammation is associated with the synthesis or release of mediators, including prostaglandins, bradykinin, substance P, and histamine (as well as other mediators not shown). The interrelationships of these inflammatory mediators form a positive feedback loop, allowing inflammation to persist far beyond cessation of the dental procedure. P_i, intrapulpal pressure; NGI, neurogenic inflammation; CGRP, calcitonin gene–related peptide; NGF, nerve growth factor; GDNF, glial cell line–derived neurotrophic factor; NPY, neuropeptide Y; NE, norepinephrine.

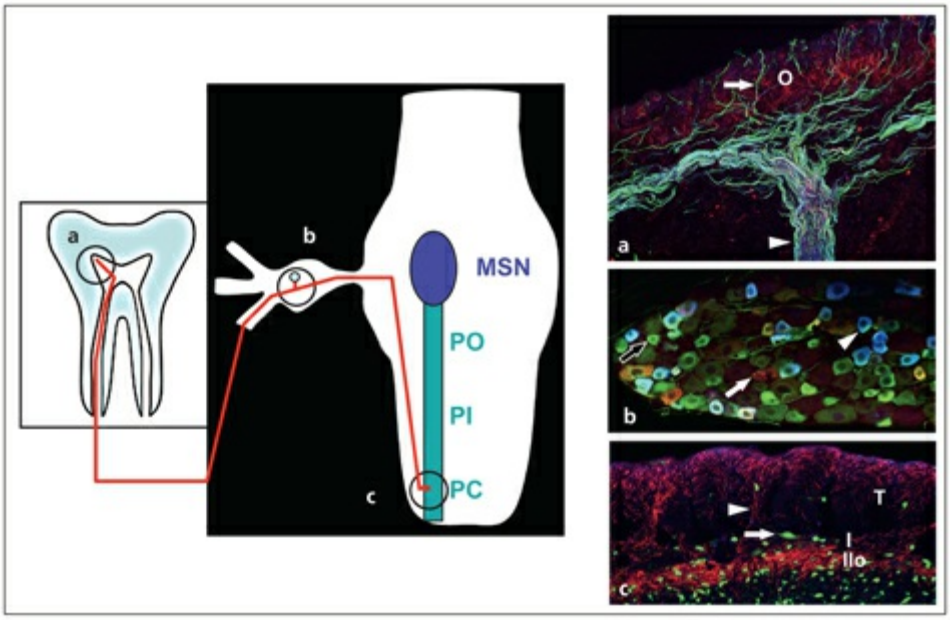

Fig 8-3 Pathway and confocal micrographs of neuroanatomical structures responsible for the transmission of pulpal nociceptive stimuli within the trigeminal system. Peripheral nociceptive nerve fibers terminate as free nerve endings within the dental pulp *(a)* and arise from primary afferent cell bodies within the trigeminal ganglion *(b)*. The central processes of these primary afferent cell bodies pass into the brainstem and enter the trigeminal tract. These fibers exit the tract to terminate within the trigeminal sensory nucleus *(c)*, composed of the main sensory nucleus (MSN) and the spinal trigeminal nucleus. The trigeminal nucleus consists of pars oralis (PO), pars interpolaris (PI), and pars caudalis (PC). *(a)* Pulpal nerve fibers are seen within the pulp horn of a human specimen and are stained with antibodies against N52 *(green)*, PGP9.5 *(blue)*, and TRPA1 *(red)*. A nerve fiber bundle *(arrowhead)* gives rise to an extensive arbor within the subodontoblastic plexus and with some fibers that enter and traverse *(arrow)* the odontoblastic layer (O). *(b)* Neuronal cell bodies are seen within the rat trigeminal ganglion and are stained with antibodies against peripherin *(green; black arrow)*, TRPV1 *(blue; arrowhead)* and CGRP *(red; white arrow)*. Larger cell bodies lack staining, while the smaller cell bodies are stained individually or multiply with these antibodies used to identify nociceptors. *(c)* Intrinsic neuronal cell bodies are stained with NeuN *(green; arrow)*, and the central processes of CGRP-containing primary afferent fibers *(red)* are seen within a transverse section of the rat brainstem at the level of caudalis. The CGRP-containing primary afferent fibers are located in the trigeminal tract (T). Some of these fibers exit the tract *(arrowhead)* to enter and terminate especially within the superficial laminae I and II outer (o) zones of caudalis, where they form synapses with processes of intrinsic and descending neurons.

Animal studies have shown that primary afferent neurons that innervate dental pulp terminate in all of the different subnuclei located within the ipsilat-eral trigeminal sensory nucleus, including prominent projections to caudalis.[24] The projection to caudalis is expected because the tooth pulp is rich in pain fibers, and trigeminal primary afferent input to caudalis has been linked to facial pain: Trigeminal tractotomy at the level of pars caudalis in humans results in the loss of pain and temperature sensation over facial structures while preserving touch sensations,[25] thus demonstrating a critical role for caudalis in facial pain perception.

Even so, additional studies have shown that neurons located in more rostral nuclei can be activated by intraoral and perioral nociceptive stimuli, even after trigeminal tractotomy at lower levels, and together these studies demonstrate a broad projection of tooth pulp nociceptors throughout the trigeminal sensory nucleus.[26] These projections form an important structural foundation for referred pain mechanisms that are of critical importance when the symptomatic patient is evaluated.

The trigeminal nucleus caudalis has been referred to as the *medullary dorsal horn* because it shares a similar laminar morphology with the spinal dorsal horn and its caudal extent merges with the dorsal horn at the upper cervical level.[27] Caudalis plays a critical role in the modulation and transmission of nociceptive information within the trigeminal sensory nucleus, and output to higher brain regions can be increased (ie, hyperalgesia), decreased (ie, analgesia), or misinterpreted (ie, referred pain) from the incoming activity.[21] These three general patterns of processing of nociceptive input (ie, hyperalgesia, analgesia, and referred pain) are reviewed in this chapter as well as in chapters 7, 9, and 19.

As viewed from a functional perspective, the trigeminal sensory nuclei have five major components related to the processing of nociceptive signals: *(1)* central terminals of afferent fibers, *(2)* local circuit interneurons, *(3)* projection neurons, *(4)* terminals from descending neurons, and *(5)* glia. Because of the importance of caudalis to the modulation and transmission of pain in the trigeminal system, including that from the dental pulp, it will be used to illustrate these components.

The first components, primary afferent endings of C-fiber and Aδ nociceptors, enter caudalis via the trigeminal tract and terminate as axonal endings (Fig 8-4; see also Fig 8-1). These axonal endings form synapses with other neural components, which include local interneurons, projection neurons, and terminals from descending neurons (discussed later). Many of these nociceptors contain neuropeptides such as calcitonin gene–related peptide (CGRP) and substance P, and they terminate mainly in the superficial laminae (I and II) of the nucleus caudalis. These sensory fibers excite intrinsic neurons by releasing the excitatory amino acid glutamate along with neuropeptides.[28]

The administration of receptor antagonists to glutamate and substance P in particular, and to CGRP to a lesser extent, reduces hyperalgesia or nociceptive transmission in animal studies.[29–32] Evidence to date strongly indicates that antagonists to the glutamate receptor *N*-methyl-D-aspartate (NMDA) are particularly effective in reducing hyperalgesia in animal studies, as discussed later. Antagonists to the NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)

classes of glutamate receptors are likely to serve as prototypes for future classes of analgesic drugs, and early clinical results in dental pain studies are promising.[12,33] Although animal studies indicate that antagonists to the neurokinin 1 class of substance P receptors block hyperalgesia, the results from clinical trials have been equivocal, and few studies have shown them to have significant effects on dental pain.[34,35]

Local circuit interneurons are a second neural component of the caudalis. These neurons may act to modulate the excitability of both primary afferents and projection neurons or can act as an intermediary for the transfer of primary afferent input to projection neurons. Thus, in both situations they ultimately regulate the transmission of nociceptive signals from the primary afferent fibers to projection neurons.[13,20,36]

Interneurons comprise a diverse class of neurons whose morphologies relate to different electrical properties.[37] Depending on their neurotransmitter systems and anatomical connections, interneurons can enhance or suppress nociceptive processing (see Fig 8-4). Examples of inhibitory influences are provided by those that contain γ-aminobutyric acid (GABA; the main inhibitory neurotransmitter in the central nervous system) or opioids[38] within synaptic vesicles located in their axon terminals and those that contact primary afferent axon terminals at axo-axonic inhibitory synapses to provide presynaptic inhibition of primary afferent input.[39,40] Other influences are provided through postsynaptic mechanisms that involve the release of neurotransmitters from primary afferent terminals and receptors located on dendrites of second-order neurons.[41] Therefore, the trigeminal nuclei are not simple relay stations between nociceptive signals from dental nociceptors to higher-order neurons (projection neurons). Instead, considerable pain-signal modulation occurs at these central sites.

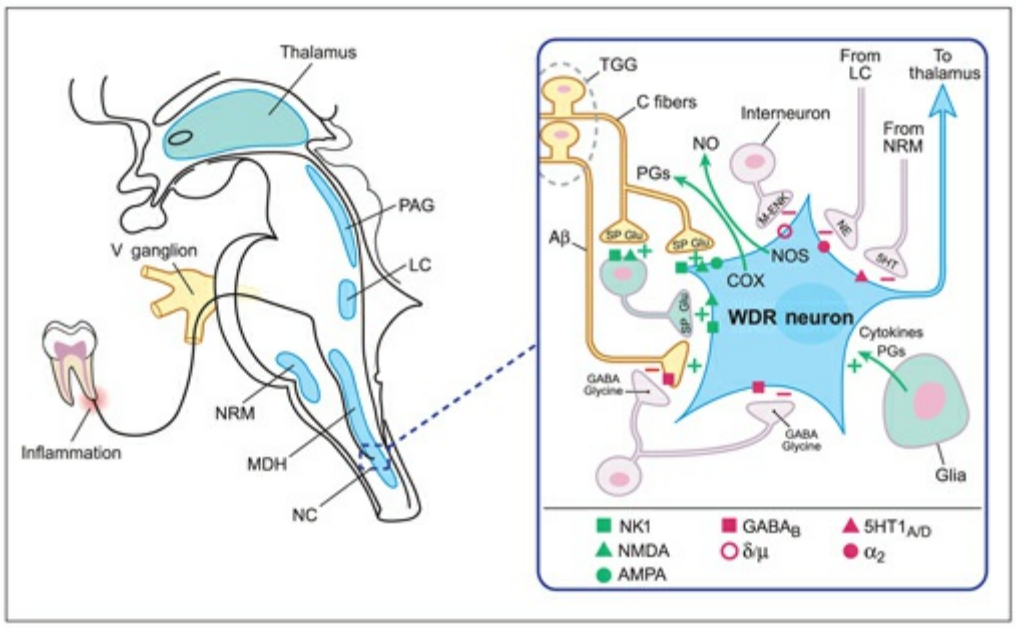

Fig 8-4 Functional processing of nociceptive input in the nucleus caudalis of the medullary dorsal horn (MDH). In this example, activation of pulpal C nociceptive fibers leads to the release of glutamate (Glu) and substance P (SP), which are conveyed across a synapse to a wide dynamic range (WDR) projection neuron. This projection neuron projects to the thalamus; the information is then relayed to the cortex. Glutamate binds and activates either *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors, while substance P binds and activates the neurokinin 1 (NK1) receptors. The sensory fibers can directly activate the WDR neuron or indirectly activate it via contacts with excitatory interneurons. Several signal transduction pathways have been implicated in modulating the responsiveness of projection neurons, including the protein kinase A (PKA) and protein kinase C (PKC) pathways. Projection neurons can themselves modulate nearby cells by synthesis and release of prostaglandins (PGs) via cyclo-oxygenase (COX) and nitric oxide (NO) via nitric oxide synthase (NOS). Glia can modulate nociceptive processing by release of cytokines (interleukin 1β and tumor necrosis factor) and prostaglandins. Descending terminals of fibers originating in regions such as the nucleus raphe magnus (NRM) or locus coeruleus (LC) can release serotonin (5HT) or norepinephrine (NE). Also depicted are the major proposed receptors for these neurotransmitters. Drugs that alter these receptors or neurotransmitters have potential as analgesics. 5HT1$_A$/D, 5HT receptor; GABA, γ-aminobutyric acid; GABA$_B$, GABA receptor; PAG, periaqueductal gray; NC, nucleus caudalis; TGG, trigeminal ganglion; M-ENK, met-enkephalin; ä/ì, äì-opioid receptor; α2, α2-adrenergic receptor.

The third neural component of caudalis is the projection neuron; the cell bodies of these neurons are within caudalis, and their axons comprise the output system for orofacial pain information because they project to other areas, including more rostral brain regions such as the thalamus. The projection to the thalamus involves the trigeminothalamic tract that crosses to the contralateral side in the brainstem before ending in different thalamic nuclei (as discussed later). Other caudalis neurons project to rostral regions of the trigeminal sensory nucleus, including oralis, where this intranuclear projection is especially important in mediating intraoral pain sensations.[26,42]

Projection neurons can receive primary afferent input directly or indirectly. An example of indirect input involves primary afferent information that ends in lamina II and is processed by interneurons before subsequent activation of projection neurons located in lamina I. Again, the excitability of projection neurons can be enhanced or suppressed by synaptic interactions provided by other neural components located within caudalis.

Three major classes of neurons within caudalis have been described, based on their physiologic responsiveness to different stimuli.[13,36] These include *(1)* the low-threshold mechanoreceptive type that is activated only by light tactile stimulation, *(2)* the nociceptive-specific (NS) type that is activated by high-threshold nociceptive stimuli, and *(3)* the wide dynamic range (WDR) type that responds to both tactile and noxious stimuli.

A large body of evidence has focused on the role of the WDR and NS neurons in encoding orofacial pain and their contributions to the development of hyperalgesic and allodynic states.[12,14,20,21,29,43,44] These studies have shown that the peripheral receptive field of NS neurons is typically smaller than the receptive field of WDR neurons. The excitability and the receptive field of the WDR neurons both increase after inflammatory insults or nerve injury, and these changes correspond to a central sensitization that is the major mechanism underlying secondary hyperalgesia following these insults.

Evidence has accumulated about the receptors and signal transduction pathways in these projection neurons that lead to increased or reduced activity.[45,46] In particular, activation of intracellular protein kinases (eg, protein kinases A and C) and elevation of intracellular calcium levels are thought to facilitate responses to nociceptive input. These projection neurons are also thought to modulate the activity of nearby cells via release of prostaglandins and nitric oxide gas[46] (see Fig 8-4). The increased responsiveness involves an unmasking of normally inactive (latent) synaptic inputs that involves the activation of NMDA receptors on these neurons.[21]

Another important physiologic feature of NS and WDR neurons relates to the issue of central convergence and involves the ability of these neurons to be excited by a wide variety of peripheral sources that include both superficial (cutaneous, mucosal) and deep (tooth pulp, muscle, jaw joint, dura cerebral blood vessels) structures. In addition, these same neurons can be activated by the application of sensory stimuli to structures that are innervated by cranial nerves VII, IX, and X and upper cervical afferents that project into the trigeminal sensory nucleus. This central convergence of peripheral inputs adds to the development of central sensitization

following inflammatory insults and tissue or nerve injury. These physiologic properties should be considered when the clinician evaluates patients with trigeminal pain, especially those patients with preexisting insults, because they may contribute to the seemingly bizarre referred pain patterns that may occur in trigeminal pain conditions such as toothache.[47]

The fourth component of caudalis consists of the terminal endings of descending neurons (see Fig 8-4). These terminals modulate (inhibit or facilitate) the transmission of nociceptive information within the other caudalis neural components.[48] One of the best-studied descending systems is the periaqueductal gray (PAG)–rostral ventromedial medulla (RVM) pathway.[49] Stimulation of different neuronal populations within the PAG can lead to either an enhancement or inhibition of activity within nociceptive neurons located within caudalis and the dorsal horn.

Activation of this pathway involves endogenous opioid peptides (EOPs), especially within the PAG, and serotonergic inputs into caudalis and the dorsal horn from neurons located in the RVM. Another descending system involves the PAG–dorsolateral pontine tegmentum pathway that provides noradrenergic inputs. The analgesic action of some nontraditional (tricyclic antidepressants) and traditional (opioids) pain medications may involve activation of these descending modulatory pathways. The endogenous opioid peptides represent a critical element of this endogenous analgesic system. The EOPs are a family of peptides that possess many of the properties of exogenous opioids, such as morphine and codeine. The EOP family includes the enkephalins, dynorphins , and β-endorphin–related peptides.

EOPs are found at several levels of the pain-suppression system. This fact underlies the analgesic efficacy of endogenous and exogenous opioids because their administration conceivably activates opioid receptors located at all levels of the neuroaxis. The EOPs are probably released during dental procedures because blockade of the actions of endogenous opioids by administration of the antagonist naloxone can significantly increase the perception of dental pain.[50–52] In animal studies, application of mustard oil to dental pulp produces a profound increase in muscle activity, and this effect is enhanced by naloxone[53] (Fig 8-5). In humans, administration of naloxone significantly increases pain perception during dental procedures[50] (Fig 8-6). Collectively, these studies indicate that orofacial pain activates an endogenous analgesic system involving the release of opioids and that blockade of this inhibitory tone by naloxone increases pain perception.

The endogenous cannabinoid system represents another modulatory system that can inhibit the central terminals of C fibers; hypoactivity of this system and

disinhibition of the central terminals may mediate some forms of chronic pain.[54–56] Cannabinoids may have profound effects for modulating pain because there are about 10 times more cannabinoid receptors than opioid receptors in the central nervous system. Additional studies have demonstrated cannabinoid receptors on sensory neurons and dental pulp, where they may act to inhibit peripheral terminals of unmyelinated nociceptors.[57–59] Further, the prominent expression of cannabinoid receptors within the descending inhibitory pathways appears to underlie the efficacy of acetaminophen (paracetamol) for treating dental pain because this widely used over-the-counter drug activates the endogenous cannabinoid system.[60,61] Therefore, the endogenous cannabinoid system represents a major pharmacologic target for the treatment of odontogenic pain.

Glia constitute the fifth component of caudalis involved in pain processing. Although glia were initially viewed as merely supportive cells, later studies strongly implicated the active participation of microglia and astrocytes in the processing of nociceptive input within caudalis and the dorsal horn.[62–65] Glia respond to nociceptive input and release substances such as proinflammatory cytokines (interleukin 1 and tumor necrosis factor α), excitatory amino acids, adenosine triphosphate, nitric oxide, and prostaglandins and express ion channels and receptors for various neuromodulators and neurotransmitters that can regulate the release of these substances. Although the signals responsible for the activation of glia are unknown, glia within caudalis contribute to pain mechanisms after both inflammatory and nerve lesion insults in the trigeminal system.[66–68]

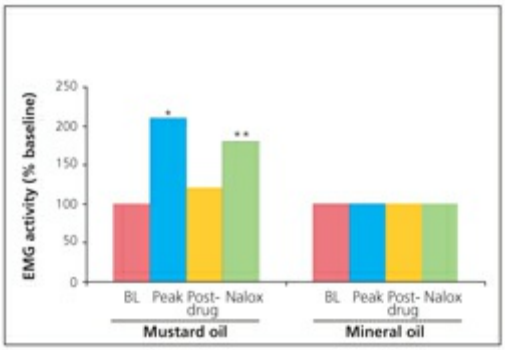

Fig 8-5 Effect of stimulation of dental pulp nociceptors on masseter muscle electromyographic (EMG) activity in anesthetized rats. Either mustard oil or its vehicle was injected into maxillary first molars. EMG data are presented as a percentage of baseline (BL) activity. Data were collected 30 minutes after drug administration (postdrug), and then both groups of animals were administered naloxone (Nalox), an opiate receptor antagonist (1.2 mg/kg, intravenously). *$P < .05$ (ANOVA) versus baseline; **$P < .05$ versus mustard oil. (Data from Sunakawa et al.[53])

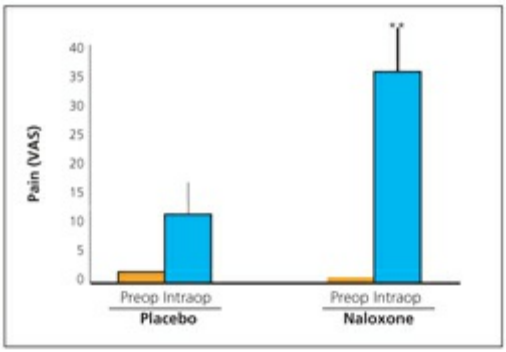

Fig 8-6 Effect of naloxone administration versus placebo on intraoperative pain levels in dental patients undergoing surgical removal of impacted third molars. Patients were anesthetized with local anesthetic and, 10 minutes into surgery, were given an intravenous injection of either naloxone (10 mg) or placebo on a double-blinded randomized basis. $^{**}P < .01$ (ANOVA). VAS, visual analog scale (0 to 100). (Data from Hargreaves et al.[50])

Some of the neurons within caudalis that are activated by nociceptive stimuli project to higher brain centers.[69] Much less is known about this topic than the local processing of nociceptive information within caudalis, but animal and human studies show projections into lateral and medial nuclei within the thalamus.[70] This segregation is important because subsequent projections from these thalamic nuclei are thought to preferentially subserve the different sensory and affective aspects of pain (discussed later in the chapter). The lateral group that receives projection from caudalis neurons includes the posterior part of the ventromedial nucleus, the ventroposterior inferior nucleus, and the ventroposteromedial nucleus (part of the ventrobasal complex). The medial group includes the medial dorsal nucleus and the central lateral nucleus.

Additionally, there are projections to brainstem structures that include catecholamine cell groups, parabrachial nucleus, PAG, reticular formation, and superior colliculus. The projection to the parabrachial nucleus is significant and provides important projections to the amygdala, hypothalamus, and the medial thalamus.[71] The projections to the amygdala and hypothalamus are noteworthy because these both project back to the PAG, where these inputs are considered critical for the control of the PAG descending system that was discussed earlier.[72]

Some of the neurons within the thalamus show properties similar to caudalis NS and WDR neurons, including sensitization following peripheral inflammatory and nerve lesion insults.

The perception of pain appears to occur within a variety of cerebral structures, and together this system represents the *pain neuromatrix*.[73] This concept is helpful to evaluate the various sensory-discriminative and affective-motivational aspects

that together create the multidimensional pain experience. This matrix involves parallel processing through a continuation of the lateral and medial pathways seen at the thalamic level. Much of this information has been gained through functional imaging studies of a variety of human pain states, including trigeminal pain conditions and tooth pain.[74,75]

Prominent projections to the lateral system include those to the primary and secondary somatosensory cortices involved in encoding stimulus duration and localization. The medial system projections include anterior cingulate and dorsolateral prefrontal cortices, insula, and the amygdala. The anterior cingulate cortex is commonly activated and has been linked to the affective component of pain, whereas the amygdala is correlated with the processing of fear. Studies have also shown the activation of subcortical structures such as the hypothalamus and the PAG that are important components of the descending modulatory pathway.[74,75]

Key findings in regards to the functional pain neuromatrix are that the psychologic context associated with the stimulus can entirely change neuronal activity, and just the anticipation of a painful stimulus can activate the matrix.[76] These findings have significant implications for the way patients should be treated. Although the lateral pathway identifies stimulus location within the body as a result of the activation of a specific region of the somatosensory cortex, there is no single area that is preferentially activated depending on stimulus location within the medial system.

Activity within the matrix can shift pathways, and the dependence on any one structure or pathway is plastic. The complex neuronal plasticity in painful states may result in short- and long-term changes in the pain-processing pathways. For example, trigeminal pain can be modulated at various locations within the pain system, including the trigeminal nucleus and other brainstem locations, subcortical sites, and cortical sites, and painful conditions can lead to significant reorganization within any of these sites.

These are all important considerations when the patient in pain is evaluated because, in reality, activity within the entire pain neuromatrix, and not just activity within the tooth itself, produces the pain associated with toothache. Nonetheless, events within the pulpodentin complex can be the first step in the activation of this trigeminal pain pathway.

Mechanisms of Dental Pain Caused by Inflammation

Inflammation is common in the diseased dental pulp and leads to the activation of pulpal nociceptors and odontogenic pain. Much of the odontogenic pain is thought to arise from the activation of unmyelinated (C-fiber) nociceptors. This hypothesis is supported by the distribution of C fibers in dental pulp, their responsiveness to inflammatory mediators, and the strikingly similar perceptual qualities of pain associated with C-fiber activation and pulpitis (ie, dull, aching pain).[77–79]

Figure 8-7 illustrates the perceptual pain response reported by volunteers in whom inorganic ions or inflammatory mediators were administered to exposed dental pulp.[80] Application of histamine and bradykinin primarily produced reports of dull, aching pain.

Similar results have been observed in electrophysiologic studies evaluating the responsiveness of Aδ and C afferent neurons to stimuli applied to deep cavity preparations[81] (Fig 8-8). The Aδ fibers respond to stimulation of dentinal tubules (eg, air blast or cold test), whereas pulpal C fibers respond to bradykinin or capsaicin.[81] Collectively, these and other studies have implicated pulpal Aδ fibers in mediating dentinal sensitivity and pulpal afferent C fibers in mediating pulpal inflammation (see Fig 8-2).

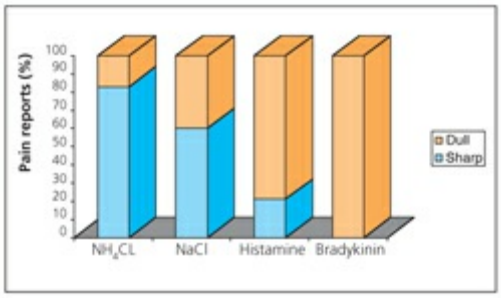

Fig 8-7 Effect of application of inorganic ions or inflammatory mediators to exposed human dental pulp. Volunteers received mepivacaine anesthesia and had their pulp exposed via a Class V cavity preparation. After the patients had recovered from anesthesia, the cavity preparations (N = 16) were dried, and test solutions of ammonium chloride (NH_4Cl; 0.77 mol/L), sodium chloride (NaCl; 0.77 mol/L), histamine (10 mg/mL), or bradykinin (10 µg/mL) were placed on the surface of the pulp. The stimulus period was less than 3 minutes, and the interstimulus interval was 5 minutes. The quality of the evoked pain was recorded after application of the test agent. (Redrawn from Ahlquist et al[80] with permission.)

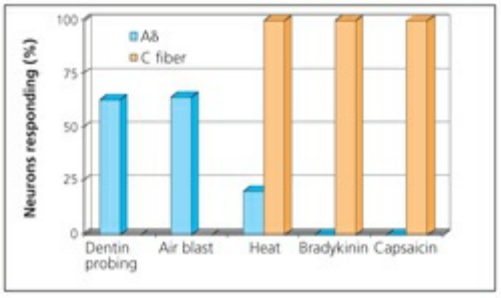

Fig 8-8 Responsiveness of pulpal Aδ afferent neurons and pulpal C afferent neurons to different stimuli applied to deep dentinal cavity preparations in anesthetized cats. (Reprinted from Närhi et al[81] with permission.)

The dental pulp is one of the most heavily innervated and vascularized tissues in the human body and is capable of exuberant inflammatory responses (see chapter 7). Microbial infections are the most prevalent etiology of pulpal inflammation, followed by traumatic injuries. The inflammatory reaction evoked by microorganisms is a complex response that involves recognition of antigenic molecular patterns and the subsequent coordinated release of multiple classes of inflammatory mediators that display distinct profiles of substance concentration over time[82] (see chapter 11).

Lipopolysaccharide (LPS; also known as *endotoxin*) is one of the most prominent microbial antigens in dental infections due to the high representation of gram-negative bacteria.[83,84] This highly potent activator of the innate immune response is recognized by a specific receptor (toll-like receptor 4 [TLR-4]) expressed on the plasma membrane of cells of the innate immune response.[85,86] Interestingly, TLR-4 is also expressed in dental nociceptors,[87] and neuronal recognition of this bacterial component activates and sensitizes these dental pain–sensing fibers, leading to increased nociceptive signals and release of proinflammatory neuropeptides[88,89] (Fig 8-9).

Given that nociceptors can extend up to 0.16 mm into dentinal tubules[90] and that LPSs diffuse apically, reaching the pulp faster than bacteria,[91] it is very likely that nociceptors in the dentinal tubules will be activated, alerting the host to an incoming bacterial infection. In addition, vasoactive peptides such as CGRP and substance P will initiate and amplify the pulpal inflammatory reaction (neurogenic inflammation), acting in conjunction with cytokines released from dendritic cells and resident immune cells. These early symptoms, when acknowledged and treated, favor the maintenance of pulpal vitality. Therefore, primary afferent terminals in the dental pulp and periradicular tissues can directly "sense" the bacterial presence by the activation of TLR-4 and subsequent activation of these nociceptors, leading to an alerting pain signal.

Also, as mentioned previously, LPS-activated immune cells release a cascade of inflammatory mediators.[92] If any given mediator reaches a concentration in the inflamed tissue sufficient to activate specific receptors expressed in nociceptors, these nociceptive neurons could become activated (ie, the membrane would be depolarized and the signal would be conducted to the central nervous system or

sensitized) (see Fig 8-2). A sensitized nociceptor displays spontaneous depolarization, reduced threshold for depolarization, and increased after-discharges to suprathreshold stimuli. Therefore, dental nociceptors can be activated directly by bacteria and their by-products and indirectly by the microorganism-mediated release of inflammatory mediators, which in turn may activate and/or sensitize nociceptors.

Some inflammatory mediators activate these terminals (eg, bradykinin, LPS), while some potentiate the effects of other inflammatory mediators (eg, prostaglandins). For example, prostaglandin E_2 substantially increases the stimulatory effect of bradykinin[93] (Fig 8-10). Therefore, the combination of mediators ("inflammatory soup") present is probably more important than the presence of any one mediator in determining the physiologic response to inflammation.

Other inflammatory mediators may produce persistent effects. For example, nerve growth factor is expressed in inflamed dental pulp (see chapter 7), and its concentration can increase up to eightfold in the inflamed dental pulp.[94] It has been demonstrated that a single injection of nerve growth factor in humans can evoke pain and allodynia that lasts up to 1 month.[95,96] This process of nociceptor sensitization has important clinical implications because it contributes to the altered pain states of hyperalgesia and allodynia.[12,97] This long-lasting effect of nerve growth factor and other mediators appears to be related to changes in gene expression leading to a different cellular physiology.[98] This change in neuronal phenotype (neuronal plasticity) has profound, long-lasting effects on the detection and processing of nociceptive signals.

The overall neuronal plasticity that results from persistent inflammation could lead to significant changes in the nociceptive pathways. For example, the expression of the voltage-gated sodium channels $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ has been found to be dramatically increased in the inflamed dental pulp[99–102] (Fig 8-11). In addition, inflammation changes the expression pattern of these channels to axonal sites different than the typical nodes of Ranvier.[103] These changes in both quantity (increased expression) and quality (expression site) have a significant effect on the transmission of action potentials generated in the innervated target tissues (dental pulp and periradicular region).

Another important example of this effect is the long-term effect of endotoxin on neuronal phenotype. As mentioned previously, pulpal nociceptors have the receptors for LPS (endotoxin), and these neurons are activated and become acutely sensitized as the result of the identification of endotoxin.[88] However, it has been demonstrated

that LPS triggers genomic changes in the dental pulp nociceptors, leading to upregulation of the expression of the transient receptor potential vanilloid type 1 (TRPV1) channel, a crucial inflammatory pain-signaling molecule.[104,105] Therefore, inflammation could lead to long-lasting changes that could persist after the removal of the inflammatory stimulus and could be involved in persistent painful states.

Signal transduction studies have revealed some of the fundamental mechanisms mediating the actions of inflammatory mediators and drugs on sensory neurons.[45,46,106–108] For example, inflammatory mediators (such as bradykinin) whose receptors couple to the G_q guanosine triphosphate binding protein, which leads to activation of the protein kinase C pathway of secondary messengers, activate nociceptors. On the other hand, inflammatory mediators (such as prostaglandins) whose receptors couple to the G_s guanosine triphosphate binding protein, which leads to activation of the protein kinase A pathway of secondary messengers, sensitize nociceptors. Finally, drugs (such as opiates, cannabinoids, and adrenergic agonists) that activate receptors coupled to the G_i guanosine triphosphate protein tend to be analgesics. This is a simplification of a very active research front that is likely to lead to fundamental advances in the knowledge of pain mechanisms and the development of new classes of analgesic drugs.

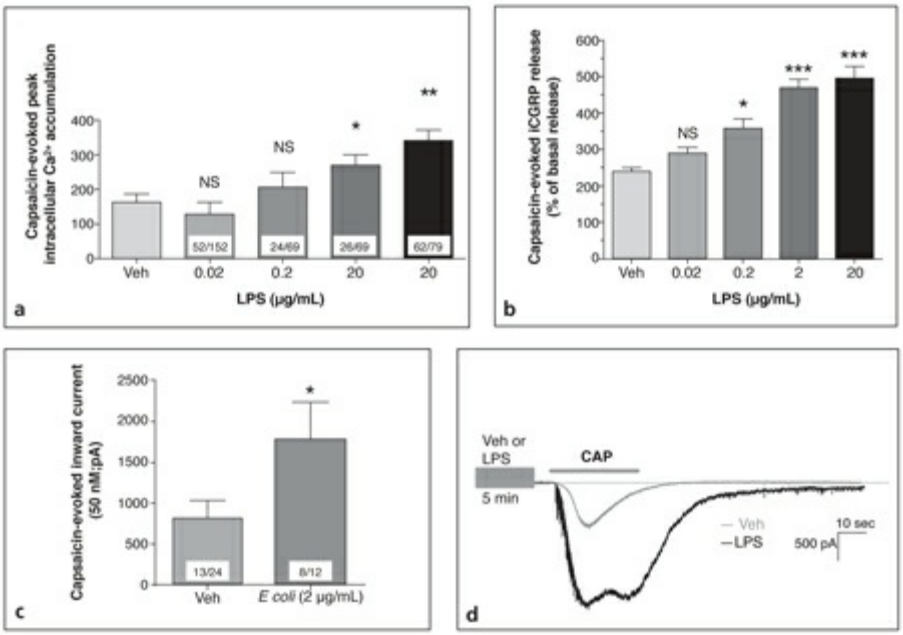

Fig 8-9 Lipopolysaccharide (LPS) sensitizes transient receptor potential vanilloid type 1 (TRPV1) responses in a concentration-dependent manner. *(a)* Pretreatment of trigeminal neurons with LPS evoked a concentration-dependent sensitization of a subsequent capsaicin-evoked accumulation of intracellular calcium. *(b)* Pretreatment of trigeminal neurons with LPS evoked a concentration-dependent sensitization of capsaicin-evoked immunoreactive calcitonin gene–related peptide (iCGRP) release. *(c)* Pretreatment of trigeminal neurons with LPS (2 µg/mL) led to the sensitization of a subsequent capsaicin-evoked inward current. *(d)* Representative traces of LPS sensitization of

capsaicin-evoked currents. Veh, vehicle; NS, not significant; CAP, capsaicin. Data are presented as mean ± standard error of the mean. *$P < .05$; **$P < .01$; ***$P < .001$. (Reprinted from Diogenes et al[88] with permission.)

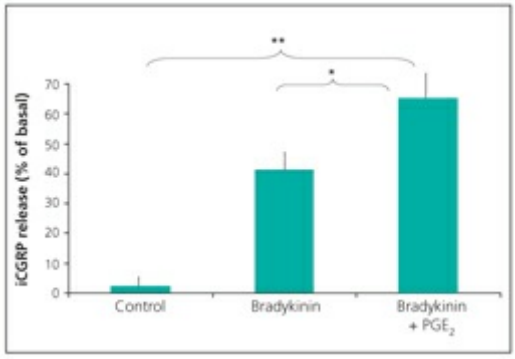

Fig 8-10 Effect of bradykinin alone and with prostaglandin E_2 (PGE_2) on the release of immunoreactive CGRP (iCGRP) from isolated superfused bovine dental pulp slices. Tissue was pretreated with bradykinin alone or with bradykinin and PGE_2, and released levels of iCGRP were measured by radioimmunoassay. *$P < .05$ (ANOVA); **$P < .01$ (ANOVA). (Redrawn from Goodis et al[93] with permission.)

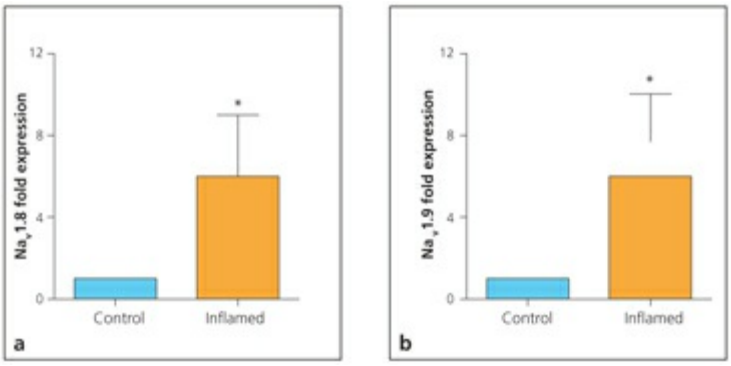

Fig 8-11 Expression of the sodium channels $Na_v1.8$ *(a)* and $Na_v1.9$ *(b)* is increased in the inflamed dental pulp. Data are expressed as fold change over control (uninflamed dental pulp). *$P < .05$. (Data redrawn from Wells et al[99] and Warren et al[101] with permission.)

Allodynia and hyperalgesia

The pain system can undergo dramatic changes in response to certain peripheral stimuli, leading to the development of allodynia and hyperalgesia.[13,20,31,32,109,110] These changes do not necessarily take weeks to develop but under certain conditions can occur within a few seconds or minutes after an appropriate stimulus. For example, extensive hyperalgesia and allodynia occur in humans a few minutes after

stimulation of cutaneous C nociceptors by injection of capsaicin.[111] These two different pain entities are detected by an increased response to a painful stimulation of the capsaicin site (hyperalgesia) and by the sensation of pain when the skin near the injection site is gently stroked with a cotton swab (allodynia).

Hyperalgesia is defined as an increase in the perceived magnitude of a painful stimulus, and *allodynia* is defined as a reduction in pain threshold so that previously non-noxious stimuli are perceived as painful. Many persons have experienced these altered pain states; common examples include sunburn or thermal injury. In sunburn, the pain experienced when a T-shirt is worn is allodynia (ie, reduced pain threshold), whereas the increased pain responsiveness experienced when someone slaps the burned skin is hyperalgesia (ie, increased pain perception).

Allodynia or hyperalgesia can occur during inflammation of pulpal or periradicular tissue. In fact, clinicians often rely on clinical testing and the patient's symptoms to detect the presence of hyperalgesia and allodynia[112] (Table 8-1). For example, thermal hyperalgesia is detected in the clinical setting when an exacerbated response is seen to the cold vitality test using carbon dioxide or 1,1,1,2-tetrafluoroethane. On the other hand, a painful response to the gentle tapping of a tooth with the mirror handle (or pain on mastication) is an indication of the presence of mechanical allodynia. Under normal conditions, this innocuous stimulation does not elicit pain. However, under conditions of acute or chronic inflammation, the mechanical pain threshold is reduced to the point at which tapping with a mirror handle is perceived as tender or painful.

In a recent prospective study including more than 3,500 patients, mechanical allodynia was detected in 56% of patients diagnosed with necrotic pulps and in 67% of patients diagnosed with irreversible pulpitis.[113] Recently, a quantitative method based on the use of a digital force transducer has been developed as an alternative to the subjective tapping of teeth with the mirror handle. This novel method is capable of quantifying the severity of mechanical allodynia.[114] It has been found that 57% of patients diagnosed with irreversible pulpitis have significant mechanical allodynia[115] and that the mechanical pain threshold was reduced by 77%.[116] Therefore, the systematic evaluation of allodynia or hyperalgesia represents the biologic rationale for endodontic diagnostic tests and provides unique information on the relative concentration of inflammatory mediators in the periradicular tissues (ie, degree of inflammation).

Similarly, studies in cats have shown that pulpal inflammation lowers the mechanical threshold of pulpal fibers to the level at which increases in systolic

blood pressure can activate pulpal neurons. The synchronous firing of pulpal fibers in response to the heartbeat is thought to mediate the "throbbing" pain of pulpitis.[77,95] Additional studies have shown that the thermal threshold of nociceptors can be reduced to the point at which normal physiologic temperature (ie, 37°C) can activate these peripheral neurons.[117,118] This may explain why some patients use ice water to relieve pain from severe, irreversible pulpitis because the neurons would be expected to stop firing when the tissue is cooled. Accordingly, the study of mechanisms and management of allodynia and hyperalgesia are important issues in managing dental pain.

Table 8-1 **Evaluation of hyperalgesia and allodynia in endodontic diagnostic tests***

Sign of hyperalgesia or allodynia	Related diagnostic test or symptom
Spontaneous pain	Spontaneous pain
Reduced pain threshold	Percussion test; palpation test; throbbing pain
Increased response to painful stimuli	Increased response to pulp test (electrical or thermal)

*Reprinted from Hargreaves et al[112] with permission.

Peripheral mechanisms of allodynia and hyperalgesia

Hyperalgesia occurs not only in the area of inflammation and tissue injury (primary hyperalgesia) but also in adjacent areas (secondary hyperalgesia).[119] Peripheral mechanisms are responsible for pain responses within the zone of primary hyperalgesia, whereas central mechanisms are responsible for the development of secondary hyperalgesia.[12–15,20,43]

Although both hyperalgesia to heat and hyperalgesia to mechanical stimuli are observed in the area of primary hyperalgesia, only mechanical hyperalgesia is seen in the zone of secondary hyperalgesia.[120] Moreover, the nociceptors that become sensitized within the primary zone of hyperalgesia produced by heat injury include a class of A-fiber nociceptors that are responsible for heat hyperalgesia, while mechanical hyperalgesia within this area is produced by the sensitization of a class of nociceptors that are typically insensitive to mechanical stimuli but respond to this stimulus after inflammation. These are called *silent nociceptors* and exist in a variety of tissue types.[121] The zone of secondary hyperalgesia that results from

pulpal inflammation has not been evaluated but most likely has clinical relevance in relation to patient symptoms and the results of certain clinical testing procedures.

Several mechanisms have been proposed to contribute to peripheral hyperalgesia (Box 8-1). As mentioned previously, the concentration and composition of various inflammatory mediators are important and can lead to activation or sensitization of nociceptors (see Fig 8-2). The concentration of the inflammatory mediator is important because tissue levels must be sufficiently high to permit binding and activation of the receptor. For example, prostaglandin E_2 levels are more than 100-fold greater in pulp samples collected from teeth with irreversible pulpitis than they are in normal control teeth[135] (Fig 8-12).

Moreover, heat-induced inflammation in pulp sensitizes pulpal afferent fibers by local release of prostaglandins; this effect is blocked by pretreatment with nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, naproxen, and diclofenac[136] (Fig 8-13). Heat-induced inflammatory changes can result clinically from tooth preparation procedures performed with insufficient water spray (additional pulpal responses are discussed in detail in chapters 6 and 13). From a clinical perspective, these studies support the use of the nonsteroidal class of analgesics to treat pulpitis[110] (see chapter 9).

Dental procedures such as the incision and drainage of an abscess or a pulpectomy may reduce pain by reducing concentrations of mediators as well as by lowering tissue pressure. Many inflammatory mediators found in inflamed pulp or periradicular tissue (see chapter 11) can either activate or sensitize nociceptors and evoke pain when administered to human volunteers. Considerable research is being directed toward clarifying the mechanisms of sensitization of nociceptors in the hope that this information will lead to the development of new classes of analgesic drugs.[137,138]

In addition to activation and sensitization, the peripheral afferent fiber responds to mediators such as nerve growth factor by increasing protein synthesis of substance P and CGRP and by sprouting terminal fibers in the inflamed tissue[95,125–127] (see chapter 7). Similar increases in neuropeptides are seen in human pulp infected by caries.[139] Sprouting increases the density of innervation in inflamed tissue and may contribute to increased pain sensitivity in chronic pulpal or periradicular inflammation.[95,125]

Box 8-1	**Peripheral mechanisms contributing to allodynia and hyperalgesia***

- Composition and concentration of inflammatory mediators[93,106–108,112]
- Changes in afferent fiber: activation and sensitization[77,111,122–124]
- Changes in afferent fiber: sprouting[125–128]
- Changes in afferent fiber: proteins[125–127]
- Tissue pressure[77,81]
- Tissue temperature[129]
- Sympathetic-primary afferent fiber interactions[130–132]
- Aβ fiber plasticity[32,133]

*Modified from Hargreaves et al[134] with permission.

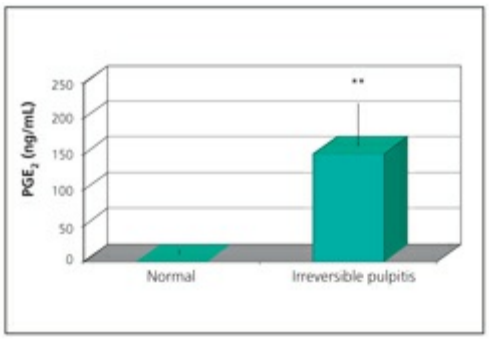

Fig 8-12 Pulpal levels of prostaglandin E2 (PGE2) in specimens taken from control pulps (normal diagnosis) (N = 21) and pulps with irreversible pulpitis (N = 21). Specimens were collected on a nylon pellet, and pulpal levels of immunoreactive PGE2 were measured by enzyme immunoassay. $^{**}P < .01$ (Mann-Whitney *U* test). (Redrawn from Nakanishi et al[135] with permission.)

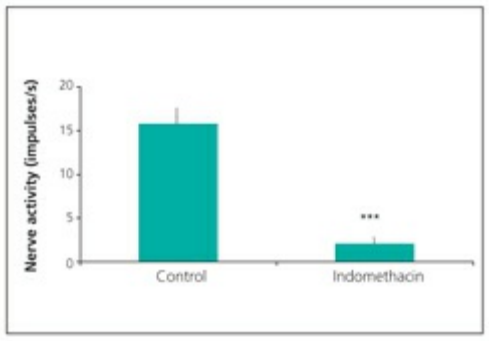

Fig 8-13 Effect of indomethacin (5 mg/kg) pretreatment on nerve activity after repeated administration of heat to the teeth of anesthetized cats. Cats were anesthetized, electrodes were placed in two dentinal cavity preparations, and heat was administered by a 1-second application of a stick of hot gutta-percha (about 90°C); the data were recorded after the third application of heat. $^{***}P < .001$ (*t* test). (Redrawn from Ahlborg[136] with permission.)

Certain afferent fibers also respond to inflammatory mediators by upregulating the expression of proteins such as voltage-gated sodium channels (Na_v) that are important in nerve impulse formation and propagation.[99,101] The different types of

sodium channels can be classified as based on sensitivity or resistance to tetrodotoxin (TTX).[140–142] An early study suggested a differential sensitivity of TTX-sensitive and TTX-resistant sodium channels to local anesthetics; the TTX-resistant subtypes ($Na_v1.8$ and $Na_v1.9$) were reportedly more resistant to lidocaine than TTX-sensitive forms such as $Na_v1.7$.143 More recent studies have shown that the $Na_v1.8$ sodium channel subtype (a TTX-resistant form) is more sensitive to lidocaine than TTX-sensitive currents, including those associated with the $Na_v1.7$ subtype.[144,145] Given these differences in the lidocaine sensitivity of sodium channel subtypes, the synthesis of new types of ion channels on sensory neurons may well contribute to the difficulty in obtaining local anesthesia that arises in certain cases of endodontic pain.

Although evidence exists for the involvement of all the major sodium channel subtypes that are preferentially expressed in the peripheral nervous system to inflammatory pain mechanisms, the $Na_v1.7$ subtype appears to be especially important.[146] An inherited mutation of this gene results in the lack of a functional current and pain insensitivity in humans, while other point mutations have been identified in two different chronic pain syndromes.[147–150] These findings suggest that $Na_v1.7$ represents a promising target for the future development of a new class of analgesics.

Several other peripheral mechanisms may contribute to allodynia or hyperalgesia. For example, Aβ fibers are normally thought to be low-threshold mechanoreceptors that do not convey nociceptive signals. However, under certain inflammatory conditions, Aβ fibers begin to express substance P and develop new central terminations in the spinal cord dorsal horn, innervating regions that contain nociceptive neurons.[133,151] Thus, it is possible that certain pain conditions may have an allodynic component because of nociceptive transmission by Aβ fibers. Other studies have implicated sympathetic fibers in the activation of nociceptive afferent C fibers after certain forms of tissue injury.[132]

Taken together, several peripheral mechanisms contribute to the development of allodynia and hyperalgesia. However, not all of these mechanisms are necessarily equally active in all acute and chronic pain states, and this factor has led to the concept of mechanistically based pain diagnoses.[112,152]

Central mechanisms of allodynia and hyperalgesia

In addition to these peripheral mechanisms, several central mechanisms of allodynia

and hyperalgesia have also been proposed[12,31,32,109,134] (Box 8-2). These central mechanisms lead to a pain state called *central sensitization* that is marked by the increased excitability of central neurons.[32] Central sensitization results from a barrage of impulses from C nociceptors. This results in the central release of glutamate and substance P (as well as other neurotransmitters), leading to activation of central receptors for glutamate (eg, NMDA and AMPA receptors) and substance P (eg, neurokinin 1 receptor) (see Fig 8-4). Under these conditions, stimulation of the normally low-threshold Aβ fibers, normally involved in the transmission of non-noxious mechanical stimulation (eg, touch), produces a much larger response resulting in pain, and this may provide a central mechanism of allodynia.

Box 8-2	**Central mechanisms contributing to allodynia and hyperalgesia***

- **Increased neurotransmitter release from primary afferent fibers**[153]
- Changes in postsynaptic receptors[154]
- Changes in secondary messenger systems[45,154,155]
- Changes in proto-oncogenes[156,157]
- Changes in endogenous opioids or cannabinoids[50,51,54,156]
- Central sensitization[12,32,158,159]
- Reduced presynaptic inhibition[32,45]
- Reduced postsynaptic inhibition[32,45]
- Windup[32]
- Dark neurons[160]
- Activation of glia[62,161]

*Modified from Hargreaves et al[134] with permission.

Another important mechanism of central sensitization is related to the endogenous inhibitory tone that descending pathways exert on the synaptic transmission. These descending inhibitory neurons originate in the locus coeruleus, amygdala, and nucleus raphe magnus and have norepinephrine and 5-hydroxytryptamine as neurotransmitters. The inhibitory nature of these neurons underlies the analgesic efficacy of some tricyclic antidepressants or serotonin norepinephrine reuptake inhibitors in certain pain states. These drugs increase the concentrations of the inhibitory neurotransmitters (norepinephrine and 5-hydroxytryptamine) and thus the endogenous inhibitory tone in the trigeminal complex, resulting in the attenuation of the ascending excitatory nociceptive signals. On the other hand, suppression of the

descending inhibitory pathways results in disinhibition of the central pain modulation and facilitation of ascending pain signals and greater pain perception (see Fig 8-4).

Several other mechanisms for central sensitization have been proposed (see Box 8-2). The concept of central sensitization is important because it implies a dynamic responsiveness of the central nervous system to peripheral input. Put another way, the same stimulus does not always produce the same response.

Activation of pulpal neurons produces a central sensitization.[20,29,53] Application of mustard oil to the pulp of a rat maxillary molar activates pulpal neurons and evokes more than a fivefold increase in the discharge rate of nociceptive-specific neurons located in the nucleus caudalis.[29] This barrage of pulpal C fibers is sufficient to produce a central sensitization because 20 minutes later there is an enhanced response to light tactile stimuli applied to the maxillary skin (Fig 8-14). This central sensitization is mediated by release of glutamate because pretreatment with an antagonist to the glutamate NMDA receptor (MK-801) abolishes this effect. Similarly, application of mustard oil to the rat molar dental pulp leads to the upregulation of NMDA receptors in the thalamus.[162,163] Thus, activation of pulpal nociceptors can induce central sensitization via central increase of the expression of NMDA receptors and the release of glutamate in both trigeminal nuclei and thalamus, and this effect is sufficient to produce allodynic-like responses.[29,163]

Pulpotomy can also evoke a central sensitization, as measured by both an expansion of receptive field sizes and an increase in spontaneous activity in the nucleus oralis[164,165] (Fig 8-15). In addition, central terminals of afferent fibers continue to exhibit increased release of CGRP, even after removal from the animal.[153] Thus, even in the absence of peripheral input, central mechanisms of hyperalgesia or allodynia can persist for some time.

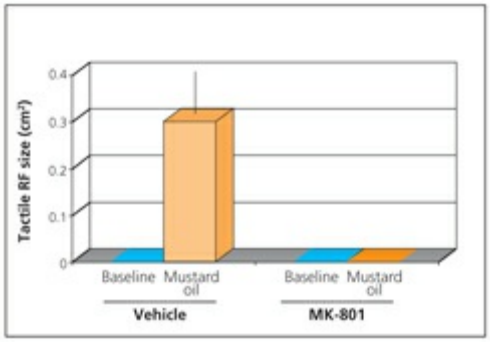

Fig 8-14 Effect of application of mustard oil on pulpal neurons in the rat. Application induces a central sensitization via glutamate release. Mustard oil was applied to the pulp of a maxillary first molar in anesthetized rats. Simultaneously, recordings were obtained from nociceptive-specific neurons in the nucleus caudalis. The area of the maxillary skin that activated the nociceptive-specific neurons after

light tactile stimulation was recorded as the tactile receptive field (RF) size. Animals were pretreated with either vehicle or an antagonist to the glutamate NMDA receptor (MK-801) before mustard oil was administered to the pulp. $^{**}P < .01$ (ANOVA) versus vehicle treatment. (Redrawn from Chiang et al[29] with permission.)

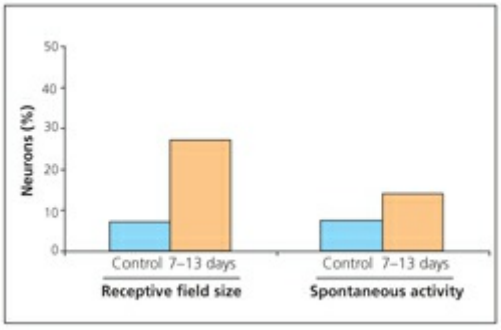

Fig 8-15 Effects of pulpotomy in a rat mandibular molar on a mechanorecep-tive field and spontaneous activity of central neurons located in the trigeminal subnucleus oralis under control conditions and 7 to 13 days after pulpotomy. $^{*}P < .05$ (chi-square test); $^{**}P < .01$ (chi-square test). (Redrawn from Kwan et al[164] with permission.)

Because at least some components of allodynia or hyperalgesia can persist even without continued sensory input from inflamed tissue, it is not surprising that up to 80% of patients experiencing pain before endodontic treatment continue to report pain after treatment.[166,167] Thus, even if the endodontic treatment has removed all peripheral factors contributing to hyperalgesia, the central mechanisms can still persist for some time. Indeed, the presence of preoperative hyperalgesia (operationally measured as preoperative pain or allodynia) is a risk factor for the occurrence of postendodontic pain[7,168] (Fig 8-16). Patients with moderate or severe preoperative pain tended to report greater pain levels for 3 days after endodontic cleaning and shaping than did patients reporting either no or mild preoperative pain.[82] The presentation of this risk factor should alert the clinician to adjust the pain-management strategy for higher levels of pain control[82] (see chapter 9).

It has been recently reported that the overall incidence of pain 6 months or more after endodontic therapy is approximately 5.3%, of which 3.4% is of nonodontogenic origin.[169] Therefore, it is possible that some of these teeth were previously misdiagnosed and underwent unnecessary endodontic therapy or that the neuronal plasticity that resulted from a prolonged inflammatory state has not yet been reversed. Conversely, patients with persistent pain after endodontic therapy that has successfully removed the obvious disease etiology (ie, infection) may have pain states other than inflammatory pain, such as neuropathic pain, dysfunctional pain, or referred pain. The alarming number of patients with persistent pain following an

otherwise successful endodontic therapy highlights the need for an astute clinician to understand the pain pathways involved in both odontogenic pain (nociceptive and inflammatory pain states) and nonodontogenic pain (neuropathic, dysfunctional, and referred pain states) in order to have a flexible pain-management plan to address these very different entities.

In summary, under normal physiologic conditions, nociceptors are only activated on suprathreshold noxious stimuli (nociceptive pain). The nociceptive pain signal acts as an important surveillance system, active when there is stimulus of enough intensity to cause the individual harm. This signal remains active for as long as the stimulus is present. However, inflammatory pain may be manifested as hyperalgesia (increased responses to noxious stimuli) and mechanical allodynia (pain after non-noxious stimuli) (Fig 8-17). In the hyperalgesic state, the individual is made aware of a disease state by a greater-than-usual painful response. In the allodynic state, further stimulation of the area of inflammation, even low-intensity stimulation, results in pain, causing avoidance behavior and protection of the inflamed site (eg, patients often avoid chewing solid foods in the quadrant of a tooth with symptomatic apical periodontitis). Therefore, nociceptive and inflammatory pain protects from actual or potential damage and promotes healing. In addition, as discussed previously, both peripheral and central mechanisms govern these two pain manifestations.

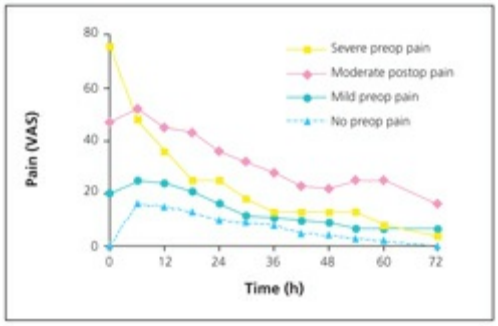

Fig 8-16 Time-response curves for postendodontic pain in patients given a placebo analgesic. Patients were subdivided into four groups based on the magnitude of their preoperative pain. Patients with moderate or severe preoperative pain tended to report greater levels of pain throughout the postendodontic (cleaning and shaping) treatment. VAS, visual analog scale (0 to 100). (Redrawn from Torabinejad et al[168] with permission.)

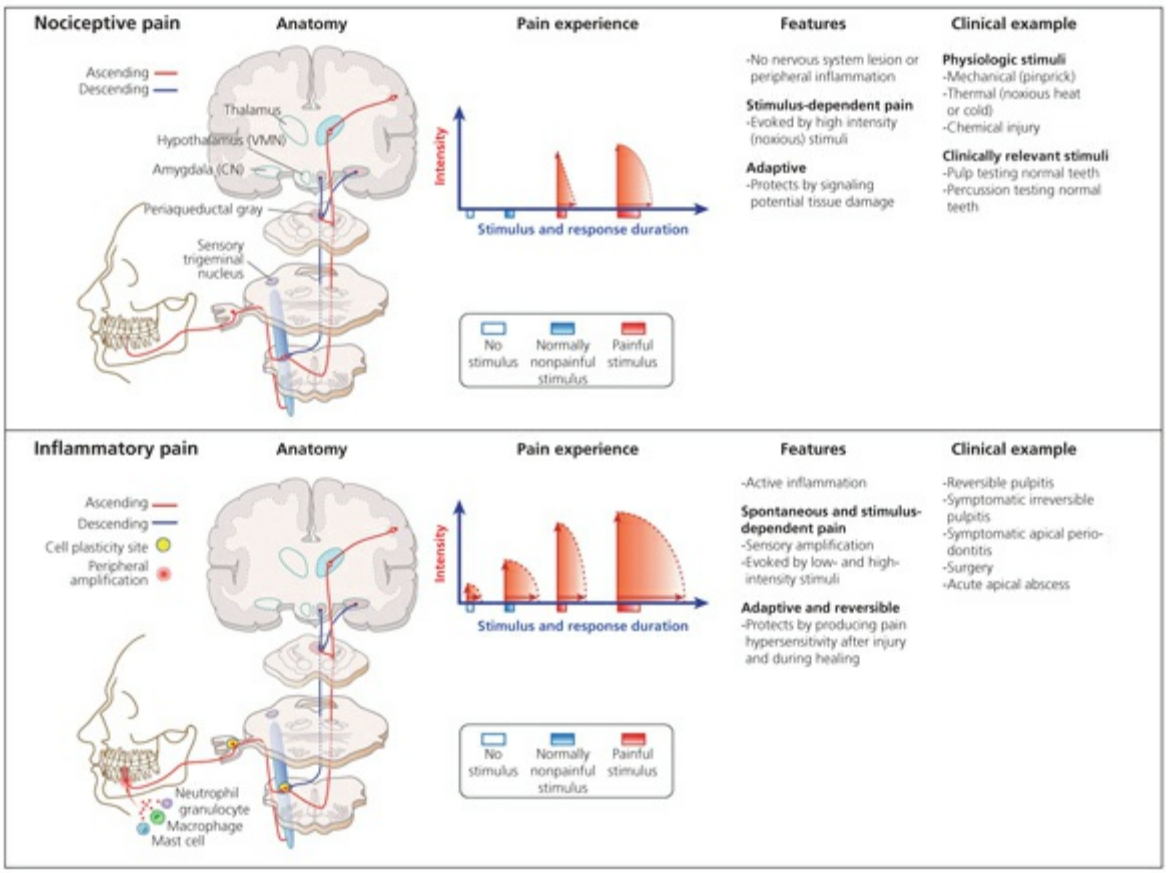

Fig 8-17 Mechanisms and characteristics of nociceptive pain and inflammatory pain. VMN, ventromedial nucleus; CN, central nucleus.

Neuropathic and dysfunctional pain

Pain states that neither protect nor promote healing may result from maladaptive plasticity of the peripheral and central nociceptive pathways. Both neuropathic and dysfunctional pain states are examples of this maladaptation of the pain pathways. Although there are similarities between these two pain states, they represent separate entities.

Neuropathic pain is typically caused by an identifiable initial presentation. The most prevalent type of orofacial neuropathic pain is trigeminal peripheral neuropathy. This painful condition could have a peripheral origination site, such as nerve damage following third molar extraction, administration of local anesthetics, implant placement, or orthognathic surgeries,[170,171] or, in the case of trigeminal neuralgia, a central origination site, such as central nerve compression, stroke, or a degenerative systemic disease (eg, multiple sclerosis).[172–174] Although resolution of

inflammatory pain is expected after the removal of the etiology (eg, infection), the same is not true for neuropathic pain. This pain state is the result of significant maladaptive neuronal plasticity, and interventions are often palliative with the goal to improve the patient's quality of life.

Further peripheral nerve injuries appear to be directly related to the incidence of neuropathic pain.[55] In fact, the risk of developing neuropathic pain after nerve injury appears to be related to the size or diameter of the injured nerve. For example, nerve injury is common in endodontic therapy because pulpal and periradicular primary afferent neurons are routinely axotomized during root canal therapy and periradicular surgeries, respectively. In this case, a very small nerve bundle is severed rather than a larger neuronal bundle, such as the inferior alveolar nerve, which, as opposed to endodontic therapies, is significantly associated with the risk of developing neuropathic pain.[171]

Although the incidence of persistent pain following adequate endodontic therapy is reported to be 5.6%, there has not been any investigation to determine whether these cases are due to a form of neuropathic pain. One important feature that aids in the diagnosis of these conditions is the quality of the perceived pain, often characterized as high-intensity "electric shock–like," "lancinating," or "stabbing" pain (Fig 8-18). These pain qualities easily differ from the usual mild-intensity "sharp" pain associated with nociceptive pain (eg, cold vitality test in an uninflamed tooth) or the "dull, aching" pain associated with inflammatory pain (eg, lingering response to cold vitality test in an inflamed tooth).

Another important feature of this painful presentation is its lack of response to anti-inflammatory drugs such as NSAIDs or acetaminophen. On the other hand, pregabalin and gabapentin, modulators of central nervous system activity, are relatively effective in the treatment of neuropathic pain states such as trigeminal neuralgia.[175–177] The effect of these drugs in persistent endodontic pain following adequate endodontic therapy has not yet been evaluated. Therefore, clinicians and researchers should be alert to the mechanisms and properties of neuropathic pain states to adequately treat and research this painful and devastating condition.

Dysfunctional pain is another maladaptive chronic pain state, but it differs from neuropathic pain in not having a known cause. However, it shares many of the features of neuropathic pain, such as temporal summation with increased responses to repeated stimuli and lowered activation thresholds (see Fig 8-18). Thus, this largely unknown condition is characterized by peripheral amplification of low- and high-intensity stimuli. Examples of this presentation include fibromyalgia and may include atypical odontalgia.[178–181]

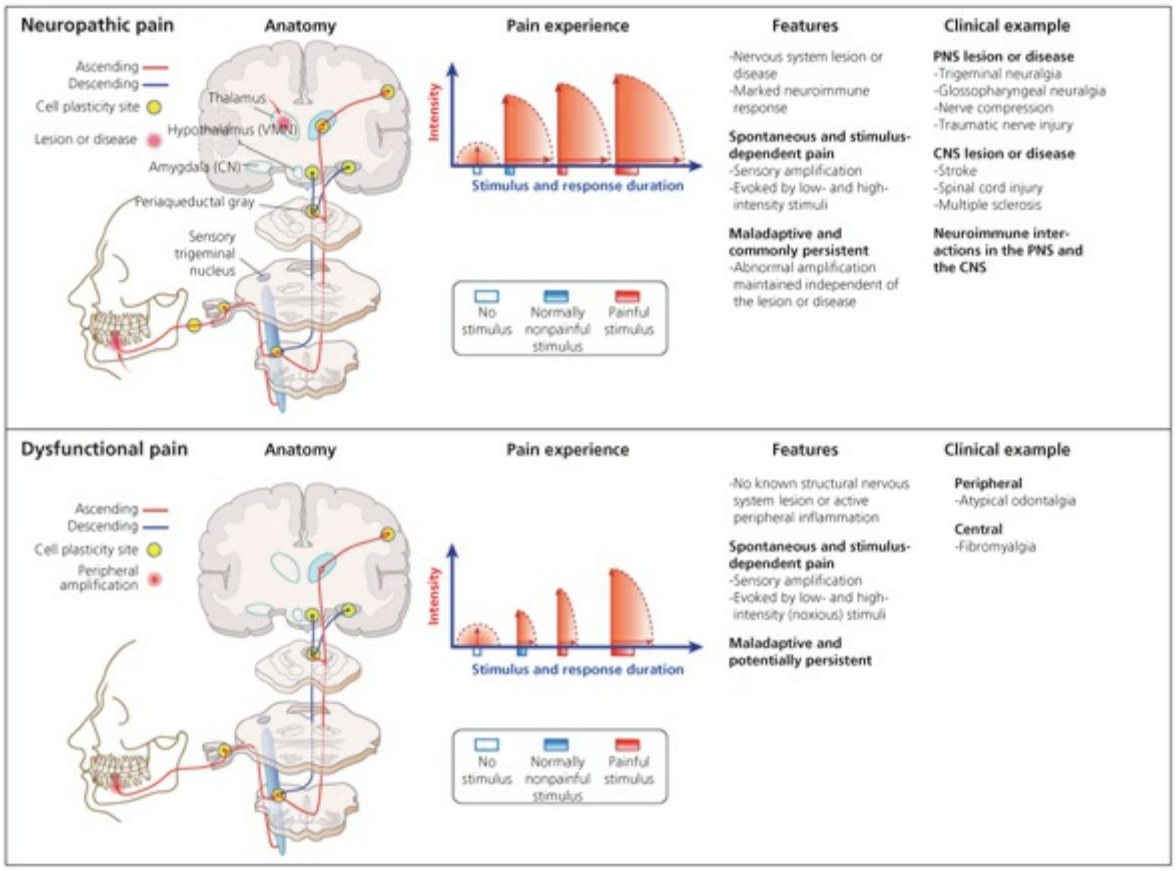

Fig 8-18 Mechanisms and characteristics of neuropathic pain and dysfunctional pain. VMN, ventromedial nucleus; CN, central nucleus; PNS, peripheral nervous system; CNS, central nervous system.

Referred pain

Referred pain is the condition in which pain is perceived to be localized in one region but is caused by nociception originating from another area. In referred pain, the region of the body where pain is perceived to occur is not the same as the region where the pain originates. Clearly, referred pain represents a diagnostic challenge to the clinician because effective treatment must be directed to the site where pain originates (see also chapter 19).

Suda and colleagues[182] have nicely summarized peripheral and central mechanisms of referred pain. Peripheral mechanisms include branching axons that innervate different structures and axonal reflexes. Central mechanisms include muscle contractions, central sensitization, memory, and convergence of primary afferents. These hypotheses are not mutually exclusive and may contribute to various

cases of referred pain. For example, there is good experimental evidence that central sensitization and convergence of primary afferents may mediate many cases of referred pain.[116,183]

Evidence shows that the central terminals of trigeminal afferent fibers converge onto the same projection neurons. For example, nociceptors in the maxillary sinus and maxillary molars may stimulate the same neuron located in the nucleus caudalis.[20,183,184] About 50% of neurons in the nucleus caudalis exhibit convergence of sensory input from cutaneous and visceral structures.[184] In one example, a single neuron in the nucleus caudalis received input from sensory neurons innervating the maxillary skin, cornea, a mandibular canine, a mandibular premolar, and a maxillary premolar[183] (Fig 8-19). Thus, the clinical problem of referred pain has a biologic basis in the convergence of sensory neurons onto the same central projection neuron, making it difficult for the patient to discern where the pain originates. Indeed, it has been reported that more than 26% of patients experiencing odontogenic pain are unable to accurately localize the painful tooth.[185]

The theory of convergence has been used to explain the clinical observation of a patient who complains of pain that originates from an inflamed mandibular molar and radiates to the preauricular region or of pain that originates in inflamed maxillary sinuses and radiates to the maxillary posterior teeth. Thus, pain can originate from the dental pulp and be referred to distant regions or, conversely, can originate from distant regions and be referred back to the dental pulp (see also chapter 19).

The latter has been demonstrated in patients with chronic orofacial pain. In patients with trigger points in the superior belly of the masseter muscle, pain can be referred to maxillary posterior teeth[186] (Fig 8-20). Conversely, in patients with trigger points in the inferior border of the masseter muscle, pain can be referred to the mandibular posterior teeth. Thus, it is important to consider multiple diagnostic tests to determine the origin of a patient's pain.

In addition to convergence, another important mechanism of referred pain is central sensitization. Nociceptive signals that originate in the periphery but have increased intensity and duration may lead to changes in the central terminals, such as upregulation of NMDA receptors for the excitatory neurotransmitter glutamate and release of substance P and other mediators, as already discussed. These changes may lower the threshold of activation of projection neurons that also receive inputs by nociceptors that innervate uninflamed regions (convergence). This decreased threshold can potentially lead to pain when uninflamed teeth experience innocuous

stimuli (eg, mastication) and low-intensity signals are amplified by a sensitized central projection neuron. Clinically, this phenomenon can be observed when a whole quadrant is tender to percussion while only a single tooth is inflamed and is the culprit for this presentation. Interestingly, mechanical allodynia has been detected using a sensitive quantitative method in otherwise normal teeth contralateral to the inflamed tooth.[116]

Both convergence and central sensitization are likely to take place concomitantly and form the basis for the diagnostic use of local anesthetics in establishing the origin of pain in patients who present with diagnostic challenges. For example, Okeson[187] described the selective injection of local anesthetics as a clinical test to distinguish the site of pain origin from the area of pain referral.

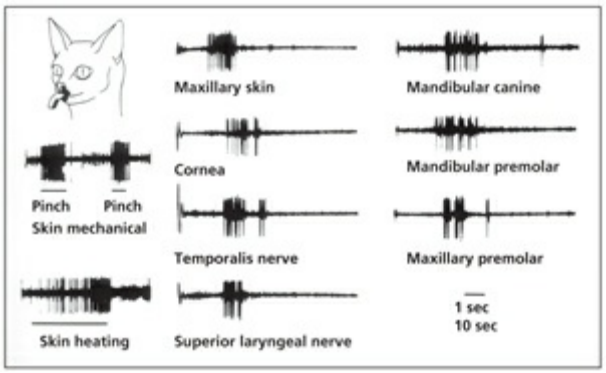

Fig 8-19 Example of convergence of multiple sensory neurons onto the same central neuron in the nucleus caudalis of an anesthetized cat. The receptive field for the neuron was in the maxillary region. The figure shows the depolarizations of the neuron in the nucleus caudalis to stimulation in various orofacial regions. (Reprinted from Sessle et al[183] with permission.)

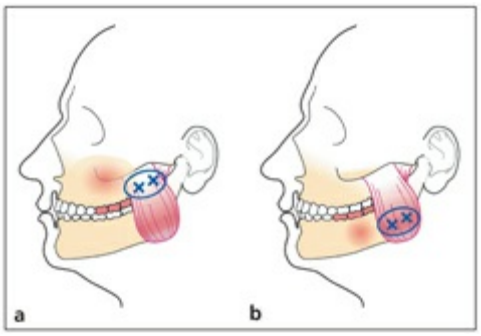

Fig 8-20 Referral patterns in patients with chronic orofacial pain. *(a)* Patients with trigger zones in the superior belly of the masseter muscle have pain referred to regions including the maxillary posterior teeth. *(b)* Patients with trigger zones in the inferior belly of the masseter muscle have pain referred to regions including the mandibular posterior teeth. (Redrawn from Travell and Simons[186] with permission.)

Mechanisms of Dentinal Hypersensitivity

Hydrodynamic theory of dentinal hypersensitivity

Dentinal sensitivity is characterized as a sharp pain that occurs soon after a provoking stimulus.[188] Dentinal tubules are well innervated near the pulp horns (see chapter 7). In this region, up to 74% of the tubules contain nerve fibers, and these fibers can extend up to approximately 200 μm into the tubule (see Fig 8-2). At the midcrown level of the pulp, fewer tubules are innervated, and the intratubule extension of the fibers is shorter. In contrast, root dentin is poorly innervated.

For all innervated tubules, the nerve fibers are in close proximity to odontoblasts, although direct connections are not evident.[95] However, the proximity of these two cell types is consistent with the hypothesis that odontoblasts and afferent terminal endings may have biochemical connections (eg, via expression of receptors and paracrine release of soluble factors) and thereby participate in the sensory transduction of noxious stimuli.

This anatomical relationship between nerve fibers and odontoblasts may be the physiologic basis for the hydrodynamic theory of dentinal pain. This theory, which has strong experimental support, postulates that movement of fluid through the dentinal tubules results in pain.[189–192] Stimuli, including air blasts, cold, and hypertonic sugars ("sweets"), can produce movement of the dentinal fluid. Movement of this fluid results in stimulation of nociceptive nerve fibers located on the pulpal side of the dentinal tubules[193,194] (Fig 8-21). The fluid movement is thought to serve as a fluid transducer, signaling the presence of stimuli at the outer opening of the dentinal tubules. Indeed, removal of dentinal fluid abolishes the ability to detect these stimuli; sensitivity returns when the fluid is replenished.[195]

It is not known how nerve fibers detect fluid movement. However, the sharp quality of the resulting pain suggests activation of Aδ nociceptive fibers[77,81,188] (see Figs 8-7 and 8-8).

Dentinal sensitivity appears to recede with age or after chronic irritation. The increase in secondary or reparative dentin during these processes is thought to diminish the flow of fluid through the tubules.[196]

Following exposure of dentin, either by loss of enamel or by loss of cementum

and gingiva, dentinal sensitivity can develop. Researchers have reported that there is a large increase in the responsiveness of Aδ fibers to dentinal stimulation after an acid-etching procedure is performed on exposed dentin[197] (Fig 8-22). The responsiveness to mechanical (eg, probing), drying (eg, air blast), and osmotic stimuli is increased. The buccal surfaces of canines and premolars are common sites of dentinal exposure, probably because of their susceptibility to toothbrush-induced abrasion.[198]

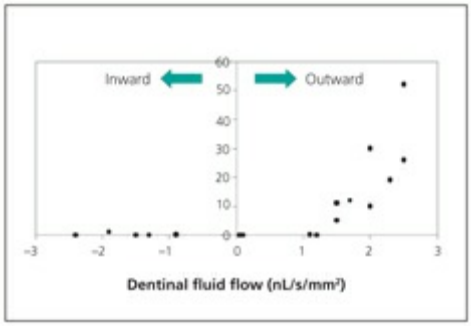

Fig 8-21 Effect of velocity of dentinal fluid flow on the responses of an Aδ fiber that innervates dentin in the anesthetized cat. (Redrawn from Matthews et al[194] with permission.)

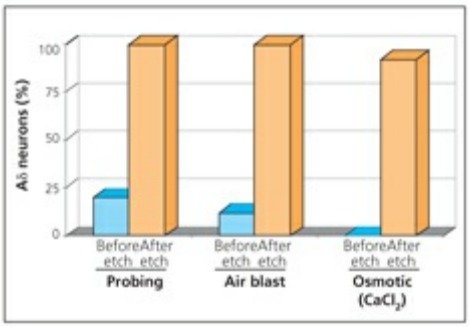

Fig 8-22 Effect of acid etching on the responsiveness of Aδ afferent fibers to stimuli applied to exposed dentin in anesthetized dogs (N = 23 to 25). CaCl2, calcium chloride. (Reprinted from Närhi et al[197] with permission.)

Effects of pulpal inflammation on dentinal sensitivity

Dentinal sensitivity is not an invariant sensation. The responsiveness to dentinal stimulation increases not only with exposure of dentinal tubules (see Fig 8-22) but also in the presence of inflammatory mediators. For example, the administration of the inflammatory mediator leukotriene B_4 to deep dentinal preparations increases the responsiveness to osmotic stimuli[123] (Fig 8-23). In an actual model of pulpal

inflammation, there is a threefold increase in the receptive field size of Aδ fibers innervating inflamed dog teeth[77,81,95,128,199] (Fig 8-24). This may result from the sprouting of Aδ fibers and the resultant increase in the area of innervated dental tubules. Thus, pulpal inflammation may predispose a tooth to enhanced dental sensitivity by reducing the threshold for activation and by increasing the area of innervated dental tubules.

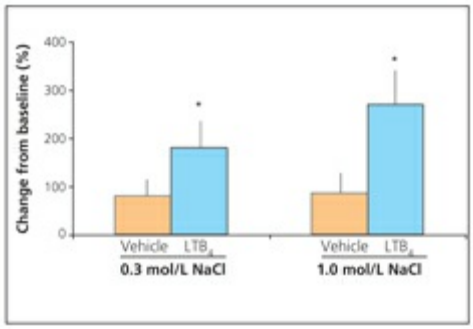

Fig 8-23 Effect of administration of leukotriene B4 (LTB4) or vehicle on dentinal sensitivity to osmotic stimuli. LTB_4 (25 mg/mL) or vehicle was applied to exposed dentin in anesthetized cats; electrodes were placed in cavities for intra-dentinal recordings. Administration of LTB_4 significantly enhanced responsiveness to osmotic stimuli (0.3 and 1.0 mol/L of sodium chloride [NaCl]) applied to the exposed dentin. *$P < .05$ (ANOVA) versus vehicle. (Redrawn from Madison et al[123] with permission.)

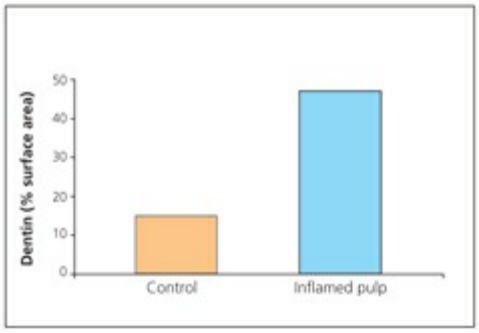

Fig 8-24 Effect of pulpal inflammation on the receptive field size of Aδ fibers innervating the dentin of dog teeth. The teeth underwent resection of the coronal portion of the crown 1 week before the experiment. On the day of the experiment, dogs were anesthetized, single units were isolated, and the exposed dentin was probed to determine the area of innervated dentin. (Redrawn from Närhi et al[128] with permission.)

Pulpal Vitality Testing

One aspect of the diagnosis of pain is the determination of pulpal vitality. The importance of assessing vascular integrity to establish pulpal vitality is discussed in

chapter 6. However, methods for assessing vascular integrity are generally not well validated or amenable to all clinical applications. Accordingly, responsiveness to various pulpal stimuli has been used as a surrogate measure of pulpal vitality.[200] In general, this involves the use of electrical or thermal testing or preparation of a test cavity; the patient's response is an outcome measure.

Accepted techniques, such as the use of control teeth interspersed with the suspected tooth, have been described in clinical texts, and the reader is encouraged to review this material.[201] This section reviews factors that modify outcomes in electrical and thermal testing and reviews studies on sensitivity and specificity.

A common method for assessing pulpal responsiveness is the use of electrical stimuli.[201,202] Under certain conditions, electrical stimuli can elicit a "pre-pain" sensation that is distinct from pain.[18] The primary factors that affect the electrical pulpal responsiveness test include electrode design (monopolar versus bipolar), electrode surface area, pulse duration, pulse strength (current and voltage), pulse frequency, electrode position (eg, incisal versus gingival), restorative status of the test teeth, and patient health. In general, the amount of current required to activate pulpal Aδ afferent fibers is only about 25% of that required to activate C fibers[203]; most clinical testing activates only Aδ fibers.

Although most commercially available electrical pulp testers are monopolar (ie, cathode on tooth and anode often on the lip), studies have shown that bipolar electrodes (ie, cathode and anode placed on tooth) provide more consistent effects.[203–205] For example, the coefficient of variation (a measure of the noise-signal ratio) for bipolar electrode stimulation of Aδ fibers is about 35% less than that for monopolar stimulation of the same units.[204]

The density of the electrical current also plays an important role in this test. A larger surface area of electrodes requires greater current to produce a detectable sensation, and, at any given electrode area, shorter pulse widths require greater current to produce a sensation[206] (Fig 8-25). This is an important concept because the density of the current is the result of these parameters as well as the pulp anatomy.

When results in different types of teeth or results from younger patients and older patients (with smaller pulp chambers) are compared, the current delivered cannot be directly compared because the pulp anatomy differs. Studies have indicated that electrical testing produces the most consistent effects when the electrode is placed on the incisal or cuspal edge of the tooth.[207,208] In a study of more than 7,000 posterior teeth, electrical testing of the mesiobuccal cusp of mandibular molars gave

the lowest response threshold, and, in general, electrical thresholds increased with patient age.[209] Other factors that influence electrical testing include the restorative status of the tooth (eg, porcelain is an insulator) and even the patient's health (hypertensive patients have significantly higher thresholds for electrical testing).[210]

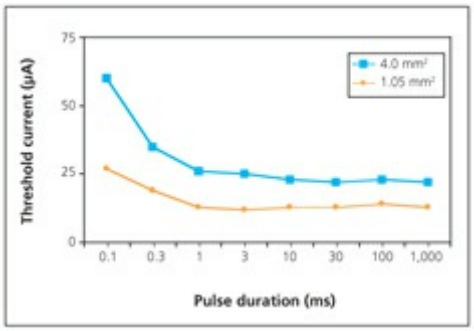

Fig 8-25 Effect of monopolar electrode and stimulation parameters on the threshold for pain perception in humans. Electrodes (1.05-mm^2 or 4.0-mm^2 surface area) were placed on exposed dentin, and the parameters were evaluated to determine if they caused positive test responses (ie, pain perception threshold of electrical stimulus). (Redrawn from Mumford and Newton[206] with permission.)

Alternative testing methods include the application of cold or hot stimuli to the tooth.[78,188,211–214] Commonly used cold stimuli include ethyl chloride spray, dichlorodifluoromethane, dry ice (ie, frozen carbon dioxide), and wet ice (ie, frozen water). In general, the response to application of cold stimuli is measured as a positive or negative reaction. This technique is probably used more often than application of hot stimuli.

Commonly used hot stimuli include electrical heat sources and heated gutta-percha (applied to a lubricated tooth surface). The initial response to heat is a sharp sensation; if the stimulus is maintained for a sufficient period of time, a dull, aching sensation is perceived. These sensations appear to be mediated by Aδ and C fibers, respectively.

All clinical tests are subject to false-positive (ie, a positive response from a necrotic pulp) and false-negative (ie, a negative response from a vital pulp) results.[215,216] One study compared the cold test (ethyl chloride) and an electrical pulp test to the "gold standard" (endodontic access and clinical verification of vitality) in 59 teeth of unknown pulpal status.[216] The probability that a negative test meant a true necrotic pulp was similar for the cold and electrical tests (89% versus 88%); the hot gutta-percha test exhibited a much lower ability to detect a true negative (48%). The probability that a positive test represented a true vital pulp was similar for the cold, electrical, and hot gutta-percha tests (90%, 84%, and 83%, respectively). Overall, the cold and electrical tests had similar accuracy values

(86% versus 81%), and both were more accurate than the heat test (71%).

Other studies have reported that the cold test is more accurate (80%) in testing human teeth (N = 50) than the electrical test (64% for monopolar electrodes).[217] In one study of more than 1,000 teeth, two different electrical testers each produced significantly more (about fivefold higher) false-positive results than did the cold test. Even when gingival controls were employed, one commercial electrical tester produced about twofold greater false-positive results than the cold test.[218]

Conclusions

The mechanisms of pulpodentinal pain have numerous clinical implications. First, dentinal pain is primarily caused when the myelinated fibers innervating dentinal tubules detect fluid movement and signal that movement back to the brain. Therapeutic reduction of dentinal fluid movement or neuronal activation can reduce dentinal hypersensitivity. Second, inflammation is detected by receptors expressed on pulpal nociceptors; the binding of inflammatory mediators onto these receptors can activate or sensitize these nociceptors. Drugs that reduce tissue levels of inflammatory mediators (eg, NSAIDs) relieve pain by reducing activation of these receptors. Third, hyperalgesia and allodynia can occur during pulpal and periradicular inflammation. Evaluation of the presence of these altered pain states provides the biologic basis for endodontic diagnostic tests. Fourth, hyperalgesia and allodynia can occur by both peripheral and central mechanisms and may persist beyond the dental appointment. Thus, patients with preoperative pain have an increased risk of experiencing postoperative pain. Fifth, maladaptive pain states such as neuropathic pain (eg, trigeminal neuralgia) and dysfunctional pain (eg, atypical odontalgia) are devastating conditions that result from significant plasticity of the peripheral and central nervous system; they represent a diagnostic and therapeutic challenge. Sixth, referred pain is due in part to convergence of multiple sensory fibers onto the same central projection neuron and may be associated with central sensitization. Seventh, pulp testing, using either electrical or thermal stimuli, requires an understanding of the mechanisms involved to allow proper interpretation of tooth and patient conditions and to minimize confusion caused by false-positive or false-negative results.

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Pharmacologic Control of Dental Pain

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A major theme of this book is the relationship between dental pulp and other tissues in health and disease. Perhaps the best example of this theme is dental pain. As detailed in chapters 7 and 8, pain is the predominant sensation following activation of those sensory neurons that innervate dental pulp and dentinal tubules. Although patients may report their pain as a "toothache," the skilled clinician understands that this single response may be an integration of pain originating from pulpal and periradicular nociceptors (eg, acute apical periodontitis) in addition to central mechanisms of hyperalgesia. Moreover, in some cases the noxious input may not even derive from the tooth in question; it may actually represent pain referred to the site from a distant tissue and integrated in the central nervous system (see chapter 19).

Given this complexity, it is perhaps not surprising that many patients view dentistry and pain as synonymous. Indeed, studies surveying more than 45,000 households in the United States indicate that odontalgia, or toothache, is the most common form of pain in the orofacial region, afflicting 12% of the study population.[1]

This percentage corresponds to about 20 million people in the United States alone.

This chapter reviews studies on treatment of the two major forms of dental pain—dentinal sensitivity and inflammatory pulpal pain. Evidence supporting pharmacologic and nonpharmacologic treatment regimens for management of dental pain are reviewed in the context of clinical interventions.

Management of Dentinal Hypersensitivity

As reviewed in chapter 8, the predominant hypothesis for dentinal hypersensitivity is Brännström's fluid flow hypothesis. Indeed, teeth with dentinal hypersensitivity demonstrate significantly greater numbers of patent dentinal tubules and significantly greater mean diameter per dentinal tubule than do control teeth[2] (Fig 9-1). Identified risk factors for dentinal hypersensitivity include erosion, abrasion, attrition, gingival recession, periodontal treatment, and anatomical defects.[3–5] Accordingly, interventions that reduce either fluid flow or the activity of the neurons that innervate dentinal tubules would be predicted to be effective in reducing dentinal hypersensitivity.

Several cross-sectional studies have reported on the clinical characteristics of dentinal hypersensitivity. The reported prevalence of dentinal hypersensitivity ranges from 3.8% (N = 3,593, United Kingdom) to 17% (N = 635, Brazil) to about 55% (N = 250, Ireland; N = 277, United Kingdom).[6–9] The wide variation in the reported prevalence may be related to cultural or genetic factors or to experimental variations in methods of assessment or sampling. Indeed, several investigators have proposed standardized methods for assessing dentinal hypersensitivity and conducting randomized controlled clinical trials.[10,11] Dentinal hypersensitivity has been reported to have a higher incidence in female patients in some studies.[5,7,8] The peak reported period for dentinal hypersensitivity is the third to fourth decades of life. Most studies report a decline in the prevalence of dentinal hypersensitivity in older patients, which may be related to reductions in dentinal tubule permeability[12] (Fig 9-2).

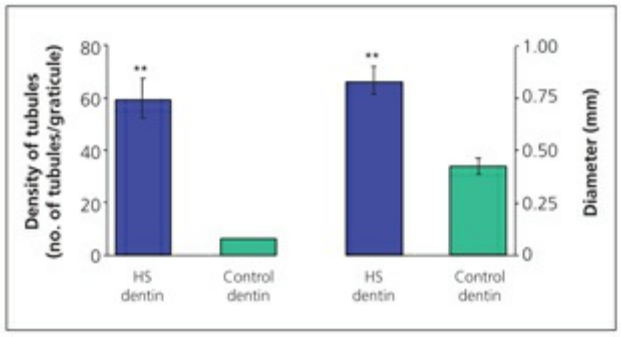

Fig 9-1 Comparison of the density of dentinal tubules and the mean diameter of dentinal tubules in teeth taken from 34 patients with hypersensitive (HS) dentin and 37 patients with normal dentin. Teeth were extracted and then examined under a scanning electron microscope. $^{**}P < .01$ versus control dentin. (Data from Absi et al.[2])

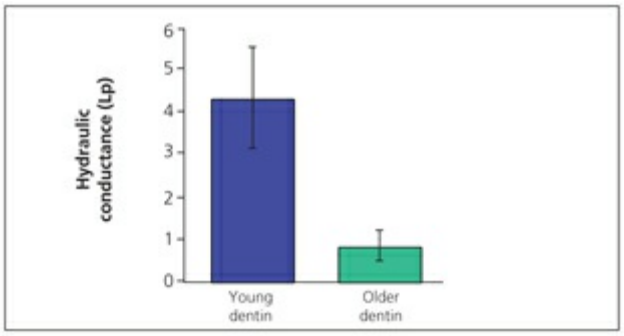

Fig 9-2 Hydraulic conductance of dentin disks taken from young (22- to 27-year-old) and older (45- to 62-year-old) patients. Lp, liquid permeability. (Data from Tagami et al.[12])

The locations most often affected tend to be the cervical region of incisors and premolars, often on the side opposite the dominant hand. This latter finding is consistent with toothbrush abrasion as an etiologic factor. Dentinal pain is elicited by cold stimuli in up to 90% of patients, although mechanical (eg, toothbrushing) and chemical (eg, candy) stimuli are also effective.[6,8,9]

Several treatments have been evaluated for management of dentinal hypersensitivity. These interventions generally focus on reducing the permeability of dentinal tubules or reducing the sensitivity of dentinal neurons.

Interventions that reduce permeability of dentinal tubules

Because fluid flow is a major stimulus for activating nociceptors that innervate dentinal tubules, it is not surprising that interventions that reduce the permeability of

dentinal tubules have been evaluated for reducing dentinal hypersensitivity. The application of resins to exposed dentinal tubules has been reported to reduce dentinal hypersensitivity of multiple etiologies at up to 12 months follow-up.[11,13–16]

One group of researchers[14] developed an experimental model of dentinal sensitivity by producing uniform dentin exposures in premolars (prior to tooth extraction for an orthodontic indication). These investigators demonstrated that the application of Concise Enamel Bond (3M ESPE) to the exposed tubules produced about a twofold reduction in dentinal sensitivity compared with control teeth (Fig 9-3). Similar results were reported by the same group for actual clinical cases of dentinal hypersensitivity.[13]

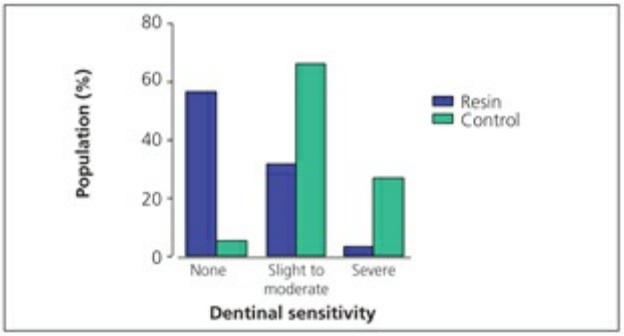

Fig 9-3 Effect of applying an unfilled resin (Concise Enamel Bond) to exposed dentin in 51 pairs of contralateral premolars in an experimental clinical model of dentinal hypersensitivity. A consistent amount of dentin exposure was made on the premolars, which were then either covered with resin or served as no-treatment controls. Dentinal sensitivity was assessed by a short pulse of compressed air on the exposed dentin. (Data from Nordenvall et al.[14])

Other interventions that block fluid flow have also been reported to be effective in treating dentinal hypersensitivity. Examples include the application of materials such as Gluma Dentin Bond (Heraeus Kulzer), oxalate salts, isobutyl cyanoacrylate, and fluoride-releasing resins or varnishes; CO_2 lasers; and the use of devices that burnish exposed dentin and even coronally positioned mucogingival flaps[16–23] (Fig 9-4). The dominant factors contributing to the efficacy of these interventions are the longevity of effective blockage of tubules and the limited potential for cytotoxicity. For example, patients who report continued efficacy of resins at 6 months after application have evidence of resin tags still blocking their dentinal tubules; patients who have recurrence of dentinal sensitivity have few or no resin tags remaining in their tubules at that point[24] (Fig 9-5).

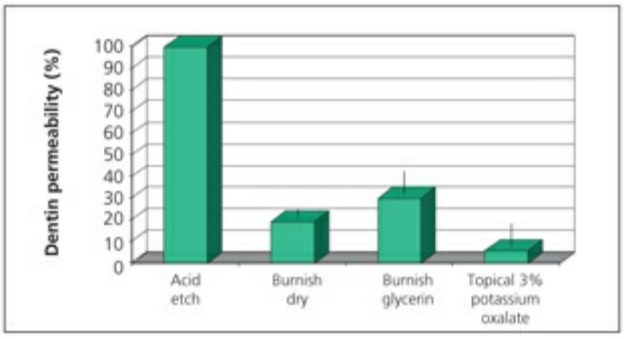

Fig 9-4 Effect of burnishing on in vitro dentin permeability. Dentin permeability after original acid etching is assigned a value of 100%. (Redrawn from Pashley et al[23] with permission.)

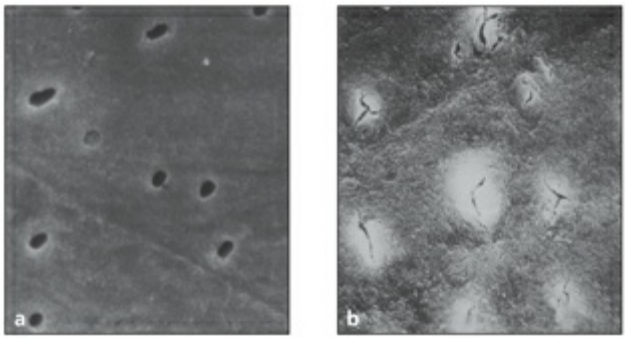

Fig 9-5 Scanning electron photomicrographs of the surface of human radicular dentin. *(a)* Hypersensitive dentin. *(b)* A tooth 6 months after application of a resin liner. Note the occluded tubules. The patient reported a lack of dentinal sensitivity after the application of resin. (Reprinted from Yoshiyama et al[24] with permission.)

However, many studies are simply before-and-after comparisons, and few studies directly compare efficacy and adverse effects among several active treatments. The lack of direct comparisons and systematic evaluations makes it difficult to determine which of the proposed treatment regimens offers the greatest efficacy and duration with the least potential for adverse effects. Thus, further research is required in this important therapeutic area.

Interventions that reduce sensitivity of dentinal neurons

The second general method to reduce dentinal sensitivity employs interventions that reduce neuronal responsiveness to dentinal stimuli. Both preclinical studies and clinical trials have been used to evaluate these agents.

The preclinical studies often employ multiunit intradental electrophysiologic

recordings of fibers that innervate dentinal tubules. For example, some researchers have used an anesthetized feline model with multiunit recordings of dentinal neurons using a pretreatment design.[25,26] In this design, desensitizing agents were applied to exposed dentinal tubules prior to the application of excitatory agents (eg, hypertonic sodium chloride). This model has been used to evaluate the efficacy of numerous compounds for reducing activation of dentinal neurons. In general, these studies indicate that application of potassium reduces neuronal activity regardless of the paired anion, that divalent cations are effective inhibitors of dentinal neurons, and that nitrate (NO_3) is not effective in altering neuronal activity[26] (Fig 9-6). However, it should be noted that the deep cavity preparations used in this model (ie, 20 to 50 mm of remaining dentin) might lead to an overestimate of drug efficacy because diffusion through dentinal fluid has been minimized. Moreover, no clinical studies to date have demonstrated that the concentrations of the test agents achieved in these experimental models are similar to actual concentrations achieved during therapeutic use in human dentinal fluid.

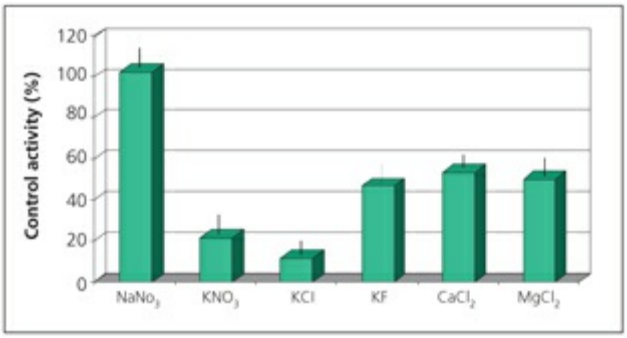

Fig 9-6 Effect of test desensitizing agents on dentinal neuron activity in exposed dentin. Data are presented as percentages of neuron activity compared with that of a control (ie, application of hypertonic [3.0 M] sodium chloride is defined as 100%). The test agents are sodium nitrate ($NaNO_3$), 1.10 M; potassium nitrate (KNO_3), 1.0 M; potassium chloride (KCl), 0.76 M; potassium fluoride (KF), 0.05 M; calcium chloride ($CaCl_2$), 0.76 M; and magnesium chloride ($MgCl_2$), 0.76 M. (Adapted from Markowitz and Kim[26] with permission.)

Several major reviews have recently summarized the outcomes of clinical trials evaluating desensitizing agents.[15,27–29] As already mentioned, variations in the methodology or design of the clinical trials (ie, techniques for assessing hypersensitivity, study duration, data analysis, comparison to active controls) may often lead to differences in study outcome. Accordingly, in an attempt to identify treatments with high efficacy, the following review focuses on those interventions that have been reported to be successful in multiple randomized, double-blind studies.

Potassium-containing dentifrices have been found to be effective in a majority of

randomized, double-blind clinical trials. The placebo controls in these studies consisted of the dentifrice without the potassium nitrate (KNO_3) component. In a review of the literature,[29]73% of formulations of potassium nitrate–containing dentifrices were significantly better than placebo at reducing dentinal hypersensitivity to application of cold air pulses (Table 9-1). When analyzed on a sample-weighted basis, the overall results indicated that placebo dentifrices reduced sensitivity to cold by about 35%; 3.75% KNO_3 dentifrices reduced sensitivity to cold by about 60.8%; and 5% KNO_3 dentifrices reduced sensitivity to cold by about 61.2% (see Table 9-1). This finding is clinically relevant because cold is reported to be the most common stimulus of dentinal hypersensitivity.[6,8,9] Moreover, 64% of formulations of KNO_3-containing dentifrices were significantly better than placebo for overall global reduction in dentinal hypersensitivity symptoms (Table 9-2). Thus, the majority of randomized, controlled clinical trials indicate that potassium-containing dentifrices are effective in reducing dentinal hypersensitivity.[30–40]

Table 9-1 **Efficacy of various potassium dentifrice formulations on cold air-induced dentinal hypersensitivity***

Study†	% Active agent (KNO_3)	N	Duration (wk)	Active (%)‡	Placebo (%)‡	Significance (*P* value)
Tarbet et al (1980)[30]	5	27	4	65	20	< .05
Manochehr-Pour et al (1984)[31]	5	75	12	63	37	NS
Manochehr-Pour et al (1984)[31]	5	75	12	52	37	NS
Silverman (1985)[32]	5	68	12	75	40	< .001
Silverman (1985)[32]	5	68	12	78	40	< .001
Salvato et al (1992)[33]	3.75	41	12	66	32	.001
Nagata et al (1994)[34]	5	36	12	80	27	< .01
Schiff et al (1994)[35]	5	60	12	61	0	< .001

Silverman et al (1994)[36]	3.75	62	8	66	28	< .05
Silverman et al (1994)[36]	3.75	62	8	61	28	< .05
Silverman et al (1996)[37]	5	220	8	54	30	< .001
Gillam et al (1996)[38]	3.75	56	6	51	48	NS
West et al (1997)[39]	5	112	6	48	53	NS
Schiff et al (1998)[40]	5	39	8	82	40	< .0001

*Data from Orchardson and Gillam.[29]
†Studies in which different formulations were compared have separate listings.
‡Percentage reduction in sensitivity to cold air stimulus from baseline values.
NS, not significant.

Table 9-2 **Efficacy of various potassium dentifrice formulations on patients' global subjective ratings of dentinal hypersensitivity***

Study†	% Active agent (KNO_3)	N	Duration (wk)	Active (%)‡	Placebo (%)‡	Significance (*P* value)
Tarbet et al (1980)[30]	5	27	4	92	21	< .001
Manochehr-Pour et al (1984)[31]	5	75	12	54	60	NS
Manochehr-Pour et al (1984)[31]	5	75	12	36	60	NS
Silverman (1985)[32]	5	68	12	75	36	< .001
Silverman (1985)[32]	5	68	12	75	36	< .001
Salvato et al (1992)[33]	3.75	41	12	75	23	< .001

Nagata et al (1994)[34]	5	36	12	82	28	< .01
Schiff et al (1994)[35]	5	60	12	52	30	< .01
Silverman et al (1994)[36]	3.75	62	8	61	32	< .1
Silverman et al (1994)[36]	3.75	62	8	52	32	< .05
Silverman et al (1996)[37]	5	220	8	55	29	<.001
Gillam et al (1996)[38]	3.75	56	6	54	29	< .001
West et al (1997)[39]	5	112	6	54	43	NS
Schiff et al (1998)[40]	5	39	8	30	19	NS

*Data from Orchardson and Gillam.[29]
†Studies in which different formulations were compared have separate listings.
‡Percentage reduction in patients' subjective ratings to overall dentinal sensitivity compared with baseline values.
NS, not significant.

The efficacy of other agents for reducing dentinal hypersensitivity has also been evaluated in clinical trials. For example, 10% strontium chloride ($SrCl_2$) has been evaluated in numerous studies. In two-cell studies (ie, $SrCl_2$ versus placebo dentifrice), the weighted mean efficacy of $SrCl_2$ was a 72.5% reduction in hypersensitivity, while the mean efficacy of the placebo dentifrice was a 34.3% reduction in hypersensitivity.[28] However, in studies consisting of three or more cells (ie, at least two active groups and a placebo dentifrice), the weighted mean efficacy of $SrCl_2$ was only 51.8% reduction in hypersensitivity, while the mean efficacy of the placebo dentifrice was a 41.0% reduction in hypersensitivity.[28] When the results of both types of study are combined, $SrCl_2$ produced about a 50% reduction in dentinal hypersensitivity. Clearly, experimental design issues contribute to the variations in efficacy estimates for desensitizing dentifrices.

The efficacy of fluoride-containing medicaments on dentinal hypersensitivity has been evaluated in several clinical trials. One meta-analysis of seven clinical trials reported that the application of 0.717% tin(II) fluoride (SnF_2) gel to exposed dentin for 3 to 5 minutes produced a significant and prolonged reduction in dentinal hypersensitivity.[41] Application of 0.4% SnF_2 gel produced a delayed effect, requiring repeated applications for several weeks. Fluoride-containing dentifrices also have been reported to reduce dentinal hypersensitivity in some but not all studies.[42–44]

Guanethidine has also been evaluated for management of dentinal hypersensitivity. In a randomized, double-blind study,[45] the application of a 1% solution of guanethidine to exposed dentinal tubules produced about a 50% reduction in sensitivity compared with baseline values (Fig 9-7). This effect was similar to a preliminary study by the same investigators.[46]

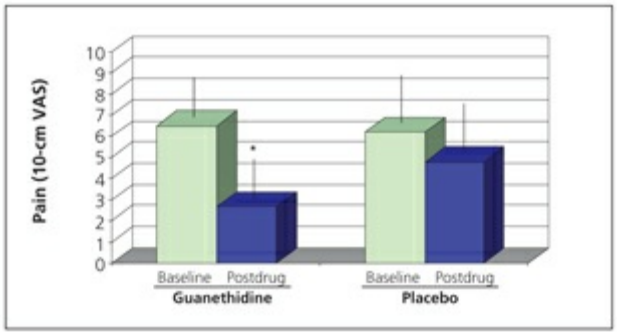

Fig 9-7 Effect of topical application of a 1% solution of guanethidine or placebo on dentinal hypersensitivity to cold air stimulation. VAS, visual analog scale. N = 39 patients; $*P < .05$ versus placebo posttreatment. (Data from Dunne and Hannington-Kiff.[45])

Guanethidine is known to act by inhibiting exocytosis from peripheral terminals of sympathetic fibers and has been shown previously to block sympathetically regulated blood flow in dental pulp.[47] However, guanethidine may also alter the activity of nociceptors. The application of guanethidine to human skin increases pricking pain thresholds, an effect not observed with other vasodilators.[48] Mashimo et al[48] concluded that guanethidine directly alters human nociceptors, a process that may involve Aδ nociceptors, because these neurons are thought to encode for pricking pain sensation. However, other studies have suggested that guanethidine also acts on the capsaicin-sensitive class of unmyelinated nociceptors.[49] Collectively, these studies suggest that further research is warranted on the potential application of guanethidine for treating dentinal hypersensitivity.

In summary, dentinal hypersensitivity represents a common, widespread form of

odontogenic pain that is believed to be mediated primarily by activation of neurons that innervate dentinal tubules. The diagnosis of dentinal hypersensitivity is based on the eliciting stimuli, the duration and location of the pain, and the absence of pulpal (ie, symptoms) or radiographic (ie, changes seen) pathoses. The management of dentinal hypersensitivity involves the application of therapies that either reduce flow of dentinal fluid (see also chapter 3) or reduce the activity of dentinal neurons.[15]

In addition, patients should be advised to avoid habits or agents that increase dentinal permeability. For example, studies have reported that tartar-control dentifrices and toothbrushing in the presence of dietary acids or certain mouthwashes (eg, those containing hexetidine or fluoride/antiseptic) may increase the risk of dentinal sensitivity by removing the smear layer of exposed dentinal tubules.[50–52]

Management of Pulpitis and Related Pain Conditions

Another common form of odontalgia is the result of pulpitis or periradicular pain pathoses. For the purposes of this chapter, this form of odontalgia includes pain resulting from activation of pulpal or periradicular nociceptors. It is appropriate to include periradicular nociceptors in this category because the accumulation of bacteria and bacterial by-products from necrotic root canal systems constitutes the predominant etiologic factor for periradicular inflammation and pain (see chapter 12), another good example of the relationship between dental pulp and other tissues during disease. Thus, relevant clinical diagnoses will include odontogenic pain of inflammatory etiologies, such as irreversible pulpitis and pulpal necrosis with acute apical periodontitis.

This section reviews both pharmacologic and nonpharmacologic methods for pain control. The clinician must use an integrated approach in combining these methods to control dental pain, which has been called the *3D method* for pain control: *diagnosis*, *definitive dental treatment* (nonpharmacologic methods), and *drugs* (pharmacologic methods).[53] This approach is summarized in Box 9-1.

Diagnosis of odontalgia

Diagnosis is of obvious importance in managing acute pain because effective treatment is directed at removing the etiology of pain. For example, nitroglycerin is effective for reducing anginal pain, yet it has comparatively little analgesic activity in most other pain conditions. Similarly, if a patient has pain associated with an abscess, then an incision for drainage may prove effective in reducing pain. In both examples, the treatments are effective because they reduce the etiologic factors that elicit the pain.

Box 9-1 **3D method for managing acute odontogenic pain***

- Diagnosis
- Definitive dental treatment:
  - Pulpotomy, pulpectomy
  - Extraction
  - Incision for drainage
- Drugs:
  - Pretreat with nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen when appropriate
  - Prescribe medications to be taken "by the clock" rather than "as necessary"
  - Use long-acting local anesthetics when indicated
  - Use a flexible prescription plan

* Based on Keiser and Byrne.[53]

For patients with acute pain, an accurate diagnosis generally leads to predictable treatment strategies, but, for patients with chronic pain, the etiologies of pain are less well understood and probably multifactorial.[54] Therefore, the first step in treating the acute pain patient is establishing the diagnosis. This information is reviewed extensively in several excellent endodontic texts and need not be covered here.[55–57] The clinician should be aware of differential diagnoses for patients who present with a chief complaint of tooth pain because etiologies, treatment strategies, and prognoses vary considerably[54,58–60] (see also chapter 19).

Nonpharmacologic methods of managing odontogenic pain

Numerous studies indicate that definitive dental treatment alone provides predictable and substantial relief from odontogenic pain. In patients with irreversible pulpitis, pulpotomy treatment, regardless of the coronal medicament used, has been reported to abolish pain symptoms in 88% of patients evaluated 1 day

after treatment.[61] The efficacy of definitive dental treatment for reducing acute pain symptoms has been confirmed by many other studies. Figure 9-8 summarizes the results in more than 1,000 patients in clinical trials to evaluate the efficacy of pulpotomy or pulpectomy for relieving odontogenic pain.[62] Because pulpotomies are only performed for vital endodontic cases (eg, pain of pulpal origin) while pulpectomies are performed to manage more complex pain conditions (ie, pain of pulpal and/or periradicular origin), it is not surprising that pulpotomies appear more effective than pulpectomies for relieving pain.[61–70]

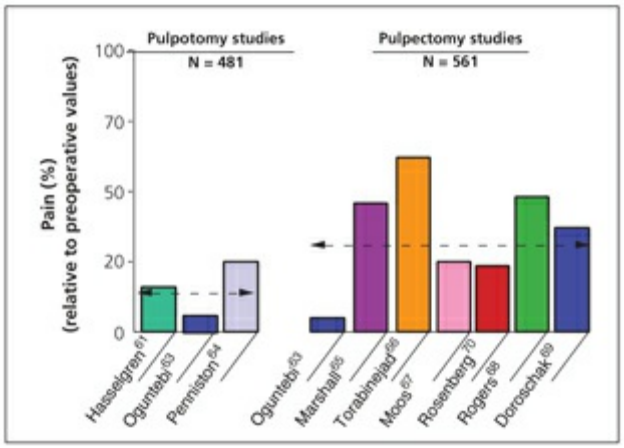

Fig 9-8 Effects of pulpotomy and pulpectomy on endodontic-related pain. Preoperative pain values are normalized to 100%. The two *dashed horizontal lines* represent the sample size–weighted mean reduction in pain for the pulpotomy and pulpectomy groups. Only the first author of each study is named. (Reprinted from Hargreaves and Baumgartner[62] with permission.)

Other forms of definitive dental treatment include occlusal adjustment, trephination, and incision for drainage procedures. In one study of 117 patients,[70] about twice as many patients who underwent occlusal adjustment reported no posttreatment pain compared to no-treatment control subjects (Fig 9-9). The authors concluded that occlusal adjustment was particularly effective for reducing pain in patients who had vital pulps and percussion sensitivity.

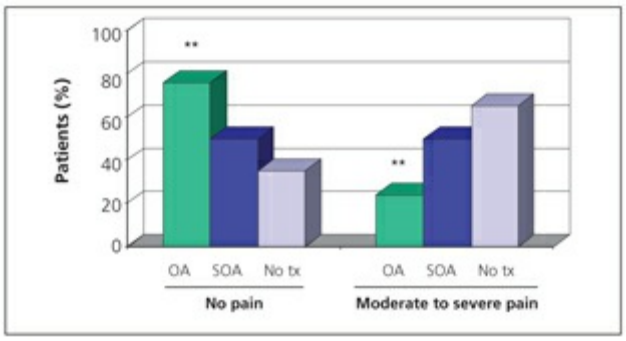

Fig 9-9 Effect of occlusal adjustment (OA), simulated occlusal adjustment (SOA, by reduction of nonfunctional cusp), or no treatment (No tx) on the incidence of posttreatment pain in 117 endodontic

patients. **$P < .01$. (Redrawn from Rosenberg et al[70] with permission.)

Another nonpharmacologic approach is to reduce intraosseous pressure. Although trephination has been advocated to reduce pain after endodontic therapy, several randomized clinical trials have failed to demonstrate a significant difference between the effect of trephination with pulpectomy and the effect of pulpectomy alone.[67,71,72] In one study, patients were randomly placed in either of two groups: pulpotomy or pulpotomy with periapical trephination. There was no difference between groups in postoperative pain, swelling, percussion pain, or use of analgesic tablets.[72] Thus, this technique does not appear to result in a clinically reproducible reduction in inflammatory mediators or interstitial pressure.

The pain-relieving benefits of these definitive dental treatments are believed to be based on a reduction in tissue levels of factors that stimulate peripheral terminals of nociceptors (see chapters 7, 8, and 11) or a reduction in the mechanical stimulation of sensitized nociceptors (eg, occlusal adjustment). Effective chemomechanical debridement of the infected root canal system combined, when indicated, with incision for drainage procedures provides predictable pain reduction in patients with an endodontic emergency. Of course, if the tooth has a hopeless prognosis, extraction will also reduce pain by reducing tissue levels of these factors. From this perspective, it can be concluded that treating the unscheduled emergency patient with drugs alone is not a definitive intervention. Instead, pharmacologic management of pain should be considered with definitive dental treatment as a combined therapeutic approach to managing odontogenic pain.

Although space limitations preclude a thorough review, other nonpharmacologic approaches have been evaluated for reduction of dental pain. These approaches include cognitive, motivational, and affective interventions and range from simple approaches such as establishing a confident doctor-patient relationship to more complex procedures.[73] For example, hypnosis has been reported to be an effective adjunct for management of dental pain in endodontic patients.[73–75] Other studies have demonstrated that instructing patients to focus on sensory stimuli significantly reduces intraoperative endodontic pain.[76,77] This effect was most evident in patients who were characterized as having a high desire for control and low perceived control over their clinical care. Thus, a number of nonpharmacologic approaches may be considered as a component of the overall strategy of managing dental inflammatory pain.

Pharmacologic methods of managing odontogenic pain

Three primary pharmacologic approaches to odontogenic pain control are reviewed in this section: *(1)* drugs that block inflammatory mediators that sensitize or activate pulpal nociceptors, *(2)* drugs that block the propagation of impulses along peripheral nerves, and *(3)* drugs that block central mechanisms of pain perception and hyperalgesia.

Drugs that block inflammatory mediators that sensitize or activate pulpal nociceptors

Although numerous clinical pharmacology studies have evaluated analgesics for treatment of dental pain, the majority of these studies employ a clinical model of acute inflammatory pain elicited by surgical trauma. Acute surgery-induced inflammation is mediated largely by release of eicosanoids such as prostacyclin and prostaglandin E_2 (PGE_2) as well as other inflammatory mediators such as bradykinin.[78] In contrast, pulpal and periradicular pain is often associated with chronic inflammation, characterized by the presence of bacterial by-products, an influx of immune cells with activation of the cytokine network, and other inflammatory mediators (see chapters 10, 11, and 12). These considerations suggest that the composition and concentrations of inflammatory mediators that activate and sensitize nociceptors likely differ in these two models of orofacial pain. Moreover, the chronicity of pulpal inflammation permits sprouting of nociceptor terminals, and thus the peripheral anatomy of the pain system changes during pulpal inflammation (see chapter 7). Therefore, it is likely that the relative efficacy of analgesics for the treatment of surgery-induced pain and pulp-related pain differs. Accordingly, this review focuses on clinical trials of endodontic pain, incorporating clinical trials of surgery-induced pain only to illustrate additional concepts.

One major class of drugs for managing endodontic pain is the non-narcotic analgesics, which include both the nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen. Studies have indicated that these drugs produce analgesia by actions in both the peripherally inflamed tissue as well as in certain regions in the brain and spinal cord.[79–81] NSAIDs have been shown to be very effective for managing pain of inflammatory origin that arises from either surgery-induced trauma or pulpal and periradicular pain.[53,62,64,66,69,79,82] Indeed, the results of several randomized, double-blind, placebo-controlled clinical trials in endodontic pain

patients[64,66,69,82] indicate that ibuprofen (400 mg), ketoprofen (50 mg), flurbiprofen (100 mg), and ketorolac (30 to 60 mg) all produce significant analgesia compared to a placebo medication (Figs 9-10 and 9-11).

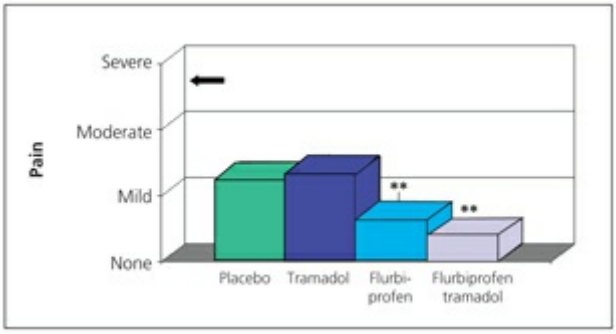

Fig 9-10 Comparison of tramadol (100-mg initial dose, then 100 mg every 6 h), flurbiprofen (100-mg loading dose, then 50 mg every 6 h), and combination flurbiprofen and tramadol (same dosages) to placebo in endodontic patients. Patients received local anesthesia, pulpectomies, and drugs. Pain levels were reported 24 hours after treatment. The *arrow* shows the mean preoperative pain level. N = 11 to 12 per group; $**P < .01$ versus placebo. (Redrawn from Doroschak et al[69] with permission.)

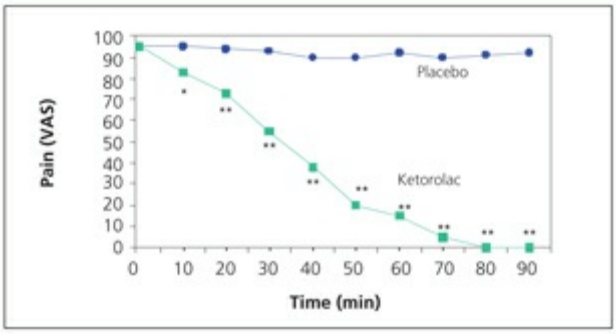

Fig 9-11 Comparison of ketorolac (60-mg intramuscular injection) to placebo for relief of endodontic pain. Patients (N = 40) completed a baseline visual analog scale (VAS) for pain, received a drug injection, and then completed postdrug pain scales. No endodontic treatment or local anesthetic was provided. $*P < .05$ versus placebo; $**P < .01$ versus placebo. (Redrawn from Curtis et al[82] with permission.)

In interpreting these studies, it is important to realize that endodontic treatment alone (eg, pulpectomy) has a major effect on reducing pain regardless of pharmacologic treatment (see Fig 9-8). This reduction in posttreatment pain combined with variable levels of preoperative pain reduces the statistical power of endodontic clinical trials for detecting active analgesics over time or in all patient groups (the so-called floor effect). This limitation causes a problem in interpreting clinical pain studies[83] in general and may explain why some endodontic clinical trials fail to detect analgesic treatment or only detect it in those patients with moderate to severe preoperative pain.[66,68]

Although many NSAIDs are available in the marketplace, comparatively few endodontic studies have directly compared one NSAID with another to determine analgesia and side-effect liability. In a study based on the oral surgery model, liquigel ibuprofen (400 mg) had superior overall efficacy and provided faster pain relief than ketoprofen (25 mg), acetaminophen (1,000 mg), and placebo.[84] In one postendodontic study, ibuprofen (400 mg) was similar to ketoprofen (50 mg) for the time course in superiority to placebo treatment.[66]

The lack of comprehensive comparative studies of endodontic pain means that only general recommendations can be made, and thus the clinician is encouraged to be familiar with several of these drugs. Ibuprofen is generally considered the prototype of NSAIDs and has a well-documented efficacy and safety profile.[85] The advantages of NSAIDs include their well-established analgesic efficacy for management of inflammatory pain. Indeed, many NSAIDs have been shown to be more effective than traditional acetaminophen-opioid combination drugs such as acetaminophen 650 mg with codeine 60 mg (for review, see Keiser and Byrne[53]).

NSAIDs have a relatively high affinity to plasma proteins, and they are preferentially distributed to inflamed tissue by local vasodilation and plasma extravasation.[86] Thus, NSAIDs are preferentially distributed into inflamed dental pulp compared with control dental pulp (Fig 9-12). This quality may improve the relative efficacy of NSAIDs compared with other analgesic drug classes.

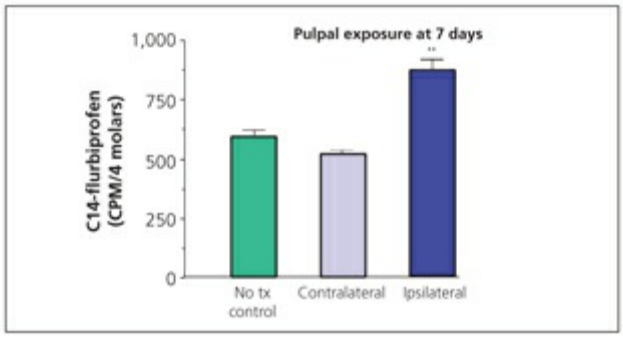

Fig 9-12 Preferential distribution of NSAIDs into inflamed pulp. Rats were anesthetized and underwent either no treatment (No tx) or pulpal exposure of the mandibular first molar. Seven days later, rats were anesthetized and received an intravenous injection of C14-flurbiprofen. No-treatment control (from a separate group of animals), contralateral (no pulpal exposure), and ipsilateral (pulpal exposure) teeth were extracted, homogenized, and analyzed for C14-flurbiprofen. CPM, counts per minute. N = 7 rats per group; $^{**}P < .01$ versus both the contralateral and no-treatment control groups. (Redrawn from Bunczak-Reeh and Hargreaves[86] with permission.)

Studies indicate that the two isoforms of cyclo-oxygenase (COX) differ in tissue distribution and potential for side-effect mediation.[87] COX-2 inhibitors may have analgesic efficacy in pulpal pain conditions because the COX-2 enzyme is elevated

in inflamed human dental pulp as compared with control pulpal tissue.[88] Celecoxib, the prototype selective COX-2 inhibitor, is cleared by the US Food and Drug Admisnistration (FDA) for acute pain. However, concern has been raised that COX-2 inhibitors may also cause at least some gastrointestinal irritation in patients with preexisting disease, suggesting these drugs should be used with some caution in certain patients.[89] More research is warranted on the potential efficacy and side-effect liability of COX-2 inhibitors for the treatment of endodontic pain.

One of the major adverse effects of NSAIDs is the risk of serious or fatal gastrointestinal bleeding.[90] The risk of developing an NSAID-associated gastrointestinal complication is highest among patients taking concurrent aspirin, steroid, or warfarin; patients with a previous history of an ulcer hemorrhage; and patients older than 65 years.[91] One of the strategies used to prevent gastrointestinal bleeding is to use a proton pump inhibitor with an NSAID. A combination of a proton pump inhibitor, esomeprazole magnesium, and naproxen (Vimovo, AstraZenica) is now available. While this is a cost-effective approach, it does not protect the lower gastrointestinal tract.[92]

Another approach is to use a histamine (H_2) receptor antagonist in combination with an NSAID. The combination of famotidene (an H_2 receptor antagonist) and ibuprofen (Duexis, Horizon Pharma) was recently cleared by the FDA. A third approach is to use a selective COX-2 inhibitor instead of an NSAID.

Although space limitations preclude an extensive review of contraindications for NSAIDs, the clinician should be aware of these issues and understand that acetaminophen, either alone or in an acetaminophen-opioid combination, may represent an alternative for patients unable to take NSAIDs.[93,94] Extensive reviews of the pharmacology and adverse effects of this important class of analgesics are available.[93–97] Other venues (eg, Internet-based drug search engines) are also available for evaluating newly released analgesics as well as potential adverse effects, contraindications, and drug interactions. These sites include www.rxlist.com, www.pharminfo.net, and www.epocrates.com, among others.

Acetaminophen (N-acetyl-p-aminophenol), used alone or in combination with an NSAID or a narcotic, is also used for pain relief. In a study evaluating pain following pulpectomy, the combination of acetaminophen (1,000 mg) and ibuprofen (600 mg) was shown to provide greater pain relief than ibuprofen (600 mg) alone.[98] Approximately 184 acetaminophen compounds are available as over-the-counter or prescription drugs.

The widespread use of acetaminophen has resulted in a substantial increase in the

number of cases of acute liver toxicity. The factors known to contribute to acetaminophen liver toxicity are listed in Box 9-2.[99,100] The FDA has taken new steps to reduce the risk of liver injury associated with the use of acetaminophen. These include limiting the maximum amount of acetaminophen in prescription acetaminophen combination products to 325 mg per tablet, capsule, or other dosage unit and requiring a boxed warning on all prescription acetaminophen products to highlight the potential risk for severe liver injury.

Box 9-2	**Factors associated with increased risk of acetaminophen-induced liver toxicity***

- Ingestion of more than 4 g of acetaminophen within a 24-hour period (this includes unintentional overdose in patients taking multiple acetaminophen combinations for different conditions and patients taking brand names of acetaminophen-containing products that do not definitively disclose that acetaminophen is an ingredient)
- Fasting or malnourishment
- Preexisting liver disease
- Genetic predisposition to liver injury
- Alcohol abuse
- Concomitant use of phenytoin, isoniazid, some protease inhibitors such as ritonavir, or zidovudine

*Information from Schilling et al[99] and Guggenheimer and Moore.[100]

Steroids, or more properly glucocorticoids, form an additional class of drugs that interfere with the production or release of mediators that activate or sensitize nociceptors. Glucocorticoids are known to reduce the inflammatory response by suppressing vasodilation, neutrophil migration, and phagocytosis and by inhibiting the formation of arachidonic acid from neutrophil and macrophage-cell membrane phospholipids, thereby blocking the cyclo-oxygenase and lipoxygenase pathways and respective synthesis of prostaglandins and leukotrienes. Thus, it is not surprising that a number of investigations have evaluated the efficacy of corticosteroids for the prevention or control of postoperative endodontic pain.

Several clinical trials have evaluated the efficacy of glucocorticoids for reducing postendodontic pain after intracanal, oral, or intramuscular administration. In general, intracanal steroids appear to provide the most consistent reductions in postoperative pain or flareups when used in vital teeth.[68,101–103] The reduced efficacy reported in necrotic teeth may be due to poor absorption via this route of administration, to diffusion of inadequate amounts of the drug into the periradicular tissue, or to other factors such as experimental design or statistical power.

Other studies have evaluated the effects of systemic administration of

corticosteroids on postoperative pain or flareups. For example, a double-blind, parallel-design clinical trial reported that pretreatment with a single oral dose of prednisolone reduced postendodontic pain for up to 24 hours in teeth with vital and necrotic pulps.[104] In general, these studies indicate that systemically administered corticosteroids reduce the severity of posttreatment endodontic pain compared to placebo treatment, but with a time course of approximately 8 to 24 hours[105–109] (Fig 9-13).

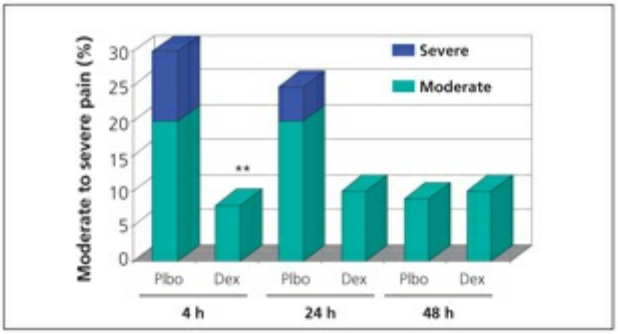

Fig 9-13 Comparison of dexamethasone (Dex) (4-mg intramuscular injection) to placebo (Plbo) for pain after endodontic instrumentation. N = 50; $^{**}P < .01$ versus placebo. (Redrawn from Marshall and Walton[105] with permission.)

As described earlier, the lack of a drug effect at later time points may be due in part to the pain-relieving effects of endodontic instrumentation. In support of this point, a study on the effects of intraosseous steroid injection on pain in patients with untreated pulpitis[110] demonstrated that steroids produced a significant reduction in pulpitis pain over a 7-day observation period (Fig 9-14). Thus, steroids can elicit long-term reduction in pulpal pain in patients who receive no endodontic treatment. Interestingly, 95% of steroid-treated dental pulps and 81% of the placebo-treated dental pulps were still vital at the 7-day observation.

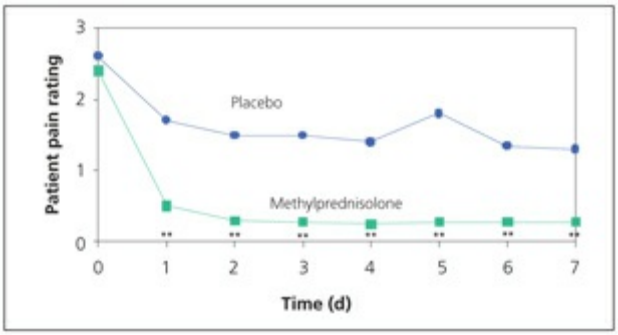

Fig 9-14 Comparison of the effects of methylprednisolone (40-mg intraosseous injection) to placebo for relief of pain due to irreversible pulpitis. After diagnosis and drug injection, patients reported pain levels for 7 days before any endodontic treatment was started. N = 40; $^{**}P < .01$ versus placebo. (Redrawn from Gallatin et al[110] with permission.)

Taken together, these studies indicate that drugs that suppress the release or actions of inflammatory mediators on nociceptors have an analgesic effect on pain caused by inflammation of pulpal and periradicular tissue. The design of a pain-management plan is considered later in this chapter.

Drugs that block the propagation of impulses along peripheral nerves

Local anesthetics represent an important component in pain control. Local anesthetics offer the benefit of prolonged pain control following completion of the endodontic appointment. Long-acting local anesthetics etidocaine and bupivacaine offer prolonged pain control 6 hours or more after injection.[111–114] However, research on pain mechanisms has revealed the existence of a central component to hyperalgesia, which can be established by an intense barrage of activity from peripheral nociceptors, particularly the unmyelinated C nociceptors (see chapter 8). The preemptive analgesia hypothesis states that clinical pain is reduced when the peripheral barrage of nociceptors is reduced.[115]

Subsequent studies on orofacial pain have provided support for this concept. For example, in a preclinical study, administration of 0.5% bupivacaine with 1:200,000 epinephrine, but not 2% lidocaine with or without 1:100,000 epinephrine, prior to pulpal exposure reduced Fos expression (a marker of neuronal activity) in the spinal trigeminal nucleus.[116] The same study showed that administration of morphine prior to pulpal exposure also reduced Fos expression, suggesting that preemptive treatment with a long-acting anesthetic and/or opioid can reduce postoperative pain.

In a clinical study, patients given an inferior alveolar nerve block injection with bupivacaine prior to an oral surgical procedure reported less pain 24 and 48 hours after the procedure than did placebo-injected patients.[117] This result is not restricted to extraction procedures because infiltration with bupivacaine prior to tonsillectomy reduced postoperative pain for 7 days compared with patients given placebo injections.[118] Similarly, other clinical studies have shown that the preemptive administration of opioids reduces postoperative pain.[119,120] Collectively, these studies indicate that measures that reduce the peripheral barrage of nociceptors, such as long-acting local anesthetics, should be considered for treatment of endodontic pain and that they may reduce posttreatment pain even days after a single administration. The clinician must be aware of the potential adverse side-effect profile of opioids and long-acting local anesthetics, including contraindications for

cardiovascular patients.[121,122]

Factors that affect the success of local anesthesia include the type of local anesthetic used, the speed of injection, the route of administration, and the presence of inflammation. Data from several clinical trials demonstrate that lidocaine provides predictable success when used for maxillary infiltration, inferior alveolar nerve block, or intraosseous injections.[123–126] A combination of lidocaine with clonidine may result in less postoperative pain and thus may be a better alternative to lidocaine with epinephrine for surgical procedures.[127]

Another commonly used anesthetic that is comparable to lidocaine is 3% mepivacaine. A randomized, double-blind clinical study compared inferior alveolar nerve blocks with 3% mepivacaine, 4% prilocaine, and 2% lidocaine with 1:100,000 epinephrine. No statistically significant differences were detected in the onset, success or failure, or duration of pulpal anesthesia among the three solutions.[128]

Articaine has a reputation for providing improved local anesthetic effect. However, it is yet to be clearly demonstrated that the use of articaine results in greater magnitude or duration of anesthesia than lidocaine. A randomized, double-blind crossover study in normal volunteers demonstrated that the effects of 4% articaine with 1:100,000 epinephrine did not differ from 2% lidocaine with 1:100,000 epinephrine when used to obtain inferior alveolar nerve blocks.[129] In a study comparing the analgesic efficacy of the two solutions when administered as a supplemental buccal infiltration following inferior alveolar nerve block with 4% articaine with 1:100,000 epinephrine, the success of pulpal anesthesia— no response to the maximum output of an electrical pulp tester—was greater with 4% articaine with 1:100,000 epinephrine (88%) than with 2% lidocaine with 1:100,000 epinephrine (71%).[130] Similar results were noted in a study comparing buccal and lingual infiltrations of 4% articaine with 1:100,000 epinephrine and 2% lidocaine with 1:100,000 epinephrine in teeth with irreversible pulpitis.[131] A meta-analysis comparing articaine and lidocaine concluded that articaine had a higher success rate than lidocaine when administered as a local infiltrate (odds ratio 3.81 [95% confidence interval, 2.71–5.36; $P < .00001$]).[132] When used for mandibular block anesthesia, articaine was superior to lidocaine only in asymptomatic or normal teeth but not in symptomatic teeth.

The speed of injection may also influence the success of pulpal anesthesia. In a randomized, double-blind, crossover clinical trial evaluating the efficacy of inferior alveolar nerve blocks, slow injection (60 seconds) of 2 mL of 2% lidocaine with

1:80,000 epinephrine resulted in a higher success rate of pulpal anesthesia than fast injection (15 seconds). Although this study was conducted on teeth with normal pulps, the success rate was not 100%.[133] A prior similar study by the same group[134] evaluating the speed of injection for incisive/mental nerve block showed no difference in the effectiveness based on speed. The reason for this discrepancy is not clear but may be related to differences in experimental design (eg, differences in injection volume).

The rate of failure of local anesthetics is higher in inflamed tissues than in normal tissues. Inflammatory mediators activate or sensitize peripheral nociceptors and thus contribute to the peripheral mechanisms of reduced pain threshold (allodynia) or increased responsiveness to painful stimuli (hyperalgesia). Mediators such as PGE_2, nerve growth factor, and serotonin increase the activity of tetrodotoxin-resistant sodium channels.[135–137] Because these channels are only one-quarter as sensitive to lidocaine as other sodium channels, their increased activity during inflammation is thought to account in part for the failure of local anesthetics in inflamed tissues.[136,138] Because PGE_2 is thought to play a key role in sensitizing this channel, it is possible that NSAIDs enhance the efficacy of local anesthetics by reducing PGE_2-mediated channel phosphorylation.[139]

Several important pharmacokinetic and pharmacodynamic characteristics of intraosseous local anesthetic injection have been determined using normal volunteers in whom pulpal anesthesia was measured with an electric pulp tester. Intraosseous injection of 1.8 mL of 2% lidocaine with 1:100,000 epinephrine provided significant anesthesia for 74% of first molars.[140] The dosage of 2% lidocaine with epinephrine produced a transient (4-minute) period of tachycardia with an average increase in rate of 28 beats per minute.[141] The intraosseous administration of a 1.8-mL volume of 3% mepivacaine produced a 45% anesthesia rate in mandibular first molars, and anesthetic success increased with a second injection of 3% mepivacaine.[140,142] The intraosseous injection of 3% mepivacaine had no significant cardiovascular effects.[141] About 5% of patients had delayed healing at the site of injection.[140] The intraosseous injection of 1.8 mL of 4% articaine with 1:100,000 epinephrine provided significant anethesia in 86% of the teeth; it produced tachycardia with an average increase in rate of 32 beats per minute.[143]

Additional studies have evaluated the efficacy of a supplemental intraosseous injection of local anesthetics in clinical patients with pain caused by irreversible pulpitis.[142,144,145] In one study of 48 patients with irreversible pulpitis in mandibular

teeth, an inferior alveolar nerve block of 2% lidocaine with 1:100,000 epinephrine was only 25% successful for pulpal anesthesia, as defined by a negative response to the electrical pulp tester and patients' ratings of no or only mild pain upon endodontic access; a supplementary intraosseous injection of 3% mepivacaine increased anesthetic success to 80%.[142] In a parallel study of 51 patients with irreversible pulpitis of mandibular teeth, an inferior alveolar nerve block of 2% lidocaine with 1:100,000 epinephrine was only 19% successful for pulpal anesthesia; a supplementary injection of 2% lidocaine with 1:100,000 epinephrine increased anesthetic success to 91%.[144] Similar results have been observed in another clinical trial of irreversible pulpitis in maxillary and mandibular teeth.[142] The supplemental intraosseous injection of 1.8 mL of 4% articaine with 1:100,000 epinephrine was 86% successful for pulpal anesthesia in teeth with irreversible pulpitis.[143] Collectively, these studies indicate that intraosseous injection of 3% mepivacaine, 2% lidocaine with 1:100,000 epinephrine, or 4% articaine with 1:100,000 epinephrine can lead to a threefold to fourfold improvement in anesthetic success in patients with irreversible pulpitis.

Space restrictions preclude a detailed review of the technique for intraosseous injection or other routes of injection and the relative indications and contraindications of various local anesthetics. The clinician is encouraged to seek this information in one of the excellent clinical texts that are available.[146,147]

Intraligamentary injections are another route for administration of local anesthetics. A randomized, double-blind crossover study compared intraligamentary injections of 2% lidocaine with 1:100,000 epinephrine and 4% articaine with 1:100,000 epinephrine in normal volunteers.[148] The incidence of moderate pain was 14% to 27% during needle insertion and 8% to 18% during solution deposition. On the day after the injection, 20% to 31% of subjects reported moderate to severe pain. No differences were noted between the two anesthetics, and there was no increase in heart rate in either group. In a parallel study, the success rates of pulpal anesthesia—defined by two consecutive negative readings on the electrical pulp tester obtained within 20 minutes— following intraligamentary injections of 1.4 mL of 2% lidocaine with 1:100,000 epinephrine or 4% articaine with 1:100,000 were similar and ranged from 74% to 86%.[149] Supplemental intraligamentary injection of 2% lidocaine with 1:100,000 epinephrine in teeth with irreversible pulpitis was 56% successful for pulpal anesthesia.[150]

One of the approaches used to increase the success of pulpal anesthesia in teeth with inflamed pulps is to administer an analgesic prior to injection of the local

anesthetic. A number of randomized, double-blind, controlled clinical trials have examined the effect of pretreatment with analgesics such as ibuprofen, indomethacin, and acetaminophen on pulpal anesthesia following administration of inferior alveolar nerve blocks. Some,[151,152] but not all,[153–155] studies have reported that preemptive administration of analgesics in patients diagnosed with irreversible pulpitis increases the chance of success of inferior alveolar nerve blocks.

Variations in methodology and design of the clinical trials may account for the differences in the study results. For example, the methods of determining pulpal anesthesia varied across the studies. Another confounding factor is how the diagnosis of irreversible pulpitis was made. In some studies, the diagnosis was based on the presence of spontaneous pain and a prolonged response to cold, whereas in others it was based only on the cold response. In addition, the response to cold was not well defined in most studies. The periapical status of the symptomatic teeth and duration of symptoms were also not factored into the data analyses. While both peripheral and central mechanisms are thought to contribute to odontogenic pain, it is likely that the central mechanisms play a greater role in patients with symptomatic periapical periodontitis or in those who have had experienced odontogenic pain for a longer duration than in those with normal periapical tissues or those with more acute onset of symptoms.

Several studies have suggested that the local application of peripheral opioids may have utility in treating pain caused by irreversible pulpitis. Opioid receptors are present on afferent neurons and undergo a peripherally directed transport.[156,157] These observations may be clinically useful because pulpal neurons express opioid receptors, and local administration of opioids is analgesic in animal models of inflammatory pain.[158,159] Therefore, clinical trials have evaluated whether intraligamentary injection of opioids is analgesic in patients with irreversible pulpitis.[161–163] The results indicated that opioids produce significant analgesia in this model (Fig 9-15) and act locally in inflamed tissue.[160]

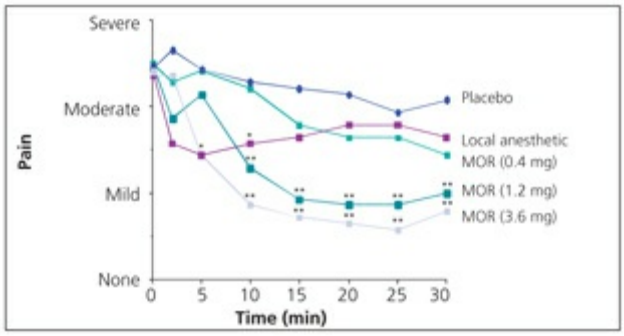

Fig 9-15 Effects of intraligamentary injection of morphine on patients' levels of pain. Patients with irreversible pulpitis reported baseline pain levels and were then injected, on a double-blind, randomized

basis, with either placebo, local anesthetic (2% mepivacaine with 1:20,000 levonordefrin), or morphine (MOR; 0.4, 1.2, or 3.6 mg) using a standard intraligamentary route of injection. N = 8 to 10 per group; *$P < .05$ versus placebo; **$P < .01$ versus placebo. (Redrawn from Dionne et al[160] with permission.)

Drugs that block central mechanisms of pain perception and hyperalgesia

Opioids are potent analgesics, often used in dentistry in combination with acetaminophen, aspirin, or ibuprofen. Most clinically available opioids activate the μ-opioid receptor. Although opioids are effective as analgesics for moderate to severe pain, their usage is generally limited by their adverse side-effect profile. Opioids induce numerous side effects, including nausea, emesis, dizziness, drowsiness, and have the potential to cause respiratory depression and constipation. Chronic usage is associated with the development of tolerance and dependence. Because the dose of opioids is limited by their side-effect profile, opioids are almost always used in combination drugs for management of dental pain. A combination formulation is preferred because it permits a lower dose of the opioid to reduce side effects.

Codeine is often considered the prototype opioid for orally available combination drugs. Most studies of surgery-induced inflammatory pain have found that a 60-mg dose of codeine produces significantly more analgesia than placebo, although it often produces less analgesia than either a 650-mg dose of aspirin or a 600-mg dose of acetaminophen.[93,94,163]

Pentazocine, a mixed agonist-antagonist opioid, has been shown to be effective in the management of orofacial pain.[164] It acts as a κ-opioid receptor agonist and a partial δ-opioid receptor agonist with mixed agonist-antagonist properties at the μ-opioid receptor. In a postendodontic study comparing the analgesic efficacy of a pentazocine (50 mg)-naloxone (0.5 mg) combination (Talwin, Sanofi) to that of ibuprofen (600 mg), female patients who took pentazocine-naloxone reported significantly greater pain relief than did male patients who took the same medication[165] (Fig 9-16). Pentazocine-naloxone could be an effective analgesic in female endodontic patients who are unable to take other analgesics such as ibuprofen and acetaminophen. Compared to other opioids, it offers the additional advantage of relatively less potential for opioid dependence and abuse.[166,167]

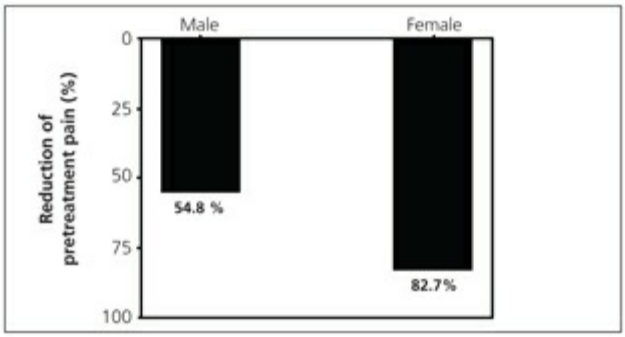

Fig 9-16 Sex difference in the analgesic effect of pentazocine-naloxone in postoperative endodontic patients. Patients used a visual analog scale (VAS) to report pain. The reduction in pain was calculated for each patient based on the following formula: ([pretreatment VAS score – postoperative VAS score] / pretreatment VAS score) × 100 at each time point and averaged over the 24-hour period. (Reprinted from Ryan et al[165] with permission.)

Pain-Management Strategies

When managing pain in an individual patient, the skilled clinician must customize the treatment plan to the patient, balancing knowledge of pulp biology with general principles of endodontics and restorative dentistry, mechanisms of hyperalgesia, pain-management strategies, and the individual's particular factors (eg, medical history, concurrent medications, etc). The following discussion reviews general considerations for pain-management strategies.

Effective management of endodontic pain starts with the three *D*s: diagnosis, definitive dental treatment, and drugs (see Box 9-1). As described earlier in this chapter, the management of endodontic pain should focus on removal of peripheral mechanisms of hyperalgesia, which generally requires treatment that removes or reduces etiologic factors (eg, bacterial and immunologic factors) and nerve terminals. Pulpotomy and pulpectomy represent rational and effective treatments for initial removal of these factors (see Fig 9-8). However, pharmacotherapy is often required to interrupt continued nociceptor input (eg, NSAIDs, local anesthetics) and to suppress central hyperalgesia (eg, opioids).

Administration of NSAIDs alone is usually sufficient for most patients who can tolerate this drug class because of its effectiveness in managing inflammatory pulpal pain and the relatively low incidence of postendodontic therapy pain. (Up to 80% of patients report pain after endodontic therapy as none to slight[168,169]). Thus, an NSAID prescription, such as ibuprofen 600 mg taken every 6 hours, is optimal

treatment for the majority of patients who can tolerate this drug class. For patients who cannot tolerate an NSAID, a 1,000-mg dosage of acetaminophen is often suitable for managing posttreatment pain.

About 20% of patients may have moderate to severe postendodontic pain,[169] and some patients may not be adequately relieved by a single-drug approach. In general, a flexible analgesic prescription strategy offers the best combination of pain relief and minimal side effects while offering the clinician a rational approach for customizing an analgesic plan to a particular patient's needs (Fig 9-17). A flexible prescription plan serves to minimize both postoperative pain and side effects.

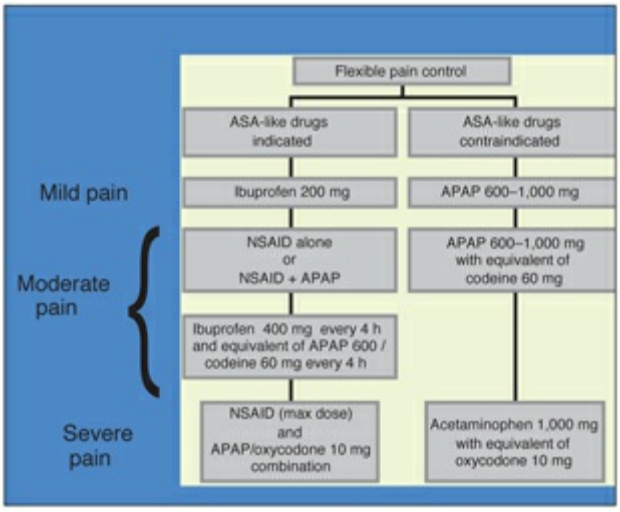

Fig 9-17 Flexible prescription plan for management of postendodontic pain based on the patient's ability to tolerate NSAIDs and designed to minimize pain and exposure to side effects. APAP, acetaminophen; ASA, acetylsalicylic acid.

With this goal in mind, the strategy is first to achieve a maximally effective dose of the non-narcotic analgesic (either an NSAID or acetaminophen for patients who cannot take NSAIDs). Second, in those rare cases when the patient still experiences moderate to severe pain, the clinician should consider adding drugs that increase the analgesia provided by the NSAID. Given its predictive value regarding the need for more comprehensive pharmacotherapy (see chapter 8), the presence of preoperative hyperalgesia may serve as an indication for considering these NSAID combinations.[53]

There are two general analgesic approaches for such patients. One approach is to coprescribe an NSAID with acetaminophen. This strategy provides predictable control for severe pain and can be used to modify a standard NSAID-only regimen if pain control is inadequate.[170,171] The concurrent and short-term administration of acetaminophen and NSAIDs appears to be well tolerated in most patients without

any apparent increase in side effects or alterations in pharmacokinetics.[170–175] A second general approach is to coprescribe an NSAID with an acetaminophen-opioid combination. All three drugs (the NSAID, the acetaminophen, and the opioid) are active analgesics that can have additive effects when combined.[170–172]

A national survey on the prescribing habits of endodontists reported that 16.76% of responders prescribed antibiotics in a scenario of irreversible pulpitis.[176] A randomized, double-blind study clearly demonstrated that administration of an antibiotic alone does not reduce spontaneous or evoked pain and has no effect on analgesic intake in patients with irreversible pulpitis.[177] As described earlier in this chapter, pulpotomy and pulpectomy represent rational and effective treatments for inflamed pulps. There is no evidence or rationale supporting the use of antibiotics in the management of irreversible pulpitis.

The information and recommendations provided in this chapter were selected to aid the clinician in the management of pulpal pain caused by dentinal hypersensitivity or pulpitis and related periradicular pain. However, clinical judgment must also take into account other sources of information, including the patient's history, concurrent medications, nature of the pain, and the comprehensive treatment plan to design the best pain-management program for an individual patient. Integration of these general principles of pain mechanisms and management with the clinician's assessment of each patient's needs is an effective approach to the successful management of pulpal pain.

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Pulpal Infections, Including Caries

José F. Siqueira, Jr, DDS, MSc, PhD

Caries is a disease of multifactorial nature, involving the interaction of an acidogenic (acid-producing) and aciduric (acid-tolerating) microbiota on a susceptible tooth surface in a conducive environment, influenced by frequent intake of rapidly fermentable carbohydrates.[1] The caries lesion develops as a result of demineralization of enamel or cementum, and later of dentin, by acids produced by plaque bacteria as they metabolize dietary carbohydrates. The initial process of enamel demineralization is usually followed by remineralization, and cavitation occurs when the former process overcomes the latter. Progressive caries lesions can lead to extensive destruction of the tooth structure (Fig 10-1).

When untreated, caries can progress through dentin and ultimately lead to pulpal inflammation, necrosis, and then infection. Caries and pulpal infections share some features with each other and at the same time present some differences from most of the other infections in the body:

1. They both are caused by inhabitants of the normal microbiota (endogenous opportunistic infections).

2. Tissues are irreversibly damaged in advanced stages of the disease.
3. Once established, neither caries nor pulpal infections can undergo spontaneous remission followed by tissue healing because of the relative inaccessibility of causative microorganisms to the host defense mechanisms.
4. Neither caries nor pulpal infections can be effectively treated by systemic antibiotics.

The third and fourth items are due to the fact that caries occurs in avascular tissues (enamel, dentin, or cementum) and pulpal infections occur in a tissue whose vascularization is compromised (during inflammation) or absent (because of necrosis). Given these characteristics, once established, caries and pulpal infections require professional intervention to receive adequate treatment.

Dental caries is certainly one of the most prevalent human bacterial infections worldwide. Although the prevalence of dental caries in children has declined markedly over the last 20 years in most Western countries, the disease continues to be a major problem for both adults and children everywhere. In reality, caries is a major health problem in most industrialized countries, affecting 60% to 90% of schoolchildren and most adults.[2,3] The map of caries indicates that higher disease experience has been disclosed in North and South America, Western Europe, and much of Africa; moderate disease experience has been reported for much of South America and Russia; and low levels of disease occur in Eastern Africa, China, Australia, and Greenland.

While the correlation between caries rates and national development is not too strong, the World Health Organization has observed that developed countries have higher rates of caries experience, while developing countries have lower rates.[2] This difference may be the result of dietary factors. In the United States, dental caries is the most common childhood disease, and recent findings by the Centers for Disease Control and Prevention reveal high ongoing prevalence of caries, with 27% of preschoolers, 42% of school-aged children, and 91% of dentate adults having caries experience.[4]

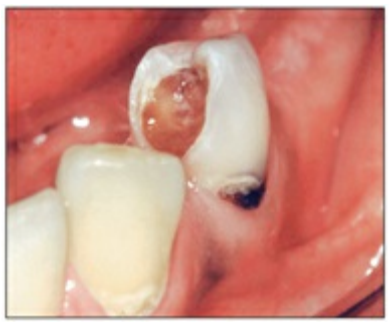

Fig 10-1 Progressive caries lesions affecting different areas of the tooth. (Courtesy of Dr Ricardo Carvalhaes, Niterói, Brazil.)

Apical periodontitis (Fig 10-2) is an inflammatory disease caused by pulpal infection, which is usually a sequel to caries. The disease is also widespread, and its prevalence increases with age; one-third of young adults are affected, while by 50 years of age values rise to about 50%. In individuals over 60 years old, the prevalence of apical periodontitis increases to 62%.[5]

Although rarely life-threatening, dental caries and its extension (pulpal infections) are major problems for healthcare service providers because of their prevalence and high treatment costs.[6] The economic burden for the treatment of caries and pulpal infections can be astounding, reaching annual costs in developed countries on the order of tens of billions of dollars.[7,8] Most of these costs are related to the restoration of carious teeth and root canal treatment, not to mention the costs related to interventions to replace restorations and re-treat root canals. Despite these enormous financial figures, they represent the cost for only those estimated 40% to 50% of the public who have access to dental treatment. Based on these data, it is reasonable to assume that dental caries and its consequences are the most expensive infections that most individuals have to contend with during a lifetime.[7]

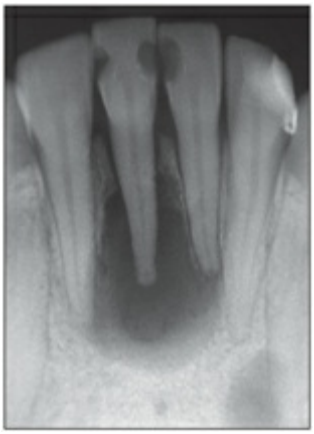

Fig 10-2 Radiograph of mandibular anterior teeth showing a radiolucent area diagnosed as an apical periodontitis lesion. The pulps of the two central incisors were necrotic.

Plaque Biofilm

Because caries is a plaque-mediated disease, several aspects of dental plaque are worth discussing to facilitate the understanding of caries pathogenesis. *Dental*

plaque can be defined as a diverse community of microorganisms adhered to the tooth surface as a complex biofilm, embedded in an extracellular matrix of polymers mostly of microbial origin (Fig 10-3). Unlike *materia alba*, which consists of an aggregation of microorganisms, leukocytes, and desquamated oral epithelium that accumulates at the surface of plaque but lacks its internal structure, dental plaque is more firmly adhered to the tooth and can withstand a strong water spray. Plaque is found naturally in health, but a shift in the composition of the plaque microbiota along with other conducive environmental factors can lead to disease.[9]

A *biofilm* is a microbially derived sessile community characterized by cells that are firmly attached to a substratum, an interface, or each other; are embedded in a matrix of extracellular polymeric substances that they have produced; and exhibit an altered phenotype with respect to growth rate and gene transcription.[10] The ability to form biofilms has been regarded as a virulence factor—a strategy that contributes to bacterial ability to establish an infection process.[11] In fact, it has been estimated that biofilm infections comprise 65% to 80% of the bacterial infections that affect humans in the developed world.[12] Given the importance of biofilms, there has been a high level of interest in the study of biofilm properties not only in medical microbiology but also in different sectors of industrial and environmental microbiology.

The vast majority of microorganisms in nature are invariably found forming biofilm structures.[13] The ability to adhere to oral surfaces and form mixed communities may be considered fundamental strategies for bacteria to be retained and survive in the oral ecosystem (Fig 10-4).

Biofilms are not merely passive assemblages of bacterial cells that are stuck to surfaces but structurally and dynamically organized complex biologic systems. As a community of bacteria, biofilms have a collective physiology, responding in concert to environmental challenges. The component species are not randomly distributed but are spatially and functionally organized, and many naturally occurring biofilms have a highly diverse microbiota. Microcolonies that form in the biofilm arise from the surface colonization by planktonic (unattached) microbial cells that then adopt a radically different biofilm phenotype. Therefore, sessile bacteria in biofilms express properties not exhibited by their counterparts growing in a planktonic state. In biofilms, these properties are usually more than the sum of the component species.

As the biofilm matures, there is continued synthesis of extracellular polysaccharides to form an extracellular matrix, until this matrix material comprises as much as 85% of the volume of the biofilm.[12] The matrix is not only important

physically as part of the scaffold that determines the biofilm structure but also biologically active and can retain nutrients, water, and essential enzymes within the biofilm.[14] The matrix can also protect the biofilm community from exogenous threats (see below). Channels traverse the biofilm matrix and create primitive circulatory systems.[15] Fluid in these channels carries substrate, end products of bacterial metabolism, and signal molecules involved in bacterial interactions.[16]

During the early stages of biofilm formation, bacteria bind to many host proteins and coaggregate with other bacteria. These interactions lead to changes in global gene transcription and expression, which equip the cells for growth and survival on a surface and enable the formation of biofilm. As gene expression is markedly altered in a biofilm, bacteria exhibit a radically different phenotype than planktonic cells. Within biofilms, sophisticated systems of cell-cell communication (quorum sensing, defined later in this chapter) are also used by some bacteria to coordinate gene expression.

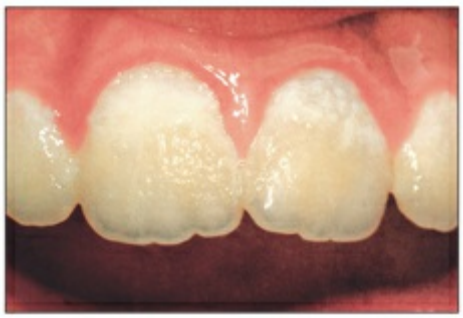

Fig 10-3 Heavy plaque accumulation on the buccal surface of the maxillary incisors of a 9-year-old child. (Courtesy of Dr Kenio C. Lima and Dr Maria Angela F. Ferreira, Natal, Brazil.)

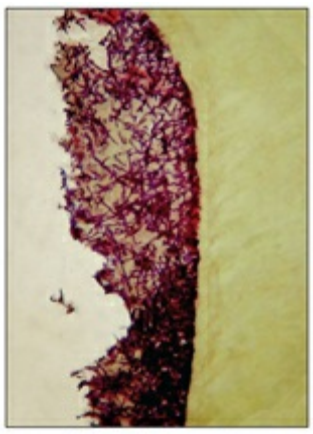

Fig 10-4 Mixed bacterial biofilm adhered to the tooth surface (Brown and Brenn stain; original magnification ×400).

Biofilm community lifestyle

Bacteria in a biofilm form a community wherein the spatially distributed populations may interact. The biofilm community lifestyle affords a number of advantages to colonizing bacteria, including[17]:

- A broader habitat range for growth. The metabolism of early colonizers alters the local environment, setting the stage for attachment and growth of latecomers (including more fastidious species). As a consequence, the diversity of the microbiota increases over time because of bacterial succession.
- Increased metabolic diversity and efficiency. Bacteria living in mixed consortia take part in a number of nutritional interrelationships so that food chains or food webs develop. In food chains, products of metabolism of one species may become the main source of nutrients for other species. In food webs, the concerted action of interacting species is required for the sequential breakdown of complex host-derived substrates to simpler products, which can favor less competent species. Many complex host macromolecules, especially glycoproteins, can only be degraded efficiently by mixed consortia of oral bacteria.
- Protection from competing microorganisms, host defenses, antimicrobial agents, and environmental stress. Neighboring cells of a different species can produce enzymes, such as β-lactamase and proteinases, that are retained in the biofilm matrix and protect other bacteria against antimicrobial agents and host defenses, respectively. The same is true for metabolites and bacteriocins, which can inhibit competing species. Bacterial cells also communicate via diffusible signaling molecules (quorum sensing) and interact with one another in biofilms via horizontal (lateral) gene transfer, including the transfer of antibiotic resistance genes. *Horizontal gene transfer* refers to the movement of genetic material between phylogenetically unrelated bacteria by mechanisms other than parent–to-progeny inheritance. The biofilm matrix also confers physical protection against phagocytosis.
- Enhanced ability to cause disease. To cause disease, bacteria must adhere to host surfaces, obtain nutrients from the host and multiply, invade tissues, overcome or evade the host defenses, and induce tissue damage. A diverse range of virulence traits are required for these particular stages of the disease process, and it is highly likely that each will require the concerted action of

bacteria in a consortium. Similarly, it is possible that certain species can have more than one role in disease, while different species could perform identical functions. This helps explain why communities with different bacterial composition can be found at sites with similar disease. Abscesses are examples of polymicrobial infections whereby bacterial species that individually are unable to cause disease can do so when in association with others as part of a mixed consortium (a concept termed *pathogenic synergism*).[18]

Resistance to antimicrobial agents

From a clinical standpoint, the increased resistance of biofilms to antimicrobial agents is of special concern. Bacteria arranged in biofilms are more resistant to antibiotics than the same cells grown in a planktonic state. The concentration of an agent that kills planktonic bacteria may have to be increased by 10 to 1,000 times to have the same efficacy on sessile bacteria in a biofilm. There are several possible mechanisms involved with biofilm resistance to antimicrobials:

- The biofilm structure may restrict the penetration of the antimicrobial agent. The agent may adsorb to and inhibit the bacteria at the biofilm surface, leaving cells deeply located in the biofilm relatively unaffected. The matrix in biofilms can also bind and retain neutralizing enzymes at concentrations that could inactivate the antimicrobial agent.
- Bacteria in biofilms can enter the stationary phase and become resistant. Many antibiotics can freely penetrate the biofilm matrix, but cells are often still protected. The occurrence of starved bacteria entering the stationary phase in biofilms seems to be a significant factor in the resistance of biofilm populations to antimicrobials. Bacteria grow slowly under nutrient-poor conditions in an established biofilm and, as a consequence, are much less susceptible to antimicrobials than faster-dividing cells. Arguably, virtually all antibiotics require at least some degree of cellular activity to be effective because the mechanism of action of most antibiotics involves disruption of a bacterial process. Therefore, bacterial cells in the stationary phase might represent a general mechanism of antibiotic resistance in the biofilm.[11]
- Persistent bacteria is ‘present. Increased tolerance of some biofilms to antibiotics may be largely due to the presence of a subpopulation of specialized

survivor cells known as *persisters*.[19] It remains unclear if these bacteria actually represent a distinct phenotype or are simply the most resistant cells selected from a population.[11]

Quorum sensing: Bacterial intercommunication

Bacteria living in biofilms can communicate with one another, enabling them to behave collectively as a group. This cell-cell communication phenomenon is referred to as *quorum sensing* and has been described in both gram-positive and gram-negative bacteria.[20–22] Quorum sensing provides a mechanism for bacteria to monitor one another's presence and to modulate gene expression in response to changes in population density. Quorum sensing involves the production, release, and subsequent detection of diffusible signaling molecules called *autoinducers*. Two predominant types of autoinducers, acyl homoserine lactones and modified oligopeptides, are used by gram-negative and gram-positive bacteria, respectively. Several other small-molecule quorum-sensing autoinducers have recently been discovered, including *Pseudomonas* quinolone signal and AI-2.[23]

As members of a bacterial population that produce and release autoinducers multiply, the extracellular concentration of these signaling molecules also increases. When autoinducers reach a crucial threshold level, the group responds with a population-wide alteration in gene expression, activating or repressing target genes.[24] Because alterations in gene expression are linked to the presence of autoinducers, bacteria can perform specific functions only when living in groups. Such behavior provides some advantages to a bacterial population, affording adaptability to and protection against threatening environments.

Quorum-sensing systems are known to regulate virulence, competence for DNA uptake, entry into the stationary phase, and biofilm formation.[23,25,26] Some opportunistic pathogens express virulence factors in response to sensing their own cell density. Using quorum sensing, bacteria can amass a high cell density before virulence factors are expressed, and in so doing the bacteria are able to make a concerted attack to overcome the host defenses and establish infection.[27] Based on these functional properties, it appears as if bacteria are able to strategically wait for a critical number of cells to be reached and then to start inducing damage, not giving

the host sufficient time to mount an efficient defense response. The process of biofilm formation, including surface attachment, proliferation, matrix production, and detachment, is also partially controlled by quorum sensing.[28] Moreover, bacteria can monitor crowding as a measure of competition for nutrients. At high cell density, there may be too much competition to utilize available nutrients for growth, and starvation is anticipated. If bacteria are already starved and crowded, as they usually are when growing as a biofilm, it may be advantageous to enter the stationary phase. Entry into the stationary phase dramatically alters patterns of gene expression to allow extended cell survival in the absence of nutrients.[29]

Cell-cell communication mediated by secreted diffusible molecules is widespread among bacteria. Several oral bacteria have been demonstrated to produce quorum-sensing signaling molecules,[30–32] indicating that bacteria in the plaque biofilm can also communicate with one another through this system.

Plaque development

Formation of the plaque biofilm on tooth surfaces involves several distinct stages[13,33] (Fig 10-5):

1. Formation of the conditioning salivary film (acquired pellicle). As the tooth erupts or is cleaned, the enamel surfaces are immediately bathed by saliva. This results in a conditioning film, composed of host and bacterial molecules, that coats the tooth surfaces. This amorphous film varies in thickness from 0.1 to 3.0 μm,7 and its molecular composition helps determine which species will specifically attach to and colonize the tooth surface.
2. Transport of bacteria to the pellicle-coated tooth surface. As saliva bathes the tooth, bacterial cells in a planktonic state are brought into contact with the tooth surface.
3. Early reversible adhesion of bacteria to the pellicle-coated tooth surface. The early colonizers can be retained near the tooth surface by nonspecific weak physicochemical interactions between the bacterial cell surface and the acquired pellicle. Although weak, this initial adhesion may facilitate the further establishment of specific and stronger interactions between bacteria and the conditioning film. Bacterial cells may also be mechanically entrapped in

retentive areas of the tooth.

4. Strong interactions between bacterial adhesins and complementary receptors in the pellicle. These interactions contribute to a specific and irreversible attachment and can explain bacterial tropisms for certain sites. Many oral bacteria possess more than one type of adhesin on their cell surface. Given the specificity of the adhesion-receptor interaction, a certain bacterial species may be excluded from the developing community if receptors specific for its adhesins are unavailable or absent on the tooth surface.[34] After specific and stronger adhesion, the early colonizers grow and modify local environmental conditions, making the site suitable for attachment and colonization by succeeding species, including fastidious bacteria. This may involve changing the local oxygen tension or pH, modifying or exposing new receptors on surfaces for attachment, removing potentially harmful metabolic products, or generating novel nutrients as end products of metabolism or as breakdown products that can be used by latecomers.[9] Early colonizers of plaque usually include streptococci, particularly *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus mitis*.
5. Binding of latecomers to the already attached early colonizers (a process termed *coaggregation*). Coaggregation is the cell-to-cell recognition of genetically distinct partner cell types and has been observed to occur between several different oral species.[35] It differs from agglutination and aggregation in that the latter two interactions occur between genetically identical cells.[36] A given pair of species can attach to each other by means of specific receptor-adhesin interactions, which are usually lectinlike interactions (attachment of a specific protein on the surface of one species to a specific carbohydrate on the surface of the other). Coaggregation can even occur between noncoaggregating species; in such cases, it is mediated by cellular constituents, such as outer membrane vesicles, of a third species. Coaggregation can favor colonization of host surfaces and facilitate metabolic interactions between the partners. The coaggregating process leads to an increase in the diversity of the biofilms and can result in the formation of unusual cellular arrangements, such as "corncobs." Coaggregation may also influence not only the spatial organization but also the functional organization of the plaque biofilm. For instance, the efficiency of metabolic interactions among bacteria in food chains may be enhanced if they are in close physical contact. Also, survival of obligately anaerobic bacteria in the presence of oxygen is markedly enhanced if they are able to coaggregate with oxygen-consuming facultative species.

6. Multiplication of the attached bacteria and biofilm formation (including the synthesis of extracellular polysaccharides). Population density increases as a result of multiplication of the already attached bacteria and the arrival of other species from saliva. In this regard, cell division is by and large more significant than coaggregation between attached cells and latecomers. Increases in bacterial diversity and density intensify interactions among members of the growing community, and there is the opportunity for division of labor. This may involve cell-cell signaling to coordinate gene expression and metabolic activities among bacteria.[35] Attached bacteria produce an extracellular polysaccharide matrix, which makes a significant contribution to the properties and structural integrity of the plaque biofilm. The overall result is the development of a spatially and functionally organized mixed-culture biofilm, in which species occupy distinct regions and modify the local environment to optimize their growth and metabolism, fitting their collective interests. The final proportions of a species within the mixed community of plaque biofilm will depend ultimately on the ability of certain species to grow and outcompete neighboring cells.
7. Detachment of bacteria from tooth surfaces. Enzymes produced by sessile bacterial cells can hydrolyze the specific adhesins that anchor cells to the surface. As a consequence, detached cells return to a planktonic state so that other sites can be further colonized.

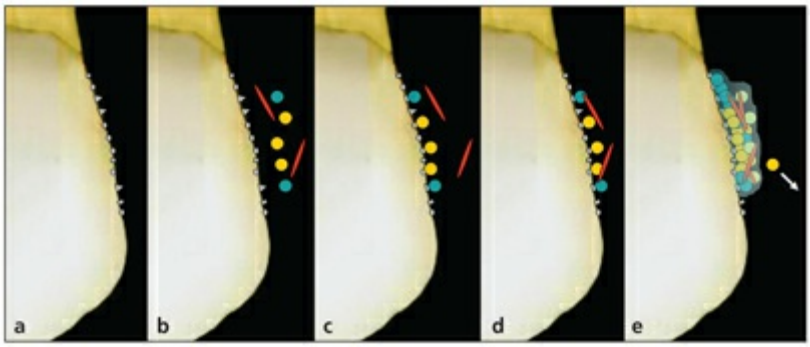

Fig 10-5 Stages of plaque biofilm formation. *(a)* Formation of acquired pellicle on the tooth surface. *(b)* Transport and early reversible adhesion of oral bacteria to the pellicle-coated surface. *(c)* Strong specific adhesion of bacteria to the tooth surface. *(d)* Coaggregation. *(e)* Multiplication of the attached bacteria, production of an extracellular polysaccharide matrix, and maturation of the biofilm. Some cells detach from the biofilm to colonize other sites.

Dental Caries

Dental caries is a biofilm-induced disease characterized by localized hard dental tissue destruction by acids produced from bacterial fermentation of dietary carbohydrates. Ecological disturbances in the plaque biofilm can favor the outgrowth of cariogenic bacteria. The disease is affected by the flow and composition of saliva, exposure to fluoride, consumption of dietary sugars, and preventive behavior.[37] The eventual outcome of caries is determined by several factors that will influence the dynamic balance between demineralization and remineralization.[38] The disease is initially reversible and can be halted at any stage, even when cavitation has developed, provided that the biofilm can be removed or effectively controlled.

Categories

The plaque biofilm tends to form and mature in certain locations on the tooth, notably the occlusal surface, the approximal surface cervical to the contact point, and along the gingival margin. These areas are relatively protected from mechanical wear by the tongue, the cheeks, abrasive food, and tooth-brushing.[39] Teeth are susceptible to caries development mostly because the plaque biofilm tends to stagnate on those surfaces and remain undisturbed for prolonged periods of time. Thus, retentive areas and surfaces that are difficult to clean are the most caries-prone sites on the teeth. Depending on the affected tooth surface, caries can be categorized as follows: occlusal (pit or fissure) caries, smooth surface caries, root surface caries, or recurrent caries.

Dental caries of the enamel is first observed clinically as a so-called white-spot lesion (Fig 10-6). This is a small area of subsurface demineralization, beneath the plaque biofilm. The lesion appears white because the loss of mineral changes the refractive index compared with that of the surrounding translucent enamel. The body of the subsurface lesion may have lost as much as 50% of its original mineral and often is covered by an apparently intact surface layer beneath the biofilm.[40] This surface layer forms by remineralization as the calcium and phosphate ions diffuse or travel out of the tooth into the overlying plaque fluid.[41] The surface continuity of enamel is not breached, and at the white-spot stage the lesion can be reversed.

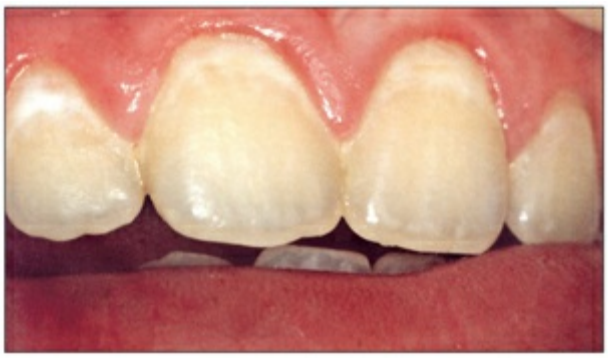

Fig 10-6 White-spot lesions on maxillary incisors. (Courtesy of Dr Kenio C. Lima and Dr Maria Angela F. Ferreira, Natal, Brazil.)

Early childhood caries is an aggressive presentation of dental caries that affects the primary teeth of infants and toddlers and typically develops in anterior tooth surfaces. It is often associated with the prolonged and frequent feeding of infants with bottles and/or pacifiers containing formulas with a high concentration of fermentable carbohydrates. The disease begins with white-spot lesions in maxillary primary incisors along the gingival margin. If allowed to continue, caries can progress and lead to complete destruction of the crown.

With the reduction in enamel caries in industrialized societies, large proportions of the public retain teeth into later life. In old age, however, gingival recession occurs and exposes the cementum surface to bacterial colonization. Root surfaces can also be exposed by mechanical injury or periodontal surgery. Cementum surfaces are especially vulnerable to demineralization by plaque acids. Not surprisingly, the prevalence of root caries increases with age. Interestingly, although root caries may appear extensive, the lesion is rarely more than 0.5 to 1.0 mm deep.[39] This slow rate of bacterial invasion and tissue destruction gives patients an opportunity to arrest such lesions by controlling the plaque biofilm with fluoride toothpastes.

The term *recurrent caries* denotes lesions that develop beneath and around previous restorations. Because this type of caries arises after the initial lesion has been removed and replaced by a restorative material, the term *secondary caries* has also been used. Recurrent caries should be differentiated from primary caries and remaining caries. *Primary caries* starts and progresses on an intact, previously unrestored tooth surface. Caries that is left behind, intentionally or unintentionally, during restorative treatment is referred to as *remaining caries*, which is more commonly found in the dentin beneath a restoration.

Recurrent caries does not develop as a result of microleakage along the tooth-restoration interface but rather is usually a surface lesion similar to primary caries

lesions on smooth surfaces.[42] Recurrent caries occurs predominantly on the gingival margins of Class II through Class V restorations but is seldom associated with Class I restorations or the occlusal part of Class II restorations.[42] Because it favors plaque accumulation, the presence of overhangs is a predisposing factor for recurrence of caries.

Pathogenesis

The mechanisms of the disease process are similar for all types of caries. The process is initiated in the plaque biofilm and can be described as follows.[38] Acidogenic bacteria in the plaque biofilm ferment carbohydrates from the diet, producing organic acids, such as lactic, formic, acetic, and propionic. The acids diffuse through the plaque biofilm into the enamel, dentin, or cementum, dissociating to produce hydrogen ions as they diffuse.[43] The hydrogen ions promote dissolution of the tooth mineral, with resulting release of calcium and phosphate into solution. Lactic acid dissociates more readily than the other acids, rapidly lowering the pH in the plaque biofilm.[43]

Demineralization can be reversed by calcium and phosphate, together with fluoride, diffusing into the tooth and depositing a new veneer on the crystal remnants in the noncavitated lesion (remineralization). The new mineral crystal surface is much more resistant to acid than was the original carbonated hydroxyapatite mineral.[38]

The demineralization and remineralization process generally takes place several times daily. In addition to the bacterial composition of the biofilm, other factors will influence the magnitude of the pH fluctuations on the tooth surface. These factors will also determine the likelihood of mineral loss and the rate at which it occurs. They include diet, fluoride concentration, and salivary function and components.[39] Over time the demineralization and remineralization process will lead to cavitation, repair, and reversal of the lesion.[39]

Frank cavitation comes after months or years of caries progression.[38] The formation of a cavity assumes significant clinical importance because now the biofilm is protected within a cavity. Because the patient is usually unable to clean this area, the caries process continues and advances toward the pulp.[39]

Etiology

Unlike most classic medical infections in the human body, in which a single pathogen is commonly isolated from a diseased site that is sterile in health, caries is a biofilm-induced disease that occurs at sites containing a preexisting normal microbiota. This feature makes the interpretation of microbiologic studies of dental caries difficult, especially for trying to establish a causal relationship between the disease and certain species.

Nonspecific and specific plaque hypotheses

There have been two main schools of thought on the role of plaque biofilm bacteria in the etiology of dental caries. The *nonspecific plaque hypothesis* claimed that disease develops as a result of the overall activity of the plaque microbiota as a whole. The *specific plaque hypothesis* considered that only one or a few specific species out of the mixed consortium that composes the plaque biofilm are involved in the pathogenesis of the disease. In fact, caries is essentially characterized by a mixed infection, in which only certain species dominate the consortium. Thus, if caries etiology is not totally specific, it shows evidence of specificity in that a limited set of bacterial species are consistently present in higher numbers in diseased sites. Also, it has become clear and undisputed that the predominant species occurring in diseased sites are rather different from those found in health.[44]

Ecological plaque hypothesis

The *ecological plaque hypothesis* is an alternative hypothesis that considers the conceptual elements of the specific and nonspecific plaque hypotheses.[1] According to the ecological plaque hypothesis, caries develops as a consequence of shifts in the natural balance of the resident plaque microbiota brought about by an alteration in local environmental conditions that favors the growth of cariogenic species[1] (Fig 10-7). This hypothesis is based on the fact that, in any ecosystem, a significant change in parameters that maintain the ecological stability of the whole community can give rise to the outgrowth of previously minor components of that community. For instance, potential pathogens may be present in low numbers in the plaque biofilm—a situation that is compatible with health. However, a major environmental change can lead to ecological pressure that allows pathogens to outcompete other members of the microbiota and then achieve levels sufficient to cause disease.[44]

Cariogenic bacteria may be found naturally in the plaque biofilm at healthy sites. At neutral pH conditions, they are usually weakly competitive and are present only at low levels as a small proportion of the total plaque biofilm community. In this situation, with a conventional diet, the levels of such potentially cariogenic bacteria are clinically insignificant, and the processes of demineralization and remineralization are in equilibrium. It has been shown that at a constant neutral pH, acidogenic and aciduric bacteria, such as mutans streptococci and lactobacilli, are noncompetitive and comprise only a minor proportion (less than 1%) of a bacterial community. When the pH drops, these bacteria gradually increase in proportions until they eventually dominate (greater than 50%) the mixed consortium at the expense of acid-sensitive species.[1] Therefore, pH can act as an important ecological determinant.

If fermentable carbohydrates are ingested more frequently, bacteria in the plaque biofilm produce acids and the pH drops and reaches critical values for enamel demineralization (pH less than 5.5). Low-pH environmental conditions favor the proliferation of aciduric bacteria, especially mutans streptococci and lactobacilli, while tipping the balance toward demineralization. As the numbers of mutans streptococci and lactobacilli in the plaque biofilm increase, more acid is produced at even faster rates, and demineralization is still further enhanced.

Other bacteria in plaque can also produce acid under similar conditions, but at a slower rate. Examples include some other streptococci, *Actinomyces* species, and *Bifidobacterium* species. These bacteria can lower the plaque pH to favor dominance by aciduric species, promote the initial stages of demineralization, or even cause lesions in the absence of other more cariogenic species.[16,17] If aciduric species were not present initially, the low-pH conditions may increase the possibility of colonization by these bacteria, including mutans streptococci and lactobacilli.

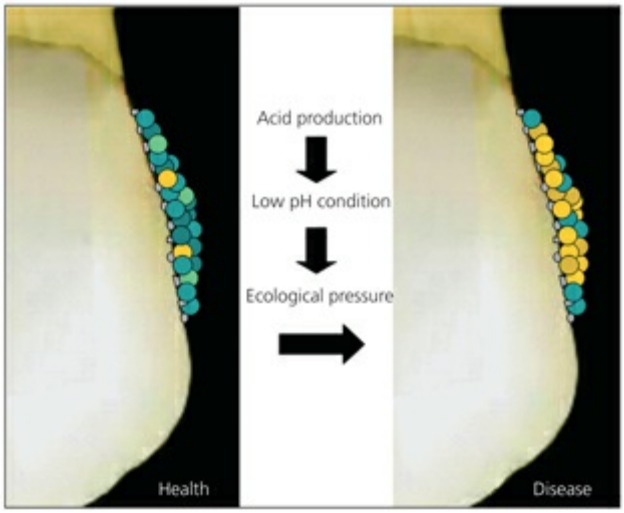

Fig 10-7 The ecological plaque hypothesis posits that caries is a consequence of shifts in the balance of the plaque microbiota induced by an alteration in local environmental conditions that favors the growth of cariogenic species *(orange)* to the detriment of beneficial species *(green)*.

Cariogenic bacteria

In his famous chemoparasitic theory of decay, Willoughby Dayton Miller[45] suggested that oral bacteria convert dietary carbohydrates into acid, which solubilizes the calcium phosphate of the enamel to produce a caries lesion. However, definitive proof for the concept that dental caries is "infectious and transmittable" only came following elegant rodent, including germ-free rat, experiments performed in the 1950s and 1960s.[46–48]

Three properties that are typical characteristics of cariogenic bacteria include the ability to *(1)* rapidly transport sugars when in competition with other plaque bacteria, *(2)* convert sugars rapidly to acid, and *(3)* maintain these activities even under low-pH conditions.[1] The ability to produce intracellular and extracellular polysaccharides can also be of some importance to cariogenicity. Synthesis and further utilization of intracellular polysaccharides enable bacteria to continue acid production even in the absence of dietary carbohydrates. Extracellular polysaccharides are mostly involved with firm adhesion of the bacteria to the tooth surface.

Few oral bacteria exhibit all these properties. Mutans streptococci and lactobacilli represent the two most important groups of bacteria that are able not only to produce acids from dietary carbohydrates but also to remain viable and continue to metabolize and multiply under low-pH conditions; that is, they are acidogenic and aciduric.[7] Mutans streptococci are also known to be high producers of both intracellular and extracellular polysaccharides.

In 1924, Clarke[49] isolated streptococci from a human caries lesion and called the bacteria *Streptococcus mutans*. More recently, similar mutans streptococci have been classified into several species; *Streptococcus sobrinus* and *S mutans* are the most frequently isolated from humans and the most often associated with the etiology of caries.[7] These bacteria are opportunistic pathogens, found commonly as minor members of the resident oral microbiota of individuals without caries, but under specific environmental conditions they can increase in levels and express their pathogenicity.

The diverse features involved with the cariogenicity of mutans streptococci have been extensively studied. Compared to other bacterial species tested in vitro, the mutans streptococci can produce acid most rapidly, produce the largest amount of acid under low-pH conditions (pH less than 5.5), and survive exposure to acidic conditions.[50,51] In such tests, *S sobrinus* can be more acidogenic and more aciduric than *S mutans*. Nonetheless, *S sobrinus* can be isolated from only a minority of individuals and is usually accompanied and outnumbered by *S mutans*. Although molecular biology methods indicate that *S sobrinus* may be more prevalent than indicated by culture studies, it is rarely present at the same levels in the plaque biofilm as *S mutans*.[52]

S mutans may use two methods of attachment to the tooth surface: sucrose independent and sucrose dependent. In the absence of sucrose, *S mutans* can adhere to salivary proteins, other bacteria, and epithelial cell-surface receptors using physicochemical and lectinlike interactions.[53] In the presence of sucrose, cell wall–associated glucosyltransferases bind to this sugar and break the disaccharide into units of glucosyl and fructosyl, the latter being transported into the cell. The glucosyl units are polymerized into extracellular polysaccharides known as *glucans* (mutan and dextran). Mutan is highly branched and water insoluble and mostly functions as a "biologic glue," strengthening the attachment of mutans streptococci to the tooth surface. Only strains of *S mutans* capable of producing glucans are cariogenic in animal models.[54]

As alluded to earlier, acid production by dental plaque is not essentially dependent on the presence of mutans streptococci. Caries may occur in the absence of these species[55] and their presence in relatively high proportions does not necessarily translate into caries activity.[56] Other oral bacteria, such as lactobacilli, nonmutans streptococci (eg, *Streptococcus gordonii*, *S oralis*, *S mitis*, and *Streptococcus anginosus*), *Actinomyces* species, and *Bifidobacterium* species, are also acidogenic, and some can be aciduric. They may outnumber mutans streptococci

in the plaque biofilm and then be involved in the initiation and/or progression of caries.

Lactobacilli are acidogenic and aciduric bacteria that have also been highly associated with caries. However, in contrast to *S mutans*, they are not high producers of extracellular polysaccharides.[57] As a consequence, lactobacilli show low adherence to smooth tooth surfaces, and their high occurrence in retentive areas such as fissures or cavities may be due primarily to mechanical retention.[58] They are usually involved more in the progression of the caries lesion rather than the initiation.[7]

Of special interest is the role of some beneficial species in plaque. Species of the genera *Veillonella* and *Actinomyces* can degrade lactic acid, thereby increasing plaque pH and reducing its cariogenicity. Another bacterial process promoting an increase in plaque pH is the production of base (eg, ammonia production by urease or arginine deiminase activities of *Streptococcus salivarius* and *S sanguinis*, which respectively act on urea and the arginine-containing peptides in saliva). This may help explain why mutans streptococci sometimes can be found in high numbers despite the apparent absence of demineralization of the underlying enamel.

Bacterial diversity in caries

Nearly all investigations into the microbial pathogenesis of caries have been conducted by culture-dependent studies. More recently, advanced culture-independent molecular methods for bacterial identification have demonstrated that the diversity and complexity of the microbiota associated with caries are far greater than anticipated. In addition to confirming the strong association between mutans streptococci, lactobacilli, and *Actinomyces* species and caries, molecular studies have included new taxa (named species or as-yet-uncultivated phylotypes) in the set of candidate caries pathogens.

It has been reported that the bacterial profiles of dental caries are heterogenous and vary from individual to individual.[59] Overall, about 40% to 60% of the microbiota occurring in caries lesions consists of as-yet-uncultivated phylotypes (eg, species that are known only by 16S ribosomal RNA gene sequences).[59–62] Among the reasons that bacteria cannot be cultivated in the laboratory are a lack of essential nutrients or growth factors in the culture medium; overfeeding conditions; the toxicity of the culture medium itself; the production of substances inhibitory to certain bacteria by other species present in the mixed consortium; metabolic dependence on other species for growth; disruption of bacterial quorum-sensing

systems induced by separation of bacteria on solid culture media; and cells in a viable but noncultivable state—that is, a state of low metabolic activity or dormancy in which cells are unable to divide or form colonies on agar plates.[63–65] Many of these as-yet-uncultivated bacteria have been suspected to play an important role in the caries process.

A molecular study confirmed the concept that dental caries is a result of an ecological disruption in plaque biofilm with a consequent decrease of bacterial diversity arising from the overgrowth of pathogenic species and elimination of beneficial species.[59,67] Plaque collected from healthy children contains significantly more taxa than white-spot lesions, cavitated lesions, and dentinal caries.

Corby et al[67] investigated the bacterial diversity in caries and observed that the top bacterial taxa found to be overabundant in caries-active subjects were *Actinomyces* species strain B19SC, *S mutans*, and *Lactobacillus* species, which exhibited an inverse relationship to beneficial bacterial species, such as *Streptococcus parasanguinis*, *Abiotrophia defectiva*, *Gemella hemolysans*, *S mitis*, *S oralis*, and *S sanguinis*. Overabundance of these beneficial species was found in caries-free subjects. *S mutans* was reported to be overabundant in about 90% of caries-active subjects and underabundant in the caries-free subjects.[67]

Molecular analyses of the bacterial diversity in progressive caries revealed that in subjects with *S mutans*, additional taxa, such as species of *Atopobium* and lactobacilli, were present at significantly higher levels.[61] Subjects with no detectable *S mutans* had a predominance of lactobacilli, *Bifidobacterium dentium*, or low-pH nonmutans streptococci. White-spot lesions and intact enamel sites in diseased subjects often did not have any detectable levels of *S mutans*. While *Actinomyces* and nonmutans streptococci seemed to be involved in the initiation of caries, *S mutans* was found at the highest levels in the later stages of disease.[59,61] Novel phylotypes of *Bifidobacterium* and *Atopobium* may also represent important pathogens in deep caries.[59,68]

Aas et al[59] reported that the microbiota of deep dentinal lesions in permanent teeth was dominated by *S mutans, Lactobacillus* species, *Propionibacterium* species strain FMA5 (currently classified as *Propionibacterium acidifaciens*), and *Atopobium* genomospecies C1. Involvement of the latter two taxa in severe caries was confirmed by other molecular studies.[66,69] Both are previously unrecognized taxa that only very recently have been associated with caries by molecular methods.

Early childhood caries, also known as *nursing (bottle) caries,* is among the most common chronic infectious diseases that affect children in developed countries. A

molecular study identified the bacterial taxa associated with severe early childhood caries in 2- to 6-year-old children and reported a significantly different microbiota when compared to caries-free children.[70] Caries-associated taxa included *Granulicatella elegans* and *Veillonella* species HOT-780. Another study used molecular methods to identify cultivated bacteria from severe early childhood caries lesions and reported that the major species associated with this disease included *S mutans, Scardovia wiggsiae, Veillonella parvula, Streptococcus cristatus,* and *Actinomyces gerensceriae.*[71] Combining culture with 16S rRNA identification identified numerous isolates of oral taxa without previously cultivated representatives. The authors concluded that the major species associated with caries were *S mutans* and *S wiggsiae,* the latter of which was suggested to be included as a candidate caries pathogen.

The complexity of the root surface microbiota suggests that root caries development involves bacterial taxa other than mutans streptococci and lactobacilli. A molecular study investigated the bacterial profiles of root caries in the elderly (between 82 and 98 years old) and observed that 54% of the identified taxa were as-yet-uncultivated phylotypes.[62] Data suggested that, in addition to *S mutans*, lactobacilli, and *Actinomyces,* other bacteria from the genera *Atopobium, Olsenella, Pseudoramibacter, Propionibacterium,* and *Selenomonas* may play an important role in root caries progression.

The bacterial taxa commonly associated with different categories of caries, as revealed by culture-dependent and culture-independent technologies, are depicted in Table 10-1.

Table 10-1 Bacterial taxa associated with different types of caries

Type of caries	Bacterial taxa detected
Occlusal caries	*S mutans* Lactobacilli *Actinomyces* species/phylotypes Nonmutans streptococci
Smooth surface caries	*S mutans*
Dentinal caries	Lactobacilli *Actinomyces* species *S mutans* *Prevotella* species/phylotypes *Bifidobacterium* phylotypes *Atopobium* phylotypes

	Scardovia species
Root caries	*Actinomyces* species *S mutans* Nonmutans streptococci *Atopobium* phylotypes *Olsenella* species *Propionibacterium* species *Selenomonas* species

Dentinal caries

As the demineralized enamel collapses and a cavity is formed, the plaque biofilm reaches dentin (Fig 10-8). The lesion then spreads laterally along the dentinoenamel junction at the edges of the cavity, as well as back through the sound, undermined enamel.[72] The central part of the cavity may be open and accessible to plaque-removal approaches. In this area, the lesion may progress slowly. However, peripheral parts of the same cavity may still be protected by undermined enamel with consequent heavy plaque accumulation. In these areas, the lesion may progress more rapidly.

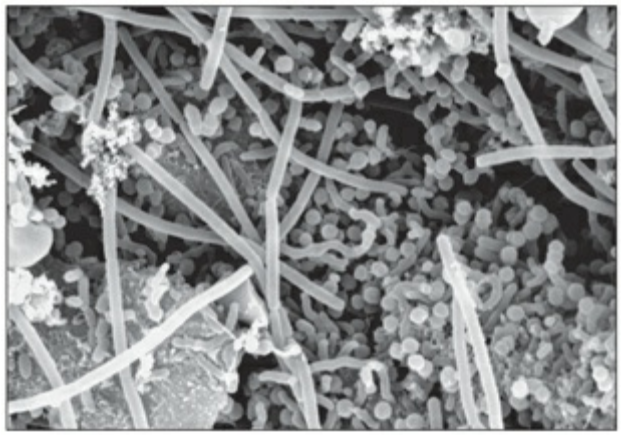

Fig 10-8 Scanning electron micrograph showing bacteria in a dentinal caries lesion. Note the presence of different bacterial morphotypes (original magnification ×4,800).

Once dentin is directly exposed to the bacterial biofilm, the most superficial part of the dentin becomes decomposed by the action of acids and proteolytic enzymes (Fig 10-9). This is called the *zone of destruction*. Beneath this zone, bacterial invasion of the dentinal tubules is frequently observed. Then, a zone of

demineralization separates the infected dentin from the sclerotic dentin, the unaffected dentin, and the pulp (Fig 10-10).

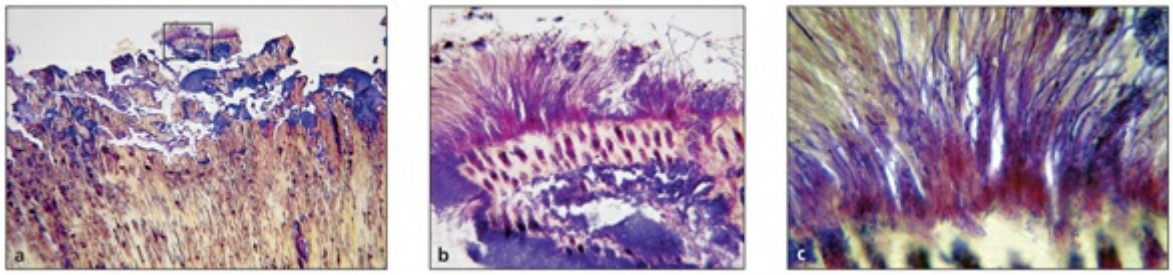

Fig 10-9 *(a)* Front of a deep dentinal caries lesion in a maxillary first molar (Taylor-modified Brown and Brenn stain; original magnification ×100). *(b and c)* Higher magnifications of the inset in *(a)*, revealing tubules filled with bacteria and a biofilm adhered to a dentin chip (original magnification ×400 and ×1,000, respectively). (Courtesy of Dr Domenico Ricucci, Rome, Italy.)

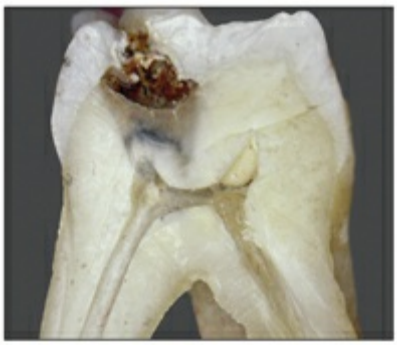

Fig 10-10 Several zones of dentinal caries. The lesion spreads laterally along the dentinoenamel junction and through the undermined enamel. Sclerotic dentin is clearly visible separating affected from unaffected dentin. (Courtesy of Dr Ricardo Carvalhaes, Niterói, Brazil.)

Dentinal destruction is maintained by the presence of a biofilm composed of species sufficiently saccharolytic to demineralize the tissue and proteolytic species that can hydrolyze the dentin collagen matrix (Fig 10-11). After the mineral part of dentin is dissolved by acids, the organic matrix is exposed to breakdown by bacterially derived enzymes. Host-derived enzymes such as the matrix metalloprotein ases, originating from both dentin and saliva, have also been suggested to play an important role in the destruction of dentin organic matrix following demineralization.[73]

As the caries lesion advances deep into dentin, the composition of the involved microbiota shifts from a predominance of facultative and saccharolytic gram-positive bacteria in shallow lesions to a predominance of lactobacilli and/or proteolytic anaerobic bacteria, such as *Prevotella*, in deep dentinal lesions.[74–76] Other taxa present in a number of deep dentinal caries lesions or occurring in abundance include *Fusobacterium nucleatum*; *Pseudoramibacter alactolyticus*;

species/phylotypes from the genera *Atopobium, Selenomonas*, *Dialister*, *Eubacterium*, *Olsenella*, *Bifidobacterium*, *Propionibacterium*, *Actinomyces*, and *Rothia*; and members of the Lachnospiraceae family.[69,77–79]

Studies have demonstrated a close association between pain and the presence of *Prevotella*, *Porphyromonas*, and *Fusobacterium* in deep dentinal caries.[76,80] Black-pigmented bacteria and *S mutans* have been positively related to pulpal pain triggered by heat, while *F nucleatum* and *Actinomyces viscosus* have been associated with cold sensitivity.[81] A molecular study found significant positive associations between *Parvimonas micra and Porphyromonas endodontalis* detection in carious dentin and irreversible pulpitis.[75] Enzymes and metabolites, such as ammonia and indole, which are produced by many anaerobic bacteria found in deep caries, can make intradental sensory nerves more susceptible to stimuli that evoke pain[82,83] and therefore may be partly responsible for symptoms associated with dentinal caries. It has been shown that, in the presence of caries, the amount of bacterial lipopolysaccharide, one of the major constituents of gram-negative bacterial cell walls, is positively associated with pulpal pain.[84] Lipopolysaccharide-mediated pain may be related to the proinflammatory effects of this bacterial component[85] or its direct effects on sensory nerve fibers.[86]

Bacteria present in the advanced front of carious dentin can be considered etiologically significant in the development of pulpitis. When caries reaches the pulp, it is likely that those bacteria are the first to invade the pulp and initiate root canal infection (Fig 10-12). Most of the species in carious dentin have also been detected in infected root canals, clearly suggesting that, in addition to being involved with pulpal damage, these dentinal lesions might well be the primary source of bacteria for endodontic infections.

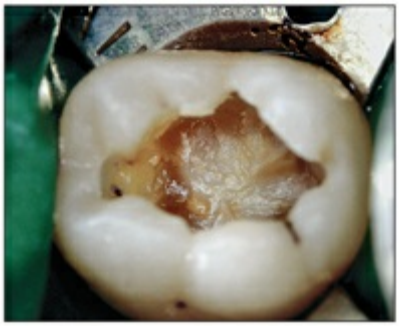

Fig 10-11 Extensive dentinal cavitation as a result of caries. Tissue destruction is mediated by the action of bacterial acids as well as bacteria- and host-derived proteolytic enzymes. (Courtesy of Dr Ricardo Carvalhaes, Niterói, Brazil.)

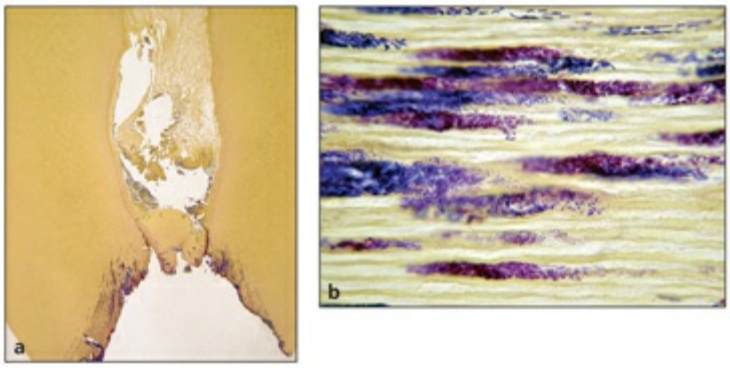

Fig 10-12 *(a)* Deep caries lesion in a maxillary second premolar associated with spontaneous pain. The pulp is severely and irreversibly inflamed after exposure. (The exposure area is not evident in this section but could be observed in other sections.) Empty spaces indicate abscess in the coronal pulp (Taylor-modified Brown and Brenn stain; original magnification ×25). *(b)* Higher magnification of the carious margin in *(a)*, showing bacteria inside dentinal tubules (original magnification ×1,000). (Courtesy of Dr Domenico Ricucci, Rome, Italy.)

Role of diet and saliva

There is a direct relationship between dental caries and carbohydrate intake. Any fermentable carbohydrate such as glucose, sucrose, fructose, or cooked starch can be metabolized by cariogenic bacteria, with consequent acid formation. Among the carbohydrates, sucrose is by far the most cariogenic because, in addition to being readily fermented by oral bacteria to generate acid, it serves as a substrate for the synthesis of extracellular and intracellular polysaccharides. The frequency of sucrose intake, rather than the total amount consumed, appears to be of decisive importance. Also important are the stickiness and concentration of the sucrose consumed, both factors influencing the period for which this sugar is retained in close contact with the tooth surface.

Saliva exerts several functions of paramount importance in the maintenance of oral health and protection against caries. The mechanical washing action of saliva is a very effective mechanism in the clearance of sugars, acids, and unattached bacteria. Salivary buffering systems, such as the bicarbonate/ carbonate system, tend to neutralize acids produced by plaque bacteria on tooth surfaces. Saliva is supersaturated with calcium and phosphate, which are important in the remineralization of white-spot lesions.[41] Antimicrobial systems in saliva entail immunoglobulins, agglutinin, lactoferrin, cystatins, lysozyme, and defensins and are involved in the first line of host defense in the oral cavity.[87]

Salivary production can be reduced as a result of head and neck irradiation or as a consequence of other diseases (eg, Sjögren syndrome) or medications.[37] Whatever the reason, reduction in salivary flow will negatively impact all of its protective functions, favoring the activity of cariogenic bacteria.[38,44] As a consequence, rapidly progressive caries can develop in many sites.

Diagnosis

Proper treatment decision making relies on accurate diagnosis. Caries diagnosis involves detecting a lesion, estimating its depth and degree of demineralization, and evaluating its activity.[39] Traditional methods of caries detection include direct visual observation, probing, and radiographs.[88] In probing, it is very useful to gently drive the probe tip across the lesion to detect a matte surface, which is indicative of an active lesion. However, it is most undesirable to jab the probe tip into the lesion because it may create an incipient breach of the enamel and spread the infection from one tooth surface to another. Sharp pain and even pulpal exposure may also be induced after probing of deep dentinal caries. Radiographs are not very helpful for anything but advanced dentinal lesions, although bitewing radiographs are still widely used and recommended to detect approximal caries in posterior teeth (Fig 10-13).

Recurrent caries associated with restorations at approximal or gingival areas can also be diagnosed by radiographs, provided that the x-ray beam is at an optimal angle in relation to the lesion.[42] However, restorative materials are radiopaque, and the radiographic image of the restoration can sometimes overlap the lesion completely or partially. Stained margins of tooth-colored restorations and ditched margins in which the probe tends to stick often are misdiagnosed as recurrent caries. In fact, only frankly cavitated caries lesions at restoration margins constitute a reliable diagnosis of recurrent caries.[42]

The current worldwide trend in caries management is to move away from the operative model toward a preventive approach, in which the initiation and progression of the disease is controlled through an individual's lifetime. Based on this, early lesions should be accurately identified so that further operative intervention is not made necessary. Early detection of caries affords the clinician the possibility to promote remineralization via enhancement of the salivary flow, use of

topical fluoride and chlorhexidine, and meticulous oral hygiene.

Nevertheless, diagnosis of initial caries lesions by traditional methods can be a challenge for clinicians, and many lesions go undetected.[89] As a consequence, new detection tools that are based on the measurement of a physical signal to identify these lesions have become available. Examples of the physical signals and detection systems that have the potential to be used include radiographs (digital subtraction radiography and digital image enhancement), visible light (fiber-optic transillumination and quantitative light-induced fluorescence), laser light (laser fluorescence measurement), electrical current (electrical conductance or impedance measurement), ultrasound, and possibly surface roughness.[89] The accuracy, safety, and clinical feasibility of most of these new technologies have yet to be demonstrated.

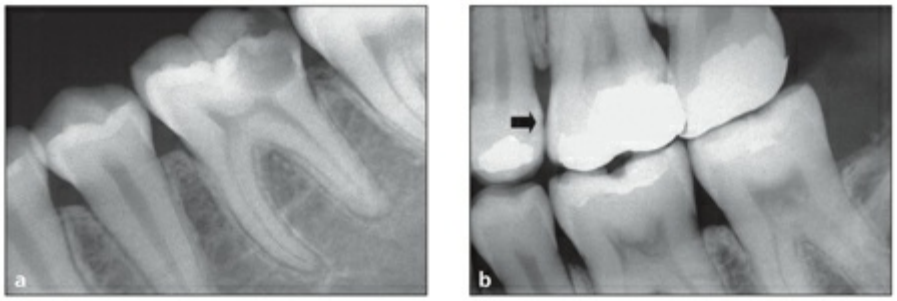

Fig 10-13 *(a)* Periapical radiograph showing an advanced dentinal caries lesion. *(b)* Bitewing radiograph showing approximal caries *(arrow)*.

Treatment

The conventional approach to the treatment of dental caries has been to remove and replace the diseased tissue with an inert restoration. The main problems with restoration without the support of a preventive approach are the short clinical durability of restorations and the propensity of recurrent caries to form at the margins of restorations. Thus, the contemporary philosophy in caries management highlights the importance of accurate diagnosis, creation of a favorable environment to arrest caries with minimal operative intervention, and active prevention.[88]

In fact, over the past three decades there has been a transition in many countries toward a largely preventive and preservative approach to caries management. Prevention of early caries lesions by meticulous removal of the biofilm, as well as application of fluoride or placement of sealants, is successful in preserving tooth

structure. When a cavity is formed, restorative intervention is needed to restore the integrity of the tooth surface so that the patient can clean. In this context, modern microrestorative techniques that use new adhesive materials are preferable to preserve tooth structure. Laser application has also been suggested for caries treatment because it has been claimed to preserve tooth structure and make the mineral highly resistant to dissolution by subsequent acid challenges.[90]

In cases of recurrent caries, repair of any localized defects at restoration margins, including the lesion, should be considered by clinicians rather than a total replacement. Repair of restorations has the advantage of preserving tooth structure.[42]

There has been a great controversy as to the need for removing all carious dentin before a restoration is placed. Important questions arise, and the answers will drive the decision making for the best approach. What is the fate of the bacteria left behind and entombed by the restoration (assuming that the restoration actually entombs them)? Do these caries lesions remain active or are they arrested? What are the effects of the remaining bacteria on the pulp tissue?

As for the progression of the caries lesion, it seems that there is no clear evidence that it is deleterious to leave infected dentin, even if it is soft and wet, prior to placement of a restoration. Indeed, sealing the dentin from the oral environment can arrest lesion progression. Reparative processes, such as tubular sclerosis and tertiary dentin formation, can be encouraged, thus reducing the permeability of the remaining dentin. The residual bacteria are now in a quite different environment, entombed by the seal of the restoration on one side and the reduced permeability of the remaining dentin on the other.[72] Provided that the restoration actually seals, these bacteria are then subject to nutrient limitation or simplification, as they no longer have access to components of diet or saliva.

A study investigated the effects of sealing infected carious dentin below dental restorations on the phenotypic and genotypic diversity of the surviving microbiota.[91] The baseline microbiota was composed primarily of lactobacilli, *S mutans*, *S parasanguinis*, *Actinomyces israelii*, and *Actinomyces gerencseriae*. None of these taxa was isolated from dentin samples taken after 5 months, with the microbiota now consisting of only *Actinomyces naeslundii*, *S oralis*, *Streptococcus intermedius*, and *S mitis*. Bacteria in the final sample exhibited a significantly increased ability to produce glycosidic enzymes, which liberate sugars from glycoproteins in the dentinal fluid. The dentinal microbiota under the restorations was subject to a significant environmental change, mostly related to the amount and type of nutrients available. The resulting changes in the microbiota were characterized by a reduction in the bacterial load in the infected dentin, a reduction in the bacterial diversity

(fewer species), and a reduction in the genotypic diversity of the surviving microbiota. Procedures and materials used to restore the teeth may also have exerted direct toxic effects on the bacteria under the restoration.

However, bacterial growth may occur beneath the restoration, although restricted to those genotypes best able to exploit that environment. Although residual bacteria will not have access to nutrients from the oral cavity, the substrate is still available, primarily from serum proteins and glycoproteins passing from the pulp through the patent dentinal tubules to the infected dentin. Thus, proteolytic anaerobic bacteria present in the deep layers of dentinal caries are expected not to be significantly affected by the seal promoted by the restoration because their main source of nutrients is relatively unaltered.

As for the effects on the pulp, those bacteria that remain viable with a sustainable source of nutrients from the pulp may maintain or exacerbate pulpal aggression. If the pulp was already exposed by the caries process, it is highly unlikely that tertiary dentin will be effectively produced to seal the exposure area, and pulpal disease will progress. Therefore, the question as to whether carious dentin must be removed or not should take into account several clinical aspects, where the ultimate goals are to arrest the caries disease process and eliminate further sources of irritation to the pulp tissue.

A recent study revealed that the pattern of gene expression in pulps associated with carious dentin differs from that in healthy pulps; several genes are downregulated and others are upregulated in disease.[92] These findings open interesting perspectives for the use of gene markers to predict pulpal status under caries lesions and establish improved diagnosis and treatment approaches.

Prevention

The major approaches to prevention of caries are:

- Elimination or reduction of between-meal ingestion of fermentable carbohydrates or use of non-cariogenic artificial sweeteners as substitutes.
- Stimulation of salivary flow after main meals (eg, by sugar-free gum).
- Reduction of the cariogenic microbiota (eg, by oral hygiene measures, antimicrobial agents, and possibly immunization).

• Use of fluoride.

Limitation of fermentable carbohydrates

Avoidance, between main meals, of foods and drinks that contain fermentable carbohydrates and the consumption of foods and drinks that contain nonfermentable carbohydrate substitutes are important to prevent caries by reducing repeated low-pH conditions in the dental plaque.[38] Foods and drinks can be prepared with compounds that are as sweet as sucrose but that cannot be metabolized rapidly to acid. Substances such as sugar alcohols (sorbitol and xylitol) or intense sweeteners (aspartame and saccharin) can stimulate salivary flow in the absence of significant acid production and can even lead to the remineralization of early lesions. Of particular interest, xylitol tastes like sucrose, is not metabolized by cariogenic bacteria, and has antibacterial properties.

Stimulation of salivary flow

In the event that mechanical removal of plaque after main meals cannot be carried out by toothbrushing or flossing, stimulation of salivary flow by chewing sugar-free gum introduces the beneficial effects of saliva, including the buffering capacity that brings the plaque pH to resting levels, mechanical removal of fermentable substrates, and promotion of remineralization.[6,44]

Reduction of the cariogenic microbiota

Mechanical removal of plaque by efficient oral hygiene measures can almost completely prevent caries. In addition, the incorporation of chemical agents with antibacterial activity into dental products has been proposed as a potential method to prevent plaque-mediated diseases.[93] Antibacterial agents, delivered either by mouthrinse or toothpaste, can be used to maintain plaque at levels compatible with oral health.[93]

Currently, one of the most successful antibacterial therapies against cariogenic bacteria is treatment with chlorhexidine gluconate rinse or gel.[90] A daily dose of chlorhexidine rinse for 2 weeks can markedly reduce the cariogenic bacteria in the oral cavity, and recolonization takes place in 3 to 6 months rather than immediately.[94] Therefore, in patients with high levels of cariogenic bacteria, chlorhexidine treatment at 3-month intervals may be beneficial.

Although the microbial etiology of dental caries is not totally specific, the concept

of immunization against caries has focused on mutans streptococci as a target. Development of a caries vaccine has faced some important drawbacks from the beginning. The potential of immunologically mediated tissue damage in humans following exposure to streptococcal antigens (as occurs in rheumatic fever) was one of the main concerns. Vaccines with whole cells of *S mutans* were shown to induce antibodies with cross-reactivity to the heart tissue.[1] Therefore, subsequent work has been directed toward characterizing the antigenic composition of mutans streptococci and selecting individual purified antigens that can confer protection but lack the risks of cross-reactivity with human tissues. Recently, the focus has been on the three potential antigens—the surface fibrillar adhesins known as *antigen I/II*, the glucosyltransferases, and the glucan-binding proteins.[95] All of them have been associated with virulence and the process of tooth surface colonization.[95] Antibodies against these antigens generally interfere with adhesion to and/or accumulation on tooth surfaces and can also promote mutans streptococci clearance from the oral cavity before colonization.

The optimal route for human vaccination has yet to be established. Possibilities include systemic injection (to generate circulating antibodies, especially immunoglobulin G, which would enter the oral cavity via the gingival fluid) and mucosal immunization (which evokes mainly a secretory immunoglobulin A response). While some early efforts utilized systemic injection with some success in rodent and primate models, most authorities have long recognized that mucosal immunization has the potential to be not only more effective but also safer.[96]

Vaccination trials on humans have been relatively unsuccessful because of the fears of possible side effects, the fact that the disease can be adequately controlled using other approaches, and the fact that the incidence of caries is decreasing in Western societies. Moreover, a major question facing health organizations is whether a vaccine is justified against a non–life-threatening disease. A caries vaccine could, however, be useful for developing countries with an increasing prevalence of caries and for prevention of disease in special high-risk groups.[88]

Use of fluoride

Because the discovery of the anticariogenic properties of fluoride represents a landmark in the history of dentistry, this agent deserves a separate section. Fluoride in the drinking water as well as in products such as toothpastes, mouthrinses, and gels for topical use has been a major contributor to the substantial reduction in caries prevalence over the last 30 years in Western societies.[90] Given its beneficial

effects, fluoride has also been added to table salt and milk.

Fluoride's extensively studied and documented principal mechanisms of anticariogenic action rely on the presence of fluoride in saliva, in the plaque biofilm at the tooth surface, and in the fluid among the crystals in the subsurface of the enamel or dentin. Fluoride in drinking water and in fluoride-containing products reduces caries via topical mechanisms. Low but slightly elevated levels of fluoride in saliva and plaque provided from these sources help prevent and reverse caries by inhibiting demineralization and enhancing remineralization. The level of fluoride incorporated into dental mineral by systemic ingestion has been shown to be insufficient to play a significant role in caries prevention.[41] Because the major effects of fluoride are related to topical mechanisms, it is needed regularly throughout life to afford protection against caries.

Fluoride primarily works through the following topical mechanisms.

Inhibition of demineralization at the crystal surfaces inside the tooth

The mineral of enamel, dentin, and bone is a carbonated hydroxyapatite. Carbonated hydroxyapatite is much more soluble in acid than is hydroxyapatite, is calcium deficient (calcium is replaced by sodium, magnesium, zinc, etc), and contains between 3% and 6% carbonate by weight, mostly replacing phosphate ions in the crystal lattice. During demineralization the carbonate is preferentially removed, and during remineralization it is excluded from the newly formed mineral. The calcium-deficient and carbonate-rich regions of the crystal are especially susceptible to attack by acids during demineralization.[97]

The crystals of enamel are about 40 nm in diameter and are clustered into enamel prisms about 4 to 5 μm in diameter. The tiny gaps that occur between the crystals are filled with protein, lipid, and water and form the diffusion routes for acids and ions (eg, hydrogen, fluoride, and calcium).[41,98] If fluoride is present in the aqueous solution surrounding the crystals, it is adsorbed strongly to the surface of carbonated hydroxyapatite and thus acts as a potent protective mechanism against acid dissolution of the crystal surface. If fluoride is in the plaque fluid at the time that the bacteria produce acid, it goes along with the acid into the subsurface of the tooth and then adsorbs to the crystal surface and protects it against dissolution.

Enhancement of remineralization at the crystal surfaces

As saliva bathes the plaque biofilm, neutralizing the acids and raising the pH, demineralization is stopped and reversed. Saliva is also saturated with calcium and phosphate, which are brought back to the tooth surface and are involved in the

remineralization process.[98] The partially demineralized crystal surfaces within the lesion act as "nucleators," and new surfaces grow on the crystals.[98]

Fluoride enhances remineralization by adsorbing to the crystal surface and attracting calcium ions, followed by phosphate ions, leading to new mineral formation. The newly formed "veneer" excludes carbonate and has a composition somewhere between hydroxyapatite and fluorapatite (ie, a fluorapatite-like mineral). The new remineralized crystal now will behave like low-solubility fluorapatite rather than the highly soluble carbonated hydroxyapatite of the original crystal surface.[90] Subsequent acid challenges must be quite strong and prolonged to dissolve the remineralized enamel.

Inhibition of bacterial metabolism

The ionized form of fluoride (F–) cannot cross the cell wall and membrane of cariogenic bacteria but can rapidly enter into the cell as hydrogen fluoride.[99] When the pH in plaque falls during acid production, a portion of the fluoride present in the plaque fluid combines with hydrogen ions to form hydrogen fluoride, which rapidly diffuses into the bacterial cell. Once inside the cell, the hydrogen fluoride dissociates, acidifying the cell and releasing fluoride ions that interfere with bacterial enzymatic activities. For instance, fluoride inhibits enolase, a bacterial enzyme involved in the metabolism of carbohydrates.

Endodontic Infections and Apical Periodontitis

Apical periodontitis is essentially an inflammatory disease of microbial etiology primarily caused by infection of the root canal system. Seminal studies about the infectious etiology of apical periodontitis are also credited to Miller.[100] However, the unequivocal role of microorganisms in the causation of apical periodontitis was definitively established only in 1965, by an elegant study in germ-free rats.[101] After exposing the pulps of conventional and germ-free rats to their own oral cavities, Kakehashi et al[101] observed that pulpal necrosis and apical periodontitis lesions only developed in conventional rats, which had a resident oral microbiota. On the other hand, the dental pulps of the germ-free rats not only remained vital but also repaired themselves by hard tissue formation, isolating the pulps again from the oral cavity. Other important studies in humans[102] and monkeys[103] have confirmed the

pivotal role of microorganisms in the etiology of apical periodontitis.

As long as the pulp is vital, it can protect itself against microbial invasion and colonization. However, if the pulp becomes necrotic as a result of caries, trauma, operative procedures, or periodontal disease, it can be easily infected. This is because host defenses do not function in necrotic pulp tissue and those in the periradicular tissues do not reach deep into the root canal. Therefore, for endodontic infections to occur, the root canal must be devoid of vital pulp tissue and its defenses, either as a consequence of pulpal necrosis or pulp removal for treatment.

Pathogenesis

Although fungi and, most recently, archaea and viruses have been found in endodontic infections, bacteria are the major microorganisms implicated in the pathogenesis of apical periodontitis. Bacteria colonizing the root canal system enter in contact with the periradicular tissues via apical and lateral foramina. As a consequence of the encounter between bacteria and host defenses, inflammatory changes take place in the periradicular tissues and give rise to the diverse forms of apical periodontitis.

Epidemiologic studies using sophisticated culture and molecular biology techniques have collectively shown that over 400 different microbial species can be found in infected root canals, usually in combinations involving many species in primary infections and a few species in secondary and persistent infections.[104] Because endodontic infections develop in a previously sterile place that as such does not contain a normal microbiota, any one of these species has the potential to be an endodontic pathogen.

Under normal conditions, the pulpodentin complex is sterile and protected from the oral microbiota by overlying enamel and cementum. In the event that the integrity of these natural layers is breached (eg, by caries, trauma, or operative procedures) or naturally absent (eg, because of gaps in the cemental coating at the cervical root surface), the pulpodentin complex is exposed to the oral environment and then challenged by microorganisms present in caries lesions, in saliva bathing the exposed area, or in dental plaque formed on the exposed area. Microorganisms from subgingival biofilms associated with periodontal disease may also have access to the pulp via dentinal tubules at the cervical region of the tooth and lateral and apical

foramina (Fig 10-14).

Fig 10-14 Routes of pulpal infection: caries lesion (via tubules or direct exposure) *(1)*; cracks in the enamel reaching dentin and exposing tubules *(2)*; periodontal disease *(3)*.

Whenever dentin is exposed, the pulp is put at risk of infection as a consequence of the permeability of normal dentin, which is dictated by its tubular structure. Dentinal tubules traverse the entire width of the dentin and have a conical conformation, with the largest diameter located near the pulp (mean of 2.5 μm) and the smallest diameter located in the periphery, near enamel or cementum (mean of 0.9 μm)[105](Fig 10-15). The smallest tubule diameter is entirely compatible with the cell diameter of most oral bacterial species, which usually ranges from 0.2 to 0.7 μm.

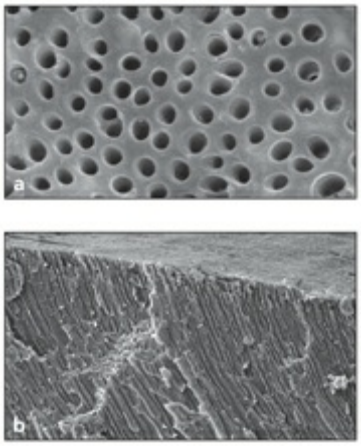

Fig 10-15 Scanning electron micrographs of dentin showing tubules in cross-sectional *(a)* and longitudinal *(b)* views (original magnification ×850 and ×130, respectively).

Thus, it might seem likely that, once exposed, dentin offers an unimpeded access pathway for bacteria to reach the pulp via tubules. Nevertheless, this is not necessarily the case. It has been demonstrated that bacterial invasion of dentinal tubules occurs more rapidly in nonvital teeth than in vital ones.[106] In vital teeth,

outward movement of dentinal fluid and the tubular contents (including odontoblast processes, collagen fibrils, and the sheathlike lamina limitans that lines the tubules) influence dentin permeability and can conceivably delay intratubular invasion by bacteria. As a consequence of the presence of tubular contents, the functional or physiologic diameter of the tubules is only 5% to 10% of the anatomical diameter visible by microscopy.[107] Moreover, other factors, such as dentinal sclerosis (see also chapter 18) beneath a caries lesion, tertiary dentin, the smear layer, and intratubular deposition of fibrinogen, also reduce dentin permeability and thereby limit or even impede bacterial progression to the pulp via dentinal tubules.[108] Host defense molecules, such as antibodies and components of the complement system, may also be present in the dentinal fluid of vital teeth and can assist in the protection against deep bacterial invasion of dentin.[109–111] Hence, as long as the pulp is vital, dentinal exposure does not represent a significant route of pulpal infection, except when dentinal thickness is considerably reduced and dentin permeability is significantly increased.

Most of the bacteria in the caries process are nonmotile, and they invade dentin by repeated cell division that pushes cells into tubules. Bacterial cells may also be forced into tubules by hydrostatic pressures developed on dentin during mastication.[112] Bacteria inside tubules under a deep caries lesion can reach the pulp even before frank pulpal exposure.[113] However, it has been assumed that the pulp will not be infected as long as it is vital. A few bacteria that reach the pulp can be of comparatively little importance because the vital pulp can eliminate such a transient infection and rapidly clear or remove bacterial products. The efficient clearance mechanism tends to prevent injurious agents from reaching a high enough concentration to induce significant inflammatory reactions.[114] On the other hand, if the vitality of the pulp is compromised and the defense mechanisms are impaired, even a very few bacteria may initiate infection.

Bacteria have been isolated from traumatized teeth with necrotic pulps and apparently intact crowns.[102,115] As described in chapter 16, it has been suggested that, in teeth whose pulps have become necrotic after trauma, bacteria from the gingival crevice or periodontal pockets might reach the root canals through severed blood vessels of the periodontium—a phenomenon called *anachoresis*.[116] However, this theory has never been supported by scientific evidence.

In fact, trauma can induce exposure of dentin by fracturing the crown or inducing the formation of enamel cracks. Macrocracks and microcracks in enamel can be present in most teeth (not only traumatized ones) and do not necessarily end at the

dentinoenamel junction, but rather deep into the dentin.[117] A large number of dentinal tubules can be exposed to the oral environment by a single crack. These cracks can be clogged with dental plaque and provide portals of entry for bacteria. If the pulp remains vital after trauma, bacterial penetration into tubules is counteracted by the dentinal fluid and tubular contents, as already discussed. In this event, pulpal health is not usually jeopardized. On the other hand, if the pulp becomes necrotic as a consequence of trauma, it loses the ability to protect itself against bacterial invasion, and, regardless of dentinal thickness, dentinal tubules then will become true avenues through which bacteria can reach and colonize the necrotic pulp.

Direct exposure of the dental pulp to the oral cavity is the most obvious route of endodontic infection. Caries is the most common cause of pulpal exposure (Fig 10-16), but bacteria may also reach the pulp via direct pulpal exposure as a result of iatrogenic restorative procedures or trauma. The exposed pulp tissue enters in direct contact with oral bacteria from caries lesions, saliva, and/or plaque accumulated on the exposed surface. Almost invariably, exposed pulps will undergo inflammation and necrosis and become infected (see Fig 10-12). The time elapsed between pulpal exposure and infection of the entire canal is unpredictable, but it is usually a slow process.[118]

Conceptually, microorganisms in subgingival plaque biofilms associated with periodontal disease could reach the pulp by the same pathways through which intracanal microorganisms reach the periodontium and thereby could exert harmful effects on the pulp. However, it has been demonstrated that although degenerative and inflammatory changes of different degrees may occur in the pulp of teeth with associated marginal periodontitis, pulpal necrosis only develops as a consequence of periodontal disease if the periodontal pocket reaches the apical foramen, leading to irreversible damage of the main blood vessels that penetrate this foramen to irrigate the pulp[119] (see chapter 16). Once the pulp is necrotic, periodontal bacteria can reach the root canal system via exposed dentinal tubules at the cervical area of the root or via lateral and apical foramina to establish an infectious process.

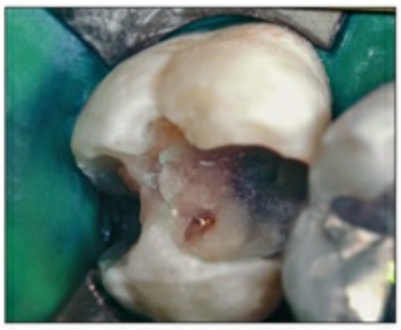

Fig 10-16 Pulp exposure by caries. This is the major route of pulpal infection. Once exposed, the pulp

tissue directly contacts oral bacteria present in the caries lesion and in saliva bathing the cavity.

Types

Endodontic infections can be classified according to the anatomical location (intraradicular or extraradicular infection). Intraradicular infections can in turn be subdivided into three categories (primary, secondary, or persistent infection), depending on the time participating microorganisms gained entry and established themselves in the root canal.[120] The characteristics of the microbiota may vary depending on the different types of infection (Fig 10-17 and Box 10-1).

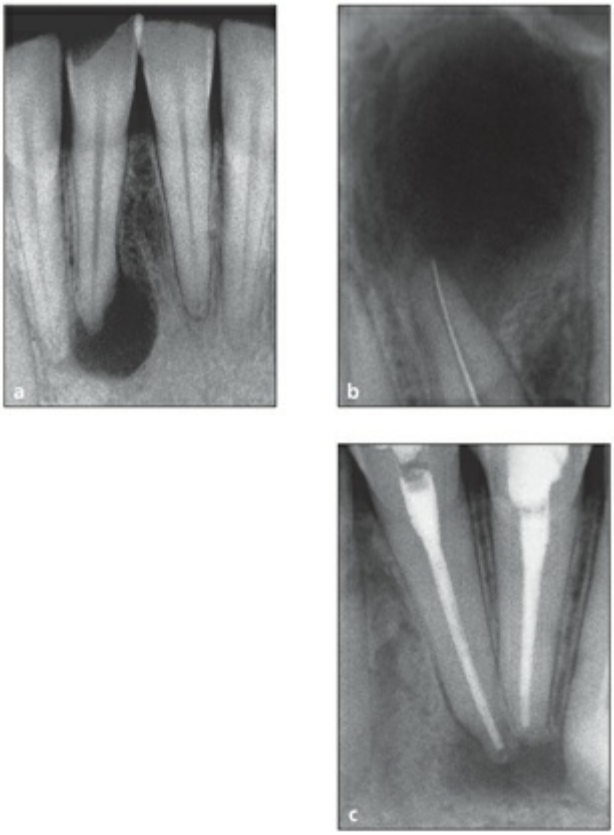

Fig 10-17 Radiographs showing presentation of each of the different types of endodontic infection (see Box 10-1) : *(a)* primary infection; *(b)* persistent infection (root canal filling stage); *(c)* persistent/ secondary infection (endodontically treated teeth).

Box 10-1	Main characteristics of the different types of endodontic infection
Primary infection • Mixed infection	

• 2–30 species per canal
• 10^3–10^8 bacterial cells per canal
• 40%–56% uncultivated bacteria
• Most frequent bacteria:

–*Treponema* species
–*Tannerella forsythia*
–*Porphyromonas species*
–*Dialister* species
–*Filifactor* alocis
–*Pseudoramibacter alactolyticus*
–*F nucleatum*
–*Synergistes species*
–*Pyramidobacter piscolens*
–*Prevotella* species
–*Olsenella* species
–*P micra*
–*Campylobacter* species

Persistent infection (root canal filling stage)
• Single or mixed infection
• 1–5 species per canal
• 10^2–10^5 bacterial cells per canal
• 42% uncultivated bacteria
• Most frequent bacteria:

–*S mitis*
–Other streptococci
–*Propionibacterium* species
–*F nucleatum*
–*Prevotella* species
–*P alactolyticus*
–*P micra*
–Lactobacilli
–*Olsenella* species
–*Actinomyces* species

Persistent/secondary infection (endodontically treated teeth)
• Single or mixed infection
• Adequate treatment: 1–5 species per canal
• Inadequate treatment: 2–30 species per canal
• 10^3–10^7 bacterial cells per canal
• 55% uncultivated bacteria
• Most frequent microorganisms:

–*Enterococcus faecalis*
–*Candida albicans* (yeast)
–*Streptococcus* species
–*P alactolyticus*
–*Propionibacterium propionicum*

–*F alocis*
–*Dialister* species
–*Actinomyces* species
–*Pseudomonas aeruginosa*
–Enteric rods

Primary intraradicular infections

Primary intraradicular infection is caused by microorganisms that initially invade and colonize the necrotic pulp tissue. It has also been referred to as *initial infection*. Participating microorganisms can have been involved in the earlier stages of pulpal invasion (usually via caries), which culminated in inflammation and further necrosis, or they can be latecomers that took advantage of the environmental conditions in the canal after pulpal necrosis.

Diversity of the endodontic microbiota

Microbiota can be defined as a collective term for microorganisms and should replace terms such as *flora* and *microflora*, which perpetuate an outdated classification of microorganisms as plants.[121] *Diversity* refers to the number of different species present (richness) and their relative abundance (evenness) in a given ecosystem.[122]

The oral cavity harbors one of the highest accumulations of microorganisms in the body. Although viruses, archaea, fungi, and protozoa can be found as constituents of the oral microbiota, bacteria are by far the most dominant microbial inhabitants of the oral cavity. There are an estimated 10 billion bacterial cells in the oral cavity,[123] and culture-independent studies (microscopy and molecular biology technologies) have shown that more than 50% to 60% of the oral microbiota still remains to be cultivated and fully characterized.[124,125] Indeed, a high diversity of bacterial species has been revealed in the oral cavity by culturing approaches, but application of molecular biology methods to the analysis of the bacterial diversity has revealed a still broader and more diverse spectrum of extant oral bacteria. More than 700 bacterial taxa have been found in the human oral cavity, and phylogenetic studies have revealed that these taxa fall into 13 separate phyla, namely Sulphur River 1 (SR1), TM7, Chloroflexi, Cyanobacteria, Deinococcus, Acidobacteria, Synergistes, Spirochaetes, Fusobacteria, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes.[126–130]

In spite of the large number of species that can colonize the oral cavity, only a limited set of bacterial species is consistently selected out of the oral microbiota for

growth and survival within necrotic root canals. Taken together, data from studies using culture-dependent or culture-independent identification approaches have suggested that a select group of bacterial species can be considered as candidate endodontic pathogens, based on both their frequency of detection and potential pathogenicity.

Sophisticated culture and molecular biology techniques have clearly revealed the polymicrobial nature of endodontic infections and a conspicuous dominance of obligate anaerobic bacteria in primary infections. Current evidence reveals that endodontic bacteria fall into 9 of the 13 phyla that have oral representatives, namely Firmicutes, Bacteroidetes, Spirochaetes, Fusobacteria, Actinobacteria, Proteobacteria, Synergistes, SR1, and TM7.[131–135] However, these figures may be underestimated; high-throughput molecular methods have demonstrated a previously unanticipated diversity of the endodontic microbiota.[136] Noteworthy is the high prevalence of as-yet-uncultivated species; about 40% to 56% of the endodontic microbiota is composed of bacteria that have yet to be cultivated and fully characterized.[132,133]

Primary infections are characterized by a mixed consortium composed of 10 to 30 bacterial species and 103 to 108 bacterial cells per canal[102,104,133,135] (see Fig 10-17a and Box 10-1). The size of the apical periodontitis lesion has been shown to be proportional to the number of bacterial species and cells in the root canal.[102] In other words, the larger the lesion, the greater the bacterial diversity and density in the canal. In addition, bacterial profiles of the endodontic microbiota also vary from individual to individual,[137,138] indicating that apical periodontitis has a heterogenous etiology in which multiple bacterial combinations can play a role in disease causation.

Bacterial genera and their common representatives in endodontic infections are shown in Table 10-2. The most prevalent named species detected in primary infections, including abscessed cases, belong to diverse genera of gram-negative (*Fusobacterium*, *Dialister*, *Porphyromonas*, *Prevotella*, *Tannerella*, *Treponema*, *Campylobacter*, and *Veillonella*) and gram-positive (*Peptostreptococcus*, *Parvimonas*, *Filifactor*, *Pseudoramibacter*, *Actinomyces*, *Streptococcus*, *Propionibacterium*, *Eubacterium*, and *Olsenella*) bacteria.[102,104,131–133,139–148] The most prevalent species found in primary infections associated with chronic apical periodontitis and acute apical abscesses are displayed in Figs 10-18 and 10-19, respectively.

About half of the bacterial taxa detected in primary infections are as-yet-

uncultivated phylotypes. Molecular studies investigating the breadth of bacterial diversity in infected root canals have disclosed the occurrence of phylotypes belonging to several genera, including *Synergistes*, *Dialister*, *Prevotella*, *Solobacterium*, *Olsenella*, *Fusobacterium*, *Eubacterium*, *Megasphaera*, *Veillonella*, and *Selenomonas,* as well as phylotypes related to the family Lachnospiraceae or the TM7 phylum.[131–134,149–152] Some uncultivated phylotypes can even be among the most prevalent bacteria in primary intraradicular infections, and others may be associated with pain.[132] Detection of as-yet-uncultivated phylotypes in samples from endodontic infections suggests that they can be previously unrecognized bacteria that play a role in the pathogenesis of different forms of apical periodontitis. The fact that they have not yet been cultivated and phenotypically characterized does not mean that they are not important.

Table 10-2 **Bacterial genera and respective common representatives occurring in endodontic infections**

	Gram-negative bacteria		Gram-positive bact	
Shape	**Anaerobic**	**Facultative**	**Anaerobic**	**Facu**
Rods	*Dialister: D invisus, D pneumosintes,uncultivated phylotypes* *Porphyromonas: P endodontalis, P gingivalis* *Tannerella: T forsythia* *Prevotella: P intermedia, P nigrescens, P baroniae, P tannerae, P denticola, P multisaccharivorax,* uncultivated phylotypes *Fusobacterium: F nucleatum, F periodonticum,* uncultivated phylotypes *Campylobacter: C rectus, C gracilis, C curvus, C showae* *Synergistes: uncultivated phylotypes*	*Capnocytophaga: C gingivalis, C ochracea* *Eikenella: E corrodens* *Haemophilus: H aphrophilus*	*Actinomyces: A israelii, A gerencseriae, A meyeri, A odontolyticus* *Pseudoramibacter: P alactolyticus* *Filifactor: F alocis* *Eubacterium: E infirmum, E saphenum, E nodatum, E brachy, E minutum* *Mogibacterium: M timidum, M pumilum, M neglectum, M vescum* *Propionibacterium: P acnes, P propionicum* *Eggerthella: E lenta* *Olsenella: O uli, O profusa, uncultivated phylotypes*	*Actin* *naesl* *Cory* *C ma* *Lacto* *saliv* *acido* *para*

	sputigena, S noxia, uncultivated phylotypes *Centipeda: C periodontii Catonella: C morbi Pyramidobacter: P piscolens*		*Bifidobacterium: B dentium Slackia: S exigua Atopobium: A parvulum, A minutum, A rimae Solobacterium: S moorei, uncultivated phylotypes* Lactobacillus: L catenaformis	
Cocci	*Veillonella: V parvula,* uncultivated phylotypes *Megasphaera:* uncultivated phylotypes	*Neisseria: N mucosa, N sicca*	*Parvimonas: P micra Peptostreptococcus: P anaerobius, uncultivated phylotypes Finegoldia: F magna Peptoniphilus: P asaccharolyticus, P lacrimalis Anaerococcus: A prevotii Streptococcus: S anginosus, S constellatus, S intermedius Gemella: G morbillorum*	*Strep mitis, S gor orali Enter faeca Gran adiac*
Spirilla	*Treponema: T denticola, T socranskii, T parvum, T maltophilum, T lecithinolyticum*	NA	NA	NA

NA, not applicable.

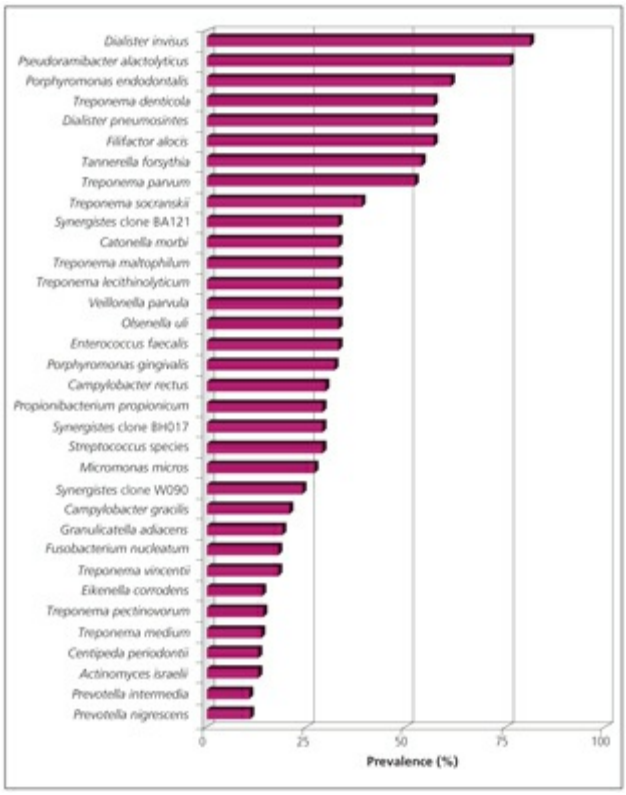

Fig 10-18 Prevalence of bacteria detected in primary infections of teeth with chronic apical periodontitis. (Data are based on studies by the author's group using a highly sensitive nested polymerase chain reaction protocol.)

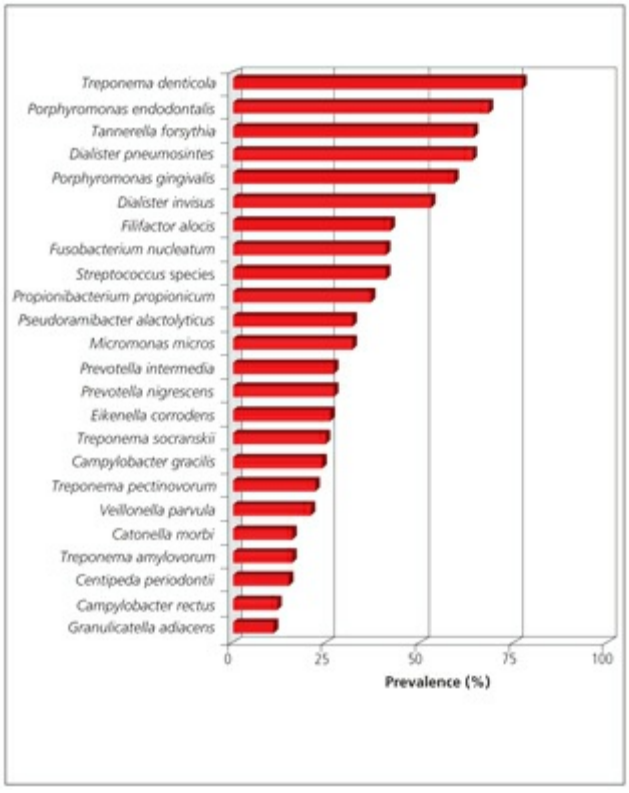

Fig 10-19 Prevalence of bacteria detected in purulent samples from acute apical abscesses. (Data are

based on studies by the author's group using a highly sensitive nested polymerase chain reaction protocol.)

Geographic influence

Findings from laboratories in different countries are often quite different regarding the prevalence of the species involved in endodontic infections. Although these differences may be attributed to variations in identification methodologies, a geographic influence in the composition of the root canal microbiota has been suspected. Studies using different molecular biology techniques directly compared the endodontic microbiota of patients residing in different geographic locations and suggested that significant differences in the prevalence of some important species can actually exist.[153–155] Analysis of the bacterial community profiles of the microbiota associated with acute apical abscesses from US and Brazilian patients also revealed a geography-related pattern, in which several species were exclusive for each location and others were shared by the two locations but showed great differences in prevalence.[138]

The composition of the oral microbiota of individuals living in a specific region may be influenced by a myriad of demographic and socioeconomic factors, race, immunologic features, quality of community water supplies, feeding habits, climate conditions, and aspects related to the use of antimicrobial agents for therapeutic purposes and/or in animal husbandry. Endodontic infections are endogenous infections caused by oral microorganisms, and, as a consequence, geography-related variations in the oral microbiota are expected to reflect in the bacterial communities participating in endodontic infections.

Some situations in endodontics may require systemic antibiotic therapy, and the choice of the most eligible agent should rely on susceptibility testing of the bacteria isolated. However, it is more commonly based on the most probable species and previous susceptibility testing. Standard protocols have been established on the basis of epidemiologic data and are proposed to treat these infections worldwide, but findings showing differences in the prevalence of several species between distant geographic locations may put any kind of standardization into question.

Future clinical trials should address the specific causes of geographic differences in the endodontic microbiota and the issue as to whether the observed differences in some way influence the outcome of standard local and systemic therapeutic procedures.

Morphology of the endodontic microbiota

Evidence indicates that apical periodontitis is also a biofilm-induced disease[156] (Fig 10-20). Morphologic studies have shown that bacteria in the root canal system can exist as planktonic cells suspended in the fluid phase of the main canal and as aggregates or coaggregates attached to the root canal walls, usually forming multilayered biofilms.[156–158] Lateral canals and isthmuses connecting main canals can also be clogged with microbial cells, primarily organized in biofilms.[156,159] Bacteria that form dense accumulations on the root canal walls are often seen penetrating the dentinal tubules[158] (Fig 10-21). The diameter of the pulpal side of dentinal tubules is large enough to permit penetration of most oral bacteria, and tubular infection occurs in about 70% to 80% of the teeth presenting with apical periodontitis lesions.[160,161] Although shallow penetration is more common, bacterial cells have been observed to reach approximately 300 µm in some teeth[158] (Fig 10-22). Bacteria that are present as planktonic cells in the main root canal may be easily accessed and eliminated by instruments and substances used during treatment, while those arranged in biofilms adhered to the canal walls or located in isthmuses, lateral canals, and dentinal tubules are definitely more difficult to eradicate and may require special therapeutic strategies.

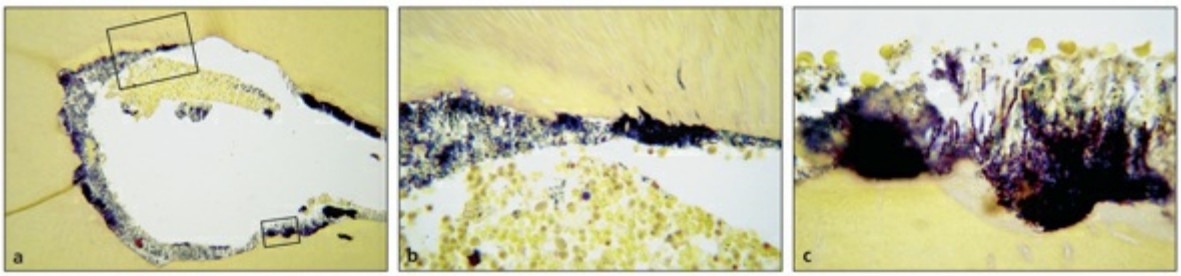

Fig 10-20 *(a)* Biofilm on the mesial root canal walls of a mandibular first molar. The tooth was symptomatic, and an apical periodontitis lesion was present (Taylor-modified Brown and Brenn stain; original magnification ×100). *(b and c)* Note the accumulation of polymorphonuclear neutrophils in the canal near the biofilm; *(b)* top inset in *(a)* (original magnification ×3,400); *(c)* bottom inset in *(a)* (original magnification ×1,000). (Courtesy of Dr Domenico Ricucci, Rome, Italy.)

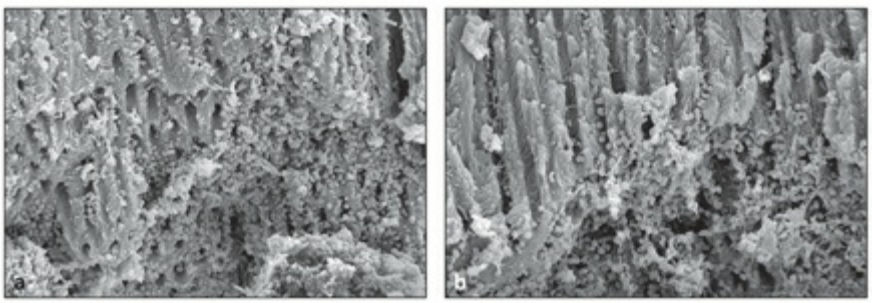

Fig 10-21 *(a and b)* Heavy infection of the root canal walls mainly by cocci and to a lesser degree by small rods. Cocci are seen invading the dentinal tubules (original magnification ×1,700 and ×2,500, respectively).

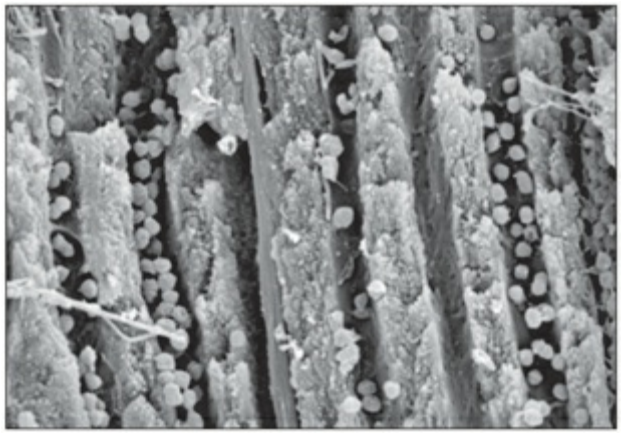

Fig 10-22 Cocci in dentinal tubules approximately 300 µm from the main root canal. Dividing cells are seen within tubules (original magnification ×5,000). (Reprinted from Siqueira et al[158] with permission.)

Ecology of the endodontic microbiota

A root canal with necrotic pulp provides a space for bacterial colonization and affords bacteria a moist, warm, nutritious, and anaerobic environment, which is by and large protected from the host defenses because of lack of active blood circulation in the necrotic pulp tissue. Intuitively, the necrotic root canal might be considered a rather fertile environment for bacterial growth and it might be realized that colonization should not be a difficult task for virtually all oral bacterial species. However, although more than 700 different bacterial species have been reported in the oral cavity and each individual's mouth can harbor about 100 to 200 species,[162] only a restricted assortment of these species is found in an infected canal. This indicates that selective pressures must occur in the root canal system that favor the establishment of some species and inhibit others.[163] The major ecological factors that determine the composition of the root canal microbiota include oxygen tension, type and amount of available nutrients, and bacterial interactions. Other factors, such as temperature, pH, and receptors for adhesins, may also be involved.

Infection of the root canal is a dynamic process, and different bacterial species apparently dominate at different stages of the infectious process. Shifts in the composition of the microbiota are largely due to changes in environmental conditions, particularly in regard to oxygen tension and nutrient availability. In the very initial phases of the pulpal infectious process, facultative bacteria predominate.[164] After a few days or weeks, oxygen is depleted within the root canal as a result of reduced blood flow secondary to pulpal necrosis and consumption by facultative bacteria. Further oxygen supply is interrupted because of loss of blood circulation in the necrotic pulp. An anaerobic milieu develops and is highly conducive to the survival and growth of obligate anaerobic bacteria. With the

passage of time, anaerobic conditions become even more pronounced, particularly in the apical third of the root canal, and, as a consequence, anaerobes will dominate the microbiota, outnumbering facultative bacteria.

The main sources of nutrients for bacteria colonizing the root canal system include: *(1)* the necrotic pulp tissue, *(2)* proteins and glycoproteins from tissue fluids and exudate that seep into the root canal system via apical and lateral foramina, *(3)* components of saliva that may coronally penetrate the root canal, and *(4)* products of the metabolism of other bacteria. Because the largest amount of nutrients is available in the main canal, which is the most voluminous part of the root canal system, most of the infecting microbiota, particularly fastidious anaerobic species, is expected to be located in this region. Bacterial species that can best utilize and compete for nutrients in the root canal system will succeed in colonization.

In addition to being influenced by variations in the oxygen levels, shifts in the microbiota colonizing the root canal system can also be dependent on the dynamics of nutrient utilization. Saccharolytic species dominate the very early stages of the infectious process but are soon outnumbered by asaccharolytic species, which will dominate later stages.[163] Although the necrotic pulp tissue can be regarded as a finite source of nutrients to bacteria, given the small volume of tissue that is gradually degraded, induction of periradicular inflammation guarantees a sustainable source of soluble nutrients, particularly in the form of proteins and glycoproteins present in the exudate that seep into the canal. At this stage of the infectious process, bacteria that have a proteolytic capacity or establish a cooperative interaction with those that can utilize this substrate in the metabolism start to dominate. Therefore, as the infectious process reaches the stage of induction of periradicular inflammation, proteins become the principal nutrient source, particularly in the apical part of the canal, favoring the establishment of anaerobic species that utilize peptides and/or amino acids in their metabolism.

The establishment of certain species in the root canal is also influenced by interactions with other species. In this regard, early colonizers play an important role in dictating which species will live along with them in the community. Bacterial interactions can be positive or negative. Positive interactions enhance the survival capacity of the interacting bacteria and enable different species to coexist in habitats where neither could exist alone. Positive interactions can be mediated when one species provides growth conditions favorable to another, for example, by reducing the oxygen tension in the environment or protecting from host defenses. Other examples of positive bacterial interactions include food webs and food chains, previously described for the dental plaque, which can also be established in the

endodontic microbiota. Thus, positive interactions increase the probability of certain species to be found together in a given habitat. Negative interactions act as feedback mechanisms that limit population densities. Examples include competition (for nutrients and space) and *amensalism* (when one species produces a substance that inhibits another species).

Symptomatic infections

Some gram-negative anaerobic bacteria have been suggested to be related to the increased probability of symptomatic apical periodontitis lesions.[102,132,141,165,166] However, several studies have found somewhat similar frequencies of the same species in both symptomatic and asymptomatic cases.[139,143–146,167] This indicates that factors other than the mere presence of a given putative pathogenic species can influence the development of symptoms. These factors include differences in virulence ability among strains of the same species, bacterial interactions resulting in synergism among species in mixed infections, number of bacterial cells (load), environmental cues regulating expression of virulence factors, host resistance, and concomitant herpesvirus infection.[120,167,169] Association of some or all of these factors (instead of an isolated event) is likely to dictate the occurrence and intensity of symptoms. As described in greater detail in chapters 7 and 8, bacterially induced symptoms can arise either from direct interaction with host nociceptive neurons (eg, lipopolysaccharide binding to toll-like receptor 4 on nociceptors) or from indirect interactions with nociceptors (eg, bacteria-evoked release of host cytokines and prostaglandins that sensitize or activate host nociceptors).

However, the precise events involved in the spontaneous conversion of an asymptomatic apical periodontitis lesion to a symptomatic one remain obscure. The possibility exists that, at a given moment during the endodontic infectious process, the microbiota reaches a certain degree of pathogenicity that elicits acute periradicular inflammation, with consequent development of pain and sometimes swelling. The structure of the endodontic bacterial communities in symptomatic teeth has been shown to be significantly different from that of asymptomatic teeth.[132,137] Differences are represented by different dominant species in the communities and a significantly higher number of species in symptomatic cases. Therefore, a shift in the structure of the microbial community is likely to occur before the appearance of symptoms. Such a shift is probably a result of environmental changes that allow either the establishment of newcomers or rearrangements in the abundance of members of the bacterial consortium. Differences in the type and load of dominant species and the resulting bacterial interactions may be responsible for differences in

the pathogenicity degree of the whole bacterial community. Thus, there is no key pathogen involved with symptomatic infections, but, along with the factors mentioned above, the whole structure of the endodontic bacterial community may exert a decisive factor in the induction of symptoms.

Other microorganisms in endodontic infections

Fungi. *Fungi* are eukaryotic microorganisms that may be encountered in the oral cavity, especially *Candida* species. However, they have been only occasionally detected in primary intraradicular infections,[170] even though a recent molecular study[171] has reported the occurrence of *C albicans* in 21% of the samples from primary root canal infections.

Archaea. Archaea comprise a highly diverse group of prokaryotes distinct from bacteria. Members of this domain have been traditionally recognized as extremophiles, but recently some of these microorganisms have also been found to flourish in nonextreme environments, including the human body. To date, no member of the Archaea domain has been described as a human pathogen. Recent studies detected methanogenic archaea in primary infected canals.[172,173]

Viruses. *Viruses* are not cells but inanimate particles composed of a nucleic acid molecule (DNA or RNA) and a protein coat. On their own they have no metabolism, and they need to infect living cells in order to use the cell's machinery to replicate the viral genome. Because viruses require viable host cells to infect and replicate themselves, they cannot thrive in the root canal with necrotic pulp. Viruses have been reported to occur in the root canal only in noninflamed vital pulps of patients infected with the human immunodeficiency virus.[174] On the other hand, human cytomegalovirus and Epstein-Barr virus have been detected in apical periodontitis lesions,[175] where living host cells are present. It has been hypothesized that human cytomegalovirus and Epstein-Barr virus may be implicated in the pathogenesis of apical periodontitis as a direct result of virus infection and replication or as a result of virally induced impairment of local host defenses, which might give rise to overgrowth of pathogenic bacteria in the very apical part of the root canal.[169]

Secondary and persistent endodontic infections

Secondary intraradicular infections are caused by microorganisms that were not present in the primary infection but that gained entry into the root canal system at

some time after professional intervention. The moment can be during treatment, between appointments, or even after root canal filling. In any circumstance, if penetrating microorganisms succeed in adapting themselves to the new environment, surviving and colonizing the root canal, a secondary infection is established. Species involved can be oral or nonoral microorganisms, depending on the cause of infection.

Persistent intraradicular infections are caused by microorganisms that can resist intracanal antimicrobial procedures and endure periods of nutrient deprivation in a prepared canal. Involved microorganisms are remnants of a primary or secondary infection. The microbiota associated with persistent infections is usually composed of fewer species than primary infections, and gram-positive facultative or anaerobic bacteria are predominant. Fungi can also be found in frequencies significantly higher than in primary infections.[176,177]

Persistent and secondary infections are for the most part clinically indistinguishable. Exceptions include infectious complications (such as an apical abscess) arising after the treatment of noninfected vital pulps or cases in which apical periodontitis was absent at the time of treatment but present in the follow-up radiograph. Both situations are typical examples of secondary infections (secondary to intervention). Both persistent and secondary infections can be responsible for several clinical problems, including persistent exudation, persistent symptoms, interappointment flare-ups, and failure of the endodontic treatment, characterized by a post-treatment apical periodontitis lesion.

Treatment failure

Persistent or secondary intraradicular infections are the major causes of endodontic treatment failures (Fig 10-23). This statement is supported by two strong evidence-based arguments. First, it has been demonstrated that there is an increased risk of adverse treatment outcome when bacteria are present in the canal at the time of filling.[178–180] Second, most (if not all) endodontically treated teeth presenting with persistent apical periodontitis lesions have been demonstrated to harbor an intraradicular infection.[181–185] Based on these arguments, studies have attempted to identify the microorganisms found at the root canal filling stage, which have the potential to put the treatment outcome at risk, and the microorganisms in endodontically treated teeth with apical periodontitis, which can be participating in the already established treatment failure.

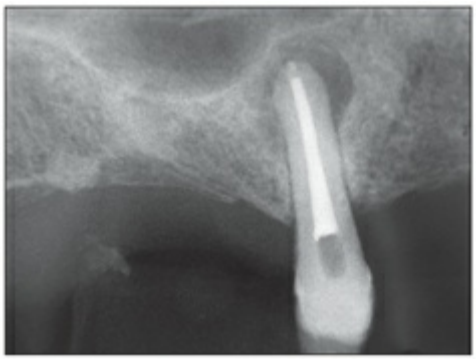

Fig 10-23 Persistence of an apical periodontitis lesion in an endodontically treated tooth. Persistent or secondary intraradicular infections are the main causative agents of endodontic treatment failure.

Bacteria at the root canal filling stage

Diligent antimicrobial treatment may fail to promote total eradication of bacteria from root canals. Persistent bacteria are either resistant or inaccessible to treatment procedures. Whatever the cause of persistence, bacterial diversity and density are substantially reduced after treatment. Root canal samples positive for bacterial growth after chemomechanical procedures, whether followed by intracanal medication or not, have been shown to harbor an average of one to five bacterial species per case. The number of bacterial cells usually varies from 102 to 105 per sample[135,179,187–189] (see Fig 10-17b and Box 10-1).

No single species has been significantly found to persist after treatment procedures. Gram-negative bacteria, which are common members of primary infections, are usually eliminated. Exceptions may include some anaerobic rods, such as *F nucleatum*, *Prevotella* species, and *Campylobacter rectus*, which are among the species found in postinstrumentation samples.[135,179,187,189–192] However, most studies on this subject have clearly revealed that gram-positive bacteria are most frequently present. Gram-positive facultatives or anaerobes often detected in post-treatment samples include streptococci (*S mitis*, *S gordonii*, *S anginosus*, *S sanguinis*, and *S oralis*), *P micra*, *Actinomyces* species (*A israelii* and *A odontolyticus*), *Propionibacterium* species (*P acnes* and *P propionicum*), *P alactolyticus*, lactobacilli (*Lactobacillus paracasei* and *Lactobacillus acidophilus*), *Enterococcus faecalis*, and *Olsenella uli*.[135,179,187–191,193–195] These findings support the notion that gram-positive bacteria can be more resistant to antimicrobial treatment measures and have the ability to adapt to the harsh environmental conditions in instrumented (and medicated) canals. Of the taxa found in posttreatment samples, 42% have been shown to be as-yet-uncultivated bacteria.[135]

For residual bacteria to be the cause of persistent apical periodontitis lesions, they have to: *(1)* adapt to the environment drastically modified by treatment, acquiring nutrients and surviving the antimicrobial effects of root canal filling materials; *(2)* reach critical numbers and exhibit virulence attributes sufficient to sustain or induce periradicular inflammation; *(3)* have unrestrained access to the periradicular tissues to exert their pathogenicity; and *(4)* induce host responses (see chapter 12). This helps to explain why some apical periodontitis lesions can heal even when bacteria are present in the canals at the root canal filling stage.[178,179] However, the fact that there is an increased risk of adverse treatment outcome when bacteria are present in the canal at the time of obturation[178–180] indicates that, in many cases, residual bacteria succeed in fulfilling the requisites to survive, thrive, and maintain an apical periodontitis lesion.

Microbiota in endodontically treated teeth

The microbiota in endodontically treated teeth with persistent apical periodontitis lesions also exhibits less diversity than that in primary infections. Canals that are apparently well treated can harbor 1 to 5 species, while the number of species in canals with inadequate treatment can reach up to 30 species, a number very similar to that found in untreated canals.[184–186,197] The number of bacterial cells varies from 103 to 107 (see Fig 10-17c and Box 10-1).[193,198]

Irrespective of the identification method used, *E faecalis* has been reported to be the most prevalent species in endodontically treated teeth, with prevalence values reaching up to 90% of the cases.[183–186,198–201] Endodontically treated teeth are about 9 times more likely to harbor *E faecalis* than are primary infections.[201] This suggests that this species can be inhibited by other members of a mixed bacterial consortium commonly present in primary infections and that the bleak environmental conditions within filled root canals do not prevent its survival.

The fact that *E faecalis* is the most commonly encountered species in treated canals and the attributes of this species that help it to survive under unfavorable environmental conditions have prompted many authors to nominate *E faecalis* as the main pathogen involved in treatment failures. The consequence of this has been an avalanche of in vitro papers focusing on this species.[202] However, findings from recent studies carried out in independent laboratories have questioned the role of *E faecalis* as the main causative agent of endodontic failures. Some studies have not detected enterococci in endodontically treated teeth with lesions[149] or have demonstrated that *E faecalis* is not the dominant species in most retreatment

cases.[197] Recent reports have demonstrated that *E faecalis* is no more prevalent in endodontically treated teeth with lesions than in teeth with no lesions.[200,203]

All these findings apparently put into question the status of *E faecalis* as the main species causing treatment failure. However, other related factors still must be clarified before this assumption turns into certainty. Further studies addressing this issue should include a precise diagnosis of apical periodontitis (circumventing radiographic biases), provide quantitative results (bacterial counts), test the association of specific clonal types with disease, and evaluate the different patterns of host response to infection.[200]

Other microorganisms found in endodontically treated teeth with apical periodontitis include streptococci, *C albicans*, and some fastidious anaerobic bacterial species: *P alactolyticus*, *P propionicum*, *Filifactor alocis*, *Dialister pneumosintes*, and *Dialister invisus*.[134,183–186,197,199] As-yet-uncultivated phylotypes correspond to 55% of the taxa detected in treated canals.[204] In fact, the bacterial community profiles in these cases can vary from individual to individual, suggesting that many distinct bacterial combinations can play a role in treatment failure.[197] All these findings strongly suggest that the microbiota of endodontically treated teeth with apical periodontitis is more complex than previously anticipated.

Extraradicular infection

Apical periodontitis is formed in response to intra-radicular infection, and in most situations it succeeds in preventing microorganisms from gaining access to the periradicular tissues. Nevertheless, in some specific circumstances microorganisms can overcome this defense barrier and establish an extraradicular infection. Thus, extraradicular infection is characterized by microbial invasion of and proliferation in the inflamed periradicular tissues and is almost invariably a sequel to intraradicular infection.

Extraradicular infection can be dependent on or independent of the intraradicular infection. The most common form of extraradicular infection dependent on the intraradicular infection is the acute apical abscess. The most common form of extraradicular infection that can be independent of the intraradicular infection is the apical actinomycosis, which can be regarded as an apical periodontitis lesion infected by *Actinomyces* species or *P propionicum*. The bacterial source for apical actinomycosis is conceivably the intraradicular infection.

Situations that can permit *Actinomyces* species or *P propionicum* to reach the periradicular tissues and establish an extraradicular infection are likely to be the

following: apical extrusion of debris during root canal instrumentation, direct advance from the infected root canal into the lumen of pocket cysts, or previous participation in acute apical abscesses followed by persistence after the acute response subsides.[205] Once in the periradicular tissues, actinomycotic colonies seem to be sufficient to sustain chronic inflammation without necessarily inducing an acute response. Apical actinomycosis corresponds to 1.8% to 4.0% of apical periodontitis lesions.[205]

The question as to whether the extraradicular infection is dependent on or independent of the intraradicular infection assumes special relevance from a therapeutic standpoint because the former can be successfully managed by conventional root canal therapy, whereas the latter can only be handled by periradicular surgery. The high success rate reported for endodontic treatment and retreatment indirectly indicates that most of the extraradicular infections observed in some studies are dependent on the intraradicular infection.

Treatment

The ultimate goal of endodontic treatment is to prevent the development of apical periodontitis or to create adequate conditions for periradicular tissue healing. Because of the microbial etiology of apical periodontitis, the logical goals of endodontic treatment are to eliminate or substantially reduce the microbial population within the root canal system (through antiseptic means) and to prevent microorganisms from infecting or reinfecting the root canal or the periradicular tissues (through aseptic means and a tight coronal seal provided by both the root canal obturation and the permanent coronal restoration). The success of the endodontic treatment will depend on how effective the clinician is in accomplishing these goals.

Apical periodontitis has a polymicrobial etiology, and the bacterial community profiles significantly vary from one individual to another.[132] Differences are even more pronounced when samples are taken from different geographic locations.[138,154] Because of these characteristics, endodontic infections should be ideally treated by using a broad-spectrum, nonspecific antimicrobial strategy, which has the potential to reach the most possible members of the endodontic bacterial communities.

Because of the privileged anatomical location, bacteria entrenched in the root

canal system are beyond the reaches of the host defenses. Therefore, endodontic infections can only be treated by means of professional intervention using both chemical and mechanical procedures. The main steps of endodontic treatment for control of the infection are the chemomechanical preparation and the interappointment medication. In this regard, the chemomechanical preparation is of paramount importance for root canal disinfection because instruments and irrigants act primarily on the main canal, which is the most voluminous area of the system and consequently harbors the largest number of bacterial cells.

Bacterial elimination from the root canal is carried out by means of the mechanical action of instruments and the flow and backflow of the irrigant solution as well as the antibacterial effects of irrigants. Several irrigants have been proposed over the years, but sodium hypochlorite (NaOCl) remains the most widely used. However, studies have revealed that instrumentation and irrigation with NaOCl per se do not suffice to predictably render root canals free of cultivable bacteria; about 40% to 60% of canals are still positive for bacterial presence after chemomechanical preparation using different concentrations of NaOCl.[179,187,189,196,206,207] Chlorhexidine has been proposed as an alternative, but clinical studies have shown that it is not superior to NaOCl with regard to antibacterial effectiveness.[136,192]

Because residual bacteria can adversely affect the treatment outcome,[178,179] the use of an interappointment medication has been recommended to supplement the antibacterial effects of chemomechanical procedures and eliminate persistent bacteria.[189,206,207] Calcium hydroxide is arguably the most used intracanal medication. The addition of other antimicrobial mixing substances can enhance the effectiveness of calcium hydroxide.[208] Studies have shown that a 7-day intracanal treatment with a calcium hydroxide paste may be necessary to supplement the antibacterial effects of chemomechanical procedures and predictably render root canals free of cultivable bacteria before obturation.[188,189,196,206]

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Molecular Mediators of Pulpal Inflammation

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Inflammation of the dental pulp is similar to that in other connective tissues in that it is mediated by cellular and molecular factors. The pulp is capable of expressing a large number of the known host mediators of inflammation, as evidenced by their identification in that pulp at the protein and/or the gene-expression levels. The principal objective of these mediators is to combat the irritating factors and minimize their harmful effects. However, in the process of mounting the innate and adaptive inflammatory mechanisms, the host factors may contribute to the injury of the pulp and escalate the ultimate demise of this tissue. Unlike the situation in other connective tissues, the pulp is enclosed in a noncompliant environment and has minimal collateral circulation. These anatomical restrictions, which become more exaggerated with advancing age, tend to intensify the injury that results from external irritation and the harmful side effects of host inflammatory mediators.

In this chapter, the data available on the contribution of molecular mediators to the inflammatory process is described and related to clinical factors important for diagnosing and managing pulpal inflammation. The inflammatory response is described as it progresses from vascular changes to the attraction and migration of inflammatory cells to the site of inflammation and finally to the actual processes that

take place in situ.

A large amount of information has been generated in recent years about the structural composition of the dental pulp and its functional responses to external irritation. It is important to recognize that there is a significant overlap in the functions of cellular elements (see chapter 4) and in the effects of inflammatory mediators produced by these cells. In this regard, it has recently become evident that the odontoblast is capable of contributing to the immune response and does not have just a structural role.

The gold standard for data generation on the inflammatory response in the dental pulp is to study these responses in humans following clinically relevant external stimulation, a condition that is frequently difficult, if not impossible, to achieve. Thus, much of the available in vitro and animal model data have to be interpreted with caution and await confirmation by clinical studies.

Odontoblasts as Defense Cells

It has been recognized for a long time that several structural cells, such as fibroblasts, epithelial cells, and endothelial cells, are capable of producing inflammatory mediators such as cytokines and defensins or expressing adhesion molecules or pattern-recognition receptors, thereby contributing to the immune response. With a function as critical as dentin formation, odontoblasts are clearly among the most important cells in the dental pulp. While odontoblasts are typically thought of as terminal protein matrix– and mineralization-mediating cells, they have been shown to participate in the expression of a number of inflammatory mediators. Given their location as the first cells encountered when caries and sulcular bacteria invade dentin, they clearly occupy a strategic location for the recognition and mediation of various host defense mechanisms. Therefore, odontoblasts should be considered members of the defense response cells in the dental pulp,[1] participating in sensing of pathogens by their toll-like receptors, production of cytokines on cell stimulation with microbial by-products, and induction of dendritic cell migration.[2]

Modulation of Vascular Flow

In the initial phases of pulpal inflammation, vasodilation occurs and blood flow increases. This increases the perfusion of the pulp, bringing needed host inflammatory factors to the area of irritation. Despite its small size, the dental pulp responds to advancing irritation in a compartmentalized manner rather than as an entire organ. Areas of the pulp closest to the irritation seem to be affected most and undergo more severe inflammatory manifestations, with resultant vascular responses, while the surrounding and distant sections may have milder inflammation or noninflamed tissue. As the inflammatory reaction in the pulp progresses, stasis in pulp vessels eventually ensues. This is related to the extravasation of proteins and cells into the interstitial tissue as well as the unyielding dentin environment that restricts tissue edema. The vascular responses of the pulp are mediated by the following vasoactive amines: histamine, serotonin, endothelin, and neuropeptides.

Histamine

Histamine is prevalent in connective tissue around blood vessels and in mast cells, basophils, and platelets. Histamine is present in cell granules and is released by cell degranulation in response to a variety of stimuli including *(1)* physical trauma, cold, or heat; *(2)* immune reactions involving binding of antibodies (immunoglobulin E [IgE]) to mast cells; *(3)* complement components called *anaphylatoxins* (C3a and C5a); *(4)* histamine-releasing proteins derived from leukocytes; *(5)* neuropeptides (eg, substance P); and *(6)* cytokines such as interleukins 1 (IL-1) and 8 (IL-8).[3,4]

Histamine is a potent vasodilator and a mediator of vascular permeability. It acts on the microcirculation via four types of G-protein–coupled receptor: H_1, H_2, H_3, and H_4 receptors. The recently discovered H_4 receptor has a higher affinity for histamine than does the H_1 receptor and appears to be more selectively expressed on hematopoietic cells. The H_4 receptor is involved in chemotaxis and inflammatory mediator release by eosinophils, mast cells, monocytes, dendritic cells, and T cells.[3,5]

Histamine was detected in small amounts in the uninflamed dental pulp.[6] Thermal injury of the pulp resulted in a fourfold increase, while stimulation with an electric pulp tester resulted in a 35% reduction in the levels of histamine.[7] Application of histamine in vivo resulted in vasodilation[8] and gradual decrease in pulpal blood flow[9] because of vascular leakage and an increase in tissue pressure in a low-

compliance environment. Similar results were produced in isolated in vitro perfused pulpal arteriole preparations.[10,11] Histamine application to cavity preparations in human dentin resulted in dull, throbbing pain and occasionally was associated with sharp, shooting pain.[12] Because histamine causes little stimulation of A or C fibers in the dental pulp, the pulp tissue must be sensitized by other inflammatory mediators, such as prostaglandins, for histamine to evoke a painful response in both types of pulp nociceptive fibers.[12]

Serotonin

Serotonin (5-hydroxytryptamine [5-HT]) is another vasoactive mediator that generally causes vasoconstriction. It is present in serotoninergic nerve terminals, endothelial cells, and platelets. Release of serotonin and histamine from platelets occurs after they come in contact with collagen, thrombin, adenosine diphosphate, and antigen-antibody complexes. Serotonin and its metabolite, 5-hydroxyindoleacetic acid–5-HT, were detected in rat incisor pulp.[13] More recently, serotonin and the enzyme monoamine oxidase, which catalyzes the oxidative deamination of 5-HT, have been localized in the linings of blood vessels in normal human dental pulp.[14]

Serotonin was shown to increase the biosynthesis of endogenous prostaglandin E_2 (PGE_2)[15] and prostacyclin (PGI_2)[16] in the rat dental pulp. It also caused significant increase in pulpal blood flow in dog canines when injected into the maxillary artery or applied locally to Class V cavities.[17] In addition, serotonin was shown to sensitize pulpal nerve fibers to various hydrodynamic stimuli,[18] indicating that it is capable of reducing the pain threshold in pulpal inflammation.

Endothelin

Endothelin is a potent, trypsin-sensitive vasoconstrictor that was first described in bovine endothelium.[19] The genes for three isoforms of endothelin (*Et1*, *Et2*, and *Et3*) have been cloned. Two types of responses to endothelin have been described that

correspond to two receptors: endothelin receptor A (ET-A), which is specific for ET-1, and endothelin receptor B (ET-B), which is specific for all isoforms.[20] Endothelin has various physiologic actions in addition to vasoactivity because receptors are found in the brain, adrenal glands, and lung and because knockout studies have found significant structural and developmental abnormalities.[19] Both receptors, as well as *Et1*, have been detected in developing rat dental issues, including odontoblasts, ameloblasts, and dental papilla.[19]

Intra-arterial delivery of ET-1 in rats produced a dose-dependent reduction of pulpal blood flow exceeding that caused by epinephrine and norepinephrine.[11] Similar effects were achieved when ET-1 was applied extraluminally to porcine pulp preparations.[21] However, blocking ET-A with an antagonist did not change basal pulpal blood flow.[22]

Neuropeptides

Different neuropeptides have been demonstrated in the dental pulp of humans and other mammals: substance P (SP), calcitonin gene–related peptide (CGRP), neurokinin A, neuropeptide K, neuropeptide Y (NPY), somatostatin, and vasoactive intestinal peptide (VIP).[23] These neuropeptides reside almost exclusively under normal conditions within the nerve endings of afferent nerves and are usually, but not exclusively, located around pulpal blood vessels. Of the 20% of pulpal blood vessels that have positive peptidergic nerve innervations, 92% expressed CGRP, 87% expressed SP, 80% expressed NPY, and 15% expressed VIP.[24] Tissue levels of SP immunoreactivity in the dental pulp were found to be the highest in the body outside the central nervous system and were higher in mature compared to immature cat pulp.[25] Recently, dental pulp fibroblasts were also found to express SP. This is indicative of their role in neurogenic inflammation in pulpal disease.[26]

Denervation experiments indicate that, in the pulp, SP-, neurokinin A–, and CGRP-containing nerve fibers originate from the trigeminal ganglion and that NPY-containing nerve fibers come from the superior cervical ganglion. The origin of VIP-containing fibers may be parasympathetic ganglia because it has been associated with acetylcholine in other tissues. The origin of pulpal neuropeptides, their receptors, and their functions are summarized in Table 11-1.

Increased production of neuropeptides plays an important role in initiating and

propagating pulpal inflammation. In the normal rat molar pulp, SP and CGRP were present in close proximity to macrophages (identified by the ED2 marker) and class II antigen–positive cells (identified by the OX6 marker)[27] (Fig 11-1). The association was more prevalent in the odontoblastic layer than in central pulp. During inflammation induced by caries, cavity preparation, or the application of orthodontic forces, sprouting of pulpal nerve fibers was shown to be associated with increased expression of neuropeptides such as SP or CGRP closely surrounding the areas of irritation[28,29] (Fig 11-2). However, severe irritation induced by pulpal exposures in the presence or absence of acid etching resulted in a drop of the overall pulpal levels of SP and CGRP. This may be due to depletion of neuropeptide stores in the nerve endings.[30]

More recent studies have also shown that the amounts of NPY or VIP in the pulp of mildly to moderately carious teeth were significantly greater than those present in noncarious or grossly carious teeth.[31,32] Conversely, a higher percentage of neuronal areas that were positively stained for SP was detected in grossly carious lesions and in painful lesions.[33] These findings were consistent at all levels of coronal pulp, subodontoblastic nerve plexus, and midcoronal pulp.

Table 11-1 **Summary of pulpal neuropeptides, receptors, and their functions***

Neuropeptide	Origin	Localization	Stimulus for release	Cell receptor
SP	Trigeminal ganglion	C and Aδ fibers	• Thermal • Mechanical • Chemical • Electrical • Caries • Capsaicin • Inflammatory mediators • Bradykinin • Prostaglandins	NK1
Neurokinin A	Trigeminal	C and Aδ fibers	• Thermal • Mechanical • Chemical • Electrical • Caries	NK2

Neurokinin A	ganglion	C and Aδ fibers	• Caries • Inflammatory mediators • Bradykinin • Prostaglandins	NK2
CGRP	Trigeminal ganglion	C and Aδ fibers	• Thermal • Mechanical • Chemical • Electrical • Caries • Capsaicin • Inflammatory mediators • Bradykinin • Prostaglandins	CGRP1, CGRP2
NPY	Superior cervical ganglion	Sympathetic fibers	• Stress • Electrical • Thermal • Caries	Y receptor
VIP	Parasympathetic ganglia	Parasympathetic fibers	• Not well elucidated • Cytokines • Lipopolysaccharides • Nitric oxide	VPAC1, VPAC2

*Modified from Caviedes-Bucheli et al[23] with permission.

Denervation of the inferior alveolar nerve depleted the pulp of its SP and CGRP[34] and increased the magnitude of pulpal degeneration following experimental pulpal exposures.[35] Addition of CGRP to human pulp cells also resulted in a twofold increase in the level of expression of bone morphogenetic protein 2, a member of the transforming growth factor β (TGF-β) superfamily that has the capacity to induce dentin regeneration[36] (see chapter 2). Teeth diagnosed with painful irreversible

pulpitis contained increased levels of substance P or its receptor, CGRP or its receptor, neurokinin A, and NPY.[23]

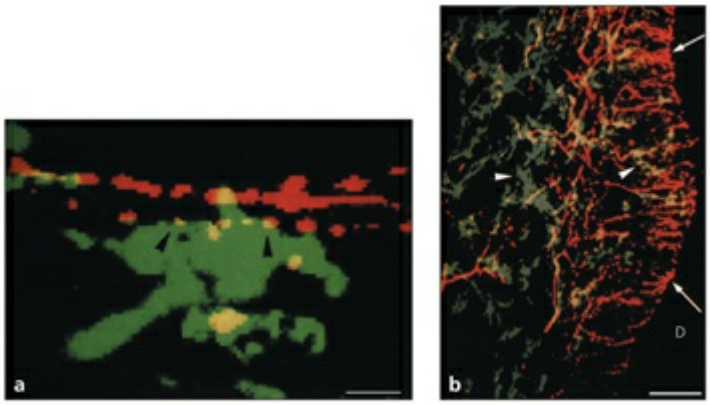

Fig 11-1 *(a)* Confocal laser scanning micrograph of the inner portion of the pulp horn of a rat mandibular first molar after double immunofluorescence staining for $ED2^+$ cells (macrophages) *(green)* and SP immunoreactive nerve fibers *(red)*. An $ED2^+$ cell shows some points of contact *(yellow, arrowheads)* (bar = 10 μm). *(b)* Confocal laser scanning micrograph of the coronal pulp of a rat mandibular first molar after double immunofluorescence staining for $OX6^+$ (rat major histocompatibility complex Class II antigen) cells *(green, arrowheads)* and CGRP-immunoreactive nerve fibers *(red, arrows)*. Close association is more frequently observed at the periphery of the pulp compared with the inner portion. D, dentin (bar = 50 μm). (Reprinted from Okiji et al[27] with permission.)

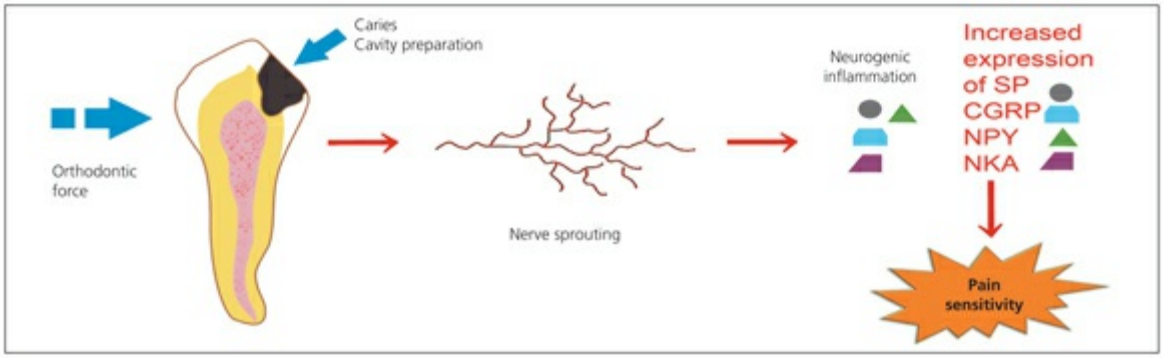

Fig 11-2 Neuronal response to injuries of the pulpodentin complex, such as caries, cavity preparation, or orthodontic forces. These insults to the pulp generate anatomical alterations in the distribution of nerve fibers (sprouts), leading to an increase in neuropeptide expression and therefore an increase in pain sensitivity as a result of peripheral sensitization. NKA, neurokinin A. (Modified from Caviedes-Bucheli et al[23] with permission.)

Immunoreactive CGRP has often been used as a marker for assessing neurogenic inflammation. Application of the excitotoxin capsaicin to its transient receptor potential vanilloid (TRPV) channel receptors resulted in increased release of immunoreactive CGRP from sensory neurons in the human dental pulp in a concentration-dependent manner.[37]

The capsaicin excitation model has often been adopted for characterization of

stimuli that regulate the release of neuropeptides from isolated tissues. For example, a decrease in pH and an increase in temperature significantly increased the effect of capsaicin on CGRP release from pulp nociceptors.[38] The results suggest that neuropeptide concentration, local inflammation, and body heat may influence the intensity of pulpal pain. Moreover, levels of SP and neurokinin A increased in the gingival crevicular fluid of teeth with painful irreversible pulpitis; the levels returned to normal 1 week after pulpectomy.[39] These findings suggest that neurogenic inflammation caused by neuropeptides plays a dynamic role in modulating pulpal inflammation and in reducing the pain threshold in pulpal inflammation.

Not all cases of irreversible pulpitis are associated with pain. This may be explained by the actions of inhibitors of neurotransmitters such as γ-aminobutyric acid or gastrin-releasing peptide. Staining identified β-aminobutyric acid–like and gastrin-releasing peptide–like immunoreactivity in the dental pulp.[40] In addition, μ-opioid receptors have been identified in human dental pulp and may be involved in suppressing pain symptoms in certain cases.[41] More recently, the Y1 receptor for NPY has been shown to co-localize with the capsaicin receptor, TRPV1, in the trigeminal ganglion and the dental pulp. Thus, activation of the Y1 receptor by endogenous NPY results in the inhibition of capsaicin-sensitive nociceptors, thereby modulating the intensity of the pain response.[42]

There are a number of mechanisms by which neuropeptides are thought to contribute to the inflammatory process. SP, CGRP, and VIP[43] are potent vasodilators, whereas NPY is a vasoconstrictor.[44] Vaso-dilation following infusion of SP or VIP in the pulp was associated with a brief increase in pulpal blood flow, which was followed by a dramatic and prolonged reduction in pulpal blood flow.[9] Intra-arterial infusion of an SP antagonist (the nonpeptide neurokinin 1 receptor antagonist SR140.333) or a CGRP inhibitor (h-$CGRP_{(8-37)}$) reduced resting blood flow and interstitial fluid pressure in the ferret canine pulp.[45] SP also increased vascular permeability and plasma extravasation.[46] The use of the β-adrenoceptors epinephrine or norepinephrine and albuterol (a β_2-adrenoceptor agonist) inhibited CGRP release from pulpal nociceptors in an ex vivo bovine pulp preparation.[47] The different stimuli that trigger neuropeptide release and their role in neurogenic inflammation are summarized eloquently in the review by Caviedes-Bucheli et al[23] (Fig 11-3).

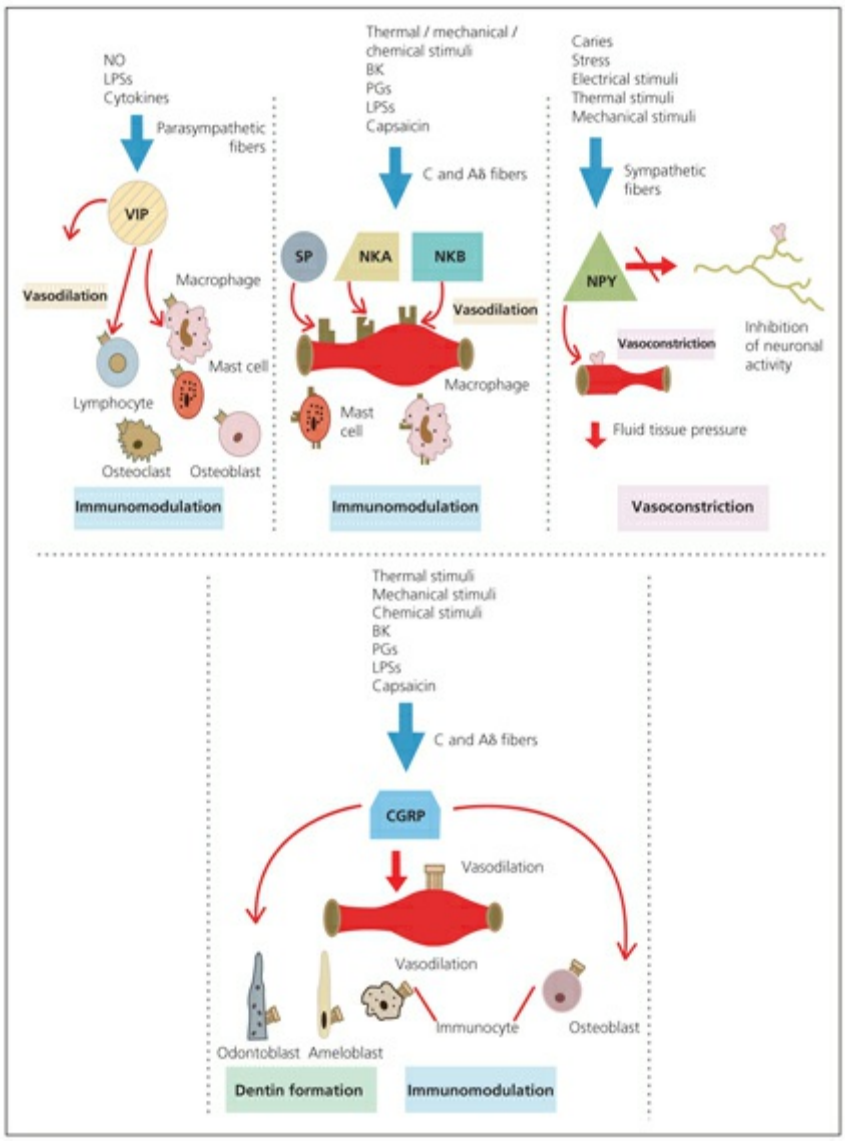

Fig 11-3 Different stimuli that trigger neuropeptide release and their role in neurogenic inflammation. VIP is released from parasympathetic fibers stimulated by nitric oxide (NO), lipopolysaccharides (LPSs), and cytokines, generating vasodilation and exerting immunomodulatory effects on different immune cells by binding on VIP/pituitary adenylate cyclase–activating peptide receptors. Tachykinins (SP, neurokinin A [NKA], and neurokinin B [NKB]) are released from C and Aδ sensory fibers stimulated by thermal, mechanical, or chemical irritants. Several inflammatory mediators, such as bradykinin (BK) and prostaglandins (PGs), as well as LPSs and capsaicin, also result in release of tachykinins, generating vasodilation and activation of some immune cells by binding to NK receptors. NPY is released from sympathetic fibers stimulated by caries, stress, and/or electrical, thermal, and mechanical irritation, generating vasoconstriction and consequently reducing fluid tissue pressure. It also inhibits neuronal activity in normal conditions. CGRP is generally coreleased with SP and NKA after stimulation of the same neurons. CGRP generates vasodilation and participates in repair processes by inducing dentin formation and modulating the action of some immune cells. (Reprinted from Caviedes-Bucheli et al[23] with permission.)

Neuropeptides may also contribute to the inflammatory process by other mechanisms. These include the release of inflammatory mediators such as histamine,

PGE_2, collagenase, IL-1, IL-6, and tumor necrosis factor (TNF); the potentiation of chemotaxis, phagocytosis, and the expression of adhesion molecules; and lymphocyte proliferation and IL-2 production. Application of SP or CGRP to human pulp fibroblasts upregulated the production of IL-1, IL-6, and TNF-α in a dose- and time-dependent manner.[48] Similar effects were shown on the production of IL-8, IL-10, cyclo-oxygenase 2 (COX-2), and monocyte chemotactic protein 1 (MCP-1).[49–51] These effects seem to be mediated by the interactions of nuclear factor κB (NFκB) and the receptor antagonist for its ligand (RANKL).[51,52] The interactions between neuropeptides and different cell populations of the dental pulp are summarized in the review by Caviedes-Bucheli et al[23] (Fig 11-4).

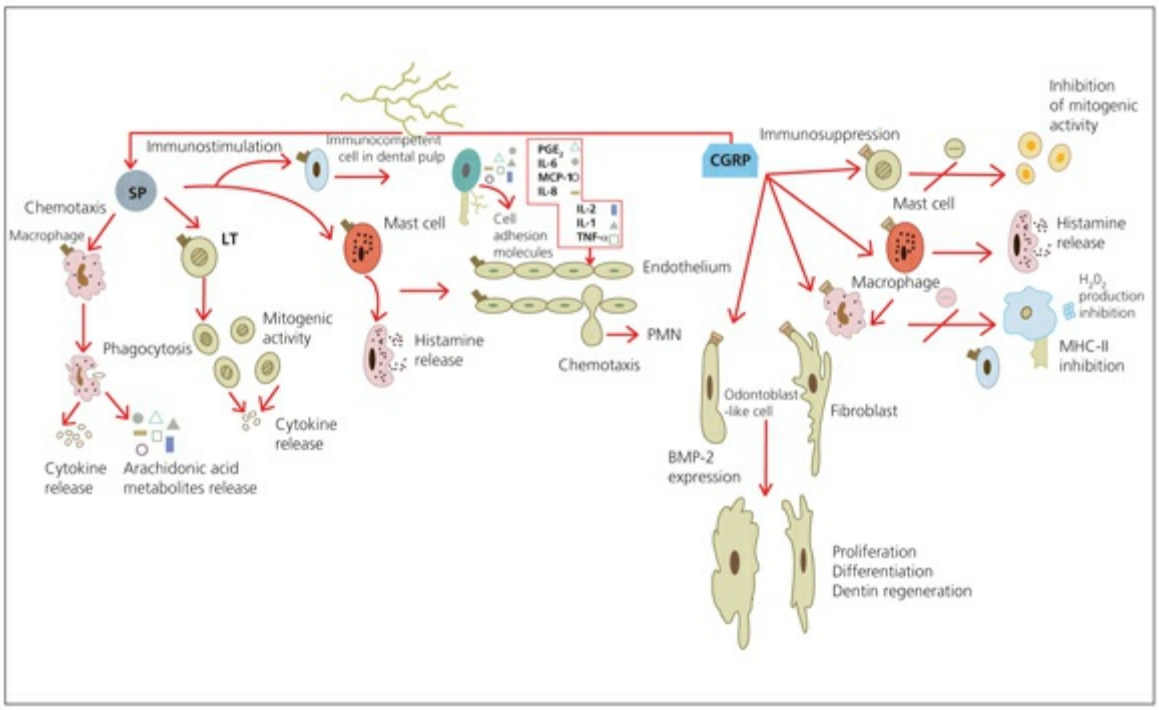

Fig 11-4 Interactions between neuropeptides and different cell populations of the dental pulp. Neuropeptides initiate and spread neurogenic inflammation. SP generates immunostimulatory effects by enhancing macrophage phagocytosis, chemotaxis, and cytokine release. In other immunocompetent cells, SP induces the release of PGE_2, IL-1, IL-2, IL-6, and IL-8, and tumor necrosis factor α (TNF-α). SP also regulates the expression of MCP-1, as well as the mitogenic activity and cytokine release of T lymphocyte (LT), and promotes polymorphonuclear neutrophil (PMN) chemotaxis. Both SP and CGRP induce histamine release from mast cells. CGRP inhibits mitogen-induced T-lymphocyte proliferation, blocks hydrogen peroxide (H_2O_2) production in macrophages, and reduces antigen presentation of Class II antigen-expressing cells (MHC-II). CGRP exerts stimulatory effects on the growth of fibroblasts and odontoblast-like cells by increasing the expression of bone morphogenetic protein 2 (BMP-2), therefore inducing dentin regeneration and lowering dentin permeability. (Reprinted from Caviedes-Bucheli et al[23] with permission.)

Leukocyte Adhesion and Transmigration

Adhesion molecules

One of the critical processes in inflammation is the delivery of leukocytes to the site of inflammation. As the blood flow slows due to vessel vasodilation and increased vascular permeability, the leukocytes assume a more peripheral position in the vessel (margination). Eventually, the leukocytes roll along the endothelial wall and finally adhere to the endothelial lining (pavementing). They then insert pseu-dopods into gaps between endothelial cells and transmigrate through the basement membrane and travel toward the site of inflammation, following the chemotactic gradient (Fig 11-5).

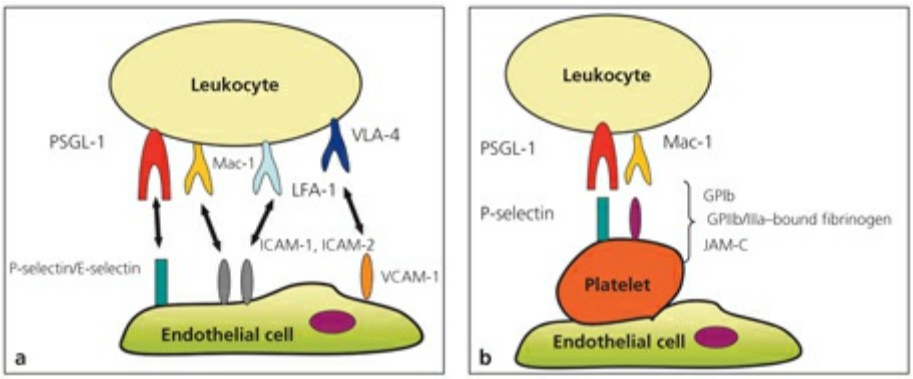

Fig 11-5 Recruitment of leukocytes to sites of inflammation depends on adhesive interactions between leukocytes and endothelial cells or endothelial cell–bound platelets. *(a)* During the course of tissue inflammation, adhesive interactions between leukocytes and the endothelium include *(1)* the initial rolling, which is the loose contact of the leukocyte with the endothelium, predominantly mediated by the binding of leukocyte P-selectin glycoprotein ligand 1 (PSGL-1) to endothelial P- and E-selectins, and *(2)* the firm adhesion of leukocytes on the endothelium, which is mediated by interactions of β_2-integrins such as (Mac-1) and lymphocyte function-associated antigen 1 (LFA-1) with the endothelial counter-receptors of the intercellular cell adhesion molecule (ICAM) family, as well as by the interaction of the β_1-integrin very late antigen 4 (VLA-4) to endothelial vascular cell adhesion molecule 1 (VCAM-1). *(b)* Leukocyte recruitment can also be promoted by endothelial-adherent platelets. In this scenario, platelets can serve as a bridge between leukocytes and the endothelium. The leukocyte-platelet interaction can be mediated by leukocyte PSGL-1 binding to P-selectin expressed on platelets as well as by the binding of β_2-integrin Mac-1 to its multiple ligands and counterreceptors on platelets, such as GPIb, GPIIb/IIIa–bound fibrinogen, or junctional adhesion molecule C (JAM-C). (Reprinted from Langer and Chavakis[53] with permission.)

Three important classes of molecules are critical for the adhesion and transmigration of leukocytes: *(1)* selectins, *(2)* endothelial adhesion molecules, and

(3) integrins. *Selectins* are the molecules that initially slow the circulating leukocytes and cause them to roll along the endothelial lining (see Fig 11-5). The L-selectins are found on leukocytes, whereas P- and E-selectins are expressed by endothelial cells. Selectins are lectins that bind to carbohydrate ligands on the corresponding cell type.[53] Mouse knockout studies have shown that the absence of either P-selectin or E-selectin individually does not cause significant disruption in cell adhesion. However, the absence of both molecules causes a condition called *leukocyte adhesion deficiency 2* (LAD-2).

In the normal dental pulp, only a few vessels located in the pulp core reacted weakly with an immunohistochemical stain for E- or P-selectin. However, in dental pulps with inflammation induced by crown preparation, dentin adhesives, or provisional cements, a large number of vessels located in the subodontoblastic layer reacted strongly with the antibody for these molecules.[54,55]

Cell adhesion molecules are members of the immunoglobulin superfamily and include intercellular cell adhesion molecules (ICAM) 1 through 5, vascular cell adhesion molecule 1 (VCAM-1), junctional adhesion molecules (JAMs), platelet-endothelial cell adhesion molecule 1 (PECAM-1), and endothelial cell adhesion molecule (ECAM).[53] In addition, apart from their well-established role as the first cellular response in the coagulation cascade, platelets are intimately involved in inflammatory reactions largely because of their direct crosstalk with leukocytes[56] (see Fig 11-5). ICAM-1 is strongly induced on leukocytes and endothelial cells. Endothelial cells constitutively express ICAM-2, VCAM-1, and PECAM-1, whereas resting leukocytes bear ICAM-3 and PECAM-1 on their surfaces. ICAM-1 interacts with the integrins lymphocyte function-associated antigen 1 (LFA-1) and macrophage 1 antigen (Mac-1); ICAM-2 and ICAM-3 interact with LFA-1; VCAM-1 interacts with very late antigen 4 (VLA-4); and PECAM-1 and JAMs are engaged in cell binding that allows the phagocyte to squeeze between or through the endothelial cells, a process called *transmigration* or *diapedesis*.[57]

After their firm adhesion, leukocytes make use of two transmigration processes to cross the endothelial barrier: the transcellular route through the endothelial cell body and the paracellular route through the endothelial junctions. Although the paracellular route of leukocyte transmigration is well known, the transcellular route of transmigration has only been recently discovered. The movement of one cell directly through another appears to be a counterintuitive process. Nevertheless, both in vitro and in vivo observations of transcellular migration have been identified and appear to involve podosome-like protrusive activities in leukocytes and specific fusogenic functions in endothelial cells[58] (Fig 11-6).

Immunohistochemical studies have localized the presence of PECAM-1, ICAM-1, and ICAM-2 on the endothelium of capillaries, arterioles, and venules in the human dental pulp. In addition, ICAM-3 was detected on peripheral blood cells and VCAM-1 weakly recognized on the venule endothelium. Selectin-P was mostly located on venule endothelium and platelets.[59]

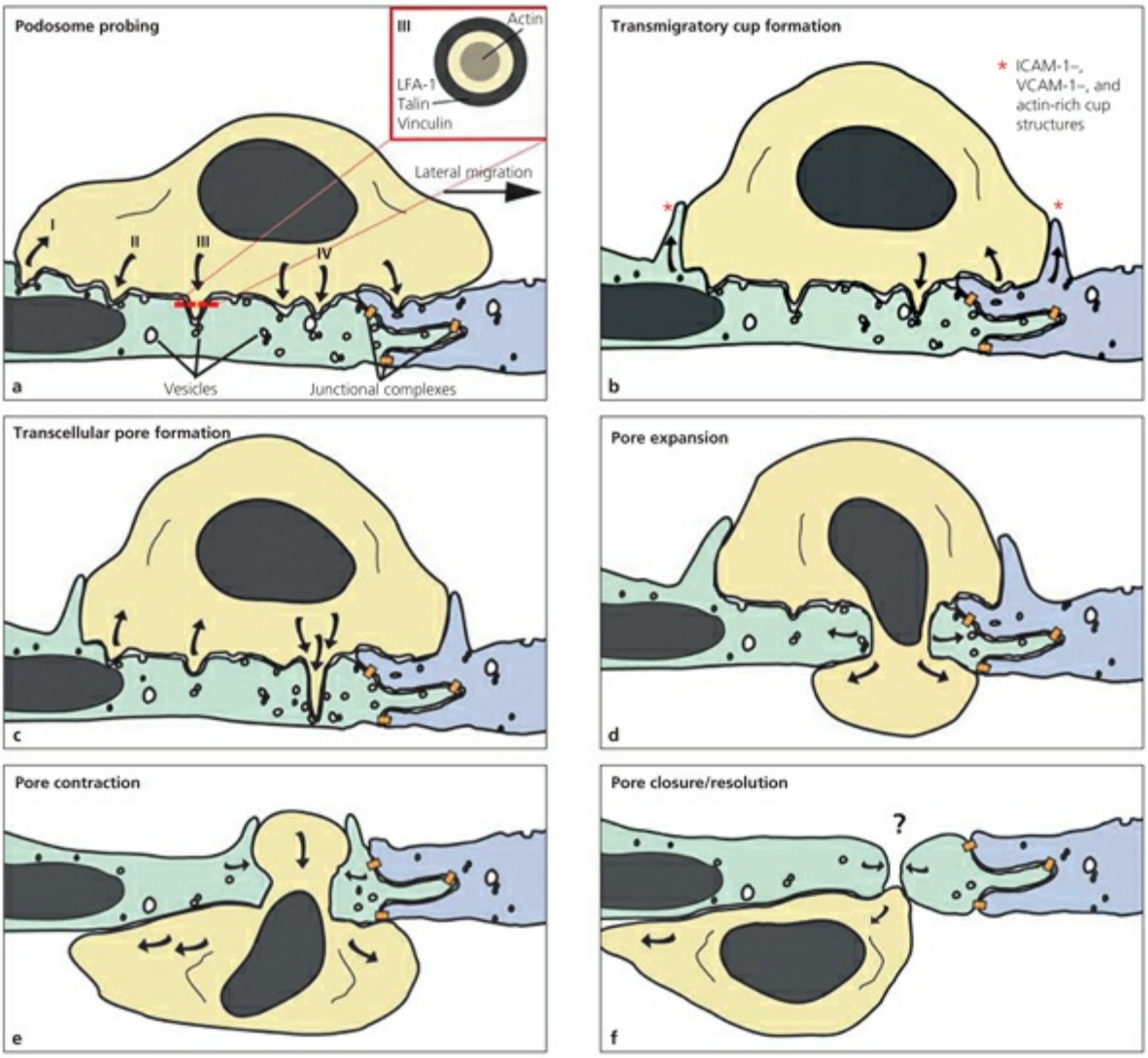

Fig 11-6 Mechanisms for transcellular diapedesis. Basic morphologic features broadly observed in vivo and in vitro as a leukocyte progressively migrates across the endothelium through a transcellular pore. A small segment of endothelium is depicted in which two individual endothelial cells are distinguished by green and blue. Locations where specific junctional adhesion complexes (ie, tight, adherens, and gap junctions) form are indicated *(orange)*. *(a)* Podosome probing. A lymphocyte laterally migrates toward an intact interendothelial junction. During migration, dozens of actin-dependent podosome-like protrusions dynamically form *(downward arrows)* and retract *(upward arrow)*, concomitantly forcing endothelial invaginations termed *podoprints*. This dynamic protrusion behavior is thought to serve in migratory pathfinding as a means of probing the endothelial surface for sites permissive to transcellular diapedesis. I, trailing edge podosome retracting; II, podosome protruding into and being frustrated by the rigid nuclear lamina; III, podosome and its cross section *(inset)*, highlighting the peripheral LFA-1 integrin–talin-vinculin zone and the actin-rich core; IV, specific podosome that progressively extends to

become an invasive podosome and facilitate transcellular pore formation in panels *(b)* and *(c)*. Endothelial vesicles, vesiculovacuolar organelles, and caveolae (vesicles) are seen enriched near or directly fused to podoprints. *(b)* Transmigratory cup formation. Overlapping temporally with podosome probing, endothelial cells proactively protrude actin-dependent, ICAM-1/VCAM-1–enriched protrusions *(asterisks)* that embrace adherent leukocytes, forming transmigratory cups that are thought to facilitate transition from lateral to transendothelial migration. Note that many podosome-like protrusions have retracted or are retracting while one continues to protrude. *(c)* Transcellular pore formation. At permissive sites, podosome-like protrusions progressively extend, transitioning to invasive podosomes, which forces the endothelial apical and basal plasma membrane into close apposition, thereby facilitating initial transcellular pore formation for diapedesis. Active fusion of endothelial vesicles at the site of protrusion may facilitate this process. *(d)* Pore expansion. The leukocyte progressively pushes across the transcellular pore, causing expansion of its diameter to as much as 5 μm. *(e)* Pore contraction. As the leukocyte completes diapedesis, the pore contracts, maintaining close endothelial cell–leukocyte contacts. *(f)* Pore closure/resolution. The leukocyte finally exits the pore completely. Substantial in vitro and in vivo data support the existence of rapid resealing of the vacated pore. However, no details currently exist on the mechanisms of this important process. (Reprinted from Sage and Carman[58] with permission.)

Integrins are transmembrane glycoproteins expressed on many cell types, including leukocytes and endothelial cells. Their cytoplasmic domains bind to the cytoskeleton. The integrin superfamily consists of about 30 structurally homologous proteins that promote cell-cell or cell-matrix binding. All integrins are heterodimeric cell-surface proteins consisting of an α chain and a β chain that are noncovalently linked. The most notable integrins found on leukocytes are LFA-1, Mac-1, and VLA-4. The major function of integrins is to mediate stable adhesion of leukocytes to endothelial cells, T cells to antigen-presenting cells (APCs), cytolytic T cells to target cells, and connective tissue cells to extracellular matrix proteins such as fibronectin, vitronectin, osteopontin, and collagen.[60]

At least 18 α- and 8 β-integrin subunits are known in humans. The sequence arginine-glycine-aspartic acid has been identified as a general integrin-binding motif (see chapter 17), but individual integrins are also specific for particular protein ligands. Immunologically important integrin ligands are the ICAMs on inflamed endothelium and APCs. On ligand binding, integrins can either transduce signals into the cell interior (outside-in signaling) or receive intracellular signals that regulate their ligand-binding affinity (inside-out signaling). Binding of integrins to leukocytes is upregulated by chemokine secretion from either the endothelial cells or the inflamed tissue.

Patients who have a defect in the biosynthesis of the β_2 chain, shared by LFA-1 and Mac-1 integrins, develop leukocyte adhesion deficiency 1 (LAD-1). Patients with LAD-1 and LAD-2 (discussed earlier) develop immunodeficiency and recurrent bacterial infections.[3]

Human dental pulp cells were found to express α_1-, α_3-, α_5-, α_6-, α_v-, and β_1-integrin subunits by immunoblot or immunoprecipitation techniques.[61,62] Anti-β_1 monoclonal antibody inhibited pulp cell adhesion to laminin but not fibronectin. Integrin chains α_1, α_2, α_3, α_5, α_6, and β_1 were also immunohistochemically demonstrated in the normal dental pulp.[63] Leukocyte transmigration is mediated by interactions between ICAM-1 and integrins, as well as PECAM-1 on leukocytes and endothelial cells.[60] In the dental pulp, integrins are essential for adhesion of odontoblasts to each other and to predentin, other pulp cells, and matrix proteins.[64,65] Mature odontoblasts were found to express $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ genes (see chapter 17). However, the exact role of these integrins is not fully understood because α_v-knockout models did not show structural changes.[65]

Chemotactic factors

After transmigration, leukocytes are attracted to the site of inflammation along a chemical gradient set by a number of chemotactic factors. Bacterial products can act as chemoattractants. Studies conducted in primates have shown that the application of bacterial components to Class V cavities causes the attraction of neutrophils to subjacent pulp areas within hours.[66]

Endogenous molecules that mediate chemotaxis include complement components C3a and C5a, leukotrienes (especially leukotriene B_4 [LTB_4]), and chemokines (such as IL-8). A more detailed discussion of these factors follows later in the chapter. It has been shown that vascular endothelial growth factor, an angiogenic growth factor that induces proliferation and migration of vascular endothelial cells, promotes chemotaxis and proliferation of human pulp cells.[67] These effects were in part mediated by the activation of the DNA-binding proteins activator protein 1 and, to a lesser degree, NFκB.

Other Mediators of the Inflammatory Response

Arachidonic acid metabolites

When cells are activated, their membrane phospholipids are rapidly remodeled to generate biologically active lipid inflammatory mediators. Products derived from the metabolism of arachidonic acid, a 20.carbon polyunsaturated fatty acid, affect a variety of biologic processes, including inflammation and hemostasis. Arachidonic acid metabolites, also called *eicosanoids*, are synthesized by two major classes of enzymes: cyclo-oxygenases (COXs), represented by prostaglandins and thromboxanes, and lipoxygenases, represented by leukotrienes and lipoxins (Fig 11-7). A third metabolic pathway, involving cytochrome P450 epoxygenases, metabolizes arachidonic acids into several epoxyeicosatrienoic acids. The epoxyeicosatrienoic acids have also been shown to play critical roles in regulating cellular proliferation, inflammation, hemostasis, and a variety of intracellular signaling pathways.

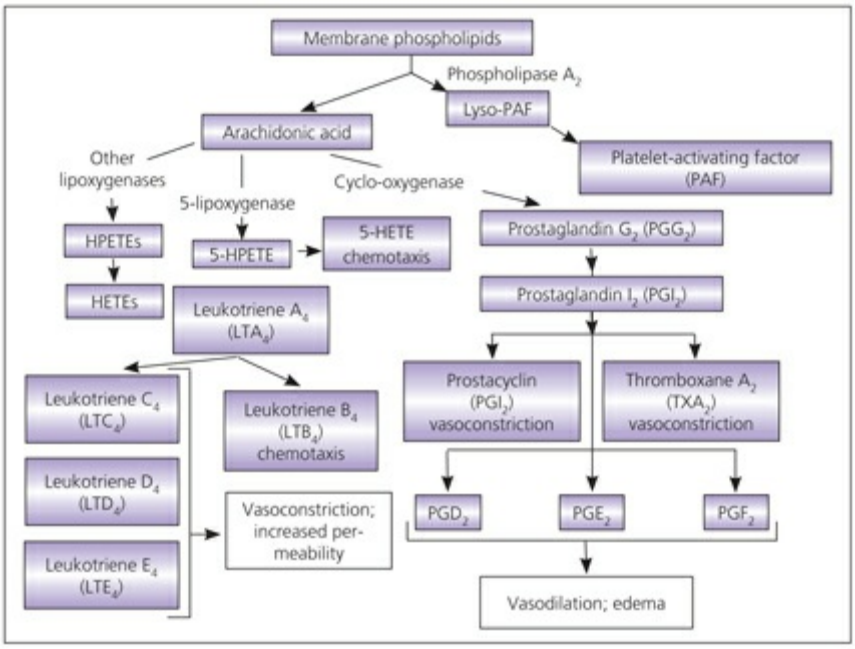

Fig 11-7 Phospholipid metabolism and arachidonic acid pathways. HETEs, hydroxyeicosatetraenoic acids; HPETEs, hydroperoxyeicosatetraenoic acids.

Prostaglandins and thromboxane

The cyclo-oxygenase pathway leads to the generation of prostaglandins. This process takes place in the normal pulp and is blocked by the addition of indomethacin, a prostaglandin synthetase inhibitor, and augmented by exogenous arachidonic acid or serotonin.[68] The most important prostaglandins associated with

inflammation are PGE_2, prostaglandin D_2 (PGD_2), prostaglandin $F_2\alpha$ ($PGF_2\alpha$), prostacyclin (PGI_2), and thromboxane A_2 (TXA_2).

It has been known for decades that prostaglandins play a critical role in the pathogenesis of pulpal disease.[69,70] Induced pulpal inflammation in the rat incisor using bacterial lipopolysaccharide (LPS) resulted in a 9.3-fold increase in PGE_2 and a 3.8-fold increase in 6-keto-$PGF_1\alpha$, a stable metabolite of PGI_2.71 Increase in pulpal levels of PGE_2, $PGF_2\alpha$ and 6-keto-$PGF_1\alpha$ content following induced inflammation has been demonstrated immunohistochemically.[72]

Prostaglandins generally cause vasodilation, while thromboxane causes vasoconstriction. Elevated PGE_2 and 6-keto-$PGF_1\alpha$ levels were associated with a significant increase in vascular permeability of the LPS-inflamed rat pulp; this increase could be inhibited in a dose-dependent manner by administration of indomethacin prior to the application of the LPS.[73] In that study, an increased level of hydroxyeicosatetraenoic acid (HETE), a lipoxygenase metabolite, was not affected by indomethacin administration.

Prostaglandins may induce the production of other inflammatory mediators or regenerative molecules. In the dental pulp, PGE_2 significantly increased the bradykinin-evoked release of immunoreactive CGRP[74] and the production of hepatocyte growth factor, a factor that stimulates DNA synthesis.[75]

Prostaglandins are also involved in the pathogenesis of pain. Intravenous administration of non-steroidal anti-inflammatory drugs (NSAIDs), which are known to block the COX pathway, resulted in significant inhibition of stimulated nerve activity in the feline pulp.[76] Furthermore, patients with severe pulpal pain had significantly greater pulpal levels of PGE_2 and $PGF_2\alpha$ than did patients with nonpainful pulpitis or normal pulp.[77]

Injection of a steroid, Depo-Medrol (Pfizer), via an intraosseous route to teeth with irreversible pulpitis significantly reduced PGE_2 after 1 day in the pulp.[78] Because prostaglandins are abundant in pulpal inflammation, it has been suggested that their level could be measured to determine the degree of pulpal inflammation of patients undergoing pulpotomy procedures in primary teeth in order to predict the long-term treatment outcomes.[79,80]

Glucocorticoids block the breakdown of membrane phospholipids to arachidonic acid by phospholipases, whereas NSAIDs block the COX pathway. Investigations have shown the effectiveness of corticosteroids[81–83] and NSAIDs[84–86] on pulpal

pain, particularly in apical inflammation that occurs following pulpectomy. The COX pathway is mediated by at least two different enzymes, COX-1 and COX-2. COX-1 is constitutively expressed and has beneficial homeostatic functions on the gastric mucosa and the kidneys, whereas COX-2 is an induced proinflammatory enzyme.[87] Several reports have documented that neuropeptides and cytokines induce COX-2 expression in pulp cells in vitro.[52,88] The inhibition of PGE_2 production in induced rat molar pulpal inflammation by the COX-2 inhibitor nabumetone was similar to that of ibuprofen.[86]

Leukotrienes and lipoxins

The lipoxygenase pathway products are only present in inflammatory cells such as neutrophils, eosinophils, mast cells, basophils, macrophages, and monocytes. This is distinctly different from COX products, which are present in all mammalian cells except erythrocytes (ie, all nucleated cells). In neutrophils, 5.lipoxygenase is the predominant enzyme. The main product, 5-HETE, which is chemotactic for neutrophils, is converted into a family of compounds collectively called *leukotrienes*. LTB_4 is a potent chemotactic agent and activator of neutrophil functional responses, such as aggregation and adhesion of leukocytes to venule endothelium, generation of oxygen free radicals, and release of lysosomal enzymes.[3,68] Inflammation induced on animal dental pulps demonstrated increased production of LTB_4[86,89] or leukotriene C_4 (LTC_4).[90] A dual inhibitor of the COX and lipoxygenase pathways inhibited LTB_4 or LTC_4 production, whereas indomethacin had no effect.[89] Minor changes in temperature increased LTB_4 expression in human primary pulp cells and pulp stem cells.[91]

The results of studies on whether leukotrienes reduce pain threshold in the dental pulp have not been conclusive. LTB_4 and LTC_4 significantly reduced spontaneous and evoked nerve excitability of the cat dental pulp in one study.[92] However, LTB_4 increased pulpal nerve excitability under similar conditions in another study.[93]

Lipoxins are a more recent addition to the family of bioactive products generated from arachidonic acid. Lipoxins A_4 (LXA_4) and B_4 (LXB_4) are generated by the action of platelet-derived 12-lipoxygenase on neutrophil leukotriene A_4. Lipoxins may be negative regulators of leukotrienes, inhibiting neutrophil chemotaxis and adhesion in acute inflammation and causing vasodilation to attenuate leukotriene LTC_4-mediated vasoconstriction.[68]

Although inflammation is the key process in host defense, the resolution of an inflammatory response is equally important to reestablish homeostasis and limit excessive tissue injury. It is now understood that the resolution of inflammation is an active instead of a passive process (ie, dilution of proinflammatory mediators) that is mediated by proresolution biochemical signaling circuits[94] (Fig 11-8). During the initial stage of inflammation, eicosanoids, including prostaglandins and leukotrienes, play important roles as proinflammatory mediators, triggering potent chemotactic leukocytic responses (see Fig 11-8a). The second stage of inflammation is coupled to the biosynthesis of lipid mediators that actively limit inflammation and promote resolution (see Fig 11-8b). These proresolution lipid mediators include endogenous lipoxins LXA_4 and LXB_4; their aspirin-triggered carbon-15 epimers (15-epi-LXA_4 and 15-epi-LXB_4); and the more recently discovered resolvins, protectins, and maresins that are derived from omega-3 fatty acid precursors.[95]

Aspirin affects lipoxin generation, leading to the production of mediators known as *aspirin-triggered lipoxins* (ATLs) through the COX-2 pathway (Fig 11-9). Thus, aspirin has the unique ability among the NSAIDs to initiate resolution of inflammation by jumpstarting the early formation of proresolution lipid mediators that would normally be produced by leukocytes later in the course of an inflammatory response.

Lipoxins are considered to act as braking signals in inflammation, limiting the trafficking of leukocytes to the inflammatory site. They also influence other target cell types that are actively involved in the resolution of inflammation and stimulating phagocytosis of apoptotic cells by macrophages[96] (Fig 11-10). Lipoxins have emerged as potential antifibrotic mediators that may influence profibrotic cytokines and matrix-associated gene expression in response to growth factors. The rapid inactivation and short half-lives of lipoxins in vivo have led to the development of three generations of lipoxin analogs that are designed to resist metabolism and to preserve their structural integrity, bioavailability, and beneficial actions.

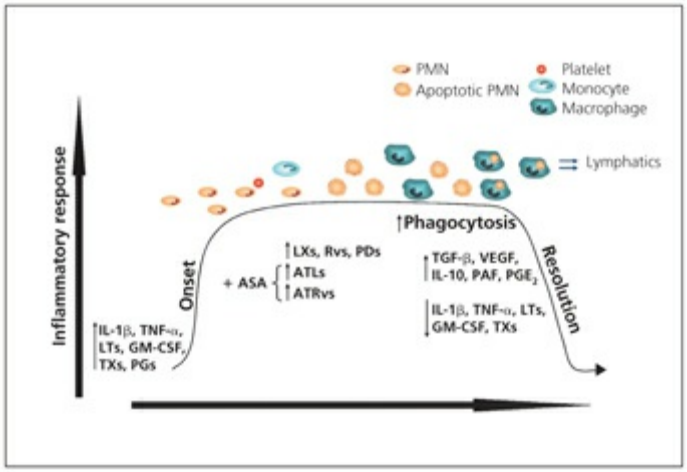

Fig 11-8a Temporal cellular and biochemical events in the onset and resolution of inflammation. The initial phase of inflammation is characterized by the release of proinflammatory mediators and extravascular accumulation of neutrophils, followed by infiltration of monocytes that differentiate into macrophages. This phase is followed by the formation of anti-inflammatory and proresolution mediators (lipoxins, aspirin-triggered resolvins, resolvins, and protectins). These mediators stop further neutrophil trafficking and facilitate the removal of apoptotic cells. The ingestion of apoptotic cells results in potent anti-inflammatory effects through the production of anti-inflammatory cytokines, such as TGF-β1, IL-10, and PGE_2, and the decrease of release of proinflammatory mediators, including IL-8, TNF-α, and TXA_2. LTs, leukotrienes; TX, thromboxane; GM-CSF, granulocyte-macrophage colony-stimulating factor; PGs, prostaglandins; ASA, aspirin; LXs, lipoxins; Rvs, resolvins; PDs, protectins; ATLs, aspirin-triggered lipoxins; ATRv, aspirin-triggered resolvins; VEGF, vascular endothelial growth factor; PAF, platelet-activating factor; PMN, polymorphonuclear neutrophil (Reprinted from Serhan[94] with permission.)

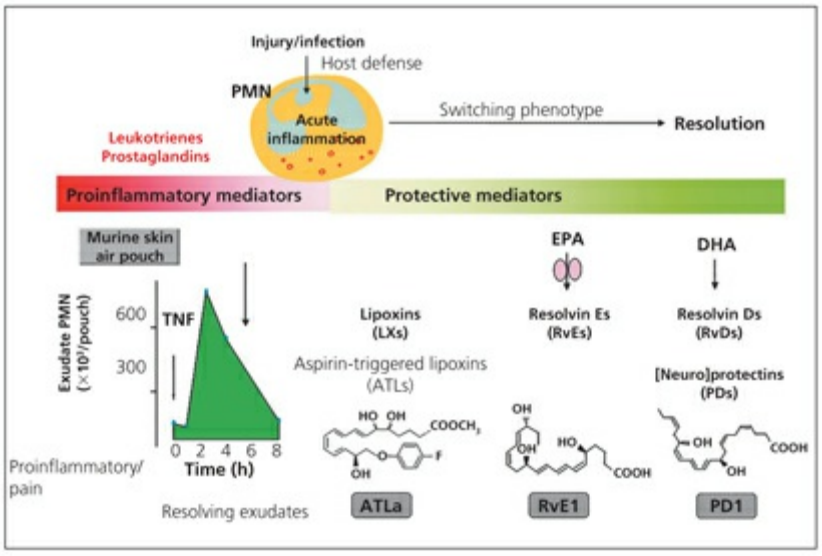

Fig 11-8b Resolution of acute inflammation is an active process. In response to injury or infection, acute inflammation is normally a protective mechanism, initiated by polymorphonuclear neutrophils (PMNs). Neutrophil-derived proinflammatory mediators, such as leukotrienes and prostaglandins, can intensify this process, potentially leading to chronic inflammation. Within the resolution phase, neutrophils can promote inflammatory resolution by changing phenotype to generate protective mediators that are derived from polyunsaturated fatty acids (PUFAs). These mediators include lipoxins

derived from arachidonic acid and aspirin-triggered epimer of lipoxins; resolvins derived from omega-3 PUFAs (eicosapentaenoic acid [EPA; resolvin E series] and docosahexaenoic acid [DHA; resolvin D series]); and protectins enzymatically derived from DHA. (Reprinted from Serhan[95] with permission.)

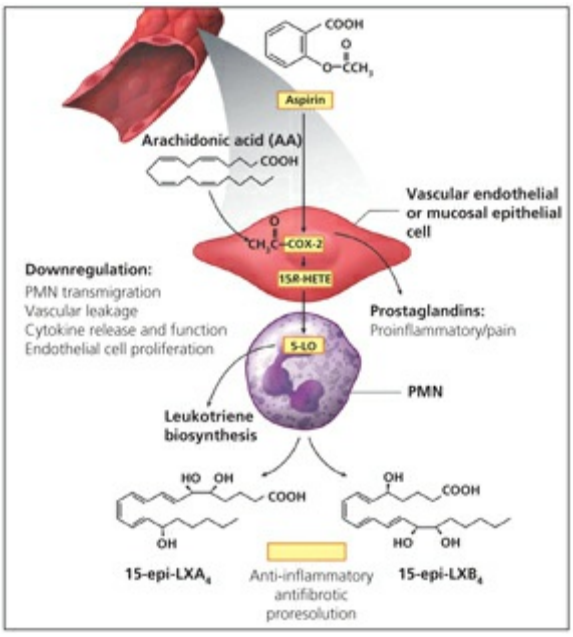

Fig 11-9 Biosynthesis of ATLs. Under this scenario, acetylated COX-2 does not produce prostaglandin (proinflammatory) intermediates but remains enzymatically active to produce 15*R*-hydroxyeicosatetraenoic acid (15*R*-HETE) from arachidonic acid (AA) that is converted by polymorphonuclear neutrophil (PMN) leukocytes to 15-epi-lipoxins (15-epi-LXA_4 and 15-epi-LXB_4). In addition, there is inhibition of leukotrienes (proinflammatory) that are normally initially generated via the 5-lipoxygenase (5-LO) pathway. (Reprinted from Serhan[94] with permission.)

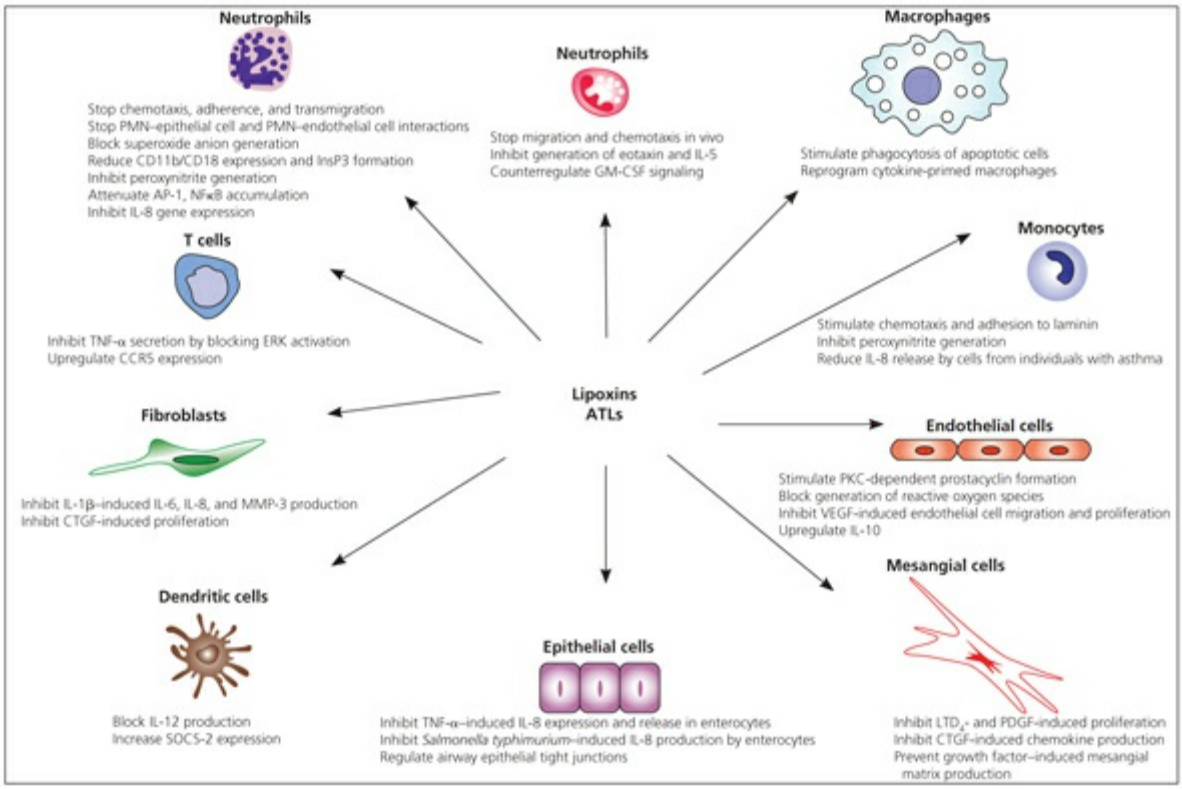

Fig 11-10 Target cells for lipoxin A_4 and ATL bioactions. AP-1, activator protein 1; CCR5, a chemokine receptor; CD, cytoplasmic domain; CTGF, connective tissue growth factor; ERK, extracellular signal–regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; LTD_4, leukotriene D_4; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PKC, protein kinase C; PMN, polymorphonuclear neutrophil; SOCS-2, suppressor of cytokine signaling 2; VEGF, vascular endothelial growth factor. (Reprinted from Maderna and Godson[96] with permission.)

Currently used anti-inflammatory therapies are directed toward the inhibition of enzymes and/or antagonism of receptors. Both selective COX inhibitors and anti–TNF-α are examples of this approach; they are used with the goal of blocking production of proinflammatory chemical mediators. The recent identification of lipoxins, ATLs, and the novel genus of anti-inflammatory specialized proresolving mediators (SPMs) has generated a paradigm shift in determining what terminates or resolves acute inflammation. Rather than targeting inhibition, the focus of contemporary research has shifted to the use of agonists to stimulate key points within the control endogenous mechanisms for resolving inflammation and has opened new frontiers in pharmacologic pursuits toward the resolution of acute inflammation, namely, resolution pharmacology.[97,98] In dermal inflammation, lipoxin stable analogs, when applied topically to mouse ears, inhibit both leukocyte infiltration and vascular permeability changes. In periodontics, experimental use of lipoxin analogs in animal models has generated positive results in the management of inflammatory periodontitis.[99] Although lipoxins have not been reported in the dental

pulp, the potential of these stable lipoxin analogs in protecting host tissues and controlling inflammation offers opportunities in designing therapeutic strategies for the resolution of pulpal inflammation.

Specialized proresolving mediators

Lipoxins were the first mediators recognized to have dual anti-inflammatory and proresolution activities. The SPMs represent a whole new genus of endogenous chemical mediators identified in inflammatory exudates. They include three distinct chemical families: resolvins, protectins, and the most recently identified maresins, which are involved in acute inflammation.[98] Each of these families is actively biosynthesized from omega-3 precursors in the resolution phase of acute inflammation. These anti-inflammatory and proresolving lipid mediators are potent agonists that control the duration and magnitude of inflammation. Their actions are agonistic in the sense that by acting on separate cell populations individually via stimulating specific receptors, they stimulate the overall resolution of inflammation.

Resolvins

Resolvins, or *resolution-phase interaction products*, are lipid mediators generated through the oxidation of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Hence, there are E series (RvE) and D series (RvD) resolvins.[100] Resolvins were initially identified by harvesting in vivo exudates during the resolution phase of acute inflammation, defined by the time period of rapidly declining neutrophil cell numbers. The mouse dorsal air pouch model was selected for isolation of these mediators because it allows the cellular and biochemical analysis of limited, self-resolving acute inflammatory responses, facilitating the isolation and discovery of molecules involved in spontaneous resolution of inflammation.[101] Resolvins are also produced by a COX-2–dependent pathway in the presence of aspirin, generating their aspirin-triggered form.

Resolvins possess potent anti-inflammatory and immunoregulatory actions that prevent excessive inflammatory responses and act via multiple cellular targets to stimulate resolution and preservation of immune vigilance.[98] They block the production of proinflammatory mediators and regulate neutrophil trafficking to the inflammatory loci. For example, RvD2 decreases leukocyte-endothelial interactions

in vivo by endothelial-dependent nitric oxide (NO) production and by direct modulation of leukocyte adhesion receptor expression. In mice with microbial sepsis, RvD2 sharply decreased both local and systemic bacterial burden, excessive cytokine production, and neutrophil recruitment, while it increased mononuclear cells and macrophage phagocytosis.[102]

Protectins

Similar to resolvins, protectins were also initially harvested in vivo from resolving inflammatory exudates. Mediators of this family are distinguished by the presence of a conjugated triene double-bond system and their potent tissue-specific bioactivity. DHA also serves as the precursor for the biosynthesis of protectins. Protectin D1 (PD1), the most potent member, is synthesized from DHA via a lipoxygenase mechanism to a 10,17-dihydroxy–containing anti-inflammatory molecule. This bioactive compound was initially called *docosatriene* but is now known as *protectin D 1* owing to its potent protective activity in inflammatory and neural systems. Protectin D1, synthesized by human peripheral blood mononuclear cells and in T_H2 $CD4^+$ T cells, blocks T cell-migration in vivo, reduces TNF and interferon γ (IFN-γ) secretion, and promotes T-cell apoptosis.

RvD1 and PD1 can serve as braking signals to prevent a runaway inflammatory response. Similar to RvE1, PD1 also shifts the onset of resolution to an earlier time point than spontaneous resolution. Both RvE1 and PD1 also upregulate CCR5 (a chemokine receptor) on neutrophils, which acts as a stop signal to chemokine signaling by clearing proinflammatory CCR5 ligands from the inflammatory milieu.[98,101]

Maresins

Maresins, or *macrophage mediators in resolving inflammation*, represent the most recently discovered class of SPMs.[103] They are produced exclusively by macrophages. Maresins were also identified from self-resolving in vivo inflammatory exudates. They are biosynthesized from omega-3 fatty acid precursors via the novel 14-lipoxygenase pathway that produces bioactive 7,14-dihydroxy–docosahexaenoic acid products. These products also possess dual anti-inflammatory and proresolving activities with a potency similar to RvE1 and PD1. Preliminary findings suggest that maresins may be involved in some of the beneficial actions of DHA and macrophages in tissue homeostasis, inflammation resolution, wound

healing, and host defense.

Platelet-activating factor

Platelet-activating factor (PAF) is another bioactive mediator derived from phospholipids (see Fig 11-7). It is secreted by platelets, basophils (and mast cells), monocytes/macrophages, neutrophils, and endothelial cells. Its actions include platelet stimulation, leukocyte adhesion to endothelial cells by integrins, chemotaxis, and the production of an oxidative burst. It causes vasoconstriction in regular doses but vasodilation that is 100 to 1,000 times more potent than histamine at very low doses.[3]

PAF stimulated the production of PGI_2 and TXA_2 by rat incisor pulp tissue ex vivo in a dose-dependent manner.[104] Furthermore, when the levels of these prostanoids increased as a result of intravenous injection of the animals with endotoxin, the addition of a PAF antagonist suppressed the increase of TXA_2.[105]

Plasma proteases

Kinin system and bradykinin

During the inflammatory process, prekallikrein is activated to kallikrein by the Hageman factor (coagulation factor XII). Kallikreins, which are specific proteases, generate vasoactive peptides from plasma proteins called *kininogens*. The most important metabolite of the kininogens is the nonapeptide bradykinin (BK). BK has four main proinflammatory actions: *(1)* vasodilation, *(2)* increased vascular permeability, *(3)* the induction of pain, and *(4)* the attraction of leukocytes.[106]

BK functions by binding with two receptors: B_1 and B_2. B_1 appears to be involved in certain forms of persistent hyperalgesia or chronic pain, whereas B_2 receptor is the main BK receptor constitutively present in normal tissues and plays a role in acute inflammatory pain.[107] B_2 is also the principal BK receptor in the dental pulp.[74,108,109]

BK is one of the main molecular mediators of inflammation in the dental pulp. In the canine model, BK, like PGE_2, increased pulpal blood flow and vascular permeability (probably at the postcapillary venule site). BK caused a smaller flow increase but produced more leakage than PGE_2.[110]

During pulpal inflammation, a complex interaction takes place between BK and a number of other molecular mediators, frequently eliciting synergy among the actions of the different mediators and an exaggeration of the inflammatory response. BK increased the release of arachidonic acid and its metabolites from a rat pulp cell line[109] by stimulating the intracellular signaling mediator cyclic adenosine monophosphate, Ca^+, and inositol phosphate.[111] BK also enhanced the formation of PGE_2 by IL-1α, IL-1β, TNF-α, and TNF-β.[108] The bovine pulp superfusion model revealed that PGE_2 increases the release of BK-evoked immunoreactive CGRP by more than 50%.[74]

Once released, BK is rapidly metabolized by specific kininases.[112] Therefore, it has been difficult to measure BK levels in vivo. However, in another study, BK levels in the human pulp interstitial fluid were measured directly using microdialysis.[113] In that study, there was a 13-fold increase in BK level in irreversible pulpitis when compared with normal pulps. Patients who had pain at the time of sampling produced significantly more BK than those who had a previous pain history (17-fold increase versus a 3-fold increase above normal levels).

Anti-inflammatory drugs such as methylprednisolone[114] or flurbiprofen[115] reduced the level of BK in inflamed tissues. Also, addition of BK to homogenized rat pulp led to the release of the endogenous opiate metenkephalin in a dose-dependent manner, an effect that was inhibited by the potent BK inhibitor des-Arg^9-[Leu^8]-BK.[116] Thus, homeostatic mechanisms may contribute to the control of BK release in vivo.

Complement

The complement system consists of 20 different plasma proteins (together with their cleavage products) that are utilized in both the adaptive and innate immune responses for lysis of microbial cells. Two complement activation pathways have been described: the classic and alternative pathways (Fig 11-11). These pathways differ in how C3b is produced but are similar after C3b production.

Complement activation promotes phagocytosis because phagocytes express receptors for C3b. The terminal components of the complement system, whose

activation is dependent on C3b, generate a lipid-soluble macromolecular protein complex called the *membrane attack complex*, which causes osmotic lysis of target cells. Peptides produced by proteolysis of C3 and other complement proteins stimulate inflammation. C3a and C5a, also known as *anaphylatoxins*, release histamine from mast cells and result in vasodilation and increased vascular permeability. C5a activates the lipoxygenase pathway of arachidonic acid in neutrophils and monocytes and is itself a potent chemotactic agent for these cells.

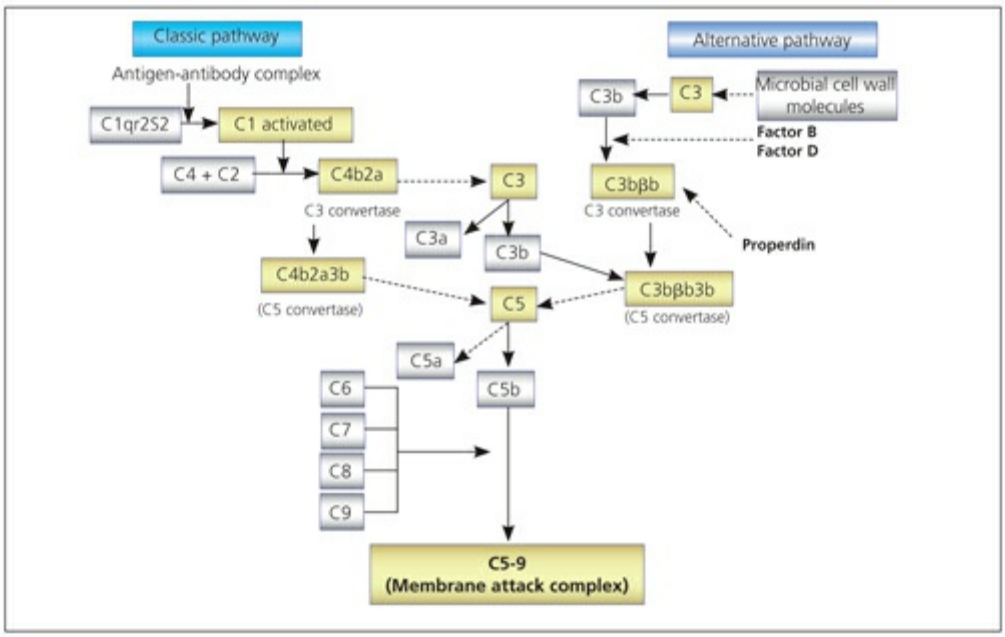

Fig 11-11 Complement cascade.

Earlier studies revealed weak complement activity in the dental pulp.[117–119] One report demonstrated positive staining for C3 and C4 complement proteins in dentin in normal and carious teeth.[120] Complement staining in caries lesions was more intense on the external surface and was probably of plaque origin.[121] The relative paucity of direct observational evidence for the presence of complement components in the pulp may be due to the fact that most complement proteins are transiently present in the inflammatory process and are easily denatured during specimen processing. The effects of bacterial irritants on the dental pulp were examined in Class V cavities prepared in primates. The experimental group was injected with purified cobra venom factor, which has known anticomplement activity. The results did not reveal histologic differences between the control and experimental groups.[122] Therefore, the role of complement activity in pulpal inflammation is not clear at this time.

Clotting and fibrinolytic systems

The clotting system is closely related to the inflammatory process. As discussed previously, prekallikrein is activated to kallikrein in inflammation by the action of the Hageman factor XII, which also starts the extrinsic pathway of the coagulation cascade (Fig 11-12). A number of intermediary steps result in the formation of thrombin (factor IIa, a serine protease) from prothrombin. In addition to activating fibrinogen to form fibrin, activating factor XIII to cross-link the fibrin polymer, and aggregating platelets, thrombin has a number of inflammatory properties such as chemotaxis, leukocyte adhesion to endothelial cells, and fibroblast proliferation.[123] The addition of thrombin to pulp fibroblasts caused a burst in the production of PGE_2 and 6-keto-$PGF_1\alpha$, an effect that was very similar to the addition of BK.[109] It was also shown that thrombin increases the DNA and protein synthesis and the proliferation of pulp fibroblasts and that these effects can be modulated by PGE_2.[124–126]

Significant advances in the understanding of blood-coagulation mechanisms over the last decade have shown that the extrinsic pathway and factor XII, as the initiator of this pathway, play significantly smaller roles in hemostasis in vivo than was previously believed. Traditionally, the coagulation process is represented by a Y-shaped cascade of proteolytic reactions that act as a biologic amplifier, with the extrinsic and intrinsic pathways meeting in a common pathway (Fig 11-13a). While this coagulation cascade provides the framework for interpreting the results of common coagulation screening tests in plasma or purified protein-based fluid systems, where the fluid is static and does not interact with vascular wall or cell surfaces, it has severe limitations as a clinical hemostasis model. The traditional cascade model suggests that the extrinsic and intrinsic pathways operate as independent and redundant pathways, but clinical manifestations of individual factor deficiencies clearly contradict this concept. For example, deficiencies in factor XII caused marked prolongation of activated partial thromboplastin time in laboratory bleeding tests but are not associated with a tendency for bleeding in humans.

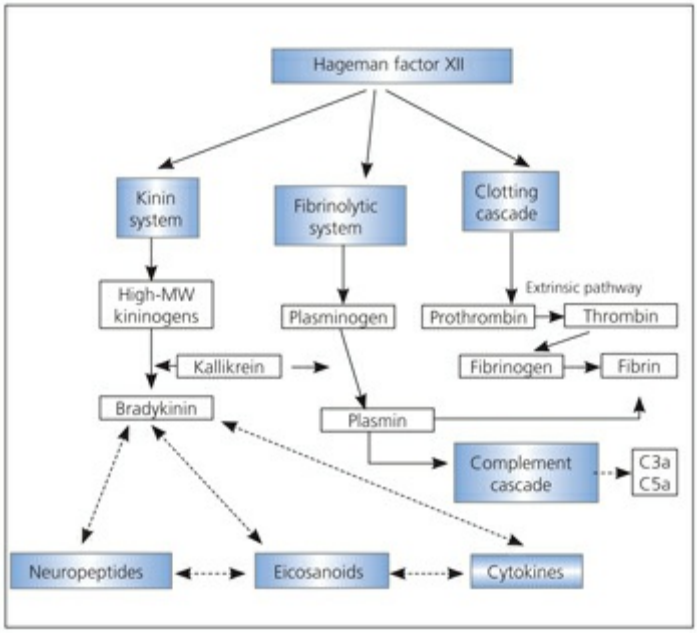

Fig 11-12 Interaction between selected groups of inflammatory systems. *Arrows* indicate synergy and/or potentiation. MW, molecular weight.

The classic cascade model of hemostasis has now been replaced by the cell-based model of coagulation that incorporates the vital role of cells in the coagulation process. This essentially represents a paradigm shift from a model that views coagulation as being controlled by the levels and kinetics of the coagulation proteins to a new concept that embraces cellular components as the key controlling elements in coagulation. The cell-based model requires the participation of two different cell types: a cell-bearing surface tissue factor and platelets. All evidence to date indicates that the single relevant initiator of coagulation in vivo is the tissue factor and that the activity of the factor VIIa–tissue factor complex is the major initiating event in hemostasis in vivo (Fig 11-13b).

The model involves four major steps: *(1)* initiation, *(2)* amplification, *(3)* propagation, and *(4)* termination. For coagulation to occur effectively in vivo, thrombin must be generated directly on the activated platelet surface and not just on the surface of the tissue factor–bearing cell, as was previously recognized in the extrinsic pathway of the cascade model. The cell-based model highlights the central role of thrombin (factor IIa) in hemostasis. This new model further suggests that there are no separate extrinsic and intrinsic pathways operating under normal conditions in vivo. The extrinsic and intrinsic systems are in fact parallel generators of factor Xa that occur on different cell surfaces, instead of being independent redundant pathways. Space limitations preclude a complete review of the detailed mechanisms involved in the cell-based model; interested readers may wish to peruse

a recent review on this topic.[127]

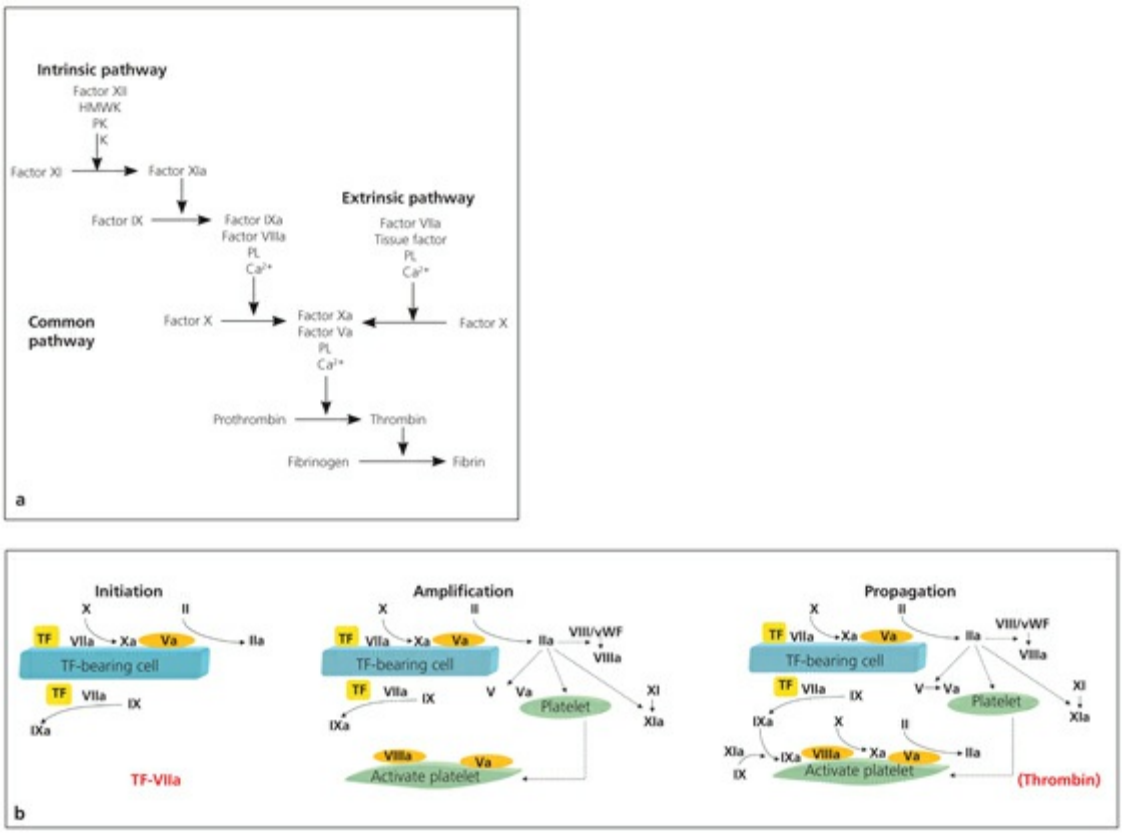

Fig 11-13 *(a)* Classic cascade model of coagulation. This model divides the coagulation system into separate redundant pathways (extrinsic and intrinsic), either of which can result in generation of factor Xa. The common pathway results in generation of thrombin (factor IIa) and subsequent cleavage of fibrinogen to fibrin. Many of the enzymes and enzymatic complexes require calcium ($Ca2^{+}$) and binding to phospholipids (PL) on active membrane surfaces for full activity. For simplicity, feedback activation of procofactors to cofactors and the many inhibitors of the various enzymes have been omitted. HMWK, high–molecular weight kininogen; K, kallikrein; PK, prekallikrein. *(b)* Cell-based model of coagulation. This model incorporates the contribution of various cell surfaces to fibrin formation. Thrombin generation occurs in overlapping phases. The initiation phase occurs on the tissue factor–bearing (TF-bearing) cell. It is initiated when injury exposes the TF-bearing cell to the flowing blood. This results in the generation of a small amount of factor IXa and thrombin that diffuse away from the surface of the TF-bearing cell to the platelet. In the second phase, amplification, the small amount of thrombin generated on the TF-bearing cell activates platelets, releases von Willebrand factor (vWF), and leads to generation of activated forms of factor V, VIII, and XI. During the propagation phase, the various enzymes generated in earlier phases assemble on the procoagulant membrane surface of the activated platelet to form intrinsic tenase, resulting in factor Xa generation on the platelet surface. Prothrombinase complex forms and results in a burst of thrombin generation directly on the platelet. When enough thrombin is generated to produce a critical mass of fibrin, these soluble fibrin molecules spontaneously polymerize into fibrin strands that result in an insoluble fibrin matrix, entrapping the activated platelets.

In the context of the present chapter, the cell-based model further incorporates the concept that some coagulation proteases, while involved in the enzymatic cascade of

coagulation, may function primarily in roles apart from hemostasis, including inflammation, vessel wall function, and cell proliferation. For example, factor XII also initiates the fibrinolytic system to dissolve the fibrin clot. A plas-minogen activator is secreted from leukocytes and endothelial cells to cleave plasminogen into plas-min, a protease. The main action of plasmin is to lyse the fibrin clot. However, it also breaks C3 into its fragments, aiding in the initiation of the complement cascade (see Fig 11-12).

Fibrinolytic activity was demonstrated in the dental pulp several decades ago.[128] A fibrin clot is formed under cavity preparations, particularly if the pulp is exposed.[129,130] Fibrinogen has been localized in the pulp and inside dentinal tubules under cavity preparations when the pulp was not exposed[131,132] and may reduce dentin permeability to advancing microbial irritants.[133] It is conceivable that the fibrino-lytic system would play an important role in the early organization of the healing pulp after injury such as that from cavity preparation. The gene-expression and protein levels[134] of tissue-type plasminogen activator were shown to increase significantly in inflamed pulp, being upregulated in the presence of proinflammatory cytokines.[134,135]

Lysosomal enzymes and metalloproteinases

Neutrophils and monocytes/macrophages have lysosomal granules containing a number of enzymes, which contribute to the inflammatory process. Neutrophils contain two types of granules: The smaller or specific granules contain lysozyme, collagenase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase, whereas the larger or azurophil granules contain myeloperoxidase, lysozyme, defensins, acid hydrolases, and neutral hydrolases such as collagenase, elastase, cathepsin G, and other proteinases.[3]

Most of these enzymes have potent antimicrobial properties, thus serving to eliminate microbial irritants. However, they can also lead to excessive tissue destruction during an inflammatory episode. Lysosomes and phagosomes have been observed in the ultrastructure of the inflamed pulp.[136] Excessive neutrophil accumulation in the pulp may increase the likelihood of tissue necrosis. Cathepsin D was observed in the normal pulp odontoblastic layer,[137] and levels of cathepsin G, elastase, and lactoferrin were shown to increase during pulpal inflammation.[138–140] The protease inhibitor α2-macroglobulin was also observed to increase with inflammation, indicating an attempt to control the tissue destruction aspect of these enzymes. A tissue inhibitor of metalloproteinase (TIMP) from cultured bovine dental

pulp was identified and was found to be destroyed by the serine proteinases, human neutrophil elastase, trypsin, and α-chymotrypsin.[141] The intensity of pulpal inflammation may determine the neutrophil and monocyte infiltration, their release of lysosomal enzymes, and the final outcome of the balance between inflammation and regeneration.

Matrix metalloproteinases (MMPs) are members of an enzyme family that require a zinc ion in their active site for catalytic activity.[142] MMPs are critical for maintaining tissue allostasis. MMPs are active at neutral pH and can therefore catalyze the normal turnover of extracellular matrix macromolecules such as the interstitial and basement membrane collagens; proteoglycans such as aggrecan, decorin, biglycan, fibromodulin, and versican; and accessory extracellular matrix proteins such as fibronectin.

The human genome has 24 matrixin genes, including a duplicated *Mmp23* gene. Thus, there are 23 members in the human MMP family, including the classic MMPs (collagenases: MMP-1, -8, and -13; gelatinases: MMP-2 and -9), stromelysins (MMP-3 and -10), matrilysins (MMP-7, -11, and -26), the membrane-bound MMPs (MMP-14, -15, -16, -17, -24, and -25), and other MMPs (MMP-12, -19, -20, -21, -23, -27, and -28). Most of the MMPs are synthesized as inactive latent enzymes. Conversion to the active enzyme is generally mediated by activator systems that include plasminogen activator or the prohormone convertase, furin.

More recently, two additional metalloproteinases have been discovered: a disintegrin and metalloproteinase (ADAM)[143] and a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS).[144] Functional ADAMs are involved in ectodomain shedding of diverse growth factors, cytokines, receptors, and adhesion molecules. An important ADAM family member (ADAM-17) is a TNF-α–converting enzyme that activates pro– TNF-α. The ADAMTSs are extracellular, multidomain enzymes. Their known functions include: collagen processing as procollagen N–proteinase; cleavage of the matrix proteoglycans aggrecan, versican, and brevican; and inhibition of angiogenesis.

Because ADAMs and ADAMTSs are new classes of metalloproteinases, they have not been reported in dental tissues. Interstitial collagenase (MMP-1) was detected in ameloblasts and odontoblasts of the developing enamel organ.[145] Gelatinases (MMP-2 and -9) were found in the dentinoenamel junction[146] and in ameloblasts, odontoblasts, and pulp of rodent incisors.[146] Another collagenase (MMP-13) was found to be present in equivalent amounts in the pulp of normal and carious teeth.[147] Enamelysin (MMP-20) was first detected in the enamel organ and

dental papilla but was also found in the pulp of mature teeth with or without caries.[148]

An extensive analysis of the presence and upregulation of MMPs in odontoblasts and the dental pulp tissue revealed that MMP-1, -2, -9, -10, -11, -13, -14, -15, -16, -17, -19, -20, and -23 were expressed by both odontoblasts and pulp tissue. MMP-7, -8, -24, and -25 were expressed only in the odontoblasts. MMP-2, -10, -11, -14, and -20 were expressed more abundantly by odontoblasts, whereas pulp tissue expressed more MMP-13 and MMP-17. TGF-β1 alone or with bone morphogenetic protein 2 significantly upregulated MMP-9 but not MMP-20 messenger RNA (mRNA) in odontoblasts; however, in pulp tissue no effects could be detected.[149]

In teeth with symptomatic irreversible pulpitis, MMP-2 and MMP-3 were found to be upregulated in one study[150] but downregulated in another study.[151] In the latter study, MMP-9 was significantly increased and correlated with an overall increase in gelatinolytic activity,[151] a finding that was corroborated by a more recent report.[152] It has also been demonstrated that painful stimulation of teeth induces an increase in MMP-8, sampled from the crevicular fluid.[153]

Host MMP-8 (collagenase) and MMP-2 and -9 (gelatinases), probably of salivary rather than pulpal origin, participated in the degradation of demineralized dentin under a caries lesion.[154] More recently, MMP-2, -8, and -9 were detected in human radicular dentin[155] and may have significance in degrading hybrid layers created by contemporary resin-based root canal sealers. Clearly, MMPs may contribute to the remodeling of dentin and pulp that take place in physiologic and pathologic situations. Clinically, the potential significance of MMP-9 as a diagnostic molecule for irreversible pulp pathosis was recently demonstrated. This molecule was found to be significantly elevated in dentinal fluid of patients with irreversible pulpitis, although this finding was not universally present in all patients studied.[156]

Protease inhibitors

Protease inhibitors serve the important function of limiting the normal proteases, including metalloproteinase, defense functions that may damage the host tissue if they are not regulated. The balance between activated MMPs and the so-called TIMPs controls the extent of extracellular matrix remodeling.[142] TIMP-1, -2, and -3 were found to be expressed by both odontoblasts and pulp tissue.[149]

Nitric oxide

Nitric oxide has received a lot of attention since its discovery in the late 1980s. Despite its high reactivity and short life, it contributes to a large array of biologic functions. NO is a soluble gas that was first identified because of its action of relaxing smooth muscle, causing vasodilation. This effect was also shown in the dental pulp.[45,157]

NO is synthesized, via L-arginine oxidation, by a family of NO synthases (NOS) and several cofactors, including nicotinamide adenine dinucleotide phosphate (NADPH). Three different isoforms of the NOS enzyme exist: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).[3] The nNOS and eNOS isoforms are constitutively expressed in the respective tissues and are calcium dependent. Conversely, iNOS is induced in macrophages and a number of other cells, primarily by cytokines such as IL-1 and TNF-α or microbial products such as LPS, and are calcium independent. The cytokines IL-4, IL-10, and TGF-β regulate the expression of iNOS in macrophages.[158]

Depending on the site of production, the amount of NO produced, and the targets within the local environment, NO can exert very different effects. A small quantity of NO released by the vascular endothelium regulates the relaxation of adjacent smooth muscle and protects against the adhesion of leukocytes and platelets to the blood vessel wall. These properties may be considered protective and anti-inflammatory (Box 11-1). In contrast, the much larger amounts of NO released by cells in response to cytokines can destroy host tissues and impair discrete cellular responses. Finally, by affecting the functions of lymphocytes and macrophages, induced NO can exert an immunomodulatory role that modifies the course of disease[159] (see Box 11-1).

Box 11-1	Modulation of inflammation by nitric oxide

Proinflammatory properties
- Promotes vasodilation and vascular leakiness
- Promotes hypotension or vascular collapse in sepsis
- Is cytotoxic
- Activates cyclo-oxygenase*
- Reacts with oxygen to form toxic peroxynitrite
- Inhibitors of NO synthesis ameliorate experimental models of arthritis
- Simulates TNF-α production by synoviocytes

Anti-inflammatory properties
- Inhibits leukocyte adhesion to endothelium
- Inhibits P-selectin expression by platelets and endothelium
- Inhibits microvascular thrombosis
- Inhibits lymphocyte proliferation
- Inhibits mast cell degranulation
- Inhibits oxidant production by phagocytes
- Inhibits cyclo-oxygenase*

* Depends on the dose.[159]

Neuronal NOS was demonstrated in feline teeth.[160] There was evidence of a dramatic increase in NO activity, as evidenced by immunoreactivity to NOS and the NADPH cofactor at the site of pulpal irritation. These activities increased to a maximum at 4 days postoperatively and declined at 14 days with evidence of necrosis[161] (Fig 11-14).

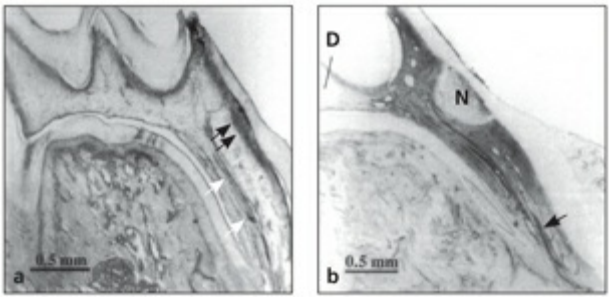

Fig 11-14 *(a)* Maxillary first molar stained for nicotinamide adenine dinu-cleotide phosphate–diaphorase (NADPH-d) activity 1 day following cavity preparation. Note the increased NADPH-d intensity in the pulp tissue adjacent to the prepared dentin *(black arrows)* and in blood vessels of the mesial root *(white arrows)*; however, only the blood vessels have statistically significantly increased intensity compared with that of controls. *(b)* Maxillary first molar stained for NADPH-d activity 4 days following cavity preparation. An area of leukocytic infiltration and necrosis (N) has developed in the mesial pulp under the prepared dentin. This area is surrounded by pulp tissue with significantly increased NADPH-d intensity in the mesial pulp horn as well as in the mesial root, while the NADPH-d intensity of the distal pulp (D) has remained at control values. Blood vessels *(arrow)* of the mesial root also have significantly increased NADPH-d intensity. (Reprinted from Law et al[161] with permission.)

In human pulp, eNOS was identified in the endothelial cells and odontoblasts of healthy tissues, and an elevation of eNOS mRNA and protein and a concomitant dilation of vessels were characteristic in inflamed tissue.[162] Healthy pulp tissue failed to exhibit any iNOS; however, acute inflammation enhanced the mRNA and protein levels of iNOS, mainly in the leukocytes.[162] Pretreatment of pulp with the NOS inhibitor N^G-nitro-L-arginine methyl ester in vivo did not affect vasodilatation but significantly potentiated SP–mediated vasodilation, possibly via increased

activity of the enzyme guanylate cyclase.[163] Likewise, SP induced NO production by activating NOS in pulpal endothelial cells.[164]

Oxygen-derived free radicals

The production of reactive oxygen species by mammalian mitochondria is important because it underlies oxidative damage in many pathologies and contributes to retrograde redox signaling from the organelle to the cytosol and nucleus[165] (Fig 11-15).

Oxygen-derived free radicals are potent inflammatory mediators released from neutrophils that are challenged with antigen-antibody complexes or chemotactic agents. The superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$) are the most important species. These can combine with NO to form peroxynitrite and other toxic nitrogen intermediates. Oxygen-derived free radicals, also known as *reactive oxygen intermediates*, upregulate the production of IL-8, and antioxidants are known to reduce this cytokine.[166]

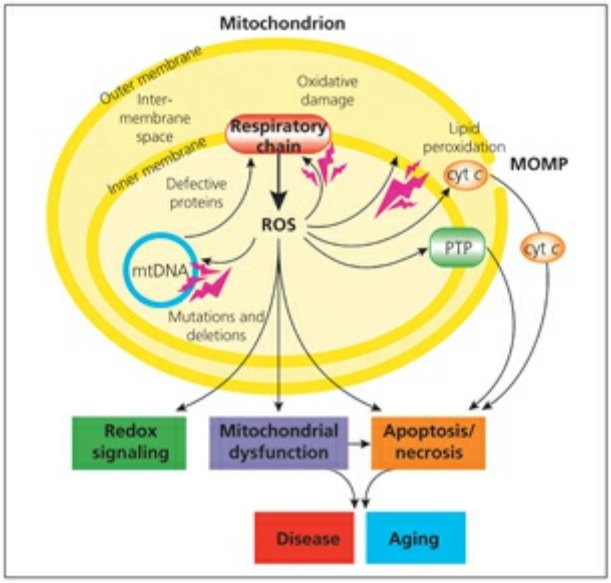

Fig 11-15 Reactive oxygen species (ROS) production by mitochondria can lead to oxidative damage to mitochondrial proteins, membranes, and DNA (mtDNA), impairing the ability of mitochondria to synthesize adenosine triphosphate (ATP) and to carry out their wide range of metabolic functions, including the tricarboxylic acid cycle, fatty acid oxidation, the urea cycle, amino acid metabolism, heme synthesis, and FeS center assembly, which are central to the normal operation of most cells. Mitochondrial oxidative damage can also increase the tendency of mitochondria to release intermembrane space proteins such as cytochrome *c* (cyt *c*) to the cytosol by mitochondrial outer membrane permeabilization (MOMP) and thereby activate the cell's apoptotic machinery. In addition, mitochondrial ROS production leads to induction of the mitochondrial permeability transition pore (PTP), which renders the inner membrane permeable to small molecules in situations such as ischemia-reperfusion injury. Consequently, it is not surprising that mitochondrial oxidative damage contributes to a

wide range of pathologic conditions. In addition, mitochondrial ROS may act as a modulatable redox signal, reversibly affecting the activity of a range of functions in the mitochondria, cytosol, and nucleus. (Modified from Murphy[165] with permission.)

The enzyme superoxide dismutase (SOD) is the main intracellular scavenger of oxygen-derived free radicals, particularly $O_2^{\bullet -}$. It has been demonstrated that the $OH^{\bullet}$ radical reduces pulpal blood flow.[167] This may be due to its effect on the endothelium or could follow the rise in tissue pressure in the low-compliance pulp environment that accompanies pulpal inflammation. Copper and zinc–containing SOD (CuZnSOD) is found in the cytoplasm, whereas manganese-containing SOD (MnSOD) is located in the nucleus or the mitochondria. In humans, CuZnSOD activity was identified in low quantities in normal pulp but increased significantly in inflamed pulp.[168] The results also indicated that the enzyme activity decreased with the advancing age of the patient, in both the normal and inflamed pulp groups. A clinical study revealed that catalase, an enzyme that reduces the highly reactive oxygen molecule H_2O_2 increases significantly in reversibly inflamed pulp, an increase that is moderated as the inflammation is diagnosed to be irreversible.[169] Areas of intense inflammation and with leukocytic infiltrates in rat pulp are associated with a dramatic increase in levels of both CuZnSOD and MnSOD activity.[170]

Cytokines

The inflammatory response represents a closely regulated balance between the proinflammatory and the anti-inflammatory mediators that is titrated to neutralize the harmful effects of the advancing irritant while minimizing damage to host tissues (Fig 11-16). This concept is very well illustrated by a discussion of cytokines in pulpal inflammation. Released from cellular components in an inflammatory process, *cytokines* are proteins that activate, mediate, or potentiate actions of other cells or tissues. Cytokines have numerous overlapping, and sometimes seemingly redundant, functions that upregulate either proinflammatory or anti-inflammatory activities to effect a closely regulated, well-orchestrated inflammatory process. Their actions can be effected in an autocrine (self-activating), paracrine (locally acting), or endocrine (systemically acting) manner.

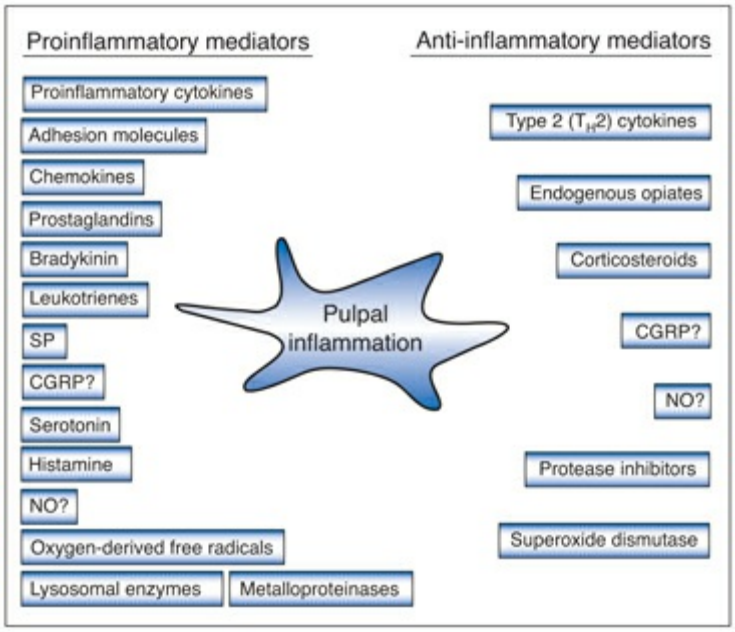

Fig 11-16 Important proinflammatory and anti-inflammatory mediators of pulpal inflammation.

Although most of the cytokines present in an inflammatory process are produced by inflammatory cells such as monocytes/macrophages, lymphocytes, and neutrophils, they may also be produced by a number of noninflammatory cells, which in the dental pulp would include fibroblasts and endothelial cells. Cytokine secretion is a brief, self-limited event, initiated by new gene transcription with mRNA encoding that is transient in the activated cell.

There are two main classifications of cytokines. A structural classification addresses the molecular structure and the types of cells that produce the cytokine (eg, type I cytokines share a four α-helical structure and receptor structure and are produced by type 1 helper T [T_H1] cells; type II cytokines are produced by type 2 helper T [T_H2] lymphocytes). A functional classification describes their role in inflammation, in that they are proinflammatory, anti-inflammatory, or effectors of chemotaxis or chemokines. A modification of the functional classification is used in this chapter.

Proinflammatory cytokines produced by innate immune cells

Interleukins and tumor necrosis factor

Cytokines secreted by innate immune cells from the dental pulp have been comprehensively reviewed.[1,171,172] Among the most important proinflammatory

cytokines are IL-1 and TNF. The actions of these two cytokines are very similar, despite the fact that they interact with structurally different receptors. IL-1 is expressed in two isoforms: IL-1α and IL-1β. IL-1 is produced mainly by monocytes/macrophages but may also be produced by polymorphonuclear neutrophils, fibroblasts, and endothelial cells.

IL-1 has several systemic effects, such as fever and production of acute phase proteins, prostaglandins, platelet-activating factor, or NO. Locally, IL-1 activates T cells and stimulates them to produce IL-2 and induces the synthesis of prostaglandins. IL-1 and TNF also activate endothelial cells and induce the expression of adhesion molecules on their membranes, thereby aiding in the recruitment of inflammatory cells to the site of inflammation.

In the pulp, it inhibits proliferation of pulp fibroblasts, and it induces the expression of collagenase from pulp fibroblasts. The effect of these cytokines on matrix production from pulp cells has been studied in vitro. In one study, IL-1β was shown to have a mild stimulatory effect on the synthesis of type I collagen in the dental pulp.[173] However, others showed that IL-1β suppressed the production of laminin, type I collagen, osteonectin, and DNA and overall protein synthesis in the pulp fibroblast in vitro; TNF-α had similar effects, except DNA and overall protein synthesis were increased.[174]

IL-1 is heavily expressed in pulpal inflammation (Fig 11-17). Furthermore, IL-1 activity was significantly higher in human dental pulps with symptomatic caries lesions than in pulps with asymptomatic carious teeth or teeth with symptomatic or asymptomatic periodontal disease. IL-1 was shown to reduce the pain threshold in peripheral tissues, primarily by increasing PGE_2 synthesis. The production of PGE_2 after stimulation of dental pulp cells with IL-1α, IL-1β, TNF-α, or TNF-β was synergistically potentiated by BK and thrombin.

The production of IL-1 and TNF in the pulp is probably a result of direct irritation by bacterial virulence factors such as cell wall products. The progression of pulpal inflammation and the tissue expression of IL-1α and TNF-β were shown to be comparable in normal mice and mice that lacked any functional T or B cells, indicating that the production of these cytokines could occur without the presence of adaptive immunity.[175] When applied to human pulp cells in vitro, LPS from *Porphyromonas endodontalis* produced IL-1β in a dose-dependent manner.[176]

Peptidoglycans and lipoteichoic acid from gram-positive bacteria were shown to have a similar, albeit less potent, effect on the production of IL-1 and TNF in other systems[177] (Fig 11-18). LPS achieves its effect by binding initially to an LPS-

binding protein (LBP) in the blood. LBP attaches to a cell-surface receptor, CD14, on macrophages. This receptor does not have a transmembrane domain to effect intracellular transduction of the signal.

The relative importance of IL-1 and TNF in mediating pulpal responses to mixed bacterial infection was investigated in the mouse model.[178] Pulpal exposures were created in mice deficient in IL-1 receptor, TNF p55 and p75 receptors, or both IL-1 receptor and TNF p55 receptors (dual deficiency). The receptor-deficient mice had a significantly faster rate of pulpal degeneration (Fig 11-19a) and microbial penetration (Fig 11-19b) than did the wild-type mice. The mice with dual deficiency had worse results than those with either deficiency. These findings indicate that, despite the proinflammatory properties of these cytokines, they have a protective role in the dental pulp against the spread of infection. It is important to recognize that the production of IL-1 and TNF from pulp macrophages in response to bacterial irritants may be suppressed by the toxic effects of dental filling materials that may come in contact with the pulp.[179,180]

A more recent analysis of the gene-expression profiles in normal and inflamed symptomatic human pulps showed that IL-1α and IL-1β were not significantly increased in the latter condition.[181] In this study, IL-6 (which has both proinflammatory and anti-inflammatory effects), IL-8 (a chemokine), and IL-18 (a proinflammatory cytokine with a profile similar to that of IL-12) were all significantly increased in teeth with painful pulpitis.

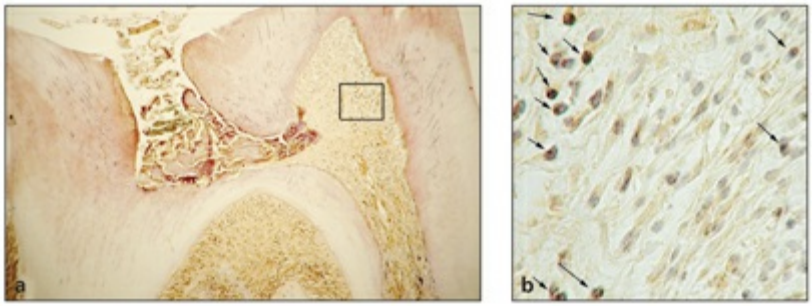

Fig 11-17 Mouse molar with pulpal exposure in the mesial pulp horn of 1 week's duration stained for IL-1α. The distal portion of the chamber and the pulp in the distal canal showed an inflammatory infiltrate. *(a)* Low-magnification view showing pulpal exposure (original magnification ×100). *(b)* High magnification view of inset from *(a)* showing IL-1α–positive cells *(arrows)* (original magnification ×1,000).

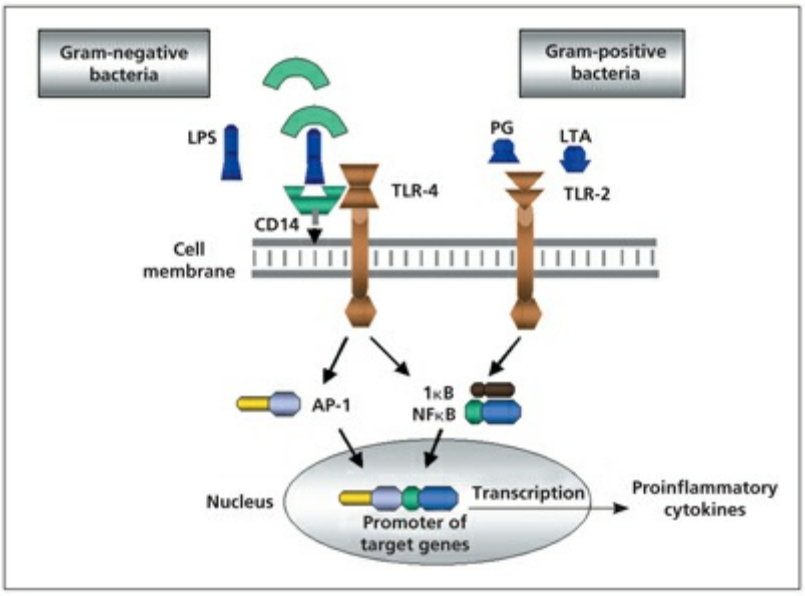

Fig 11-18 Pathway for proinflammatory cytokine production after stimulation by bacteria cell wall products. AP-1, activator protein 1; PG, peptidoglycan; LTA, lipoteichoic acid; TLR, toll-like receptor.

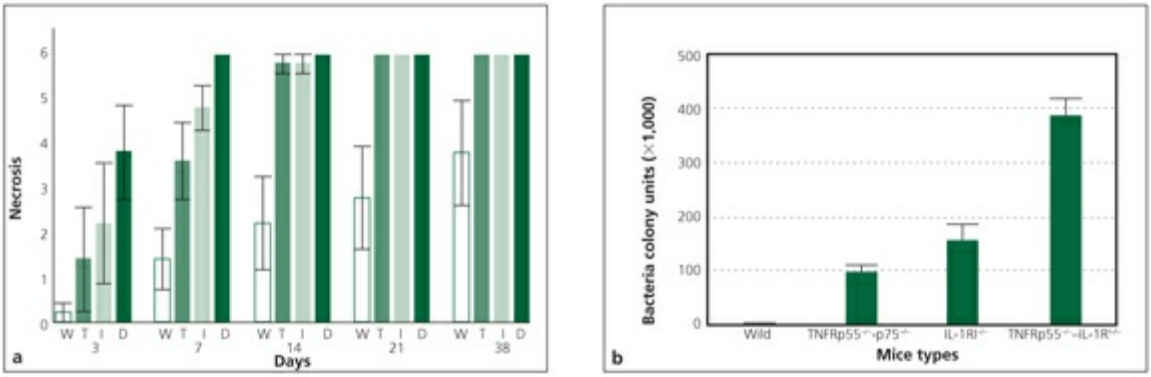

Fig 11-19 Quantitative analysis of tissue necrosis in mice lacking response to IL-1 and/or TNF. Surgical pulpal exposure followed by inoculation with six oral pathogens was carried out. Hematoxylineosin–stained cryostat sections were examined for the presence of tissue necrosis in the dental pulp. This tissue was divided into three equal parts: coronal third, middle third, and apical third, which follows the path of necrosis from the coronal third to the apical third of the dental root. *(a)* Under microscopic examination (magnification ×3,400), the following scale was used: 0, no necrosis; 1, partial necrosis of coronal third; 2, total necrosis of coronal third; 3, partial necrosis of middle third; 4, total necrosis of middle third; 5, partial necrosis of apical third; and 6, total necrosis of apical third. The highest score for the specimen represented the necrotic status of that tooth and was used in statistical analysis. Each value represents the mean ± standard error of the mean; $n = 5$ for each time point. Statistically significant differences ($P < .01$) were noted between all receptor-deficient and wild-type mice at 7, 14, 21, and 38 days after bacterial challenge. There were no statistically significant differences between any of the groups 3 days after pulpal insult. W, wild type; T, TNFRp55$^{-/-}$-p75$^{-/-}$; I, IL-1RI$^{-/-}$; D, TNFRp55$^{-/-}$–IL-1RI$^{-/-}$. *(b)* Bacterial penetration at tissue is greater in mice lacking IL-1 and/or TNF activity. The number of bacterial colonies that could be cultured from the apical portion of the distal root 8 days after exposure and inoculation with six oral pathogens was determined. Values represent the mean ± standard error of the mean; $n = 10$ for each group. All receptor-mutant mice showed statistically significant differences ($P < .01$) when compared with wild-type mice. Significantly

($P < .01$) larger values were observed in TNFRp55$^{-/-}$-IL-1RI$^{-/-}$ mice compared to IL-1RI$^{-/-}$ and TNFRp55$^{-/-}$-p75$^{-/-}$ mice. IL-1RI$^{-/-}$ and TNFRp55$^{-/-}$-p75$^{-/-}$ mice had values that were not significantly different from each other ($P < .05$) but were significantly higher than those of wild-type mice ($P < .01$). (Reprinted from Chen et al[178] with permission.)

IL-12 is a cytokine produced by monocytes, macrophages, and dendritic cells and is thought to be essential for T_H1 cell differentiation and proliferation. It is also essential in the production of IFN-γ by T_H1, $CD8^+$, γδ T cells, and natural killer cells.[1] Peripheral blood mononuclear cells challenged with *Streptococcus mutans*, which is associated with superficial caries, produced significantly more IFN-γ and IL-12 than those challenged with *Lactobacillus casei*, which is associated with deep caries.[182]

Toll-like receptors

In addition to CD14, the effects of bacterial cell wall products are mediated by pattern-recognition molecules collectively known as *toll-like receptors* (TLRs). Identification of TLRs has clarified the mechanisms by which the innate immune system recognizes nonself and the important role played by TLRs in detection of invading pathogens. Innate immunity has traditionally been viewed as a nonspecific process and somewhat simple compared to adaptive immunity, mediated via the engulfment and lysis of microbial pathogens by phagocytic cells such as macrophages and neutrophils and involving no complex protein-protein interactions. With the emergence of TLRs, innate immunity is now recognized as a highly complex process, in line with adaptive immunity.

TLRs also play a crucial role in linking innate and adaptive immunity through action on T cells and particularly via interactions on dendritic cells (Fig 11-20). These start out as immature cells with low T-cell activation potential and function to detect, capture, and phagocytose pathogens, leading to TLR activation. On signal transduction through TLRs, dendritic cells undergo maturation characterized by upregulation of cell-surface major histocompatibility complex (MHC) molecules that enhance their ability to activate T cells.[183]

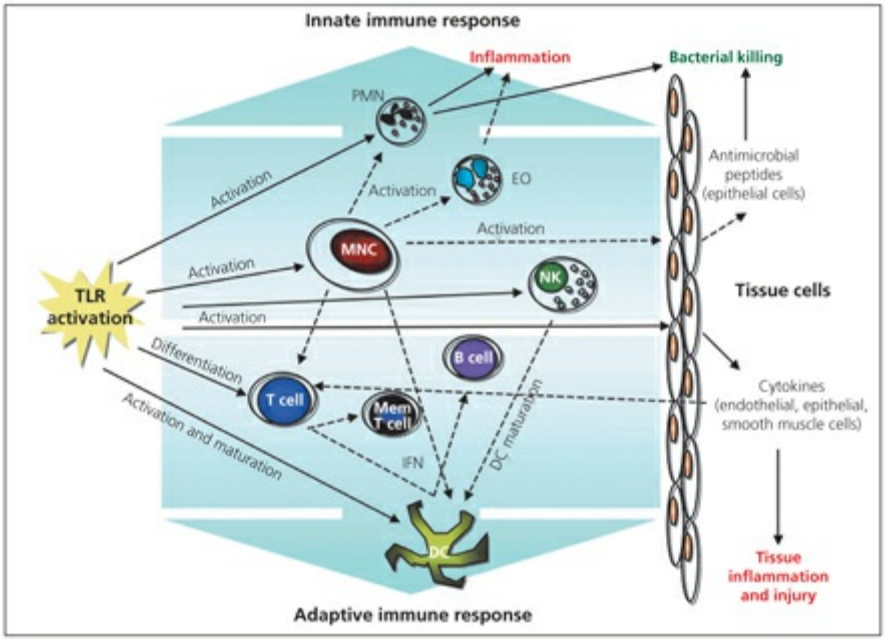

Fig 11-20 Immune pathways activated by TLR signaling. TLR signaling activates a number of apical pathways that result in the stimulation of both the innate and adaptive immune responses. Mononuclear cells (MNCs) in particular serve to amplify TLR activation by the production of cytokines and growth factors. These mediators facilitate communication between different cell types, coordinating the recruitment and activation of the immune response network as a whole. *Solid arrows* illustrate effects of direct TLR activation; *dashed arrows* represent paracrine actions resulting from TLR activation of an intermediary cell. PMN, polymorphonuclear neutrophil cells; EO, eosinophils; NK, natural killer cells; DC, dendritic cells; Mem T cell, memory T cell; IFN, interferon. (Reprinted from Parker et al[183] with permission.)

TLRs belong to a family of pattern-recognition receptors that recognize conserved parts of microbial components (pathogen-associated molecular patterns). The name *toll* stems from the recognition of the *Drosophila* toll protein in fighting fungal infections. Human TLRs can be divided into five subfamilies: TLR-2, TLR-3, TLR-4, TLR-5, and TLR-9. The TLR-2 subfamily consists of TLR-1, -2, -6, and -10; the TLR-9 subfamily is composed of TLR-7, -8, and -9. The other three subfamilies, TLR-3, TLR-4, and TLR-5, are each represented only by one family member. TLR-1, -2, -4, -5, -6, and -10 receptors are expressed in cell membranes (Fig 11-21). TLR-3, -7, -8, and -9 receptors are not located in cell membranes; however, they fulfill their functions intracellularly.[184] For example, TLR-3 is important in the induction of antiviral defense mechanisms and the recognition of nucleic acids.

Two important cell-surface TLRs are TLR-4, for which the ligand is LPS,[185] and TLR-2, for which the ligands are peptidoglycans and lipoteichoic acid.[186] TLRs cause the translocation of several nuclear factors, including NFκB and activator protein 1, to the nucleus and subsequently cause the transcription of the gene signal

to produce proinflammatory cytokines.[187] LPS (endotoxin) was reported to be present in large amounts in carious dentin. It was also reported that superficial caries had more LPS than did deep caries and that the LPS content correlated with the incidence of pain.[188] Human trigeminal neurons that were TRPV1-positive nociceptors reportedly expressed TLR-4 and CD14.[189] In inflamed pulp tissue, these receptors co-localized with N52, which is a marker of myelinated sensory neurons.[189] In addition, LPS from *Escherichia coli* or *Porphyromonas gingivalis* directly activated rat trigeminal neurons and sensitized TRPV1 via a TLR-4–mediated mechanism.[190,191] TLR-2[192] and TLR-4[193] were also identified in odontoblasts. It is of interest that these receptors are identified in such superficial locations in the pulp, in which it is critical to detect bacteria and mount an immune response to them in the pulp at an early stage. Not only do odontoblast-like cells express CD14 and TLR-4, but also the latter appears to mediate the upregulation of vascular endothelial growth factor by these cells when they are stimulated by LPS.[194]

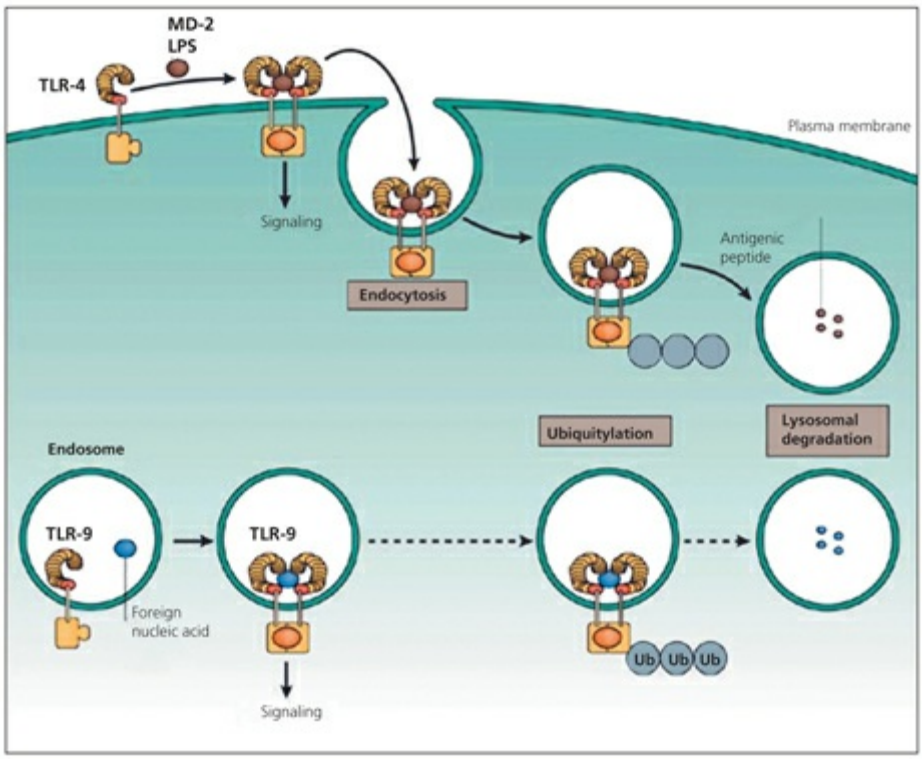

Fig 11-21 TLRs can be divided into two groups according to their cellular localization. They are either surface exposed or localized in vesicles from the endoplasmic reticulum and the Golgi apparatus. TLR-4 is found on the cell membrane and reacts to extracellular stimulation with LPS, whereas TLR-9 occurs in endosomal vesicles and is stimulated by foreign nucleic acids. TLR-4 signaling is terminated by endocytosis, ubiquitylation (posttranslational modification of a protein by the covalent attachment of one or more ubiquitin [Ub] monomers) and lysosomal degradation, a mechanism that is likely to be shared by all TLRs. *Dashed arrows* indicate pathways that have not been completely defined. MD-2, myeloid

differentiation protein 2. (Modified from Gay et al[184] with permission.)

TLR-2 was found to be strongly expressed, and TLR-4 was faintly expressed, in human dental pulp fibroblasts.[195] This suggests that fibroblasts also contribute to innate immunologic surveillance of the dental pulp, apart from their anabolic and catabolic roles in maintaining the structural integrity of the extracellular matrix.

Nucleotide-binding oligomerization domains

In addition to the TLRs that occupy a cell-surface location, nucleotide-binding oligomerization domains (NOD1 and NOD2) are intracellular receptors for two active entities of peptidoglycans containing diaminopimeric acid and N-acetylmuramyl-L-alanyl-D-isoglutamine muramyldipeptide (MDP), respectively; they activate NFκB.[195] These entities are found in cell walls of both gram-positive and gram-negative bacteria. Human pulp fibroblasts were found to express both NOD1 and NOD2 and, upon stimulation, produced various proinflammatory cytokines. Furthermore, NOD2 was found to act synergistically with TLR-2 but not with TLR-4.195 More recently, NOD2 gene expression was found to be significantly upregulated in patients with symptomatic irreversible pulpitis compared with normal patients.[196] In the same study, it was reported that lipoteichoic acid (LTA) from gram-positive bacteria augmented NOD2 gene-expression and protein levels in odontoblast-like cells in vitro. In blocking experiments, both NOD2 and TLR-2 acted synergistically in response to stimulation with LTA.[196]

Other proinflammatory cytokines

T_H1 cytokines, including IFN-γ, IL-2, and TNF-β (also known as *lymphotoxin* [LT-α]),[197] are also considered to be proinflammatory cytokines. These cytokines are mostly involved in cell-mediated hypersensitivity, self-activation of T cells (IL-2), and activation of B cells and macrophages (IFN-γ). IFN-γ is the key cytokine produced by T_H1 cells. It augments TNF activity and induces NO release.[187] It is noteworthy that IFN-γ is produced by natural killer cells in addition to being a T_H1 cytokine. This finding may explain the production of proinflammatory cytokines in T-cell– deficient mouse models and illustrate the multitude of cellular sources for key inflammatory cytokines.

IL-2 was detected in normal vital pulp and in one earlier study was significantly elevated in histologically verified cases of symptomatic irreversible pulpitis.[198]

More recently, IL-2 was shown to be present at comparable levels in normal, inflamed, and necrotic pulps.[199] Shallow caries lesions mostly colonized by *S mutans* were shown to elicit a strong type 1 cytokine response.[182] The expression of IFN-γ mRNA in human pulp samples was found to be significantly greater than that of IL-10 and IL-4 and to be significantly greater in superficial than in deep caries.[182,200]

Immunoregulatory or anti-inflammatory cytokines

T_H2 cells produce IL-4, -5, -6, -9, -10 (also secreted by T_H1 and macrophages), and -13. Both T_H1 and T_H2 produce IL-3 and granulocyte-macrophage colony-stimulating factor. T_H2 cytokines are mostly involved in humoral immunity (production of neutralizing antibodies), mast cell degranulation (IgE production), and eosinophil activation. They also serve the very important function of inhibiting most of the proinflammatory functions caused by other cytokines, thus maintaining the homeostasis of the immune response.[197] This last function is primarily regulated by IL-10, -4, and -13.

Peripheral blood mononuclear cells challenged with *S mutans* produced a dose-dependent increase in IL-10 production but no IL-4 production.[182] The levels of mRNA expression of IL-10 and IL-4 were significantly lower than those of IFN-γ in shallow caries lesions, but this difference disappeared in deep caries lesions.[182] There is probably an increased need for these two immunoregulatory cytokines in deep caries lesions because more proinflammatory cytokines are produced.

IL-6 was initially thought to be proinflammatory but is now recognized to be an immunoregulatory and an anti-inflammatory cytokine. The anti-inflammatory functions of IL-6 are caused by suppression of IL-1 and TNF, the induction of glucocorticoid release, and the induction of natural antagonists of IL-1 (IL-1 receptor antagonist) and TNF (soluble TNF receptor p55).[201] Peptidoglycan from the cell wall of *L casei* was shown to increase the IL-6 production by human pulp cells in a dose-dependent manner.[202] Likewise, LPS from *P endodontalis* produced IL-6 in human pulp cells. The latter preceded and was independent of IL-1β production.[203]

The mean protein level of IL-6 in patients with carious, symptomatic teeth was found to be 36.0 ± 3.9 pg/mg. This was less than that in periradicular lesions (mean: 78.1 ± 9 pg/mg) but much greater than that in normal pulp (mean: 0.01 ± 0.02 pg/mg).[204] These findings are corroborated by the more recent findings that IL-6 is

significantly increased in teeth with symptomatic pulpitis.[181]

IL-6 causes the formation of acute phase proteins in the liver such as fibrinogen and C-reactive protein. C-reactive protein functions as an opsonin by binding to C1q or Fcγ receptors or by activating the complement classic pathway. The dental pulp with irreversible pulpitis has significantly elevated C-reactive protein compared to uninflamed pulp, a finding that did not correlate with the patients' serum levels of C-reactive protein.[205]

Another cytokine that is considered an immunosuppressant as well as an inducer of extracellular matrix production is TGF-β1. This cytokine is a member of the TGF-β superfamily, which includes TGF-βs, activins, and bone morphogenetic proteins. However, TGF-β1 has been shown to play a fundamental role not only in the formation of enamel and dentin matrix but also in the prevention of spontaneous pulpal inflammation.[206] In that study, mice deficient in TGF-β1 (but treated with corticosteroids to keep them alive) developed very thin enamel and dentin with significant attrition and spontaneous pulpitis and pulpal necrosis. Crossing this species with the RAG-2 mouse that is deficient in T- and B-cell immunity resulted in a breed that showed the same hard tissue defects but had normal pulp structure. The results indicate that TGF-β1 stops spontaneous inflammation mediated by the adaptive immune response.

TGF-β1 is significantly increased in inflamed pulp, but only in the odontoblastic-subodontoblastic layer rather than the stromal pulp tissue.[207] Interestingly, in vitro application of TGF-β1 to pulp tissue or odontoblasts upregulated proinflammatory and some anti-inflammatory cytokines, mostly IL-6, -7, and -8.[208] The reader is referred to three recent reviews for a more detailed description of the adaptive cytokine response in the dental pulp.[171,172,209]

Chemokines

Chemotactic cytokines, or *chemokines*, are potent proinflammatory cytokines that are structurally homologous and act by mediating leukocyte movement and chemotaxis of inflammatory cells to the inflammatory site.[210] They are also involved in angiogenesis. They may be classified based on functional criteria: Inflammatory chemokines are expressed by circulating leukocytes and other cells only on activation, whereas homeostatic chemokines are constitutively expressed to promote constant monitoring of at-risk areas.

A large number of inflammatory chemokines have been described, including IL-8 (CXCL8) for neutrophils; RANTES (CCL5) for monocytes and T cells; MCP-1

(CCL2), MCP-2 (CCL8), MCP-3 (CCL7), and MCP-4 (CCL13) for monocytes, basophils, and T cells; and eotaxin (CCL11) for eosinophils.[1,211] Seventeen chemokine genes and 9 chemokine receptor genes were expressed in healthy human dental pulps.[2] Dental pulp cells exposed to endodontically relevant species of gram-positive or gram-negative bacteria expressed IL-8 and MCP-1 at both the protein and the mRNA levels.[212] Human odontoblasts cultured in vitro are capable of expressing IL-8 at both the protein and mRNA levels.[213] Patients with symptomatic or asymptomatic irreversible pulpitis had an almost 23-fold increase in IL-8 levels in the pulp,[214] a finding that was recently supported by another study of symptomatic pulpitis.[181] In addition, as noted previously, SP was found to be a potent inducer of IL-8, and to a lesser degree of MCP-1, in pulp cells and pulp tissue explants.[49]

Differentiated odontoblasts that were exposed to LTA in vitro expressed their own receptor, TLR-2, as well as the chemokines CCL2 and CXCL10.192 CXCL10 was also found to be upregulated in inflamed human pulp, primarily in macrophages, endothelial cells, and fibroblasts, whereas its receptor, CXCR3, was found on T cells.[215]

Chemokines not only induce cell locomotion but also influence angiogenesis. Among the chemokines expressed by odontoblasts in vitro, CCL2, CXCL2, and CXCL12 are proangiogenic, whereas CXCL4, CXCL10, and CXCL14 are angiostatic. Production of angiostatic chemokines in the healthy dental pulp in vivo might be involved in the maintenance of blood vessels beneath the odontoblastic layer. During inflammation caused by caries, the number of capillaries increases in the pulp under the lesion, and some of them penetrate the odontoblastic layer. The proangiogenic chemokine CXCL2 was strongly upregulated in LTA-stimulated odontoblasts and might contribute to the increased vascularization in inflammatory conditions by binding to CXCR2 receptors that are highly expressed on endothelial cells.[2]

Other molecules of innate and adaptive immunity

Class I and Class II major histocompatibility complex molecules

The innate and adaptive immune responses work in synchrony to recognize and

destroy foreign antigenic material. One of the fundamental mechanisms underlying this process is mediated by the Class I and Class II MHC molecules. Class I MHC is expressed on all nucleated cells, whereas Class II MHC is expressed on a small group of cells collectively called *antigen-presenting cells* (APCs). APCs include macrophages, dendritic cells, Langerhans cells, B cells, endothelial cells, and a few other cell types, although only MHC-1–positive dendritic cells and macrophages have been demonstrated in the dental pulp.[216]

Class I MHC is recognized by $CD8^+$ or cytolytic T cells; the cells are referred to a s *Class I–restricted*. This process is best known for viral infection of any nucleated cell, in which the viral antigens are recognized by the Class I–restricted $CD8^+$ cells. However, APCs process many other antigens, including bacterial antigens, and present them to MHC Class II–restricted $CD4^+$ helper T cells. The processed antigens are recognized in the context of the Class II MHC molecule on the APC and the T-cell receptor on the T cell. This mechanism is fundamental for the development of specific immunity to the presented antigen and the activation of T cells, macrophages, and B cells that follow. The expression of MHC molecules is mediated by cytokines, most notably IFN-γ.

Class II APCs were observed in normal human dental pulp, where they were localized to the odontoblastic layer and the central pulp.[217] It was further shown that dendritic cells were much more important than macrophages in providing the antigen presentation signals to costimulate T cells in the rat pulp.[218] In the rat molar pulp, a dramatic increase in the number of Class II APCs was seen following cavity preparation alone or with the application of *P gingivalis* LPS.[219,220] There was also a significant reduction in the number of these cells to baseline levels when the cavity preparations were immediately restored with a self-curing dental adhesive resin.[219] A possible functional relationship was demonstrated between pulp APCs and sensory nerve fibers and their products. Sensory nerves were in close histologic proximity to the APCs, and in vitro experiments revealed that SP potentiated and CGRP suppressed the proliferation of stimulated T lymphocytes in the presence of APCs.[27]

An analysis of human teeth with superficial and deep cavity preparations, caries, and its treatment revealed that Class II antigen-presenting dendritic cells started accumulating in the odontoblastic area with deep cavities. While not detectable when caries was restored, they reappeared 6 months after treatment.[221] This suggests that levels of these cells are closely related to the degree of external insult due to caries, cavity preparation, or microleakage after function. Taken together, these

studies suggest that these immunocompetent cells play an important role in pulpal inflammation and interact with neuropeptides.

Immunoglobulins

In addition to the specific cellular immunity factors already discussed, the dental pulp has long been shown to express humoral immunity under inflammatory conditions, whereby B cells differentiate into plasma cells that undergo clonal selection and produce the immunoglobulins G (IgG), A (IgA), E (IgE), and M (IgM).[117,118] Immunoglobulins serve in both the recognition and effector functions. When antibodies are first expressed in a membrane-bound form on B cells, they recognize antigens in their environment. After their release, these antibodies mediate cytotoxic reactions by innate immune cells such as natural killer cells, opsonize bacterial antigens and other antigens to enhance phagocytosis, and form antigen-antibody complexes that activate the classic pathway of the complement cascade. This leads to the secretion of anaphylatoxins and lysis of membranes of target cells, as described previously. In the case of IgE, antibodies cause the degranulation of mast cells.

The processes of opsonization and complement fixation take place via the Fc portion (non–antigenbinding portion) of the antibody molecule, which is unique for each antibody isotype. Antibodies bind to Fc receptors on the surface of phagocytic cells for opsonization or to the C1q complement protein for complement fixation.

IgG1 was found to be the predominant immunoglobulin in normal dentin close to the pulp as well as in uninfected dentin under caries. When the pulp was sampled directly during pulpectomy procedures, significantly higher levels of IgG, IgA, and IgM were reported in inflamed pulps than in normal pulps,[222] indicating that the experimental technique may have influenced the findings in these studies.

An additional protective function of immunoglobulins in the pulpodentin complex was proposed with the finding that IgG, IgA, or IgM reduced fluid filtration through dentin in vitro.[223]

Defensins

Human α-defensins and β-defensins belong to a large number of molecules that include LL-37, histatin, and other peptides with potent antimicrobial activities. These peptides have been shown to suppress gram-positive and gram-negative bacteria, fungi, and viruses. Human β-defensins were first described in relation to

epithelial cells, including oral mucosal and gingival keratinocytes. Some inflammatory cells, such as neutrophils, produce human α-defensins.

Recently, a study reported that odontoblasts express human β-defensin 1 and 2, thus confirming the role that these cells play in the innate immune response of the dental pulp.[224] Furthermore, it was shown that human β-defensin 2 causes downregulation of human β-defensin 1 and upregulation of IL-6 and IL-8.[225] These findings illustrate the diversity of immunologic reactions within the pulp and the powerful interplay of molecular mediators to mount an effective host response.

Conclusion

The dental pulp develops a diverse number of substances that support both inflammatory and healing responses found in reversible and irreversible diseases. A great number of functions are ascribed to these substances, many of which may act both in propagation of disease and healing of diseased tissue. Although application of these principles may be complicated, a better understanding of the functions of these substances may eventually lead to the development of better diagnostic tools of pulp conditions and to pharmacologic therapies that may reverse disease processes and return the dental pulp to its original form and function.

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Interrelationship of the Pulp and Apical Periodontitis

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Infections of the dental pulp occur as a consequence of caries, operative dental procedures, and trauma and involve a mixed, predominantly gram-negative, anaerobic bacterial flora.[1] These infections initially elicit an immune response in the dental pulp, which is ineffective in eliminating the invading microorganisms (see chapters 10 and 11). Consequently, these infections typically progress to cause total pulpal necrosis and subsequently stimulate a secondary immune response in the periapical region. The latter is commonly referred to as a *periapical lesion* but in fact represents a protective response to the bacteria in the necrotic pulp and root canal system.

Both pulpal and periapical immune responses initially involve innate immunity, particularly the influx of phagocytic leukocytes and the production of proinflammatory cytokines. As infections become more chronic, adaptive immune elements are also activated and become superimposed on the innate response, including T and B cells and their specialized subsets, leading to a typical "mixed"

inflammatory cell response. In this milieu, a complex array of immunologic mechanisms is activated, some of which act to protect the pulp and periapical region, while others mediate tissue destruction, particularly periapical bone resorption. The latter include the expression of numerous host-derived factors, including cytokines, arachidonic acid metabolites, and neuropeptides that contribute to or modulate apical periodontitis.

Although many gaps in knowledge still remain, understanding of these processes is rapidly increasing through the application of new biologic tools such as genetically engineered mice, recombinant proteins, neutralizing antibodies, and new model systems. This chapter reviews the immune mechanisms that protect the host against pulpal infections as well as those that contribute to periapical tissue destruction. The ultimate goals are to better understand the immunobiology of apical periodontitis that occurs in response to pulpal infection and ultimately to use this knowledge in the rational design of clinical procedures.

Pulpal and Periapical Immune Responses

Immune cells in pulpitis

The dental pulp, similar to most connective tissues, has certain immunocompetencies that facilitate the host response to noxious stimuli, including bacteria (see also chapter 11). For example, antigen-presenting dendritic cells are present in the odontoblastic layer, and macrophage-like cells are found centrally in the pulp.[2–4] A small number of T cells are present in the normal pulp, primarily in blood vessels; these likely represent recirculating T cells that serve an immunosurveillance function. In contrast, B cells are extremely rare or undetectable,[2,5] and their plasma cell progeny are absent.[6]

The earliest pulpal response to frank bacterial infection or to the diffusion of bacterial antigens through dentinal tubules includes the infiltration of polymorphonuclear neutrophils (PMNs) and monocytes.[7–9] The cellular infiltrate intensifies as the infection progresses, leading to induction of elements of the adaptive response, including helper T (T_H) cells, cytotoxic T (T_C) cells, regulatory

T (T_{reg}) cells (formerly known as *suppressor T cells*), B cells, and, in later stages, antibody-producing plasma cells. Innate cells, including PMNs, monocytes, and natural killer (NK) cells, continue to be present.[5,6] As described later, the levels of locally produced immunoglobulins (IgG and IgA)[5] are elevated,[10] and antibodies that react with microorganisms isolated from deep caries are present.[11]

These mechanisms are usually incapable of clearing the infection. Tissue destruction subsequently proceeds, beginning with the formation of small abscesses and necrotic foci in the pulp and eventually resulting in total pulpal necrosis.[5]

Immune cells in periapical lesions

Periapical immune responses (also known as *apical periodontitis* o r *periapical lesions*) may be viewed as a second line of defense, the purpose of which is to localize the infection within the confines of the root canal system and prevent its egress and systemization[12–15] (Fig 12-1). Periapical immune responses to bacterial infection essentially recapitulate the pulpal response already described, with the additional feature that periapical bone is destroyed and, in severe cases, some root resorption may occur (Fig 12-2).

In experimental rodent models in which the timing of pulpal infection is controlled, the earliest periapical response involves an influx of PMNs and monocytes 1 to 3 days after pulpal exposure[4,16,17] (Fig 12-3). Periapical inflammatory cell infiltration, increased numbers of osteoclasts, and bone destruction are apparent well in advance of total pulpal necrosis (Fig 12-4), at a time when vital pulp is still present in the apical root canal.[18,19] In a mouse model, increased osteoclastogenesis was observed in the periapical region by day 3 after pulpal exposure; however, most inflammatory cell accumulation occurred in the root canal system.[20]

These data explain the clinical observation that vital tissue and pain are often present even in teeth with periapical radiolucencies. A further implication is that pulpal infections cause periapical tissue destruction indirectly and from a distance via stimulation of soluble host-derived mediators rather than by the direct effects of bacteria on bone. Another implication is that rapid osteoclastogenesis in these models seems to be dependent on innate immune and early inflammatory responses in the dental pulp and/or periapical region; adaptive responses have not yet been

fully established.[21]

The inflammatory cell infiltrate in chronic periapical lesions in both humans and animals has been extensively studied and characterized. As with pulpal responses, a mixed infiltrate of T and B lymphocytes, PMNs, macrophages, dendritic cells (DCs), plasma cells, NK cells, eosinophils, and mast cells is present.[17,22–31] There remains some disagreement about the predominant infiltrating cell type in periapical lesions: Lymphocytes[23,24,32–34] or macrophages[17,22,28] are variously reported to be the more numerous. Large numbers of PMNs are also present,[33,34] and T cells have consistently been reported to outnumber B cells.[26,33,34]

Among the T lymphocytes, both $CD4^+$ T_H and $CD8^+$ T_C cells have been identified.[24,27–29,33,35–38] In several studies, a lower T_H-to-T_{reg} ratio than that seen in peripheral blood (< 2.0) has been reported in chronic lesions.[26,29,33] Kinetic studies suggest that, after the first days of infection, few temporal differences in the cell infiltrate remain after pulpal exposure. One notable exception is an increase in T_C cells as the lesion becomes more chronic[17,29] (Fig 12-5). This may reflect the evolution of a specific T-cell response; T_H cells are initially activated to proliferate by exposure to antigen-presenting cells (APCs) and subsequently induce T_C cells that may serve other protective functions.[39]

In human periapical lesions, immature and mature myeloid-type DCs, plasmacytoid DCs, and monocyte-derived DCs have all been identified.[31] As antigen-presenting cells, $HLA\text{-}DR^+$ DCs seem to play a much more important role in local and lymph node T-cell activation than do macrophages.[40]

T_H17 cells are a recently identified T_H-cell subset and are distinct from classic T_H1 and T_H2 cells. T_H17 cells are characterized by their expression of interleukins: IL-17 (also termed *IL-17A*), a second isoform known as IL-17F, IL-21, and IL-22.41 T_H17 as a proinflammatory cell type was first described by its role in the induction of experimental allergic encephalitis, a model for multiple sclerosis. Experimental allergic encephalitis was found to require T-cell–derived IL-17, but not classic T_H1 cytokines, for its induction.[42] In addition, T_H17 cells are involved in the exacerbation of rheumatoid arthritis.[43] In contrast, T_H17 cells may be protective against bacteria. Transforming growth factor β (TGF-β) and IL-6 regulate the differentiation of T_H17 cells.[44] However, the T_H17 phenotype appears to be somewhat plastic because these cells can be converted into $T_H17/1$, which

expresses both IL-17 and interferon γ (IFN-γ), and into T_H1 by IL-12 and IFN-γ.[45,46]

The presence of T_H17 cells has been reported in human periapical lesions.[31] In this report, T_H17 cells were found to coexpress IFN-γ; therefore, these cells appear to be $T_H17/1$ cells induced by IL-12 and IFN-γ.[45] The function of T_H17 cells in the context of periapical immunopathology is discussed below.

Other T-cell subsets are also present. T_{reg} cells are a $CD4^+$ subset that also express the transcription factor forkhead box P3 (FOXP3), may express CD25 (IL-2 receptor), and secrete large amounts of the immunosuppressive cytokines TGF-β and/or IL-10.[47,48] T_{reg} cells are clearly critical in controlling overexuberant immune and inflammatory responses.[49] $FOXP3_+$ T_{reg} cells have been identified in periapical lesions and draining lymph nodes in a mouse model.[50] The number of T_{reg} cells increased with the progression of periapical inflammation (Fig 12-6). In contrast, T_{reg} cells were not found in periodontal ligament tissue associated with uninfected teeth.

Other recent studies of human clinical samples suggest that T_{reg} cells are present in higher numbers in periapical granulomas from asymptomatic teeth than they are in symptomatic lesions or radicular cysts.[51] This suggests that T_{reg} cells may act to counterregulate excessive proinflammatory immune responses in periapical lesions.

T_{reg} cells are divided into two groups by the mechanism of their development.[52] Natural T_{reg} (nT_{reg}) cells are developed as a "natural" cell population during the normal process of T-cell maturation in the thymus. $FOXP3^+CD25^+$, $CD8^+CD122^+$, and natural killer T (NKT) cells are members of the nT_{reg} family. These cells are capable of regulating the activity of APCs by cytokines they secrete. $FOXP3^+CD25^+$ and $CD8^+CD122^+$ produce IL-10,[53,54] which downregulates the function of APCs and subsequent inflammatory responses. In contrast, NKT cells produce proinflammatory IFN-γ.[55] NKT cells also produce IL-4; however, NKT-derived IL-4 has only a minor effect on T_H2 cell differentiation.[56] nT_{reg} cells bridge innate immunity (APCs) and acquired immunity via effects on T-cell differentiation and activation (Fig 12-7).

On the other hand, inducible (or adaptive) T_{reg} (iT_{reg}) cells are distinguished from nT_{reg} cells in the process of acquired immunity.[52] Their development and proliferation are the result of antigen-specific responses. $FOXP3^+$ T_H3 cells mainly

produce TGF-β, which regulates proliferation and survival of T cells.[57,58] Furthermore, in orchestration with other cytokines, TGF-β induces the differentiation of T_H17 (with IL-6) and iT_{reg} cells (with IL-2).[59] Type 1 regulatory (Tr1) cells secret immunosuppressive IL-1060 and appear to prevent extreme proinflammatory responses. Although Qa-1 is a member of the class lb major histocompatibility complex family, Qa-1a– restricted $CD8^+$ cells appear to regulate delayed-type hypersensitivity via production of TGF-β and IFN-γ.[61,62]

Other T_H-cell subsets include follicular helper T (T_{FH}; $CXCR5^+CD4^+$) cells, which are found in lymphoid tissues and play an important role in the development of T-cell–dependent antibody responses. T_{FH} cells potently stimulate the differentiation of B cells into antibody-producing plasma cells via IL-21 signaling.[63] As discussed in the next section, humoral immunity is important in preventing dissemination of endodontic pathogens in vivo.[14] Although the role of many of these novel T-cell subsets has not yet been confirmed in periapical immune pathogenesis, it may be speculated that these cells are likely to be involved in the regulation of the inflammatory status of periapical lesions and bone loss.

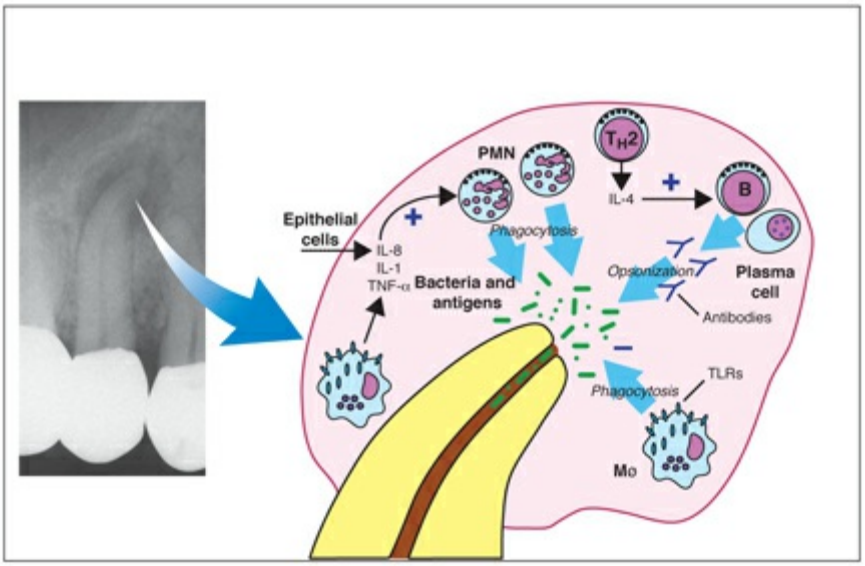

Fig 12-1 Major immunologic mechanisms that kill and eliminate microorganisms or antigens derived from the root canal system. Specific experiments and references supporting this network are described in the text. IL, interleukin; MØ, macrophage; PMN, polymorphonuclear neutrophil; TNF, tumor necrosis factor; TLR, toll-like receptor; T_H2, helper T cell; B, B cell.

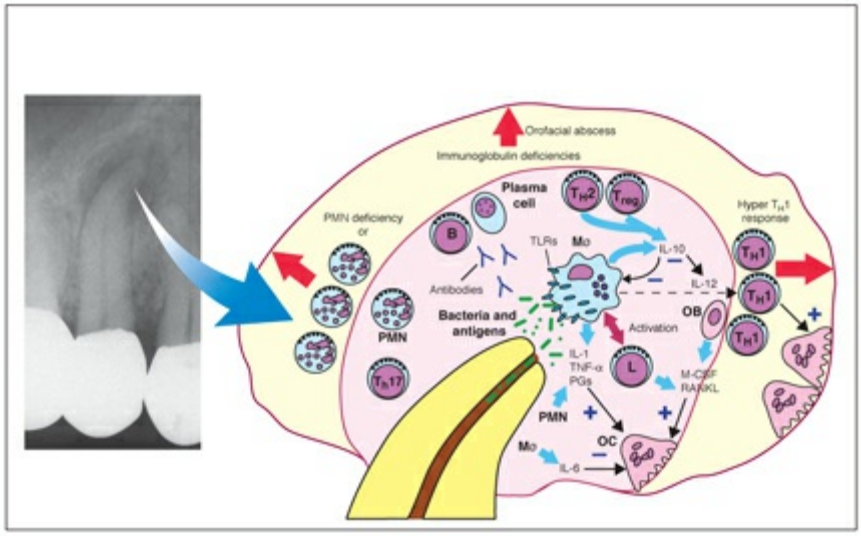

Fig 12-2 Major immunologic mechanisms that mediate the development of apical periodontitis elicited by microorganisms or antigens from the root canal system. Specific experiments and references supporting this network are described in the text. IL, interleukin; PGs, prostaglandins; M-CSF, macrophage colony-stimulating factor; MØ, macrophage; PMN, polymorphonuclear neutrophil; TLR, toll-like receptor; RANKL, receptor activator of nuclear factor κB ligand; T_H, helper T cell; TNF, tumor necrosis factor; T_{reg}, regulatory T cell; B, B cell; OB, odontoblast; OC, osteoclast; L, lymphocyte.

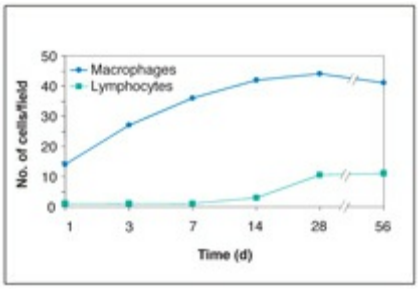

Fig 12-3 Effect of pulpal inflammation and necrosis on accumulation of macrophages and other phagocytic cells (labeled with ED1 antibody) and T lymphocytes (labeled with OX19 antiserum) into periapical tissue of rats. The dental pulp underwent total necrosis by 14 to 28 days after pulpal exposure. (Redrawn from Kawashima et al[17] with permission.)

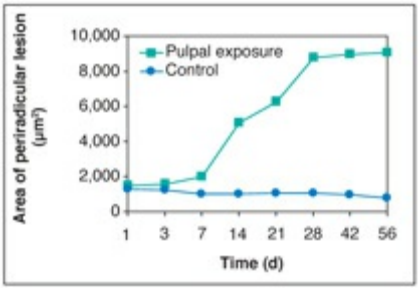

Fig 12-4 Effect of pulpal inflammation and necrosis on the area of periradicular lesion in rats after pulpal exposure or in no-treatment controls. The dental pulp underwent total necrosis by 28 days after pulpal exposure. (Redrawn from Yamasaki et al[18] with permission.)

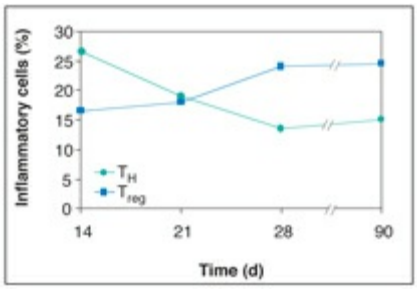

Fig 12-5 Effect of pulpal inflammation and necrosis on subpopulations of T lymphocytes in rat periradicular lesions after pulpal exposure. Tissue levels of T_H and T_{reg} lymphocytes were determined by immunohistochemical markers. (Redrawn from Stashenko and Yu[29] with permission.)

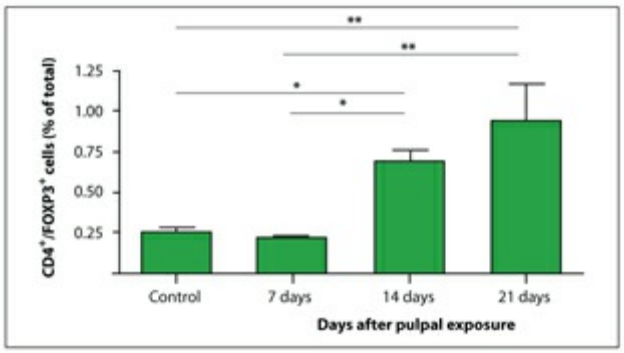

Fig 12-6 Kinetic quantitation of T_{reg} cells in periapical lesions after pulpal infection in mice. Cells were extracted from periapical lesions at the indicated times, and T_{reg} cells ($CD4^+/FOXP3^+$ cells) were quantified by flow cytometry. Controls were unexposed and uninfected; other groups were exposed and infected. Bars represent the mean ± standard error of the mean. $*P < .05$; $**P < .001$. (Redrawn from Alshwaimi et al[50] with permission.)

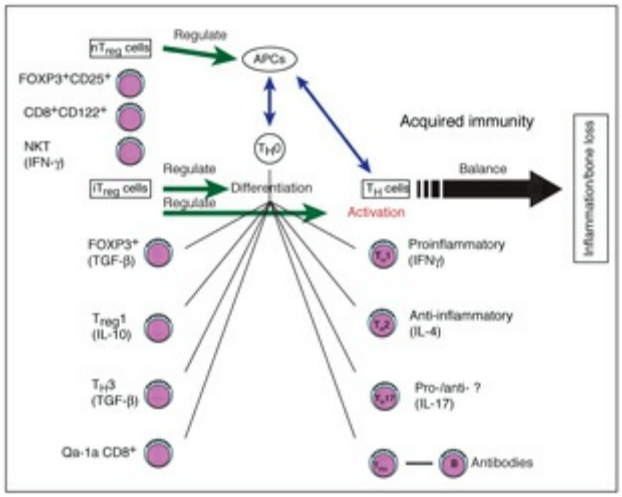

Fig 12-7 Possible regulating mechanism of periapical inflammation and bone loss by acquired immunity. Acquired immunity has an important role in the establishment of periapical inflammation and bone loss. Periapical inflammation and bone loss are regulated by a network of APCs, T_{reg} cells and T_H cells. T-cell–derived cytokines play important roles in determining the directionality of T-cell differentiation and proliferation and the balance of proinflammatory and anti-inflammatory responses, but the role of these

T-cell subsets is not yet fully understood. NKT, natural killer T cells; T_{FH}, follicular helper T cells.

Humoral immunity in pulpal and periapical inflammation

Bacterial infection from dental caries stimulates the local production of antibody responses in the dental pulp.[6] In a recent study, immunoglobulin levels in the pulpodentin complex were quantitatively determined.[64] In the absence of caries, only IgG1 and IgA1 were detectable in the dental pulp. However, IgA2 and IgM, in addition to IgG1 and IgA1, were detected when dental caries resulted in microbial penetration into dentin. The level of immunoglobulins was significantly higher than it was in uninfected teeth. IgG1 was the predominant immunoglobulin, followed, in decreasing amounts, by IgA1, IgM, and IgA2. The effect of caries severity on the levels of immunoglobulins (except for IgA2) was unclear. These immunoglobulins were found to be reactive with microorganisms isolated from caries lesions, including *Streptococcus mutans* and *Prevotella intermedia*. [11]

Chronic periapical lesions contain many immunoglobulin-producing plasma cells. Of these, IgG-positive cells are most prominent (70%), followed by IgA (14%), IgE (10%), and IgM (4%).[22,38,65–67] Of the IgG subclasses, IgG1 is produced in largest quantity, followed by IgG2, and then IgG3 and IgG4, the last two in about equal amounts.[68] Some of the antibody produced in lesions is also reactive with infecting microorganisms.[69,70] Explant cultures of periapical tissues produce antibody against common endodontic pathogens, including *P intermedia*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Peptostreptococcus micros*, *Actinomyces israelii*, *Staphylococcus intermedius*, *Fusobacterium nucleatum*, and other species. It has long been known that antigens within the root canal are also capable of stimulating a systemic antibody response. Introduction of bovine serum albumin and sheep erythrocytes into the root canals of monkeys induced systemic antibody formation against both antigens.[71] Systemic antibody responses have also been shown against lipopolysaccharide (LPS) and other bacterial antigens.[72,73] These observations suggest that both locally and systemically produced antibacterial antibodies could protect the host against bacterial egress from the root canal into the tissues and circulation, likely via opsonization.

It has also been suggested that antibody-mediated mechanisms could actually contribute to periapical bone destruction.[74] However, there is at present no

compelling evidence that these mechanisms actually stimulate bone resorption in vivo. These mechanisms include the binding of antibodies to bacterial antigens to form antigen-antibody complexes, which have been found capable of stimulating periapical bone destruction in experimental systems.[74]

Complement components are present in lesions.[65,66] Complement fixation and the generation of cleavage products (C3a, C5a) may stimulate PMN chemotaxis. Potentially destructive by-products of PMNs include elastase, cathepsin G, and leukotriene B_4, all of which are elevated in inflamed pulps.[75,76] However, periapical lesions in complement C5– deficient animals are similar in size to those found in wild-type controls, suggesting that complement probably does not play a major role in pathogenesis. The presence of mast cells in combination with IgE suggests that anaphylactic hypersensitivity reactions may also occur periapically, although their significance is unclear.[30,65,77,78]

Arguing against a role for antibody-mediated phenomena in the pathogenesis of apical periodontitis is the lack of correlation between the number of antibody-producing cells and the expansion of the periapical lesion.[16,34] More importantly, antibody protects against disseminating dentoalveolar infections in immunocompromised animals and reduces bone resorption in animals that develop abscesses.[13–15]

Resident cells in pulpal and periapical inflammation

In addition to immune cells, resident cells in the dental pulp and periapical tissue may play important roles in regulation of immune and inflammatory responses. Odontoblasts are the primary cells that encounter exogenous stimuli, including bacteria, in the pulpodentin complex. In addition to dentin formation, odontoblasts potentially regulate inflammatory responses because these cells constitutively express toll-like receptors (TLRs) that interact with bacterial components to initiate inflammatory responses.[79] In this study, exposure of odontoblasts to bacterial lipoteichoic acid activated an inflammatory pathway that is dependent on upregulation of TLR-2 and resulted in expression of chemokines CCL2 and CXCL10. Concomitantly, lipoteichoic acid inactivated pathways associated with dentin formation, including the expression of type I collagen, dentin sialophosphoprotein, and TGF-β. In another study, the expression of TGF-β and

SMADs (TGF-β signal transduction molecules) was also significantly downregulated in mouse incisor dental pulp stimulated with LPS.[80] Odontoblast-specific TGF-β receptor 2–deficient mice exhibited extremely rapid and severe inflammatory cell infiltration and tissue destruction in responses to LPS stimulation, whereas wild-type controls exhibited only mild inflammatory responses.[81] This finding demonstrates an important anti-inflammatory action of TGF-β. Taken together, these findings suggest that TLR-induced inflammatory responses in odontoblasts may play a role in immunopathology in the pulp.

Other resident cells, including fibroblasts, osteoblasts, and cementoblasts, also express TLRs and may participate in infection-stimulated inflammatory bone loss, particularly via TLR-2.[82–84] Human periodontal ligament fibroblasts exhibited greater expression of TLR-2 and released high levels of IL-8 in response to TLR-2–stimulating peptidoglycan from *Staphylococcus epidermidis*, but not TLR-4–stimulating LPS, than did human gingival fibroblasts.[82] In osteoblasts, in vitro stimulation by LPS or IFN-γ significantly reduced the expression of osteoprotegerin, which is the decoy receptor of bone resorptive receptor activator of nuclear factor κB ligand (RANKL), and increased expression of IL-6 and prostaglandin E_2 (PGE_2), which promote bone resorption.[83] In cementoblasts, expression of RANKL, monocyte chemoattractant peptide 1, IL-6, CCL5, and macrophage inflammatory protein 1α was significantly upregulated in response to TLR-2–stimulating *P gingivalis* LPS and TLR-4–stimulating *Escherichia coli* LPS.[84] These findings indicate that resident cells in dental pulp and periapical tissue may play important regulatory roles in immunity and inflammation in these tissues.

Protective Immunity to Pulpal Infections

From the foregoing discussion, it is apparent that the mixed inflammatory cell infiltrate in both the dental pulp and in periapical lesions is potentially capable of mediating the entire spectrum of immunologic responses. These include antibody-mediated phenomena (antigen-antibody complex formation, complement-dependent cell lysis and chemotaxis, and immediate-type hypersensitivity), delayed-type hypersensitivity, cytotoxicity, and cytokine and prostaglandin production (Box 12-1). However, the critical question is not which antibacterial responses are present but rather which antibacterial responses actually function to protect the host as well as

the pulpal and periapical tissues. To answer this question, animals with various immunodeficiencies have been studied to identify which immune functions are critical in reducing or preventing infection and periapical bone resorption.

Box 12-1	Functions mediated by cells that infiltrate periapical lesions

- Neutrophils: Phagocytosis (bacterial killing); cytokine production (IL-1, TNF-α)
- Monocytes: Phagocytosis (bacterial killing); immune induction; cytokine production (IL-1, TNF-α, IL-6, IL-10, IL-12); PGE production
- Dendritic cells: Antigen presentation; T-cell activation; cytokine production (IL-12, IFN-α)
- Eosinophils: Histamine release; immediate-type (anaphylactic) hypersensitivity
- B cells: Differentiation to plasma cells; low-level antibody production
- Plasma cells: Large-scale antibody production
  - IgM: Complement-mediated lysis; chemotaxis stimulated by C3a, C5a
  - IgG: Opsonization; immune complex formation; complement-mediated lysis; chemotaxis
  - IgA: Adhesion inhibition
- T cells
  - CD4+
    - TH1: Delayed-type hypersensitivity; IFN-γ, IL-12, TNF-α
    - TH2: "Help" for antibody production; anti-inflammatory modulation (IL-4, IL-5, IL-6, IL-10, IL-13)
    - TH17: Phagocytosis enhancement; proinflammatory modulation; IL-17, IL-22
    - Treg: Regulation of T-cell proliferation; inflammation control; TGF-β, IL-10
  - CD8+
    - Cytotoxic lymphocytes (TC): Cytotoxicity; suppression

Immunodeficiencies fall into two broad categories, depending on whether they primarily affect innate or specific (adaptive) immune responses. In general, humans and animals with defects in phagocytic leukocytes, including neutrophils and monocytes, have increased susceptibility to bacterial infections. Although the effect of these deficiencies on pulpal infections has not been reported in humans, they clearly increase the severity of marginal periodontitis.[85,86] In contrast, patients with defects in specific immunity and/or diminished T- or B-cell numbers or function exhibit marginal periodontitis that is similar to or milder than that seen in normal age-matched individuals.[87–91] An exception may be the marginal periodontitis that occurs in some individuals infected with human immunodeficiency virus (HIV), although the disease appears to be somewhat atypical and may not be identical to other periodontal diseases.[92]

Deficiencies in innate immune responses

Individuals with PMN defects, including chronic granulomatous disease, cyclic neutropenia, Papillon-Lefèvre syndrome, Chédiak-Higashi syndrome, and leukocyte adhesion deficiencies (LADs), have an increased incidence and severity of bacterial infection, including oral (periodontal) infections.[93–95] Best studied are the LADs, of which there are two recognized types.[96–98] LAD-1 is due to a genetic defect in the β chain of integrins, which are important for leukocyte transmigration across the blood vessel wall. LAD-2 is due to a defect in the sialyl Lewis X ligand on leukocytes to which P- and E-selectins on endothelial cells bind, an interaction that mediates the initial "rolling adhesion" of leukocytes to the blood vessel wall (see Fig 11-2). In both conditions, the adhesion deficiency significantly reduces the ability of PMNs to migrate from the vascular system into tissues.

Patients present with severe infections and elevated numbers of circulating PMNs and macrophages (leukocytosis), yet no pus is formed. In addition, they exhibit early-onset or prepubertal marginal periodontitis.[98–102] Based on these considerations, it is not surprising that a recent case report of a patient with an LAD-1 immunodeficiency described numerous examples of infected, necrotic teeth with apical periodontitis.[103] Others have provided case reports of patients with cyclic neutropenia who showed an increased prevalence of apical periodontitis.[104]

Older studies have attempted to determine the role of innate immune cells such as PMNs and monocyte/macrophages in periapical responses to infection. The administration of cyclophosphamide, which causes severe neutropenia, was reported to increase periapical bone destruction.[105] In cyclophosphamide-treated animals, bacteria were observed both in the pulp and in the periapical lesion, suggesting increased bacterial invasion in the absence of PMNs. However, methotrexate treatment, which also causes neutropenia, has been reported to inhibit the development of apical periodontitis.[106] The reason for these differences is unclear; however, although the effects of the immunosuppressive agents are correlated with neutropenia, both cyclophosphamide and methotrexate also profoundly affect the production and responses of lymphocytes, so these effects could not be solely attributed to PMNs.

Knockout mice deficient in both P- and E-selectins ($P/E^{-/-}$) have been developed as a model of human LAD-2. $P/E^{-/-}$ mice have defective rolling adhesion and leukocytosis and increased susceptibility to various infections, but few reported defects in adaptive immunity.[107] Interestingly, $P/E^{-/-}$ mice have been shown to develop much larger periapical lesions than their normal, wild-type counterparts (Fig 12-8), which correlated with both decreased PMN infiltration of periapical

tissues and increased periapical expression of the bone resorptive cytokine IL-1.[108]

Another experimental approach has been to use immunomodulators to increase the number and function of innate immune cells and subsequently to determine the effect on pulpal and periapical resistance to infection. Granulocyte colony-stimulating factor (G-CSF) is a cytokine that stimulates the production of granulocytes by the bone marrow and increases the antimicrobial function of mature neutrophils. The effect of G-CSF was recently examined in a rat pulpitis model following methotrexate-induced neutropenia.[109] Pulpal inflammation, necrosis, and abscesses rapidly progressed in animals with neutropenia, and pulpal inflammation rapidly extended to the periapical area after pulpal exposure. In contrast, G-CSF injection increased PMN counts and reduced pulpal necrosis and limited intrapulpal abscesses to a small area adjacent to the site of pulpal exposure. However, generalized pulpal and periapical inflammation was absent.

Poly-β-1-6-glucotriosyl-β-1-3-glucopyranose (PGG) glucan is a biologic-response modifier derived from yeast that effectively increases host antibacterial responses without inducing inflammation, including a complete lack of proinflammatory cytokine production (IL-1 and tumor necrosis factor α [TNF-α]) by macrophages and other cells.[110–112] Systemic administration of PGG glucan increased PMN production and primed phagocytic and bactericidal activity in vivo[113] and prevented postsurgical infections in humans.[114]

In the pulpal exposure model, PGG glucan reduced periapical bone destruction by 40%[19] (Fig 12-9). Animals in which PGG glucan was administered had increased numbers of circulating PMNs and monocytes, which possessed enhanced phagocytic activity ex vivo. The protective effect on periapical bone was secondary to decreased pulpal necrosis; only 3% of pulps exhibited complete pulpal necrosis in PGG glucan–treated animals compared with 41% of pulps in control animals. These results clearly indicate that PMNs are predominantly protective against pulpal infections and as a consequence reduce periapical bone destruction.

Osteopontin (OPN) is a multifunctional cytokine that regulates inflammation, bone metabolism, tumor progression, and metastasis. $OPN^{-/-}$ mice exhibit larger periapical lesions than do wild-type animals (Fig 12-10); this difference is accompanied by a larger area of PMN infiltration and upregulation of neutrophil elastase gene compared to that found in wild-type controls.[20] However, OPN also affects the development of T_H1 and possibly other immune responses, so it is possible that the increased PMN infiltration in this study was compensating for the lack of another protective immune function. Further studies are needed to dissect the

mechanism underlying the observed increase in bone loss.

PMNs clearly protect the host and host tissues against pulpal infections.[115,116] At the same time, recent data indicate that LPS-stimulated PMNs have the capacity to express surface RANKL via TLR-4 signaling, which potentially could stimulate osteoclastogenesis and bone resorption.[117] Furthermore, PMNs may play a role in neurogenic inflammation, which has been described in inflamed pulp and periradicular lesions.[118–121] Substance P, which is secreted by sensory nerves, affects vascular permeability and the release of histamine from mast cells.[122] Substance P also enhances immune complex inflammation.[123] This mediator is highly expressed by PMNs in human periapical lesions.[119] Therefore, PMN-derived substance P could mediate neurogenic inflammation via this pathway, leading to a hyperoxidative burst[124] and upregulation of proinflammatory cytokines.[125]

Given that these data represent correlations that have not yet been confirmed by functional studies, it is uncertain at this time whether PMN RANKL expression or neurogenic inflammatory responses play a major role in periapical pathogenesis.

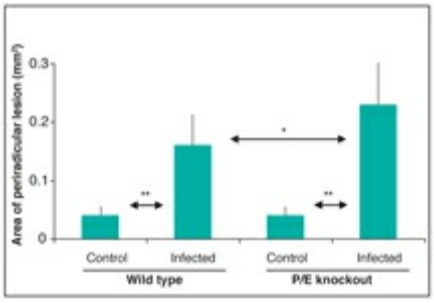

Fig 12-8 Effect of deletion of the P- and E-selectin genes on the size of periradicular lesions after pulpal exposure in mice. The endothelium of $P/E^{-/-}$ mice lacks the constitutive P-selectin and the inducible E-selectin and therefore lacks the critical initial step of rolling adhesion of leukocytes to endothelium in inflamed tissue. $*P < .05$; $**P < .01$. (Redrawn from Kawashima et al[108] with permission.)

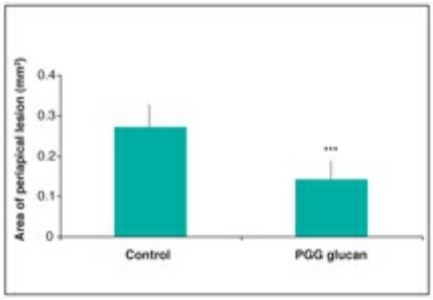

Fig 12-9 Effect of the biologic-response modifier PGG glucan on the size of periapical lesions after pulpal exposure in rats. Animals were administered PGG glucan or a vehicle starting 1 day before pulpal exposure. PGG glucan increases host immunologic responsiveness by increasing neutrophil production and by priming phagocytic and bactericidal activity. $***P < .001$. (Redrawn from Stashenko et al[19] with

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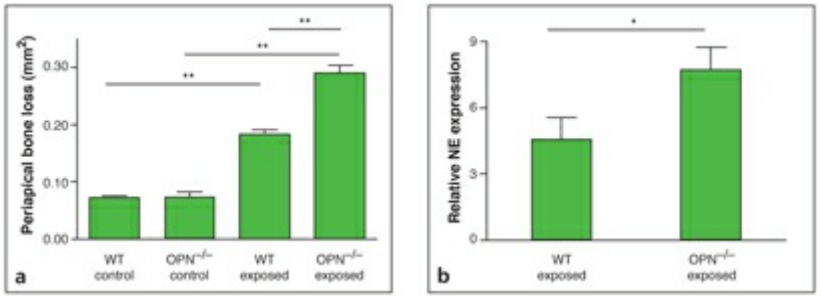

Fig 12-10 Effect of OPN deficiency on infection-induced periapical bone loss and neutrophil elastase gene expression after pulpal exposure. *(a)* Quantification of bone loss in wild-type controls (WT; n = 3) and $OPN^{-/-}$ animals (n = 5). $^{**}P < .01$. *(b)* Neutrophil elastase (NE) was measured by quantitative polymerase chain reaction in RNA from infected mandibles 3 days after infection and normalized to control genes. $^{*}P < .05$; n = 6 or 7. (Redrawn from Rittling et al[20] with permission.)

Deficiencies in specific (adaptive) immune responses

A number of human immunodeficiency diseases affect specific (adaptive) immunity. These include severe combined immunodeficiency (SCID), DiGeorge syndrome (thymic aplasia resulting in a decrease or absence of T cells), hypogammaglobulinemias, and selective IgA and IgG deficiencies.

SCID is characterized by profound defects in both humoral and cellular immunity. Because most patients do not survive into adulthood, reports of oral manifestations are limited. Although no studies are available on the effects of SCID on periapical destruction in humans, some potentially important data have been derived from studies in genetically engineered SCID mice.[13,126,127] In these animals, functional T and B lymphocytes are totally absent, while cells involved in innate immunity, including PMNs and monocytes, are unaffected. Following pulpal exposure and infection with a mixture of endodontic anaerobes, approximately one-third of SCID mice developed large orofacial abscesses, splenomegaly, weight loss, and in some cases septic shock, whereas immunologically normal animals were always able to localize the infection to the root canal system[13] (Fig 12-11).

These phenotypes of SCID mice were recently confirmed in a *Treponema denticola* monoinfection model.[128] Abscessed SCID mice exhibited more local periapical bone destruction than did SCID mice without abscesses or normal controls. Fouad[127] also found that SCID mice displayed bone resorption similar to

that of controls but did not observe abscess formation. These findings are reminiscent of those in immunized monkeys with periapical lesions, which, although not different in size, were more circumscribed than those in nonimmunized animals.[129] Furthermore, nonimmunized animals had an inflammatory infiltrate resembling osteomyelitis that extended to the trabecular system of the bone.[129]

Studies have since shown that B-cell–deficient, but not T-cell–deficient or complement-deficient, mice are susceptible to disseminating infections. This was shown directly in experiments in which antibody against infecting endodontic pathogens was transferred subcutaneously to SCID mice prior to pulpal exposure.[14] The dissemination of the pulpal infection to orofacial tissues (see Fig 12-11) was found to be largely prevented by antibody transfer, leading to a reduction in frequency of orofacial abscess formation from 73% to 25% ($P < .05$). When the antibodies were fractionated into IgG- and IgM-enriched preparations, IgG was found to be more effective in preventing orofacial abscess formation than IgM (Fig 12-12). This finding further indicates that IgG antibody probably exerts protection by opsonizing bacteria, leading to their more efficient phagocytosis and killing by neutrophils and macrophages in the pulp and periapical lesion (see Fig 12-1). The finding that complement-deficient animals were not susceptible to dissemination of infection indicates that bacteriolytic mechanisms are probably not important in protection.

The results of these studies are consistent with studies of the effect of pure T-cell deficiencies on pulpal and periapical destruction. Congenital thymic aplasia (DiGeorge syndrome) results in drastically reduced or absent T cells and decreased antibody levels, secondary to a lack of T_H activity for B cells. Affected individuals mount normal immune responses against common bacterial infections but are extremely susceptible to viral, protozoan, and fungal infection.[130] There are as yet no reports of increased susceptibility to oral infections in patients with DiGeorge syndrome. In animal models that mimic DiGeorge syndrome, athymic *nu/nu* animals have yielded conflicting results; two studies indicated no effect of T-cell deficiency on periapical destruction,[131,132] but another study reported decreased destruction.[133]

In progressive HIV infection, there is a profound depression in $CD4^+$ T cells and a lack of cell-mediated immunity. Several infections affect the periodontium in such individuals, resulting in a wide range of clinical presentations.[134,135] However, several cross-sectional studies have failed to find an increase in the frequency of acute root canal reinfection (flare-ups) during endodontic treatment in HIV-infected individuals.[136,137]

As detailed earlier, T cells are not a monolithic cell type but rather include many T-cell subclasses, each of which is now known to regulate periapical immune and inflammatory responses in a differential manner. For example, as discussed later, IL-$10^{-/-}$ mice are highly susceptible to infection-induced bone loss caused by hyperactivation of T-cell responses. The T_{reg} subset is a major source of IL-10. Induction of T_H1-type immune responses by preimmunization with an endodontic pathogen with IL-12 or CpG resulted in severe periapical inflammation, including significant upregulation of IFN-γ and IL-1 and extensive bone loss, in wild-type mice[138] (Fig 12-13). These findings suggest that periapical inflammation and bone loss are strongly enhanced by overactivated T_H1 responses.

Individuals with pure antibody deficiencies are not at a greater risk of developing marginal periodontitis or dental caries than age- and sex-matched controls.[86,91,139] Little is known about the effects of pure antibody deficiencies on periapical pathogenesis, although results with B-cell–deficient animals suggest increased severity and possible infection dissemination.[14] However, because most hypogammaglobulinemic patients also receive long-term administration of antibiotics and/or gamma globulins, the oral flora may be significantly suppressed and root canal infections ameliorated, reducing inflammation.[91,140]

Taken together, these data strongly indicate that specific immune responses mediated by B cells and antibodies protect against the regional and/or systemic spread of endodontic infections by localizing the infection to the root canal system. Antibody responses otherwise have relatively minor effects on localized periapical bone destruction. T cells in the aggregate have only a marginal effect on the extent and severity of periapical inflammation, but individual T-cell subsets clearly upregulate or downregulate inflammation and periapical bone destruction.

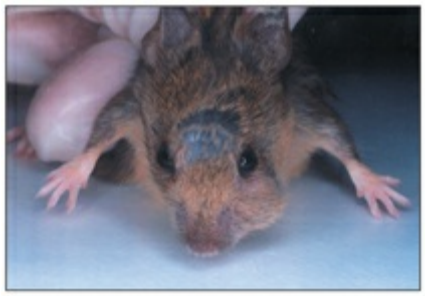

Fig 12-11 Development of an orofacial abscess of endodontic origin after pulpal exposure in a RAG-2 mouse. RAG-2 animals are genetic knockouts for the recombination activator 2 gene (RAG-2) and are thus unable to generate immunoglobulins or T-cell receptors. RAG-2 knockouts are a model of SCID because they have substantial defects in both humoral and cellular immunity. (Reprinted from Teles et al[13] with permission.)

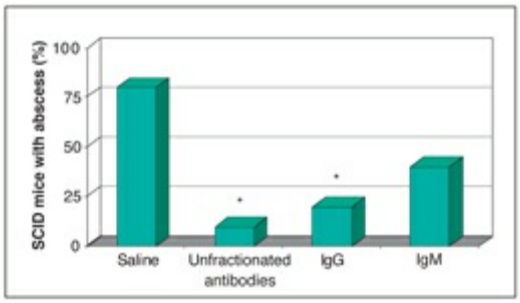

Fig 12-12 Effect of subcutaneous administration of saline, an unfractionated mixture of antibodies, or purified IgG or IgM antiserum on the incidence of disseminated orofacial abscesses in SCID mice after pulpal exposure. *P < .05 versus saline; n = 10 or 11 per group. (Redrawn from Hou et al[14] with permission.)

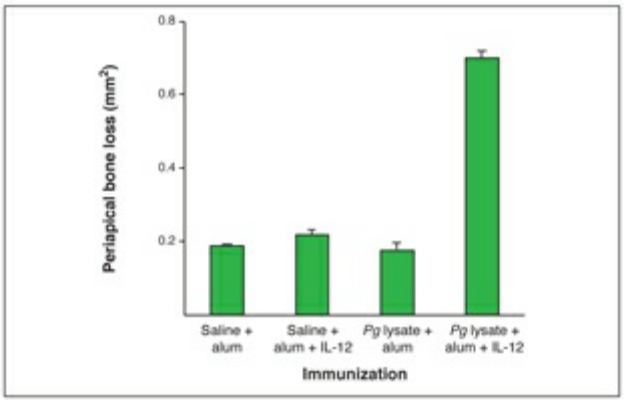

Fig 12-13 Effect of T_H1- or T_H2-biased immunization on periapical bone resorption caused by *P gingivalis*. C57BL/6 mice (10 per group) were immunized with 10 µg of *P gingivalis* soluble lysate (*Pg*) preparation mixed with 25 µg of alum (for T_H2 response) or with 25 µg of alum plus 1 µg of the cytokine IL-12 (for T_H1 response). Four weeks after a second immunization with the same antigen/adjuvant formulations, the mice were subjected to pulpal infection with *P gingivalis*. Periapical bone resorption was analyzed by microcomputed tomography after 21 days. Bars represent the mean ± standard deviation. (Redrawn from Stashenko et al[138] with permission.)

Mediators of Periapical Inflammation

Most evidence suggests that periapical inflammation and bone destruction are stimulated primarily by host-derived mediators that are induced by infection rather than by direct interaction of bacteria with osteoclasts and other host cells. These mediators help to combat infection but appear to do so at the price of stimulating concomitant tissue degradation. In this section, the activity of various mediators of periapical inflammation and destruction is discussed.

Proinflammatory cytokines

As noted, the earliest periapical responses to pulpal infection involve the migration of PMNs and monocytes. Chemokines, including IL-8 and monocyte chemoattractant peptide 1, are produced locally and are likely to be involved in regulating PMN and monocyte infiltration.[141–143] IL-8 also helps to "prime" PMNs for an elevated oxidative burst that is important in bacterial killing.

Once activated by bacterial components, macrophages and PMNs express a cascade of proinflammatory cytokines (see Fig 12-2). The proximal members of this cascade include IL-1α, IL-1β, and TNF-α. IL-1α and TNF-α may further stimulate each other's expression in an autoregulatory fashion. Downstream, the cytokines IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are induced as secondary mediators. GM-CSF stimulates bone marrow production of PMNs and monocytes and primes PMN activation. As discussed later, IL-6 can synergize with PGE_2 to increase some inflammatory responses, but its predominant action overall in the periapical milieu is anti-inflammatory.

As already mentioned, macrophages and other cell types express an evolutionarily conserved family of TLRs that recognize bacterial structures, including LPSs, lipopeptides, peptidoglycans, lipoteichoic acid, flagellin, and microbial DNA.[144] To date, 13 TLRs are recognized in both humans and mice.[145] Activation of TLRs triggers signaling pathways that result in the expression of IL-1, TNF-α, and other inflammatory cytokines (see Fig 12-2). Thus, TLR activation explains the observation that IL-1 and TNF-α are induced in periapical lesions in the absence of T or B cells.[82] Mice deficient in TLR-4, the primary receptor for most forms of LPS,[146,147] exhibit reduced inflammatory cytokine responses and decreased periapical bone resorption[15] (Fig 12-14). Although in vitro data suggest that TLR-2 is also involved in periapical immune responses,[148–150] the functional role of TLRs, including TLR-2, has not been directly examined in vivo.

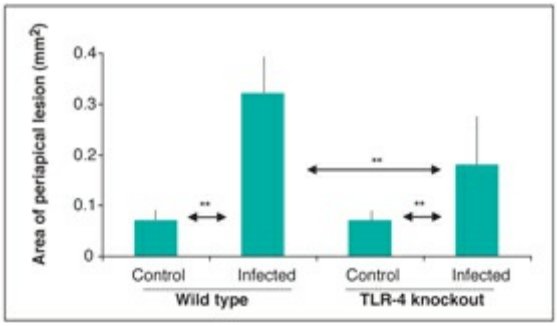

Fig 12-14 Effect of deletion of the TLR-4 gene on the size of periradicular lesions after pulpal exposure in mice. The TLR-4 gene binds to bacterial LPSs and contributes to the innate immune response of macrophages and other cells to bacteria. Activation of TLR-4 leads to synthesis of cytokines such as IL-1 and TNF. $^{**}P < .01$. (Redrawn from Hou et al[15] with permission.)

At sites of pulpal and periapical inflammation, IL-1α, IL-1β, and TNF-α messenger RNA and protein are expressed in the first few days following infection (Fig 12-15).[151–154] These mediators are largely derived from macrophages and PMNs.[151] IL-1β is present in inflamed human pulp,[155] periapical lesions,[156] and cysts.[157] Higher levels of IL-1β are present in periapical lesions from more severely affected, symptomatic teeth.[158,159] The level of IL-1β in human periapical exudates was twice that of IL-1α.[160] Interestingly, IL-1β declined following treatment, whereas IL-1α increased, suggesting that IL-1β may be primarily associated with pathogenesis. TNF-α has been identified in periapical exudates.[161] IL-6, which induces osteoclast formation but does not appear to stimulate activation, is expressed by macrophages, PMNs, and T cells and has been detected in periapical tissue and radicular cysts.[162–166] IL-11 is present in periapical lesions, but its role is unclear.[108]

Proinflammatory cytokines and periapical bone loss

Because proinflammatory cytokine expression, particularly IL-1, is associated with the severity of periapical bone loss, these molecules have been implicated in periapical bone loss. IL-1α, IL-1β, TNF-α, and TNF-β stimulate bone resorption by osteoclasts. Collectively, these mediators generate the activity formerly termed *osteoclast-activating factor* (*OAF*).[167–170] It is now known that this OAF activity is due to the ability of these mediators to induce the expression of RANKL by osteoblasts and stromal cells in bone.

Both IL-1 and TNF-α are produced in large quantities by macrophages and other cells, including fibroblasts,[171] keratinocytes,[172] osteoblasts,[173] and neutrophils.[174] In addition to bone resorption, IL-1 and TNF-α possess overlapping activities relevant to periapical tissue destruction, including induction of PGE_2,[175] proteinase production,[176] and inhibition of bone formation.[168,177] IL-6 and IL-11 have also been reported to increase bone resorption, but this effect is probably secondary to their ability to stimulate osteoclast formation from precursor stem cells rather than to activate preexisting osteoclasts.

Among the proinflammatory cytokines, IL-1 appears to play a central role in

stimulating periapical bone resorption by osteoclasts. In humans, IL-1β constitutes most OAF activity, reflecting both its high level of expression and its pharmacologic potency.[167,177,178] In this regard, IL-1β has been shown to be approximately 500-fold more potent than TNF in stimulating bone resorption.[179]

Bone-resorbing activity is present in extracts of both human and rat periapical tissues.[180,181] In rodent experimental models, in which the timing of lesion induction can be controlled,[182] the highest levels of resorbing activity are present in periapical tissues during the active phase of lesion expansion (3 to 14 days after pulpal exposure). Most of this activity can be resolved to a peak molecular weight of 15,000 to 20,000, consistent with the size of IL-1. Moreover, this activity is neutralized primarily by anti–IL-1 but not by anti–TNF-α antibodies.[182,183] Thus, despite its fairly high level of expression in periapical lesions, TNF-α does not exert significant resorptive activity because it is less potent in stimulating osteoclasts than IL-1. Only 10% to 15% of resorptive activity in lesions is due to the action of PGE_2.

Specific inhibition of IL-1 in vivo by infusion of rats with an IL-1 receptor antagonist (IL-1ra)[184] over a 14-day period after pulpal exposure decreased lesion size by approximately 60%[174](Fig 12-16). IL-1ra, another member of the IL-1 family induced during inflammatory responses, binds to IL-1 receptors but fails to activate cellular responses. Consequently, it acts as a competitive inhibitor of both IL-1α and IL-1β. The expression of endogenous IL-1ra is induced following infection and has been found in periapical lesions.[185] Interestingly, the ratio of IL-1ra to IL-1β in periapical exudates from symptomatic lesions was threefold lower than the ratio in exudates from asymptomatic lesions, suggesting that the balance between inhibitor and agonist may determine lesion progression.

The central role of IL-1 in infection-stimulated bone resorption is further underscored by findings in models of periodontal disease. Thus, treatment of monkeys with soluble IL-1 receptors (which block IL-1 activity) reduced periodontal bone loss by 60% to 70%.[186]

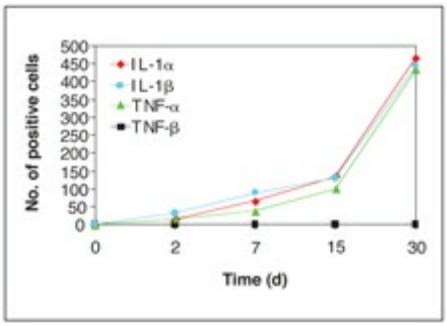

Fig 12-15 Effect of pulpal inflammation and necrosis on the number of cytokine-expressing cells in the

periradicular tissue of rats following pulpal exposure. (Redrawn from Tani-Ishii et al[151] with permission.)

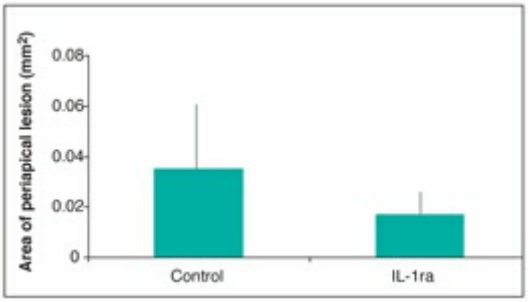

Fig 12-16 Effect of administration of an IL-1 receptor antagonist (IL-1ra) or a vehicle control on the size of periapical lesions after pulpal exposure in rats. (Redrawn from Stashenko et al[174] with permission.)

Protective effects of IL-1

The effect of a lack of IL-1 and/or TNF receptor signaling has been studied with respect to periapical destruction. Surprisingly, mice deficient in the primary receptor for IL-1 (IL-1 type I receptor [IL-1RI]) or for both receptors for TNF-α (TNFR), p55 and p75, were reported to have greater periapical bone resorption than wild-type controls.[187] Mice deficient in both IL-1RI and TNFR p55 exhibited even more periapical bone resorption. This finding suggests a protective role of IL-1 and TNF-α. The increased bone destruction correlated with more rapid bacterial penetration through the radicular pulp and increased pulpal destruction, despite the presence of increased numbers of infiltrating PMNs and monocytes in receptor-deficient animals.[187] Mice deficient in IL-1RI, but not TNFR p55 and TNFR p75, also exhibited soft tissue abscess formation and increased mortality.[188]

These provocative findings have been confirmed using infusion of neutralizing anti–IL-1 antibodies. In this study, IL-1α, IL-1β, or both IL-1α and IL-1β were neutralized in vivo using specific antibodies in wild-type mice, and periapical bone loss and abscess development were assessed. Whereas neutralization with either anti–IL-1α or anti– IL-1β antibodies alone had no effect, treatment with both antibodies simultaneously resulted in orofacial abscess formation, disseminating infections, and sepsis.[189] Only male mice were susceptible to abscess formation, whereas female mice were resistant. However, female mice that were ovariectomized to eliminate estrogen production also exhibited abscess formation and disseminating infections. This susceptibility was reversed by estrogen-containing implants. As in IL-1RI– and TNFR-deficient animals, the numbers of

infiltrating PMNs in lesions were increased. However, the PMNs were less able to ingest and kill bacteria than were the PMNs in controls when tested ex vivo.

Taken together, these data strongly indicate that although IL-1α and IL-1β play a role in stimulating periapical bone destruction in chronic infections, they also have a critical host-protective function, particularly early after initial infection. This protective effect involves the "priming" of PMNs by IL-1 and TNF for enhanced phagocytic and bactericidal activity. As lesions progress, however, the effect of IL-1 is clearly destructive, as discussed earlier.[190,191]

Arachidonic acid metabolites

Products of arachidonic acid metabolism have also been correlated with pulpal and periapical inflammation. PGE_2 has been shown to be present in higher concentrations in symptomatic pulps[192] and in acute periapical lesions[193] (Fig 12-17), and levels of PGE_2 in periapical exudates decrease following root canal treatment.[194] Experimentally induced inflammation of the dental pulp increases the production of 6-keto prostaglandin $F_1\alpha$, thromboxane B_2, and leukotriene C_4.[195] Similarly, the application of bacterial LPSs to pulps increases production of all arachidonic acid metabolites.[196] Prostaglandins increase vascular permeability[197] and stimulate bone resorption, and, as previously noted, PGE_2 is directly responsible for 10% to 15% of total bone-resorbing activity in extracts of rat periapical tissues.[181] However, approximately 60% of the bone-resorbing activity stimulated by IL-1 is inhibited by indomethacin. This finding reflects the fact that IL-1–induced bone resorption is partly dependent on PGE_2 synthesis by cells present at inflammatory sites (Fig 12-18).

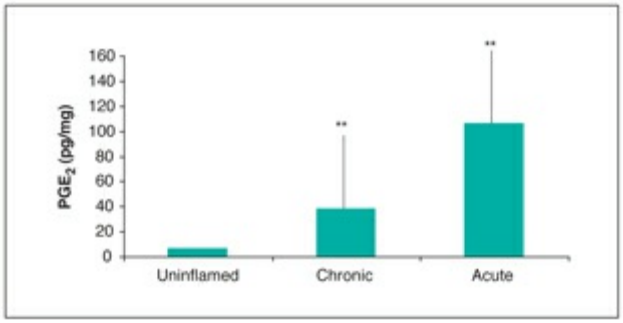

Fig 12-17 Prostaglandin levels in periapical tissue collected from endodontic surgery patients. Uninflamed tissue samples consisted of periapical tissue taken from clinically normal unerupted third

molars. Chronic inflamed tissue was taken from teeth diagnosed as having chronic apical periodontitis. Acutely inflamed tissue was taken from teeth diagnosed as having acute apical lesions. $^{**}P < .01$ versus uninflamed tissue; n = 16. (Redrawn from McNicholas et al[193] with permission.)

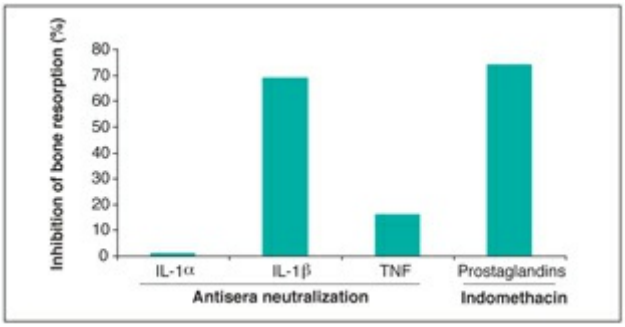

Fig 12-18 Inhibition of the bone-resorbing activity of extracts taken from human periradicular lesions. Cytokine activity was blocked by immunoneutralization with antisera to IL-1α, IL-1β, and TNF-α or TNF-β. Prostaglandin activity was blocked with indomethacin. (Redrawn from Wang and Stashenko[181] with permission.)

IL-1 and PGE_2 have been shown to synergize with respect to bone resorption.[34,179,198] Indomethacin was found to block the progression of periapical bone destruction in experiments in which the deposition of immune complexes into root canals of cats induced such lesions.[74] The participation of PGE_2 in periapical destruction in vivo is also supported by the finding that indomethacin-treated rats displayed milder inflammation and less bone resorption than controls.[199] This finding is consistent with periodontal disease studies in which long-term administration of the cyclo-oxygenase (COX) inhibitor flurbiprofen suppressed naturally occurring periodontal destruction in beagles by 60% to 70%.[200]

Paradoxically, infusion of PGE_2 in vivo has actually been found to increase bone formation rather than to stimulate bone resorption,[201] and inhibitors of PGE_2 production (such as ibuprofen) inhibit fracture repair.[202] PGE_1 has been shown to induce regeneration of cementum, alveolar bone, and periodontal ligament in dogs.[203] Because prostaglandins appear to act largely through synergy with other mediators, it may be that their primary effect is to increase the sensitivity of osteoblasts and osteoclasts to other signals, regardless of whether they stimulate bone resorption, such as proinflammatory cytokines, or bone formation, such as growth factors.

Arachidonic acid metabolism leads to the generation of prostaglandins and leukotrienes through COX and lipoxygenase pathways and is generally proinflammatory.[204] However, alterations in arachidonic acid metabolism can

induce beneficial anti-inflammatory lipid mediators. Aspirin, a nonsteroidal anti-inflammatory drug, has the ability to inhibit prostaglandins but also acetylates COX-2 and triggers production of 15-epi-lipoxins, which are lipid mediators that possess anti-inflammatory and proresolving actions.[205] Furthermore, acetylated COX-2 produces an array of autacoids, termed *resolvins*, from the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid.[205]

Resolvins potently reduce vascular permeability, PMN infiltration, chemotaxis, and clearance of apoptotic PMNs.[206–209] In a rabbit model of marginal periodontitis, topical application of the stable analog 15-epi-16-phenoxy-para-fluoro-LXA4 dramatically reduced leukocyte infiltration, alveolar bone loss (by greater than 90%), and gingival inflammation.[210] These substances are likely to play important roles in the context of resolution of pulpal and periapical inflammation but have not yet been thoroughly investigated in this system.

Regulation of proinflammatory cytokines: The cytokine network

The expression and action of proinflammatory and bone-resorbing mediators is modulated by a network of other regulatory cytokines. Although complex, this network is best understood in the context of the four major subpopulations of T-helper cells, which include T_H1, T_H2, T_{reg}, and T_H17 subsets (see Fig 12-7). Each of these cell subtypes possesses distinct immunoregulatory functions and may be characterized by its gene-expression profile as well as certain specific markers.

T_H1 cells are proinflammatory[211–214] and produce IFN-γ, IL-2, and TNF-α. T_H1 cells are induced by IL-12 and IL-18 and mediate delayed-type hypersensitivity reactions through the activation of macrophages mainly by IFN-γ. They also increase inflammation and inhibit the responses of T_H2 and T_{reg} cells.

Conversely, T_H2 cells are anti-inflammatory and produce IL-4, -5, -6, -10, and -13. T_H2 cells stimulate the production of most subclasses of antibody by B cells, decrease inflammation, and inhibit the responses of T_H1 and T_H17 cells.

T_{reg} cells are perhaps the predominant anti-inflammatory T-cell subset and exhibit the activities formerly attributed to “suppressor” T cells, which in the past were

believed to be $CD8^+$. As described earlier, T_{reg} cells are identified by their expression of CD4 and the transcription factor FOXP3. T_{reg} cells induce their immunosuppressive effects by secretion of TGF-β and IL-10 and, in some cases, by direct cell-cell contact.

T_H17 cells are a more recently described subset that are proinflammatory in some contexts, notably in autoimmune diseases. However, T_H17 cells may be protective against bacteria, including pulpal infections. Their differentiation is induced by TGF-β and IL-6,[44] and their proliferation is enhanced by IL-23.[215] T_H17 cells produce IL-17, which induces chemokines that stimulate PMN chemotaxis.

These T-cell subsets derive from nonpolarized precursor cells (T_Hp), which are directed to differentiate into these various subsets, largely as a consequence of the local cytokine milieu. In many cases, the cytokines that determine T_H-subset commitment are produced by macrophages following their stimulation by bacteria. As noted earlier, the macrophage-derived cytokines IL-12 and IL-18 favor the development of T_H1 cells,[216] whereas IL-10 promotes T_H2 responses.[217–220] In addition, IL-4 and IL-13 derived from other T cells, basophils, and NK cells are also important determinants of T_H2 commitment. The mechanism of T_{reg}-cell induction is not fully understood; however, TGF-β plays a key role.[221]

These subsets regulate the activities of other immune cell types, notably B cells and macrophages, and control other T-cell subsets. T_H1 and T_H2 cells are cross-regulatory and their cytokines are antagonistic, acting to inhibit the proliferation and cytokine production of the opposing subset.[222] Data from periodontal disease models suggest that inflammation and bone resorption are increased by T_H1 responses and decreased by T_H2 responses. Thus, transfer of T_H1 clones exacerbates periodontal bone resorption, whereas T_H2 clones are protective.[223,224] A model for the operation of such a network in periapical inflammation predicts that the T_H1 subset increases IL-1 and other proinflammatory cytokines, whereas inhibitors of IL-1 are related to the T_H2 subset (see Fig 12-2). Many of these regulatory cytokines have been identified in mouse periapical lesions, including IFN-γ, TGF-β, and IL-2, -4, -6, -10, -12, and -13.[225]

Following pulpal infection in mice, T_H1-type cytokines IL-2, IL-12, and IFN-γ showed a linear increase within periapical lesions up to day 28. In contrast, T_H2-type cytokines IL-4, IL-6, and IL-10 were increased following pulpal exposure, but

levels declined by day 28, suggesting possible inhibition by T_H1-type mediators. Significant correlations were observed between levels of IL-1 and T_H1 cytokines in periapical tissues, whereas there was a lack of correlation between IL-1 and T_H2-type mediators. These results demonstrate that a cytokine network is activated in the periapex in response to infection and that T_H1-modulated proinflammatory pathways predominate in early pathogenesis.

In chronic human periapical lesions, IFN-γ and IL-2, -4, -6, and -10 have been identified by immunohistochemistry.[226] In contrast to findings in the mouse, human lesions exhibited greater expression of IL-4, -6, and -10 than IL-2 and IFN-γ, which suggests that T_H2-type mediators predominate and may act to stabilize the size of chronic lesions.

As discussed earlier, TGF-β, IL-6, and IL-23 are the key cytokines in differentiation and proliferation of T_H17 cells. T_H17-derived IL-17 plays a proinflammatory role in induction and progression of autoimmune diseases such as experimental allergic encephalitis and rheumatoid arthritis. In contrast, IL-17 may play a protective role in bacterial infection. The role of IL-17 against various bacteria species has been investigated.[227] Major mechanisms of IL-17– mediated protections are prevention of colonization, reduction of bacterial burden, and enhancement of bacterial clearance. IL-17 is also protective against *P gingivalis* in a marginal periodontitis model that is dependent on PMN-mediated protection.[228] In these processes, IL-17 mediates chemokine expression and subsequent attraction of PMNs, which are protective against pulpal infections.

This cytokine is also protective against common human endodontic pathogens including *P intermedia*, *F nucleatum*, *P micros*, and *S intermedius* in a mouse periapical lesion model. The mechanism of this protection appears to be IL-17–mediated downregulation of macrophage/monocytic inflammation, including IL-1 and macrophage inflammatory protein 2, which is a distinct mechanism from that of the periodontitis study that was already cited.[229]

Functional studies of regulatory cytokines

The hypothesis that emerges from these findings is that regulation of periapical bone resorption is mediated principally through the effects of a mediator on RANKL expression, either via IL-1 expression by macrophages and other cell types or other pathways. This scenario is clearly an oversimplification, however, because a number of cytokines, including IFN-γ and IL-4, -8, -10, and -18, have been reported

to directly inhibit osteoclasts at least in vitro. Again, the question is what these cytokines actually do functionally within developing periapical lesions rather than what they can do in other systems. Experiments to address this question have either employed genetic knockouts of individual cytokines or inhibition of cytokines with specific inhibitors, antagonists, or neutralizing antibodies. Such analyses have now been carried out for many of the key regulatory cytokines expressed in periapical lesions.

Knockout animals have been used to determine the effect of a lack of the prototype T_H2 anti-inflammatory cytokines IL-4, -6, and -10.[230,231] The results demonstrated that IL-10–deficient mice had dramatically increased periapical bone destruction, by more than 250% (Fig 12-19), whereas, somewhat surprisingly, IL-4–deficient animals had bone loss similar to that of controls.[230] IL-$6^{-/-}$ animals and animals treated with anti–IL-6 antibodies also had increased resorption, suggesting an anti-inflammatory effect of this cytokine, although this increase (approximately 50%) was moderate compared to that seen in IL-$10^{-/-}$ animals.[231] The increased bone destruction in the IL-10 knockouts correlated with a tenfold elevation in the periapical levels of IL-1α, whereas IL-1α was increased twofold in IL-$6^{-/-}$ animals but not increased in IL-$4^{-/-}$ mice. Macrophage IL-1α expression was inhibited to a significant degree by IL-10 and IL-6 but not by IL-4 in vitro.

These data demonstrate that endogenous IL-10 has a strong (and nonredundant) inhibitory effect on periapical bone destruction and that proinflammatory pathways predominate in its absence. IL-4, although a potent inhibitor of inflammation in other systems, has no effect on bone resorption in vivo.

This finding also contrasts with its reported inhibition of bone resorption in organ culture.[232] The role of IL-6 is interesting because it is often considered to be proinflammatory. Part of this misconception is due to the fact that IL-6 increases osteoclastogenesis, and its absence might be expected to decrease osteoclast formation as well as resorption. However, IL-6 also possesses many anti-inflammatory effects. These include the induction of acute phase proteins, many of which are themselves anti-inflammatory, as well as a weak inhibition of IL-1 expression. Thus, the predominant actions of IL-6 are anti-inflammatory, consistent with its categorization as a T_H2 cytokine.

TGF-β is produced by many cell types, including macrophages, epithelial cells, and T_{reg} cells, and is also a powerful negative regulator of inflammation. Thus, animals lacking TGF-β have multifocal inflammation and exhibit severe pulpal and periapical inflammation.[212,233,234] The role of TGF-β in periapical resorption

induced by infection has not yet been determined.

The functional role of T_H1-inducing IL-12 and IL-18 and prototype T_H1 cytokine IFN-γ, which may be expected to increase IL-1 expression and resorption, has also been analyzed in vivo using genetic mutant mice.[235] However, none of the animals—whether IL-$12^{-/-}$, IL-$18^{-/-}$, or IFN-$\gamma^{-/-}$ knockouts— showed significantly decreased periapical bone loss. These cytokine deficiencies did not alter the level of IL-1α in periapical lesions. These findings indicate that endogenous expression of T_H1-related cytokines as single-gene deficiencies has at most minor effects on periapical inflammation and bone loss in vivo, likely due to the redundancy in proinflammatory pathways.

However, recent studies using double- or triplegene knockout mice demonstrate that the expression and biologic function of these genes appear to be strictly regulated by endogenous IL-10. Although macrophage-specific IL-10 signal–deficient (Stat3 mutant) mice exhibit spontaneous colitis similar to IL-$10^{-/-}$ mice, this characteristic phenotype was not observed in TLR-4/Stat3, IL-12/Stat3, or RAG-2/Stat3 mutant mice.[236] Similarly, the upregulated periapical and alveolar bone loss in IL-$10^{-/-}$ mice is dependent on a hyperinflammatory T-cell response mediated by macrophage-derived IL-12.237 Indeed, IL-10/IL-$12^{-/-}$ mice are resistant to both periapical lesion and periodontitis.[132,237] These studies demonstrate that a functional cytokine network between proinflammatory and anti-inflammatory cytokines regulates periapical bone destruction.

The functional role of IL-17 in periapical lesions was recently assessed using IL-$17^{-/-}$ mice.[238] In this study, the extent of periapical bone loss in IL-$17^{-/-}$ animals was similar to that in wild-type controls. However, in a more recent study using IL-17 receptor–deficient (IL-$17R^{-/-}$) mice, the extent of bone loss was significantly greater than that observed in wild-type controls. A similar result was seen when IL-17 neutralizing antibodies that block the function of the IL-17A ligand were used. The greater bone loss was strongly associated with upregulated IL-1 and macrophage inflammatory protein 2. In addition, IL-17R deficiency induced upregulation of marginal alveolar bone loss compared with that exhibited by wild-type controls,[228,239] apparently as a response to reduced PMN traffic. The susceptibility of IL-$17R^{-/-}$ animals to infection-induced bone loss is caused by impaired recruitment of PMNs because IL-17 is largely involved in the chemotaxis and activation of PMNs. At this time, the reason for the discordance in findings between IL-$17^{-/-}$ and IL-$17R^{-/-}$ mice is uncertain, and further studies are needed to resolve this question.

A number of other cytokines with potential modulating activity on periapical resorption have not yet been assessed in these in vivo systems. These include the macrophage-derived T_H1-inductive mediators IL-15, T_H2 cytokine IL-13, which is also produced by NK cells, and IL-22. Other soluble cytokine receptors (eg, sIL-4R and sIL-6R) may affect the network by antagonizing regulatory cytokines.[240–243]

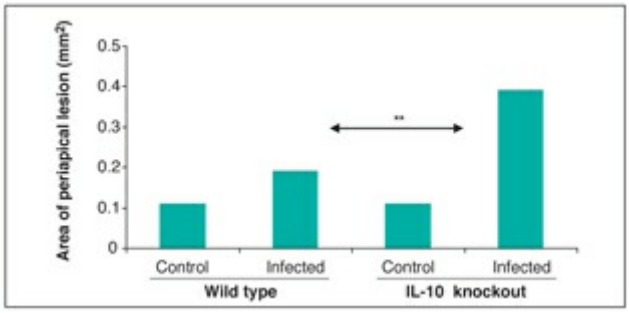

Fig 12-19 Effect of deletion of anti-inflammatory cytokines on the size of periapical lesions in mice after pulpal exposure. Mice were engineered to have knockouts of the IL-10 gene. The cytokine IL-10 promotes the anti-inflammatory response of lymphocytes and inhibits osteoclast activity. $^{**}P < .01$. (Redrawn from Sasaki et al[230] with permission.)

Conclusion

There has been significant progress in recent years in the understanding of the pathogenesis of apical periodontitis, particularly with respect to its modulation by the immune system. The elements of innate and specific immunity that protect against pulpal infections and prevent their dissemination have been defined, at least in broad terms. A number of the key mediators of periapical bone resorption have been elucidated. Future studies will seek, first, to completely characterize the protective versus destructive components in this system and, second, to learn how to control them through immunotherapy.

Furthermore, it seems likely that research in the field of dental pulp biology will continue to progress rapidly as a result of advances in science and technology. Translational dental medicine, which bridges the basic findings reviewed in this chapter with patient information, is also essential. This review of the pathogenesis of apical periodontitis has several clinical implications. First, patients with diseases or patients taking medications that suppress certain immune functions may experience a greater risk of developing apical periodontitis or exhibit apical periodontitis that is more resistant to endodontic treatment compred to healthy patients. The known effects of certain immunodeficiencies have been noted

previously in this chapter. Second, the primary etiologic factor for apical periodontitis is pulpal bacterial infection and the resulting immune response. Accordingly, those endodontic procedures that have the greatest effect in reducing bacteria and their antigens and products in the root canal system are likely to have the greatest success in preventing reinfection and resolving apical periodontitis.

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Repair of Pulpal Injury with Dental Materials

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Vital pulp therapy includes direct and indirect pulp capping, pulpotomy, and other therapies that minimize pulpal injury by protecting the pulp from the toxic effects of chemical, bacterial, mechanical, or thermal insult.[1] Vital pulp therapy therefore is aimed at treating reversible pulpal injuries by sealing the pulp and stimulating the formation of tertiary dentin.[2] Pathologic stimuli that can induce reversible pulpitis include attrition, erosion, caries, restoration procedures, and restoration placement.[3]

This chapter first explores the factors that affect the success of vital pulp therapy and then specifically discusses calcium hydroxide ($Ca[OH]_2$) treatment, which is compared with treatments that use other materials developed and evaluated for vital pulp therapy.

The dental pulp is vulnerable to injury for three reasons: *(1)* It is a relatively large volume of tissue with a relatively small volume of blood supply; *(2)* it is a terminal

circulation with few, if any, collateral vessels; and *(3)* it is confined in calcified tissue walls (dentin). Therefore, the pulp is considered a low-compliance system that does not tolerate injury easily. Nevertheless, the dental pulp has the capacity to heal itself. Like many other tissue systems in the body, the dental pulp can answer an adverse stimulus with an immune response that allows the dental pulp to protect itself and survive. This capacity may depend on age because the volume of the coronal pulp and root canal system decreases with age.

The effects of dental materials on the dental pulp have fascinated investigators since teeth were first subjected to restorative procedures. From the time that caries was thought of as being caused by "worms,"[4] many materials have been suggested not only to replace lost or prepared tooth structure but, more importantly, to protect and preserve the vitality of the dental pulp. This innate repair system is influenced by the clinician's choice of material and technique and other factors influencing case selection. The dental pulp, when exposed by caries or mechanical, chemical, or physical trauma, may respond favorably to application of a variety of materials used in pulp capping procedures.[5] Many studies have confirmed the formation of hard tissue over the site of the exposure (see section on calcium hydroxide). The dental pulp thus can be said to demonstrate an intrinsic capacity to heal.

However, in the clinical situation, where a pulpal exposure leads to long-term irritation and inflammation at the exposure site, the outcome of hard tissue formation is not predictable.[5] Some factors relating to pulpal defensive reactions and healing after capping procedures are understood, but the mechanisms of other factors are less well known, including those that regulate the inflammatory response and possible resultant necrosis. Improved understanding of the regulation of healing and wound closure (hard tissue formation) may allow the development of improved treatment procedures, leading to more predictable outcomes.

The clinician should understand how restorative materials aid in the recovery of the pulp tissue in the face of reversible and irreversible tissue changes that threaten its vitality (see also chapters 14 and 15). In order to anticipate the reaction of the dental pulp to various materials and to the preparations for their placement, the clinician must investigate and understand:

- The ability of the available materials to stimulate tissue repair
- The methods used to test the materials, both in the laboratory (in vitro) and the clinic (in vivo)
- The ability of the materials to seal the interface between the margin of the preparation and the margin of the restorative material

- The role of microorganisms (bacteria) in pulpal disease
- The potential for tooth preparation procedures to cause pulpal disease
- The effects of mechanical or pathologic exposures that allow contact of pulp tissue with the oral environment as well as restorative materials
- The potential for a medicament, a material, or a combination of both to obdurate a pulpal exposure and allow the pulp to recover its natural form, function, and vitality

Influence of Bacteria on Pulpal Healing

The classic studies of Kakehashi et al[6] clearly showed the pathologic role of bacteria in pulpal exposures. In the presence of bacteria, exposed rat pulp tissue is partially necrotic by 8 days (Fig 13-1) and completely necrotic with formation of periradicular abscesses by 14 days (Fig 13-2). This response is not seen in germ-free animals with pulpal exposures. Figure 13-3 shows the dental pulp at 7 days after exposure in germ-free animals; although food debris has been impacted in the dental pulp, the tissue appears normal. By 32 days after pulpal exposure in germ-free rats, an intact dentin bridge has developed with normal dental pulp tissue beneath the newly formed dentin (Fig 13-4). Thus, bacterial infection of dental pulp constitutes a critical etiologic factor for pulpal necrosis (see also chapter 10). Vital pulp therapy therefore must include materials and methods that reduce or eliminate bacteria.

This is an important consideration because success in direct pulp capping procedures is not predictable. There are conflicting studies concerning the procedures and materials that offer the greatest chances for success with pulp capping. A pioneering study[7] described the placement of phenol at the site of pulpal exposure prior to the pulp capping procedure. The study also examined the direct use of either $Ca(OH)_2$ or zinc oxide–eugenol at the site of exposure with prior use of phenol. Healing occurred when $Ca(OH)_2$ was used as the capping material but not when zinc oxide–eugenol was used. Phenol neither interfered with nor enhanced pulpal healing. An important principle to remember is that pulpal healing occurs in an environment free from the presence of microorganisms.

Two issues modify pulpal healing and therefore must be considered in the

development of guidelines for vital pulp therapy: *(1)* How does the duration of pulpal exposure to the oral environment modify the success of pulp capping procedures? *(2)* What is the relative importance of the capacity of a material to seal against bacterial invasion compared to its cytotoxicity?

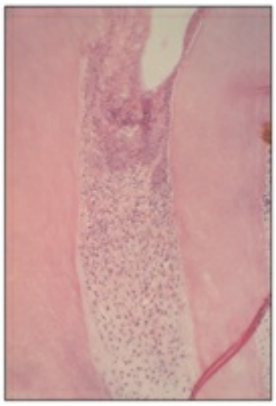

Fig 13-1 Dental pulp 8 days after pulpal exposure in conventional rats. Note the necrotic and vital pulp tissue juxtaposed in the root canal system (hematoxylin-eosin [H&E] stain; original magnification ×100). (Reprinted from Kakehashi et al[6] with permission.)

Fig 13-2 Dental pulp 14 days after pulpal exposure in conventional rats. Note the complete necrosis of the dental pulp with the development of a periradicular abscess (H&E stain; original magnification ×40). (Reprinted from Kakehashi et al[6] with permission.)

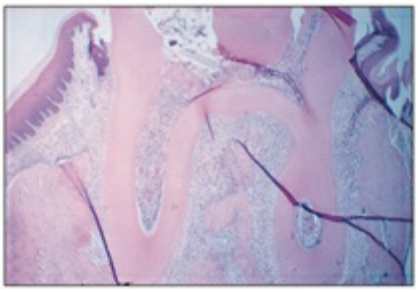

Fig 13-3 Dental pulp 7 days after pulpal exposure in germ-free rats. The dental pulp remains vital even though food debris has been impacted into the exposure site and pulp tissue (H&E stain; original magnification ×340). (Reprinted from Kakehashi et al[6] with permission.)

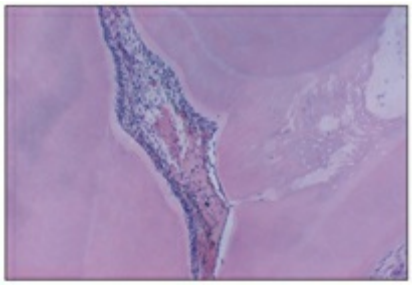

Fig 13-4 Dental pulp 32 days after pulpal exposure in germ-free rats. Note the formation of a dentin bridge across the exposure with vital and uninflamed dental pulp beneath the bridge (H&E stain; original magnification ×3,100). (Reprinted from Kakehashi et al[6] with permission.)

Duration of pulpal exposure

The duration of pulpal contamination is an important factor in the success of pulp capping procedures. Many clinicians believe that only uncontaminated pulpal exposures should be treated and that longer periods of contamination by oral microorganisms and debris reduce the chance of success.[8–11] This view is reinforced by results from animal studies that indicate that the success of $Ca(OH)_2$ pulp capping is reduced from 93% to 56% when microbial contamination is extended from 1 hour to 7 days.[11] However, clinical studies in younger patients have shown that the superficial pulp is resistant to bacterial invasion and that partial pulpotomy with $Ca(OH)_2$ dressing can provide a 93% radiographic success rate at a mean follow-up of more than 4.5 years.[12,13] In these studies, treatment of pulp that was exposed for up to 3 months had success rates similar to those associated with exposures of a shorter duration.[12] Collectively, these studies illustrate that the duration of contamination remains an important yet controversial factor in terms of successful pulp capping.

Bacterial microleakage versus material toxicity

A second issue in pulpal healing is the relative importance of bacterial microleakage and material toxicity. Various materials differ in these properties; some (eg, zinc oxide–eugenol) show greater ability to prevent microleakage, while others (eg, zinc

phosphate cement) were thought to show greater tissue toxicity. Early research focused on the tissue toxicity of materials, but in 1971 Brännström and Nyborg[14] demonstrated that infection caused by the microleakage of microorganisms around the restoration provided the greatest threat to pulpal health.[15] Other investigators[16–18] have suggested that pulpal devitalization following a restorative procedure is likely to result from the combined effect of bacteria, the mechanical injury induced during cutting of the tooth substance, the extent and depth of the cavity preparation, and the toxicity of the restorative materials (see also chapter 14). However, bacteria are believed to be the main factor. Pulpal mechanisms proposed to reduce bacterial invasion include increased outward flow of dentinal fluid (see also chapter 4), emigration of neutrophils into dentinal tubules, and the sequestering of toxic substances (of either restorative or bacterial origin) by their binding to dentin.[18]

Taken together, these studies indicate that both bacteria and material toxicity contribute to the development of pulpal pathosis. An obvious relationship has been established between the presence of microorganisms and the degree of pulpal response.[6,10] In an excellent review, Bergenholtz[18] indicated that even a thin wall of primary dentin, if intact, often prevents the deleterious effects of both toxic materials and bacterial leakage. The biocompatibility of materials is directly affected by bacterial contamination (eg, leakage) as well as their intrinsic toxicity (eg, effects of constituents such as acids and components such as catalysts and photoinitiators). Therefore, both the sealing ability and the toxicity of the material are important factors in predicting pulpal responses to vital pulp therapy.[18,19]

Factors That Influence the Outcome of Pulp Capping and Repair

Size of pulpal exposure

Several studies suggest that the size of the pulpal exposure may influence case selection. Many dentists believe that for pulp capping to be successful the exposure must be less than 1.0 mm in its major dimension and the patient must be young.[20,21]

Pulpal exposures that are too large may have greater risk of adversely reacting to microleakage and may be very difficult to restore. However, partial pulpotomies after traumatic crown fractures have been shown to produce a 96% success rate with an average 31-month follow-up, even for pulpal exposures ranging from 0.5 to 4.0 mm in size.[12] Thus, the size of the exposure, within this range, does not appear to be a major factor in the success of vital pulp therapy. Similar success rates were observed in teeth with immature and mature roots.[12,21,22]

Presence of dentin chips

Pulpal exposures are often contaminated with dentin chips, or debris, resulting from the use of rotating instruments in caries removal and tooth preparation procedures (Fig 13-5). The question of whether these dentin chips promote or retard healing remains controversial. Some researchers believe that dentin chips encourage the formation of a dentin bridge.[23–25] However, if dentin chips are forced into the deeper coronal pulp tissue by a rotating instrument, they may produce a pulpitis with abscess formation, especially if contaminated with oral microflora. One study found that unintentional deep impaction of the medicament and dentin chips in primary teeth led to an increased inflammatory response.[26]

Ideally, any capping agent should be placed gently on the exposed pulp surface and not in the deeper pulp tissue because deep impaction of particles of the pulp capping material can also reduce the success of dentin bridge formation and healing. Control or limitation of impaction is a clinical challenge and one that is not always achieved (see Fig 13-5). Studies have shown that particles of certain $Ca(OH)_2$ formulations can be phagocytosed. Particles that are no longer chemically active can be retained indefinitely in macrophages and giant cells in the healed area beneath the bridge and adjacent normal areas.[21]

One study used a cell culture model system to evaluate the ability of $Ca(OH)_2$ to alter macrophage function.[27] Inflammatory macrophages were obtained from rats, and substrate adherence capacity assays indicated that $Ca(OH)_2$ decreased substrate adherence in a time- and dose-dependent manner. Thus, $Ca(OH)_2$ inhibited macrophage function and reduced inflammatory reactions when used in direct pulp capping and pulpotomy procedures. This may explain, at least in part, the mineralized tissue induction property of the agent.

In another study, an adhesive system applied to exposed human pulp tissue caused large areas of neutrophil infiltration and death of odontoblasts.[28] The inflammatory infiltrate was subsequently replaced with fibroblasts, macrophages, and giant cells in the coronal pulp tissue; this response inhibits pulp repair and dentin bridging. Together, these studies suggest that the sealing ability of the agent, the method of placement (eg, minimizing the impaction of pulp capping agents in dental pulp), and the chemical nature of the pulp capping material are all critical factors in pulpal healing.

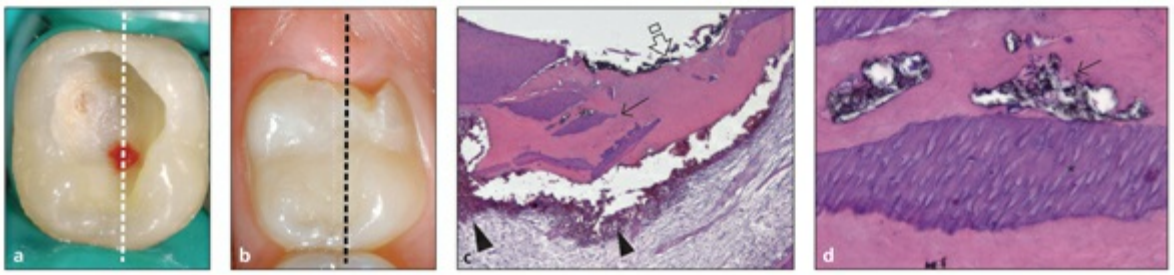

Fig 13-5 *(a)* Mandibular left second molar of a 17-year-old girl. Caries excavation resulted in exposure of mesiobuccal and distolingual pulp horns. After control of hemorrhage, pulp capping was performed with Dycal (Dentsply Caulk) and followed by the placement of a light-cured resin-modified glass-ionomer liner (Vitrebond). The cavity was restored with resin composite. *(b)* The patient presented 19 months later with fracture of the distal marginal ridge. No symptoms were present. The tooth responded normally to pulp tests. Radiographically, the margin of the fracture was close to the periodontal bone. After different treatment options were discussed, the tooth was extracted to allow eruption of the third molar. *(c)* The tooth was processed for light microscopy for serial sectioning. This section represents the one that passed through the mesiobuccal exposure site. A calcified barrier is present and dentin chips *(arrow)* are embedded within the barrier. $Ca(OH)_2$ remnants *(open arrow)* are present on top of the calcified tissue, which exhibits tubular characteristics. The pulp tissue is uninflamed and appears detached from the hard tissue because of hemorrhage *(solid arrowheads)* that resulted from a crack created during extraction (H&E stain; original magnification ×50). *(d)* Higher magnification of the area indicated by the arrow in *(c)*. The dentin chip *(asterisk)* is completely surrounded by an amorphous eosinophilic calcified mass. In addition, $Ca(OH)_2$ remnants *(arrow)* that were previously pushed into the exposed pulp are trapped within the calcified mass (H&E stain; original magnification ×400). (Courtesy of Dr Domenico Ricucci, Rome, Italy.)

Hemostatic control of hemorrhage and plasma exudate

The need for hemorrhage control was first investigated by Marzouk and Van Huysen[29] in 1966, and others have since confirmed its necessity.[30–32] Chiego[33] has suggested that operative trauma may evoke very rapid changes in the dental pulp, leading to permeation and leakage of plasma proteins out of the tubules to the cut dentin surface. Such leakage could inhibit wound healing (ie, dentin bridge

formation).

Hemorrhage of exposed dental pulp tissue is in part due to the inflammatory response of the pulp to bacteria and their by-products from carious dentin. The trauma of caries removal leading to the exposure may increase the amount of bleeding encountered. It is apparent from several studies that the materials placed against a bleeding pulp will not lead to subsequent tertiary dentin formation and bridging and may not lead to maintenance of vital pulp tissue (see next section). Studies have examined the use of hemostatic agents placed over the exposure to halt hemorrhage and allow capping materials to be placed in a relatively dry environment. Sodium hypochlorite (NaOCl) has been suggested to remove the coagulum, control hemorrhage, remove dentin chips, and aid formation of a dentin bridge.[34]

In one study, Class V cavities (n = 90) with mechanical pulpal exposures were prepared in adult monkey teeth. Hemorrhage was controlled with a 3% solution of NaOCl.[35] All-Bond 2 (Bisco Dental; n = 22), One-Step and Resinomer (Bisco Dental; n = 26), and $Ca(OH)_2$ and amalgam (n = 42 positive controls) were used as pulp capping agents. The pulp tissue was examined at 7, 27, and 90 days. Eighty-six percent of pulps irrigated with NaOCl prior to adhesive placement showed normal soft tissue reorganization and dentin bridge formation.

In another study, several hemostatic agents were used when Class II preparations were placed in 25 human teeth scheduled for removal for orthodontic reasons.[36] Teeth capped with an adhesive system after hemorrhage control with saline, ferric sulfate, 2.5% NaOCl, and a $Ca(OH)_2$ solution showed a tissue response that varied from acute inflammation to necrosis and no dentin bridge formation. The group with saline rinses to control hemorrhage and $Ca(OH)_2$ as a capping agent demonstrated bridge formation. In a subsequent study that utilized $Ca(OH)_2$ instead of a dentin adhesive as the pulp capping agent,[37] the authors also observed that the use of saline as a hemostatic agent resulted in significantly better pulpal response and dentin bridge formation than did the use of 2.5% NaOCl.

Saline (n = 14), 5.25% NaOCl (n = 16), and 2% chlorhexidine digluconate (n = 15) were used for hemorrhage control in another study,[38] and the exposures were covered with a hard $Ca(OH)_2$ cement. The results demonstrated that the three hemostatic agents did not impede the healing process of the dental pulp to $Ca(OH)_2$ capping. It is apparent that more work is needed to better define the use of these agents prior to capping, especially when they will be used in combination with other

materials, such as mineral trioxide aggregate (MTA), now suggested for these procedures.

The development of pulpal edema can have several deleterious effects, including extrusion of pulp tissue, dislodgment of the pulp capping material, loss of an effective seal against bacterial invasion, development of a chronic inflammatory infiltrate, and inhibition of tertiary dentin formation. Accordingly, the use of a hemostatic agent may be recommended in the future for all vital pulp procedures. Ideally, clotting of the capillaries within the subjacent pulp tissue should occur.[21]

The presence of open or cut vessels can carry capping material particles into the deeper pulp tissue. In a large mechanical exposure, especially one resulting from a traumatic injury or following a pulpotomy, many vessels may be dilated or transsected. Sometimes particles of the capping material may enter these vessels and travel until lodged in the vessel as it diminishes in size near the apical portion of the root canal (Fig 13-6). At these sites, the chemical or caustic effects of agents such as the fresh particles of $Ca(OH)_2$ (if still chemically active and especially if from high-pH formulations) produce perivascular foci of mummification and inflammation. If the particles are from low-pH $Ca(OH)_2$ formulations, they merely block the vessels and decrease pulpal blood flow, which may lead to delayed or inadequate healing.

Quality of the dentin bridge

If dentin bridge formation is essential to the success of pulp capping procedures, then the presence and quality of the dentin bridge are important prognostic factors for clinical success. A dentin bridge will form with appropriate $Ca(OH)_2$ treatment, permitting intimate contact with remaining pulp tissue (Fig 13-7). Although the integrity of a dentin bridge may be suspect (see also chapter 2), it nevertheless serves as a physical barrier to protect the pulp. Figure 13-8 shows a thin, porous dentin bridge that formed in 4 weeks. Figure 13-9 represents an example of a thick, dense bridge that formed over a period of 90 days. Thus, dentin bridges may continue to form over time, and most pulps survive despite the presence of a porous dentin bridge[21] (Fig 13-10). However, incomplete dentin bridges may occasionally result in the breach of the physiologic seal, bacterial ingress, formation of microabscesses, and persistent inflammation adjacent to the exposed part of the dentin bridge (Fig 13-11).

Additional studies have shown that exposed pulps survive even in the absence of dentin bridges. The theory is that acid etching and bonding techniques adequately seal the exposure sites from bacterial invasion so that the inflammatory pulpal response to bacteria or their by-products does not occur, obviating the need for a dentin bridge. This theory is based on the observation that the newer dentin bonding agents can prevent recontamination of the exposed pulpal surface by forming a seal that protects against bacterial invasion. The bonding agent will penetrate the dentinal tubules approximating the exposure site and create an impenetrable hybrid layer that prevents subsequent microleakage when followed by placement of a permanent restoration.

This theory is no longer acceptable in light of more recent findings on the adverse effects of direct resin pulp capping. Rather, the results of preliminary studies have indicated that the application of adhesive systems in direct contact with healthy pulp tissues does not result in the expression of proteins or signals that are essential for pulp repair,[41] that is, signaling molecules that replace the original ectomes-enchymal signals necessary for inducing odontogenesis (see chapter 2). For example, MTA also provides a good seal but produces dentin bridges,[42] probably due to its ability to dissolve bioactive dentin matrix components[43] and the activation of transcription factors[44] (see chapter 1) that act as signaling molecules for pulp repair.

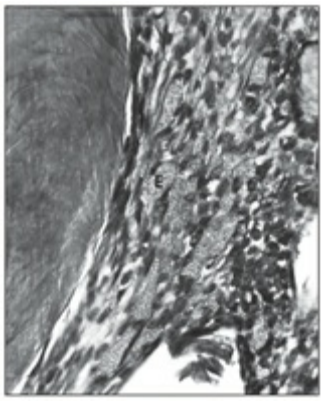

Fig 13-6 Emboli (E) of $Ca(OH)_2$ (Prisma VLC Dycal [Dentsply]) in numerous blood vessels far removed from the exposure surface (H&E stain; original magnification ×480). (Reprinted from Stanley and Pameijer[39] with permission.)

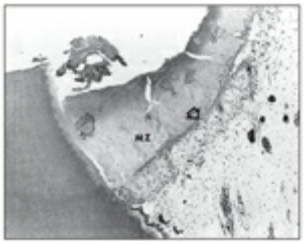

Fig 13-7 Pulpal response to a $Ca(OH)_2$ saline paste at 7 days. Note the thickness of the mummified

zone (MZ). The *arrow* denotes the line of demarcation between the mummified zone and the dental pulp. A new layer of odontoblast-like cells is forming subjacent to the mummified zone (H&E stain; original magnification ×80). (Reprinted from Turner et al[26] with permission.)

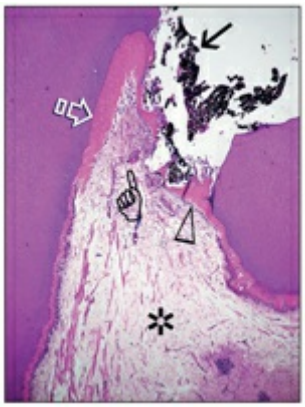

Fig 13-8 Example of a porous dentin bridge created in human pulp tissue that was mechanically exposed and capped with Dycal for a 30-day period. A partial hard tissue barrier *(open arrowhead)* was present underlying the capping agent *(solid arrow)*. Additional reparative tertiary dentin had been formed adjacent to the site of pulpal exposure *(open arrow)*. A mild inflammatory pulpal response is present immediately below the pulp capping site *(pointer)*. The subjacent pulp tissue exhibits normal histologic characteristics with no inflammatory response *(asterisk)* (H&E stain; original magnification ×32). (Courtesy of Drs Carlos Alberto de Souza Costa and Josimeri Hebling, Sγo Paulo, Brazil.)

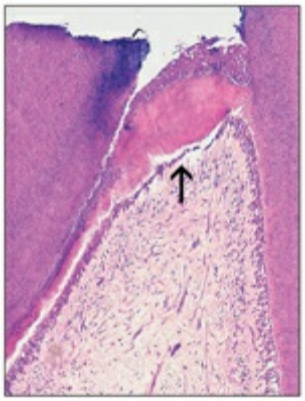

Fig 13-9 Example of a high-quality, dense dentin bridge in human pulp tissue that was mechanically exposed and capped with Dycal for a 90-day period. New odontoblasts (odontoblast-like cells *[arrow]*) were present underlying the complete hard tissue barrier. The subjacent pulp tissue exhibits normal histologic features (H&E stain; original magnification ×32). (Courtesy of Drs Carlos Alberto de Souza Costa and Josimeri Hebling, Sγo Paulo, Brazil.)

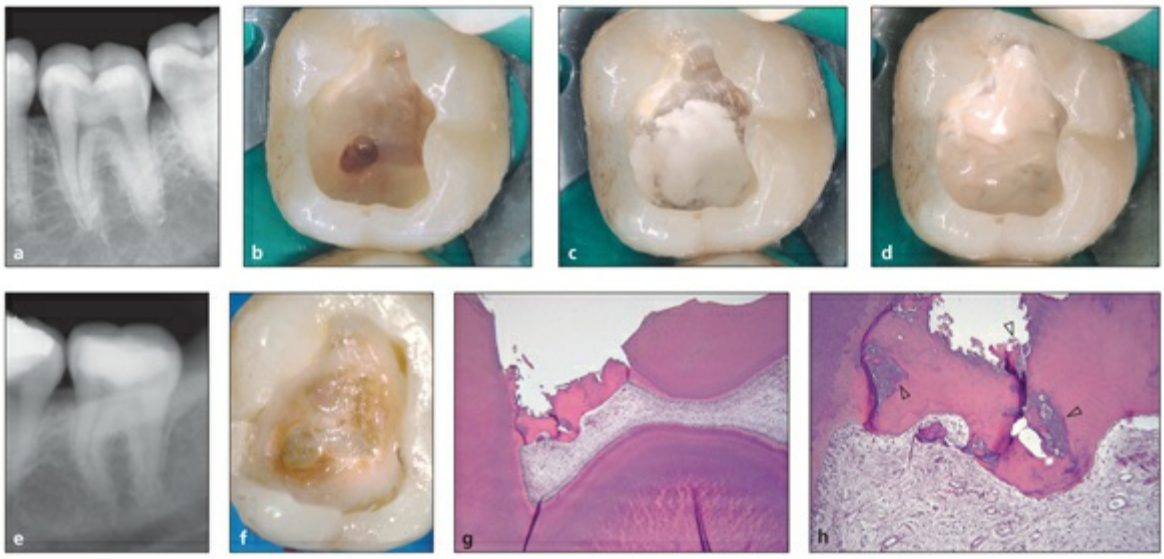

Fig 13-10 *(a)* Radiograph of a mandibular molar of a 26-year-old man showing deep occlusal caries. The patient was asymptomatic, and sensitivity tests yielded normal responses. *(b)* During caries removal, a large exposure of the lingual pulp horn was produced. *(c)* Following hemorrhage control, pulp capping was performed with $Ca(OH)_2$ powder. *(d)* The powder and the remaining dentin were covered with Dycal. The cavity was restored with IRM (Dentsply Caulk). *(e)* The patient never returned for the final restoration. He presented after 2 years 4 months with acute pericoronitis. The restorative material was in good clinical condition with little marginal deterioration. The tooth responded normally to sensitivity tests. A radiograph showed normal periapical conditions. *(f)* The patient decided to have the tooth extracted. Restorative materials were removed after extraction and a hard tissue barrier was present in the previous exposure site. *(g)* The tooth was processed histologically. Sections were taken on a mesiodistal plane passing through the exposure site. An irregular barrier is present covering the defect (H&E stain; original magnification ×25). *(h)* Detail of the calcified tissue in *(g)*. The calcified barrier shows tunnel defects containing necrotic tissue *(open arrowheads)*. These spaces provide pathways for bacterial leakage. The calcified tissue mass does not resemble dentin in that it does not have dentinal tubules. In addition, no odontoblasts are seen underlying the calcified tissue while they form a normal layer on the adjacent mesial root canal wall. The pulp tissue beneath the barrier is uninflamed (H&E stain; original magnification ×100). (Reproduced and modified from Ricucci[40] with permission.)

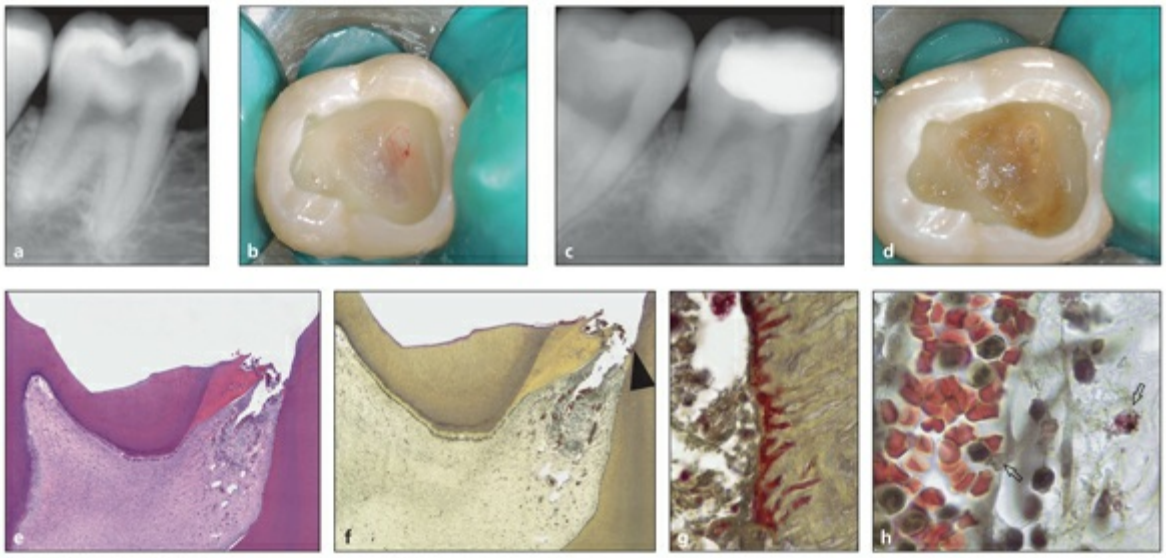

Fig 13-11 Molar of a 32-year-old man who presented with pain on mastication, lingering sensitivity to cold stimuli, and episodes of spontaneous pain. *(a)* A radiograph showed deep occlusal caries approximating the mesial pulp horns. *(b)* Caries excavation resulted in exposure of the mesiolingual pulp horn. Pulp capping was performed with $Ca(OH)_2$ powder followed by Dycal. The cavity was restored with IRM, and the patient was asked to return after 3 months. *(c)* The patient presented after 7 months because of severe, spontaneous pain. The radiograph did not show periapical changes. *(d)* A decision was made to examine the cavity floor. After removal of all the restorative materials, calcified tissue was seen at the site of the previous exposure, but a sharp probe could penetrate into the subjacent pulp. Probing did not provoke bleeding. The patient did not accept any further treatment and requested extraction. *(e)* Histologic section passing through the exposure site. The overview confirms the incompleteness of the calcified barrier and an abscess in the mesial pulp horn (H&E stain; original magnification ×16). *(f)* Proximal section. Note the transition between the abscess and the relatively normal pulp tissue (Taylor's modified Brown & Brenn stain; original magnification ×16). *(g)* Higher magnification of the area from the mesial wall indicated by the arrowhead in *(f)*. Bacteria are present within the dentinal tubules (Taylor's modified Brown & Brenn stain; original magnification ×1,000). *(h)* Higher magnification from the inflamed area under the perforation showing the cellular aspect of acute inflammation. The lumen of a vessel is congested with erythrocytes and polymorphonuclear leukocytes. An elongated inflammatory cell is visible; its cytoplasm adheres to the vessel wall in the process of transmigration through the endothelial cell or cell junction. Outside the vessel, the cytoplasm of a cell is filled with phagocytosed foreign bodies that appear to be bacterial fragments *(arrows)* (Taylor's modified Brown & Brenn stain; original magnification ×1,000). (Modified from Ricucci[40] with permission.)

Calcium hydroxide treatment

From a historical perspective, the introduction of $Ca(OH)_2$ products played an important role in the development of vital pulp therapy. The first materials to show promise as pulp capping agents were dentin chips and pastes utilizing $Ca(OH)_2$.[45–47]

Numerous later studies have demonstrated dentin bridge formation in 50% to 87% of teeth treated with various $Ca(OH)_2$ formulations.[11,48–52] However, despite its long history, the use of $Ca(OH)_2$ in vital pulp therapy remains controversial.

Part of this controversy concerns the caustic actions of $Ca(OH)_2$. When applied to dental pulp in the pure state, rather than functioning merely as a biologic dressing, $Ca(OH)_2$ actually destroys a certain amount of pulp tissue. Because $Ca(OH)_2$ is also extremely toxic to cells in tissue culture, this destructive characteristic has triggered efforts to find a formula that can stimulate reparative dentin bridging without inducing this caustic effect.

Numerous studies have shown that $Ca(OH)_2$ is capable of promoting the formation of reparative dentin at the junction between the caustic zone and vital tissue in human subjects.[53,54] The caustic actions of the high-pH formulations of $Ca(OH)_2$ reduce the size of the subjacent dental pulp by up to 0.7 mm; the thickness of the resulting dentin bridge also reduces the pulp size.[55] In contrast, the low-pH formulations of $Ca(OH)_2$ have only a minor effect because only the thickness of the dentin bridge reduces the bulk of the remaining vital pulp tissue.[56]

One advantage of $Ca(OH)_2$ is its antimicrobial characteristics. Classic studies have demonstrated that bacteria represent the primary etiologic agent of pulpal necrosis[6,10] (see Figs 13-1 to 13-4), suggesting that antimicrobial properties may confer therapeutic advantages. In one study, canine pulps were exposed to *Streptococcus sanguinis* for 2 days prior to placement of $Ca(OH)_2$; nonetheless, thick dentin bridges formed 10 weeks later.[49] In a primate study with a 1- to 2-year follow-up, $Ca(OH)_2$-induced dentin bridge formation occurred in 78 (85%) of 91 exposed and contaminated dental pulps, while 10% of the pulps in the study sample became necrotic.[52] Well-controlled clinical trials evaluating this effect are usually not possible because it would be necessary to contaminate the pulps of human subjects.

Another advantage of $Ca(OH)_2$ is its ability to extract, from mineralized dentin, "fossilized" growth factors and bioactive dentin matrix components that induce dentin regeneration at the site of pulpal exposure.[57] More recently, it has been shown that calcium ions released from $Ca(OH)_2$ stimulate fibronectin synthesis by dental pulp cells, which in turn may induce the differentiation of pulp progenitor cells into mineralized tissue-producing phenotypes.[58]

Other studies have evaluated the ultrastructure of $Ca(OH)_2$-induced dentin bridges

to determine whether the structure was permeable and yet still provided satisfactory pulpal protection.[59] Scanning electron microscopy was employed to evaluate dentin bridges formed 4 to 15 weeks after pulp capping of deliberately exposed human premolars and third molars (teeth scheduled for removal for orthodontic reasons) with a $Ca(OH)_2$ paste.[60] Results suggested complete bridging and increasing thickness over longer posttreatment periods. Cross sections of pulps treated for more than 6 weeks demonstrated a superior amorphous layer of tissue debris and $Ca(OH)_2$, a middle layer of a coarse meshwork of fibers identified as fibrodentin, and an inner layer showing tubular osteodentin. In a later study using microradiographic techniques, the same three layers in the dentin bridge were observed; the middle tubular layer exhibited the highest mineral content.[61]

Another study comparing the hard tissue barrier formed following short-term applications of either cyanoacrylate or $Ca(OH)_2$ in pulpotomized monkey teeth showed that $Ca(OH)_2$ increased the incidence of a continuous dentin barrier below the level of the original wound. The condition of the pulp was negatively affected by the presence of bacteria and positively influenced by high continuity of the hard tissue, and results suggested that low-grade irritation was responsible for hard tissue barrier formation.[62]

However, other studies have reported porosity in $Ca(OH)_2$-induced dentin bridges, and the term *tunneling* has been used to describe incomplete dentin bridge formation (Fig 13-12). A summary of several primate studies involving direct pulp capping with $Ca(OH)_2$ reported a number of inflamed and infected pulps after a follow-up period of 1 to 2 years.[64] The authors proposed that these findings resulted from deterioration of the overlying restorations and subsequent migration of microorganisms through tunnels within the dentin bridges. Of 192 dentin bridges in primate teeth, 172 (90%) contained tunnel defects. The authors also questioned the long-term efficacy of commercially available $Ca(OH)_2$ bases, particularly in light of the potential for microleakage.[64]

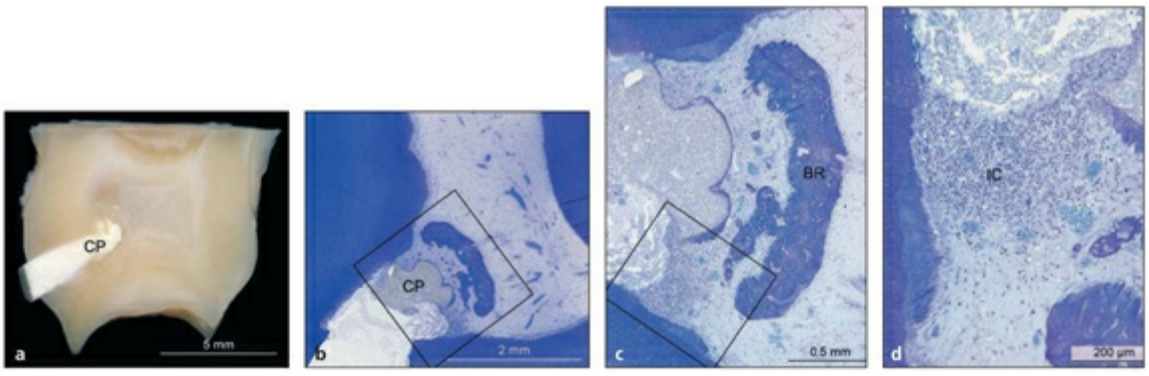

Fig 13-12 Mixed pulpal response to Dycal capping after 3 months of observation. *(a)* A distal macrophotographic view of the mesial half of a maxillary right third molar shows the remnants of the restorative and capping material (CP) as a white plug in the cavity preparation and pulp chamber. *(b)* This photomicrograph is a segment of a histologic section of the specimen in *(a)*. Note the cavity opening into the pulp chamber and remnants of the capping material (CP). *(c)* Higher magnification of the rectangular area demarcated in *(b)*. The distinct but incomplete hard tissue bridge (BR) reveals gaps on either side. *(d)* Higher magnification of the rectangular area demarcated in *(c)*. An infiltrate of chronic inflammatory cells (IC) is visible in the gap (periodic acid–Schiff and methylene blue–azure II stains; original magnification ×6, ×18, ×46, and ×120, respectively). (Reproduced from Nair et al[63] with permission.)

Molecular Responses to Pulpal Exposure

Vital pulp therapy attempts to maintain pulpal vitality and function and restore normal tissue architecture subsequent to the pulp capping procedure.[65] For a successful outcome, the healing response must have the following three characteristics: *(1)* rapid formation of hard tissue to protect the pulp from other stimuli (oral bacteria); *(2)* formation of a barrier that prevents secondary pulp infections; and *(3)* induction of hard tissue formation at the pulp-material interface, not within the pulp itself, to avoid obliteration of the pulp. These characteristics represent the desirable healing process that should occur when any substance is applied directly to the pulpal exposure site that is capable of stimulating dentinogenesis.[66]

Despite the successful use of $Ca(OH)_2$ as a pulp capping agent for 60 years,[7] predictable outcomes remain a problem. For example, a retrospective study that examined $Ca(OH)_2$ pulp capping of carious exposures in 123 teeth revealed that 45% failed in the 5-year group and 80% failed in the 10-year group.[67] In that study, the placement of a definitive restoration within the first 2 days after pulpal exposure was found to contribute significantly to the survival rate of those teeth. A more recent study that examined the treatment outcomes of 248 teeth that had undergone direct pulp capping with $Ca(OH)_2$ for 0.4 to 16.6 years showed that the overall survival rate was 76.5% after 1.3 years.[68] In that study, the pulps of 60-year-old patients showed a less favorable outcome than did the pulps of patients younger than 40 years. Also, the likelihood that a tooth would become nonvital after direct pulp capping was higher within the first 5 years after treatment than it was more than 5 years after treatment.

Apart from the contribution of microbial leakage to the failure of direct pulp capping, another important issue is likely to be the lack of appropriate stimulating factors for dentin formation. Researchers have begun to unravel the molecular biologic responses of pulp cells and their responses to injury and pulp therapy. This approach holds great promise, but no clear guidelines have been developed at this point because of the complexities involved. Semiquantitative reverse transcription polymerase chain reaction analysis confirmed that markers preferentially expressed in odontoblasts, namely dentin sialophosphoprotein (DSPP) and nestin, amplified more readily from the extracted pulp-odontoblast complex than from pulp tissue alone. In addition, analysis characterizing the expression of members of the transforming growth factor (TGF) and bone morphogenetic protein (BMP) families and their receptors indicated that these genes, which were expressed by healthy odontoblasts, were upregulated in both pulpal cells and odontoblasts in response to pulpal injury.[69] Certain inflammatory and antimicrobial peptides produced by neutrophils, such as macrophage inflammatory protein-3α and β-defensin-2, have the ability to stimulate odontoblast differentiation via upregulation of *DSPP* gene expression.[70]

The pulp contains progenitor cells that can differentiate into odontoblast-like cells in the event the original odontoblasts are so severely injured that they undergo necrosis. Recombinant human BMP-2 promoted the differentiation of human pulp cells into odontoblasts[71] but did not affect cell proliferation. A study aimed at identification of the changes in gene-expression profiles in the dental pulp under caries lesions identified 445 genes with twofold or greater differences in expression levels; 85 genes were more abundant in healthy pulp tissue, while 360 were more abundant in diseased tissue. Real-time polymerase chain reaction analyses of adrenomedullin and dentin matrix protein 1 showed increased expression of adrenomedullin in neutrophils activated by bacterial products, while dentin matrix protein 1 was expressed by cells in healthy pulp tissue.[72]

Evolutionarily conserved pathways such as Notch signaling control the developmental fate of multipotent stem cells into functional cell types (see chapter 1). In a study of adult rats, in situ hybridization of exposed maxillary first molars that were pulp capped with $Ca(OH)_2$ revealed an increased expression of Notch 1 signaling genes on day 1 that decreased on day 3. Notch 2 expression increased in areas of tissue surrounding coronal odontoblasts, while Notch 3 expression increased in areas of perivascular cells. These results indicated that Notch signaling is activated in the dental pulp in response to injury and is associated with differentiation of pulpal stem cells into perivascular cells and odontoblast-like

cells.[73]

Apoptosis (programmed cell death) is associated with wound healing and regeneration of tissue. Examination of the effects of heat stress on a clonal dental pulp cell line (RPC-C2A cells) was carried out by exposing the cells to heat stress of 43°C for 45 minutes. Apoptosis was induced in some cells, but other cells remained alive and acted as scavenger-like cells and engulfed apoptotic cells.[74] This suggests a possible pathway for partial healing of pulpal wounds created by heat generated during cavity preparations. In another study, primary pulp cells and dental pulp stem cells were cultivated and exposed to heat shock between 37°C and 45°C for 5- to 10-second durations. Thermal stimulations enhanced leukotriene B_4 (LTB_4) synthesis for both cell cultures at all temperatures.[75] The arachidonic acid mediator LTB_4 has the capacity to induce inflammatory reactions and sensitize nociceptive nerve endings (see chapter 11). The study demonstrated that pulp cells have the capability to synthesize LTB_4 in response to minor temperature changes and that the generation of this inflammatory mediator may inhibit wound healing after cavity preparations and pulpal exposures.

Extracellular matrix proteins such as fibronectin and tenascin are important for cell adhesion during the early stages of wound healing. $Ca(OH)_2$ was placed over intentional exposures in third molars and covered with zinc oxide–eugenol cement. Teeth were removed at times between 1 and 30 days. Immunohistochemical analysis showed increased expression of both glycoproteins, indicating their role in the healing of dental pulpal exposures.[76]

Although the information presented by the aforementioned studies appears disconnected, the results serve to illustrate the complexity of the processes involved in pulpal wound healing and repair at the gene-expression and protein-transcription levels following pulpal exposure and direct pulp capping. Much more work will be required to understand these molecular events before they can be incorporated into predictable pulp therapy techniques for restoring the dental pulp to its preinjury form and function. Nevertheless, with the recent advances in molecular biology and pulpal regeneration research, it is possible to envision the future availability of materials, medicaments, and delivery techniques, perhaps applied based on a controlled-release approach, that will ultimately address the clinical problem of pulpal exposure (see chapter 2).

Comparison of $Ca(OH)_2$ and Other Materials for Vital Pulp Therapy

Studies utilizing $Ca(OH)_2$ as the sole pulp capping agent appear to be of little interest to investigators today, leading to newer studies comparing it to other materials. Researchers have investigated the concept that the pulp capping agent itself can serve as the equivalent of a reparative dentin bridge, thereby reducing the loss of remaining vital pulp tissue.[56] This theory has been previously tested with dentin chips,[23] synthetic hydroxyapatite,[77,78] and Bioglass (US Biomaterials).[79,80] The results of these earlier studies, in general, indicated that the tested materials demonstrated limited pulp repair. Healing was consistently hampered by continuing inflammatory responses with little, if any, tertiary dentin formation. Conversely, the $Ca(OH)_2$ control groups demonstrated formation of tertiary dentin without significant inflammatory responses.

Many studies, beginning in the mid-1990s, have involved the use of glass ionomers (GIs) and dentin adhesives as direct pulp capping agents. In general, results support the following statements:

- Both systems may work well when there is no pulpal exposure and there is at least a minimum amount of remaining dentinal thickness.
- In direct exposures, results remain controversial at this time.
- If there is bacterial microleakage, vital pulp therapy will fail, whereas $Ca(OH)_2$ is sufficiently bactericidal and usually leads to dentin bridge formation.

More recently, there has also been great interest in the use of MTA for direct pulp capping and vital pulp therapy. These newer materials are reviewed in the following sections.

Resin-modified glass ionomers

Pulpal responses to indirect pulp capping of deep dentin and caries lesions with GIs and resin-modified glass ionomers (RMGIs) are covered in chapter 14. RMGIs have

been used as definitive restorative materials to decrease microleakage because of their capacity to bond to the tooth structure[81] and their antimicrobial effects.[82]

Although they have been successful as indirect pulp capping agents even in cavities with minimal remaining dentinal thickness,[83,84] the histopathologic responses to RMGIs as direct pulp capping agents remain controversial because there are very few human clinical studies available that involve the application of GI systems directly to exposed pulps.[85] An earlier primate study that involved the placement of an RMGI as a direct pulp capping material failed to demonstrate any sign of dentin bridge formation.[86] A subsequent long-term primate study showed that the use of an RMGI (Vitrebond, 3M ESPE) as a direct pulp capping agent for more than 2 years resulted not only in dentin bridge formation but also in less bacterial leakage, fewer tunnel defects within dentin bridges, and less pulpal inflammation than did the use of $Ca(OH)_2$ as a pulp capping agent.[87]

Contrary to these favorable responses reported with the use of RMGIs in primates, poor responses have been reported in human teeth. Human teeth with intentional mechanical exposures that were capped with Vitrebond were found to exhibit moderate to intense inflammatory pulpal responses, including large necrotic zones, lack of dentin bridge formation, and impaired healing.[88] Thus, RMGIs do not appear to be appropriate materials for direct pulp capping in human teeth.

Dentin adhesives

A number of usage and clinical studies in the late 1990s and early 2000s have reported that exposed nonhuman and human dental pulps and periradicular tissues will heal when capped with etch-and-rinse dentin adhesives[89–92] (Figs 13-13 and 13-14). Most of the adhesive usage studies from that era reported a lack of bacterial staining along the cavity walls or within the pulp at long-term intervals. These results were confirmed in a subsequent long-term primate study showing that direct pulp capping with dentin adhesives achieved significantly better results than did the use of $Ca(OH)_2$ as a direct pulp capping agent.[87] The collective biologic assessment suggested that most adhesives are biologically compatible when placed directly on exposed vital pulp tissue and are comparable to or even better than $Ca(OH)_2$ controls.

Opposing results, however, were demonstrated in other studies. The pulp horns of 51 sound human premolars were gently exposed (exposures irrigated with sterile saline) and capped with either an adhesive resin or $Ca(OH)_2$.93 In the short term, the adhesive-treated teeth exhibited dilated, congested vessels with a moderate inflammatory response that included blanching of pulp cell nuclei. Long-term results revealed no evidence of healing or dentin bridge formation and the presence of a persistent inflammatory response; microabscesses were associated with bacterial infiltration (Fig 13-15). With time, macrophages and giant cells engulfed resinous materials that had been displaced into the pulp space. Conversely, pulps capped with $Ca(OH)_2$ showed an initial organization of elongated pulp cells beneath coagulation necrosis, eventual pulp repair, and complete dentin bridging.

Other studies indicate that adhesives allow greater bacterial penetration and cytotoxic effects than those observed with $Ca(OH)_2$[94,95] and that light-cured resins should be avoided for direct pulp capping.[96] Adhesive systems placed in direct contact with mechanically exposed pulp in healthy dog teeth did not lead to acceptable repair.[96] Compared with dentin adhesives, $Ca(OH)_2$ remains the agent of choice for mechanically exposed human dental pulp.[97] Similar conclusions have been reached about the use of the recently available self-etching adhesive systems for direct pulp capping. Intense, unresolved inflammatory responses and minimal pulp tissue repair were unanimously reported in those studies.[98–101]

Furthermore, many of the resin components employed in etch-and-rinse and self-etching dentin adhesives are vasorelaxants.[102–104] These resin components promote bleeding and impair healing when placed directly on exposed pulps after hemostasis has been successfully achieved with hemostatic agents. The accompanying plasma extravasation may also compromise polymerization of the dentin adhesives, resulting in increased cytotoxicity to the dental pulp.

It is apparent from the more recently available data that dentin adhesives are unacceptable and contraindicated as direct pulp capping agents. The critical issue that argues against the use of adhesive resin pulp capping procedures is not the presence or absence of a hard tissue barrier but the persistence of fairly intense inflammation and foreign body reactions that frequently accompany the application of such procedures.

The necessity of a physiologic hard tissue barrier is debatable because it is not totally impervious to bacterial invasion in the presence of tunnel defects.[64,83] This physiologic barrier may well be replaced by a synthetic, noncytotoxic hard barrier in the event that an intact, durable seal is established with resinous materials.

However, delayed healing is frequently observed even after the creation of an intact resinous seal over the exposed pulp. Recent studies have further indicated that this resinous seal is not as durable as it was previously conjectured[105] and that the resin-dentin bonds degrade over time (see chapter 14).

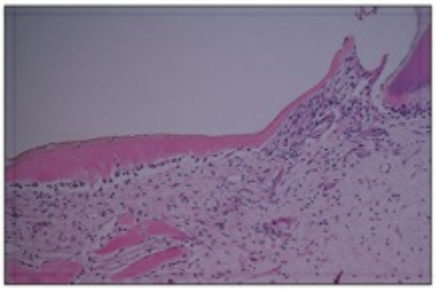

Fig 13-13 Histologic section of a nonhuman primate exposure that was capped directly with Resinomer for 27 days. A new (pink-stained) dentin bridge is seen directly at the material interface. New odontoblas-toid cells are located along thicker portions of the new dentin. Some operative debris chips remain in the lower left of the pulp. A thin, light, pink-stained mass of reparative dentin (mid-right) is seen at the pulp-dentin interface of the cavity wall. The deeper pulp is normal with no resin particles. No $Ca(OH)_2$ was employed in this procedure (H&E stain; original magnification ×125). (Courtesy of Dr Charlie Cox, Birmingham, AL.)

Fig 13-14 Histologic section of a nonhuman primate pulpal exposure that was treated with 5% NaOCl for 30 seconds to remove operative debris and control hemorrhage. The exposure was capped with resin for 7 days. No clot, coagulum, or operative debris is present. The pulp has migrated to the clear interface on the left, which was previously filled with resin. There is only a minimal presence of inflammatory cells. Odontoblasts are present along the remaining dentin, and a very thin zone of reactionary dentin has formed (H&E stain; original magnification ×50). (Courtesy of Dr Charlie Cox, Birmingham, AL.)

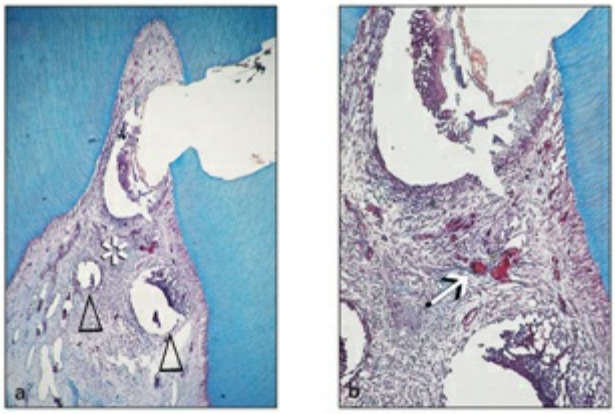

Fig 13-15 Unlike nonhuman primate exposures, mechanical exposures performed on noncarious human teeth have demonstrated unacceptable results following pulp capping with dentin adhesives. *(a)* A mechanical exposure that was capped with Single Bond (3M ESPE) for 90 days reveals no sign of dentin bridge formation; a persistent, moderately intense inflammatory infiltrate *(asterisk)*; and microabscess formation *(open arrowheads)* (Masson trichrome stain; original magnification ×10). *(b)* Higher magnification shows congested blood vessels *(arrow)* in the inflamed part of the pulp and delayed healing of the adjacent pulp tissues (Masson trichrome stain; original magnification ×20).

Mineral trioxide aggregate

While $Ca(OH)_2$ has been the material of choice in deep cavities and exposed pulps, MTA, a more recently developed Portland cement–based material, has gained great popularity. This material was first suggested as the material of choice for correcting procedural errors (canal system perforations and apical root stripping) that occurred during cleaning and shaping procedures in endodontic therapy. The material is often used in apicoectomy procedures as a root end filling material. In both instances, the material has been shown to be tissue compatible, encouraging the formation of new cementum-like hard tissue with restoration of the periodontal ligament, and is considered to have significant osteogenic potential.

MTA has now taken its place with $Ca(OH)_2$ as a material of choice in direct pulp capping procedures, and investigations into its ability to act in a manner compatible with formation of dentin bridges and retention of pulpal vitality have been encouraging, especially in the area of dentinogenesis. MTA has been compared to bioactive glass, ferric sulfate, and formocresol as a pulpotomy agent in rats.[106] After 80 rat maxillary first molars were exposed, pulpotomies were performed and capped with one of the materials. Histologic sections after 2 weeks showed that some MTA specimens displayed acute inflammatory responses with evidence of

macrophages in the radic-ular pulp. Dentin bridge formation and normal pulp biology was a finding at both 2 weeks and 4 weeks. Ferric sulfate resulted in moderate pulpal inflammation with widespread coronal necrosis. Formocresol resulted in zones of atrophy, inflammation, and fibrosis, and there was evidence of calcification in some samples. Bioactive glass induced inflammation at 2 weeks, but it was resolved at 4 weeks.

MTA was compared with $Ca(OH)_2$ in young permanent teeth undergoing apexogenesis (coronal pulpotomy, retention of root system vital pulp tissue, and immature root formation).[107] Two of 14 teeth in the $Ca(OH)_2$ group failed because of pain and swelling, while all in the MTA group appeared to be successfully treated. Calcific metamorphosis (mineralized tissue extension from the exposure site into the body of the pulp) was a radiographic finding in two teeth treated with $Ca(OH)_2$ and four teeth treated with MTA. When MTA and $Ca(OH)_2$ were compared in direct pulp capping procedures in dog teeth,[108] MTA presented a higher success rate than $Ca(OH)_2$, with a lower occurrence of infection and pulpal necrosis. No report was made concerning the loss of provisional restorations in any of the test teeth.

In some areas of the world, carious pulpal exposures of young permanent first molars are relatively common, leading to the use of MTA in large populations of young patients. Thus, 30 young permanent asymptomatic first molars were pulp capped with MTA. The teeth were assessed clinically with pulpal sensitivity testing and periodic radiographic observation, but no apparent controls were used. At 24 months, both the sensitivity and radiographic evaluations showed a 93% success rate.[109]

A similar study was conducted in 23 patients with carious exposures in 31 permanent first molars in patients 7 to 13 years of age. Pulpotomies were performed, and MTA was placed against the wound and covered with a GI. The teeth were restored with amalgam or stainless steel crowns and assessed radiographically and clinically over a 24-month period.[110] Twenty-two teeth showed no radiographic sign of disease and responded to clinical vitality testing. The remainder did not respond to testing but appeared normal radiographically. Again, proper controls were omitted in this study.

White ProRoot MTA (Dentstply Tulsa Dental) was compared to hard-setting $Ca(OH)_2$ (Dycal) for capping procedures (n = 24 for each group). A light-cured copolymer was placed over the pulp capping material, and a resin composite adhesive was used to restore the teeth.[111] The teeth were removed at 7, 30, or 136

days and evaluated histologically. Findings at 136 days showed that 20 teeth capped with MTA and 18 teeth capped with $Ca(OH)_2$ had developed a dentin bridge. No significant differences were found for superficial or deep inflammatory cellular responses at any of the time periods. Another study compared the same two materials, again using young permanent first molars. MTA and $Ca(OH)_2$ were judged to have comparable success rates.[112]

A more recent randomized clinical trial compared the pulpal responses to iatrogenic pulpotomy performed in healthy human teeth using MTA or Dycal.[63] Pulpal wounds treated with MTA were mostly free from inflammation after 1 week (Fig 13-16) and became covered with a compact hard tissue barrier within 3 months following capping (Fig 13-17). The control teeth treated with Dycal revealed distinctly less consistent formation of a hard tissue barrier that had numerous tunnel defects. The presence of pulpal inflammation up to 3 months after capping was identified as a common feature in the Dycal specimens (see Fig 13-12).

A similar study performed on intentionally exposed immature human permanent premolars also showed that slightly better results were achieved with white MTA than with $Ca(OH)_2$ in regard to the number of teeth that demonstrated definitive dentin bridge formation.[113] In a randomized controlled prospective study, no differences were identified in the histologic responses to pulp capping between two commercially available gray MTA formulations, MTA-Angelus (Angelus Soluções Odontológicas) and ProRoot MTA.[114] Analysis indicated that 94% of the human teeth capped with MTA-Angelus and 88% of those capped with Pro-Root MTA exhibited total or partial dentin bridge formation. Collectively, the results of all these studies indicate that MTA is as successful as or even more successful than $Ca(OH)_2$ in vital pulp therapy procedures.[115,116]

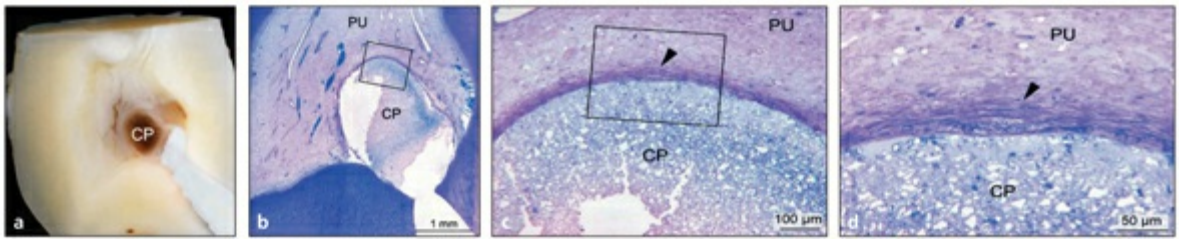

Fig 13-16 Pulpal response to MTA capping after 1 week of observation. *(a)* Distal macrophotographic view of the cut face of the mesial half of a maxillary left third molar with remnants of the restorative and capping (CP) material (white plug in the cavity preparation and pulp chamber). *(b)* This photomicrograph represents part of a histologic section of the specimen in *(a)*. Note the cavity opening into the pulp chamber, remnants of the capping material (CP), and healthy remaining pulp (PU). *(c and d)* Higher magnifications of the rectangular areas demarcated in *(b)* and *(c)*, respectively. The interface *(arrowheads)* of the pulp (PU) and cap (CP) shows fibrous encapsulation. Note the absence of pulpal inflammation (periodic acid–Schiff and methylene blue–azure II stains; original magnification ×7, ×18,

×90, and ×220, respectively). (Reproduced from Nair et al[63] with permission.)

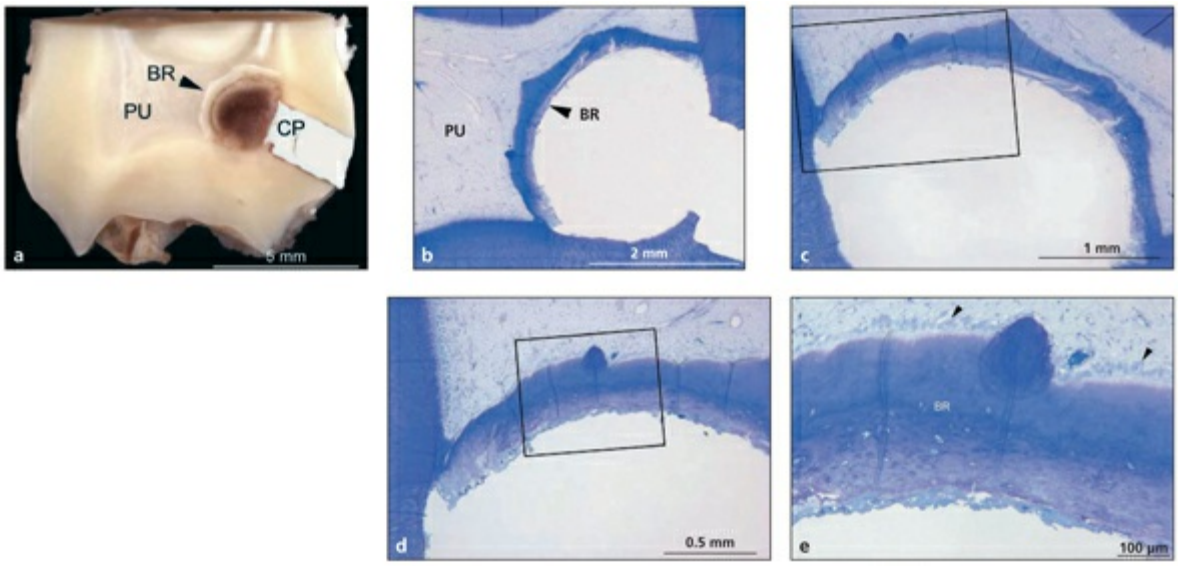

Fig 13-17 Pulpal response to MTA capping after 3 months of observation. *(a)* Distal macrophotographic view of the mesial half of a maxillary left third molar shows the remnants of the restorative and capping material (CP) and a distinct hard tissue bridge (BR) across the exposed pulp (PU). *(b and c)* These photomicrographs are part of a histologic section of the specimen in *(a)*. Note the mineralized hard tissue barrier (BR) stretching across the full length of the exposed pulp (PU). *(d)* Higher magnification of the rectangular area demarcated in *(c)*. *(e)* Higher magnification of the rectangular area demarcated in *(d)*. Note the cuboidal pulpal cells *(arrowheads)* lining the bridge (BR) and the absence of pulpal inflammation (methylene blue–azure II stains; original magnification ×6, ×8, ×23, and ×200, respectively). (Reproduced from Nair et al[63] with permission.)

Experimental bioactive molecule–containing materials

$Ca(OH)_2$ causes inflammatory responses in pulp tissue that may induce dentin bridge formation and pulpal healing if the restoration is sealed. Despite the controversial nature of this idea,[117] the last several years have seen a change in thinking from attempting to induce pulp repair through irritation to the use of substances that mimic normal developmental processes in response to cellular signaling mechanisms. These strategies involve selective activation of genes and other proteins that are necessary in dentinogenesis and may allow translation of findings from laboratory biomedical research into clinical practice. Investigations of these bioactive substances, while still in their infancy, include the roles of stem cells and genetic recruitment. This section examines studies that have employed bioactive molecules in experimental direct pulp capping procedures. Understanding the fundamental

mechanisms of biomineralization is of broad biologic interest, and it is envisaged that such mechanisms will eventually be used clinically to effect pulp repair and healing.

To evaluate the effects of bioactive molecules in pulpal wound healing, cavities were prepared and pulp tissues exposed in maxillary rat molars.[118] Comparisons were made between *(1)* dentin and predentin shavings implanted in the pulpal exposure and evaluated at 8, 14, and 28 days; *(2)* a carrier without bioactive substances; *(3)* $Ca(OH)_2$; *(4)* bone sialoprotein (BSP); *(5)* different concentrations of BMP-7 and osteogenic protein 1; and *(6)* N-acetyl cysteine, an antioxidant that prevents glutathione depletion. Of the first four agents examined after 28 days, BSP was the most efficient and induced homogenous and well-mineralized reparative dentin. BMP-7 induced formation of reparative osteodentin coronally and homogenous mineralized structure in the root canal. These findings indicate that the coronal and radicular parts of the pulp bear their own specificity. BSP and BMP-7 were superior to $Ca(OH)_2$, presenting larger areas of mineralization with fewer pulp tissue inclusions. The mineralization process appeared to take place by mechanisms that involved recruitment of cells that differentiated into osteoblasts, producing a mineralized extracellular matrix. Reparative dentin formation was also induced by N-acetyl cysteine.

The results of this study have been far-reaching in providing new avenues for direct pulp capping treatment. Nevertheless, a healthy pulp model was employed in the study.[118] This underestimated the potential difficulties encountered with the use of bioactive molecules for reparative dentinogenesis in inflamed dental pulps. Ferret dental pulps with inflammation induced by the use of lipopolysaccharides responded differently to exogenous BMP-7 and did not exhibit reparative dentinogenesis.[119]

Smith et al[120] adopted a different approach by using growth factors based on the TGF-β family. These bioactive molecules have been shown to signal cellular events leading to reactionary and reparative tertiary dentinogenesis. They may be released during active caries or other pulpal injuries and through subsequent tooth preparation procedures. Thus, this approach supports the regenerative potential of inflamed pulps and has led to more biologic approaches to clinical treatment of dental disease.

Smith[121] reiterated his belief that growth factors are key mediators in health and disease. Magloire et al[122] considered two major growth factors that may be implicated in the control of odontoblast activity: TGF-β1 released from demineralized dentin and nerve growth factor released from the pulp. Tooth slices

were immersed in either one of the two molecules and cultured at 4 and 7 days. Binding sites for the two molecules were detected on odontoblasts, suggesting that odontoblasts respond to factors expressed from dentin and pulp during tissue repair.

During odontogenesis, amelogenins from preameloblasts are translocated to differentiating odontoblasts in the dental papilla. The effect of enamel matrix derivative (eg, Emdogain gel in propyleneglycol alginate, Biora AB) on the healing of pulpal wounds was examined by placement of the molecule through Class V preparations and pulpal exposures in premolars of miniature swine.[123] Contralateral teeth were used as controls, and all preparations were sealed with GI. At 2- and 4-week intervals, the teeth were harvested and subjected to histologic analysis. The teeth treated with enamel matrix derivative displayed large amounts of newly formed dentin-like tissue; associated formative cells outlined the pulpal wound and separated the wound area from the underlying pulp tissue. Inflammatory cells were present in the exposure area but not subjacent to the newly formed hard tissue. The amount of new tissue was more than twice that found in $Ca(OH)_2$-treated controls.

Porcine enamel matrix was found to weakly enhance the formation of both reparative dentin and dentin bridges during wound healing of amputated rat molar pulp.[124] A 23–amino acid peptide derived from matrix extracellular phosphoglycoprotein, Dentonin (Acologix), was used successfully to stimulate dental pulp stem cell proliferation and/or differentiation.[125] Dentonin primarily affects the initial cascade of events leading to pulpal healing.[126]

However, application of bioactive molecules that is intended to stimulate repair of the exposed pulp could result in eventual mineralization and occlusion (closure) of the entire pulp canal system.[127] This event has been shown to occur with the use of BSP, BMP-7, Dentonin, and two small amelogenin gene splice products (A+4 and A–4). These splice variants cause proliferation and cell recruitment toward an odonto-osteogenic phenotype.[128] The same researchers implanted the gene splice products in the mucosa of the cheeks in mice. Immunohistochemical analysis at 3, 8, and 30 days revealed expression of osteochondrogenic markers.[129]

Goldberg et al[130] summarized what is known and/ or assumed concerning the biologic mechanisms of bioactive molecule therapies. It is generally believed that the repair of dental pulp by implantation of bioactive molecules implies a cascade of four steps: moderate inflammation, commitment of adult reserve stem cells, their proliferation, and their terminal differentiation.[131,132] The link between the initial inflammation and cell commitment is not yet well established but appears to be a potential key factor in the reparative process. Either the release of cytokines in

response to inflammatory events activates resident stem (progenitor) cells, or inflammatory cells or pulp fibroblasts undergo a phenotypic conversion into osteoblast/odontoblast–like progenitors implicated in reparative dentin formation. Activation of antigen-presenting dendritic cells by mild inflammatory processes may also promote osteoblast/ odontoblast–like differentiation and expression of the bioactive molecules implicated in mineralization. Recognition of bacteria by toll-like odontoblast and fibroblast membrane receptors (see also chapter 11) triggers an inflammatory and immune response within the pulp tissue that will also modulate the repair process.

In summary, dentin contains many peptides and signaling molecules within a mineralized matrix. These molecules are released in response to pulpal injury.[133] They include many of the same molecules that are expressed during embryonic tooth development and are again expressed in dental tissues in response to pathologic conditions.[134] The pulpodentin complex demonstrates great regenerative potential in response to injury and contains a population of multipotent mesenchymal progenitor cells (dental pulp stem cells) that can be recruited to produce new hard tissue. Therefore, these substances will generate new, biologically based approaches to pulpal healing.[135] These approaches hold the promise of leading to more predictable solutions to the vexing and unpredictable problem of direct capping of the dental pulp.

Indirect Pulp Treatment

Although not a new concept, indirect pulp treatment continues to elicit a great deal of controversy. Many clinicians believe that the pulp may be so diseased beneath caries lesions that resolution of established lesions may not be possible. A review of the events occurring during development of caries is necessary to understand the histopathologic events involved in indirect pulp capping (see also chapter 14).

In 1969, Keyes[136] described three factors essential to the etiology of caries: *(1)* a susceptible host, *(2)* cariogenic microflora, and *(3)* a suitable substrate. For caries to occur or progress, all factors must interact simultaneously. It has been hypothesized that if the source of nutrition for the cariogenic bacteria could be eliminated, the organisms would die, thus arresting the caries process.

Caries penetrates dentin at an average rate of approximately 1 mm every 6

months.[137] In untreated carious teeth, the relationship of bacterial penetration to pulpal pathosis is quite predictable. The intensity of the pulpal response to bacterial penetration of dentin is substantial, regardless of whether the penetration is 3 mm or 1 mm from the pulp. However, when the bacterial penetration comes within 0.75 mm of the pulp or when the bacteria invade previously formed reparative dentin, the degree of pulpal disease becomes extreme and, most likely, irreversible. In other words, although the practitioner cannot evaluate this measurement clinically, the pulp remains reasonably intact if there is at least 0.75 mm of intact, bacteria-free dentin between the caries lesion and the pulp.

The reason for this abrupt change in the intensity of the response in the last millimeter is that before the bacteria reach the pulp, their by-products (enzymes, toxins, and organic acids) can penetrate the remaining tubular distance and cause pulpitis. In addition, the number of tubules per unit area and the tubule diameter increase closer to the pulp,[138] providing an easier path for penetration. If all of the caries is removed except for the last deep layer overlying some intact, relatively bacteria-free primary, secondary, or tertiary/reparative dentin, then the bulk of the lactic acid–producing complex is eliminated.

When the inflammation that previously prevented the continual formation of tertiary dentin has been eliminated, tertiary dentin can be formed by either the reactionary mechanism (via surviving postmitotic odontoblasts) or the reparative mechanism (see chapter 2). The few persisting cariogenic organisms that may filter through the remaining dentin are phagocytosed by neutrophils in the rejuvenated pulp tissues. The process has shifted from favoring the caries lesion and a gradually dying pulp to one that favors the potential for complete resolution of the pulpal lesions, unless abscess formation has occurred.

In an indirect pulp treatment procedure, debridement of the carious layers in a manner that minimizes mechanical trauma is the first step toward pulpal recovery. Application of zinc oxide–eugenol, which is antibacterial and of low pulpal toxicity if there is a layer of intact dentin, reduces the bacterial threat. Its ability to seal the tooth from further ingress of bacteria is an important property. Another argument for its use is the possibility that the partially decalcified dentin would remineralize after bacteria are deprived of a substrate, with a subsequent return to normal tissue pH.[139]

An alternative method is to place resin composite systems directly on carious dentin or on pulp tissue beneath deep cavity preparations, as previously stated. The success of these materials depends on two factors: *(1)* the ability of the pulp to respond to materials put in contact with or in close proximity to it and *(2)* the ability of the restorative material to seal the interface between it and the preparation (hence

the terms *total etch* and *total seal*).[105] Most reports of successful total seal appear to be empirical and/ or anecdotal; therefore, more work is necessary to make valid determinations.[18] Some studies[137,140] support the occurrence of at least some of these conditions.

However, other studies have shown that resin-dentin bonds created in vivo are susceptible to degradation by endogenous, dentin matrix–bound matrix metalloproteinases (MMP-2, -3, -8 and -9) that are activated during the application of mildly acidic dentin adhesives.[141,142] As complete infiltration of caries-affected dentin by both etch-and-rinse and self-etching adhesives has never been demonstrated, the loss of bond integrity caused by degradation of resin-sparse, water-rich collagen fibrils within the bonded caries-affected dentin jeopardizes the long-term seal of the resin-dentin interface. More recently, cysteine cathepsins have also been reported in intact dentin but were more abundantly (approximately tenfold) identified from carious dentin.[143] Similar to MMPs, cysteine cathepsins may be activated in mildly acidic environments. Acid activation of dentin-bound cathepsins may further result in activation of matrix-bound MMPs. Taken together, salivary MMPs, dentin matrix–bound MMPs, and cysteine cathepsins may contribute to the degradation of bonds created by dentin adhesives in caries-affected dentin.[144]

Another approach to caries control has gained relatively wide acceptance in underdeveloped parts of the world. In the atraumatic restorative technique, severe caries is removed with hand instruments and the cavity is restored with a GI restoration. Although not technically an indirect pulp capping procedure, the atraumatic restorative technique may accomplish the same objective. Clinical trials have shown the success of this method, particularly in large Class I restorations, which is comparable to or better than the success rates for traditional restorative procedures for periods up to several years.[145–147] A study in older adults, in whom Class V preparations were placed on root surfaces, found no differences in survival rates between conventional and atraumatic restorative procedures at 12-month examinations.[148]

A 10-year clinical trial compared the marginal integrity of three types of restorative preparations: *(1)* a conventional Class I preparation extended for prevention into noncarious fissures, with removal of all caries and placement of an amalgam restoration; *(2)* a more conservative preparation, with removal of all caries, restoration with amalgam, and use of pit and fissure sealant; and *(3)* preparation of a 45- to 60-degree bevel in the enamel surrounding a frankly cavitated caries lesion; the deep soft portions of the caries remained untouched. The

lesion extended no deeper than halfway into dentin between the dentinoenamel junction and the pulp chamber, and resin-based composite was used.[137] At the 10-year follow-up period, open margins were found on 8% of the resin-based composite restorations, 9% of the sealed amalgam restorations, and 29% of the unsealed amalgam restorations. However, the most important result was that the caries lesions beneath the sealed resin-based composite restorations ceased to progress. Not one pulp became nonvital in the 10-year study. These results have contributed to a new understanding of the caries process and the value of indirect pulp capping.

A review of the literature indicates that the majority of the most recent studies are dedicated to preserving the primary dentition (and permanent first molars in preteenagers) and maintaining proper spacing for the eventual loss of these teeth to their permanent successors. Studies dedicated to the latter were carried out, for the most part, in primary molars, which tended to be the last primary teeth lost.[149,150]

Stepwise excavation of caries lesions in the permanent dentition involves initial removal of gross caries and subsequent placement of a material that is used in an attempt to remineralize the remaining caries lesion to prevent a direct carious pulpal exposure. At a later time (6 months to a year), the previously placed restoration and indirect capping material are removed with the expectation of finding a sterile, hard, mineralized tissue that can be left with the expectation that caries will not invade the coronal pulp. When incomplete caries excavation was performed in 32 teeth with deep caries lesions,[151] changes found on reentry were subjected to radiographic density assessments by digital image subtraction. At reentry, the dentin was dry and hard in 80% of the teeth, and there were statistically significant increases in radiographic density.

However, in vitro studies of severely carious teeth found that clinical observations of dentin color changes and increases in mineral in the remaining carious dentin do not always represent a change in the bacterial content.[152] While the microbiologic bioburden may be reduced, it is still in place. These studies must be carried out over a longer time period to ensure that remaining caries-causing microorganisms are not present to cause further tooth breakdown.

Chlorhexidine has been suggested as a cavity varnish. Compared with a 1% thymol–containing varnish and demeclocycline hydrocortisone ointment, chlorhexidine varnish was less effective in reducing the total anaerobic microorganisms associated with carious dentin.[153] In another study, the antimicrobial effect of chlorhexidine was more effective when it was combined with a GI.[154]

Collectively, these studies indicate that microbiologic bioloads are reduced during stepwise excavation procedures and the remaining carious dentin becomes darker, harder, and drier, allowing for further removal of carious tooth structure if deemed necessary at a subsequent visit. The final excavation has two aims: *(1)* to verify the arrest of the caries process and *(2)* to remove a darker, harder dentin when necessary.[155] However, a meta-analysis recently conducted for the Cochrane Database[156] led to the following conclusions: First, there were too few studies that could be included in the review because of the many different variables found in the studies. Second, the findings of the studies reviewed did not suggest that any significant changes should be made from accepted conventional practice procedures. The reader is encouraged to review the entire report so that judgments can be made about the need for direct and indirect pulp capping, pulpotomy or pulpectomy, diagnoses of reversible versus irreversible symptoms, and the presence of radiographic evidence of periradicular changes.

Conclusion

To improve the success rate of vital pulp therapy, a concerted effort must be made on the part of pulp biologists, dental researchers, and clinicians to recognize the progress that has been achieved in vital pulp therapy and to incorporate the latest available information into practice and clinical training. A review of the treatment techniques and other considerations associated with removing all caries (leading to an exposure) or retaining a layer of carious dentin (indirect procedures) presents the dilemma most clinicians face in deciding how to treat these lesions. Continued research and clinical trials are needed to develop the appropriate case selection guidelines, treatment approaches, and materials needed to maximize clinical success. Significant advances in the understanding of the molecular basis of the pulpal healing response are currently underway and should lead to significant new, biologically based pulp therapies.

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Caries, Restorative Dentistry, and the Pulp

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Restorative dentistry has undergone a fairly drastic change in the past 30 years, from restorations that are cemented or retained primarily by mechanical undercuts to adhesive restorations that rely primarily on bonding. This change gained momentum in the 1980s with the development of improved dentin adhesives for resin composite restorative materials. Prior to that time, enamel bonding was well established, but the consensus was that application of acids to dentin did nothing to potentiate bonding and was injurious to the pulp. Resin adhesion to dentin was first achieved in 1982 through mechanical hybridization between resin and collagen fibrils using an adhesive resin that contained a functional hydrophilic monomer.[1] Subsequent studies showed that the pulp was not injured by acids if the surface was sealed adequately.[2]

Adhesive restorations allow the preservation of tooth structure. Removal of tooth structure is primarily used to obtain access to remove caries and to allow adequate thickness for restorative materials. It is not necessary to remove tooth structure to achieve mechanical retention and resistance form. The minimal removal of tooth

structure helps to preserve the structural integrity of the tooth and lessens the chances of irreversible pulpal changes.

Restorative materials can be used conservatively to restore caries lesions and for cosmetic restorative procedures. Adhesive luting cements are used for intracoronal and extracoronal indirect restorations. They have allowed the development of porcelain veneers, which are more esthetic and conservative of tooth structure than crowns. Glass-ionomer materials form chemical bonds to tooth structure and can be used in some clinical situations without tooth preparation, such as in noncarious cervical lesions. Glass-fiber posts are employed in conjunction with adhesive luting cements in the restoration of teeth that have undergone root canal treatment.

Adhesive dentistry is not without its limitations, however.[3] Adhesive procedures require strict isolation and are more technique sensitive than traditional restorative procedures. Failure to perform the adhesive procedures properly is likely to result in clinical failure of the restoration, with potentially serious implications for the pulp.

Another change in recent years has been in the management of caries. Whereas complete removal of caries was a basic principle strictly taught for many years in dental schools, the emphasis in recent years has shifted to strategies to preserve dentin and protect the pulp, sometimes with incomplete removal of caries.[4]

This chapter summarizes contemporary adhesive restorative treatment philosophies regarding the effects of caries, tooth preparation, and restoration and how to minimize potential negative effects on pulp tissue.

Structure and Physiology of the Pulpodentin Complex

The dentin and pulp function physiologically as a single unit, the *pulpodentin complex* (see chapter 3). The net effect of a restorative procedure on the pulp is thus the result of a complex interaction of many factors: the thickness and permeability of the intervening dentin, the health of the underlying pulp, any mechanical injury to odontoblast processes during cavity preparation, the possible toxicity of the restorative material, and any microbial leakage.[5] Apart from their role in primary and reactionary dentinogenesis, odontoblasts also play important roles as defense cells in the innate immunity system[6] (see chapter 10) and as thermal and mechanical sensory receptors[7,8] (see chapter 8). In view of the recent discovery that

odontoblasts function as sensory cells, the classic hydrodynamic theory of activation of Aβ and Aδ sensory nerve receptors by inward and outward fluid shifts as the mechanism of dentin hypersensitivity during cavity preparation procedures may have to be modified.

The effects of restorative procedures on the dental pulp may be understood only within the context of the role of intervening dentin[9] and, more specifically, of the dentinal tubules. Acute effects of cavity preparation result from a hydrodynamic effect of fluid flow within tubules (and direct injury to the odontoblast process if the cavity is deep enough). Diffusion of bacteria or bacterial toxins associated with microleakage occurs via fluid-filled tubules. Part of the protective response of the pulp involves occlusion of tubules and a reduction in dentin permeability.

Normal structure and permeability

In human dentin, dentinal tubules converge as they approach the pulp, so tubule density increases from approximately 20,000/mm^2 in the outer dentin to 45,000/mm^2 adjacent to the pulp. The cross-sectional area of dentin occupied by tubules varies from less than 1% near the dentinoenamel junction to more than 20% close to the pulp. Hence, when dentin is cut, the permeability to noxious agents is much greater close to the pulp than in outer dentin. Permeability is not uniform throughout the tooth but greater overlying the pulp horns and on axial walls compared to the occlusal surface.[10]

A positive hydrostatic pressure from the pulpal circulation results in outward fluid flow when tubules are exposed. Outward fluid flow may contribute to pulpal protection because as a transudate of plasma it contains proteins (immunoglobulins and fibrinogen) and minerals (calcium and phosphate).[11] Outward fluid flow also limits the rate of diffusion of noxious agents in a pulpal direction.[12]

When the pulp is subjected to a gradually progressive insult, a major part of the pulp's response is the deposition of minerals within the dentinal tubules, occluding the tubule against further ingress of noxious stimuli. Recent advances in the understanding of the structure and composition of peritubular dentin indicate that it is completely devoid of type I collagen and consists of much smaller apatite crystallites than are present within the intertubular dentin. Those smaller apatites are deposited within a phospholipid-proteolipid complex.[13] Noncarious cervical lesions

(abrasion or abfraction lesions) also show occluded dentinal tubules and a surface hypermineralized zone[14] (Fig 14-1). Carious dentin is classified into two layers: demineralized, bacteria-containing *infected* dentin and a deeper, *affected* layer that includes a transparent zone in which tubules are occluded by mineral deposits.[15] Regardless of the stimulus, occlusion of tubules reduces dentin permeability. From a clinical standpoint, it is important to preserve this layer because it protects the pulp against restorative insult.

Odontoblasts, with processes extending into dentinal tubules, are the first cells to encounter an external insult and may be directly injured during cavity preparation. Odontoblasts show a gradation in their response to injury. With a gradual, progressive insult that does not directly involve the odontoblast process (caries or abrasion), tubules become progressively occluded by mineral deposits, which serve to wall off the underlying pulp. In the case of caries, it is uncertain whether tubule occlusion is the result of an active defense mechanism or the reprecipitation of mineral dissolved during the caries process.[15]

Additional dentin, commonly termed *tertiary dentin*, may also be deposited on the pulp surface underlying the injury. The latter may be further classified into *reactionary dentin* or *reparative dentin* (see also chapter 2). Depending on the severity of injury, the odontoblasts will survive to deposit reactionary dentin, the tubules of which are continuous with those of overlying primary and secondary dentin.[16] If odontoblasts are irreversibly damaged, reparative dentin is laid down following the differentiation of new odontoblast-like cells from the dental pulp.[17]

The most important determinant of the severity of odontoblast injury and the extent of tertiary dentin deposition appears to be the trauma of the cavity preparation rather than effects of restorative materials.[16] In experimental studies involving cavity preparations in sound dentin of previously intact teeth, the concept of remaining dentinal thickness (RDT) as a factor in restorative injury to the pulp has been investigated extensively.[9] RDT may well be a surrogate for direct injury to odontoblast processes, although it is recognized that dentin permeability also increases with decreasing RDT.

Mature dentin is normally approximately 3.0 mm thick, and odontoblast processes extend from 0.1 to 1.0 mm into tubules.[11] Murray et al[16] reported that odontoblast cell numbers were unaffected by deeper cavity preparations with as little as 0.5 mm RDT, while the quantity of reactionary dentin increased with decreasing RDT. The response was also brief; reactionary dentin deposition ceased within 28 days. Deeper cutting (less than 0.3 mm from the pulp) results in direct odontoblast injury,

including displacement of cell bodies into tubules and cell death. This necessitates the activation of dental pulp progenitor or stem cells before reparative dentin deposition can begin.[18]

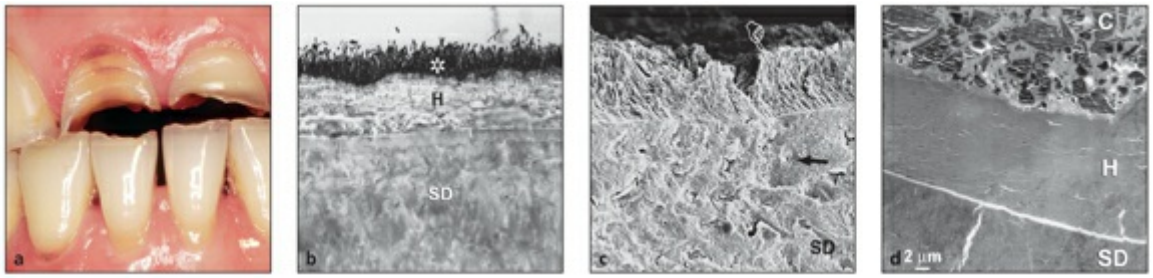

Fig 14-1 *(a)* Noncarious cervical lesions. Despite the loss of tooth structure, dentinal sclerosis and tertiary dentin deposition have protected the pulp from injury. *(b)* Light microscopic image of the shiny surface of a representative noncarious cervical lesion, showing the presence of a hypermineralized surface layer (H) along the surface of the sclerotic dentin (SD). The surface of these lesions may be colonized by a bacterial biofilm *(asterisk)* (original magnification ×40). *(c)* Scanning electron micrograph of the hypermineralized surface layer *(pointer)*. Dentinal tubules in the underlying sclerotic dentin (SD) are completely occluded with mineral deposits *(arrow)*, reducing the permeability of dentin in these lesions (original magnification ×2,000). *(d)* Transmission electron micrograph of the hypermineralized surface layer (H) between the sclerotic dentin (SD) and resin composite (C).

Effects of caries and cavity preparation on the dentin and pulp

The pulpodentin complex is a dynamic tissue that can respond to mechanical, bacterial, or chemical irritation in a number of ways that serve to decrease that irritation.[19] The vitality and dentin-repair potential of the pulp are dependent on the survival of the odontoblasts beneath the site of injury.[20] When the cellular and defense responses of the pulp to injury are discussed, it is important to discriminate between effects that directly arise from a caries insult and those that can occur in the absence of caries. The latter would include injurious events that occur after deep cavity preparation[21] or bacterial leakage from restorations.[22]

Characteristics of Caries Lesions and Pulpal Responses

Primary caries and secondary caries, respectively, have been cited as the principal

reasons for the placement of initial restorations and the subsequent replacement of failed restorations. Acids are unable to destroy highly cross-linked dentin type I collagen, which can only be degraded by sodium hypochlorite and proteolytic enzymes.[23] There is increasing evidence that host-derived proteolytic enzymes are responsible for the degradation of carious dentin.[24] Matrix metalloproteinases (MMPs) are a class of zinc- and calcium-dependent hydrolases that consist of 23 well-characterized members (see chapter 11). They function in hydrolysis of extracellular matrices and play a central role in tissue development, matrix remodeling, angiogenesis, wound healing, and malignancy progression.[25]

Host-derived MMPs that are of relevance to dentinal caries may originate from saliva and dentin. As saliva penetrates the cavitated dentin, host-derived MMPs from saliva may have direct access to the demineralized dentin. This source of salivary MMPs probably originates from gingival crevicular fluids and includes collagenases, such as MMP-8, as well as gelatinases (MMP-2 and MMP-9). Unlike bacterial collagenases, these enzymes are activated by an acidic pH. Once activated, they are able to digest the demineralized dentin matrix after pH neutralization by salivary buffers.

MMPs that have the potential to attack the dentin extracellular matrix (ie, MMP-2, -8, -9, and -20) may be incorporated into the mineralized dentin[25–27] during primary and secondary dentinogenesis. Recently, another class of collagen-degrading enzymes, the cysteine proteinases (cathepsins), was also identified from mineralized dentin.[28]

Host-derived MMPs and cysteine cathepsins may be released and activated during caries demineralization of dentin. Because dentin MMPs are activated by mild acids, increases in collagenolytic and gelatinolytic activities of mineralized dentin in a pH-dependent manner also occur when mineralized dentin is treated with mildly acidic etch-and-rinse adhesives or self-etching primers.[3] Activation of dentin MMPs may also be involved in the degradation of collagen fibrils that are incompletely infiltrated by resins within the hybrid layers of resin composite restorations.[29,30] Thus, they are at least partially responsible for adhesive restoration failures and the lack of longevity of resin-dentin bonds.[1,3]

The combined effects of acid demineralization and matrix degradation in advanced dentinal caries lesions result in the ultrastructural appearance of two distinct layers with different microscopic and chemical structures: the outer layer of caries-infected dentin, which is highly infected, completely demineralized, and grossly denatured (Fig 14-2), and the inner layer of caries-affected dentin, which has

a partially demineralized, remineralizable collagen matrix. The philosophy of caries removal in restorative dentistry has shifted from G. V. Black's classic concept of complete removal of all infected and affected dentin[31] to the complete removal of the caries-infected dentin and retention of the caries-affected dentin.[32] More recently, there has been a further paradigm shift to the ultraconservative approach of partial caries removal, in which most, but not all, of the bacteria-infected carious dentin is removed.[3,33] There is little evidence that caries-infected dentin must be removed before the tooth is sealed. Retention of bacteria-infected dentin does not seem to result in caries progression, pulpitis, or pulpal death.[34]

A related technique for treating deep caries lesions is the use of a stepwise excavation approach (ie, indirect pulp capping) to minimize the risk of pulpal exposure.[35] The rationale of the two-step excavation approach aims at converting a rapidly progressing active lesion into a slowly progressing arrested lesion.[36] To date, it is unknown whether it is necessary to reenter a tooth that has undergone indirect pulp capping for further caries excavation because studies in which teeth were not reentered did not report adverse consequences.[33]

Caries-affected dentin is generally less stiff and contains more water than sound dentin.[37] These properties correspond with the ultrastructural appearance of irregular islands of increased porosities within the caries-affected intertubular dentin. These porosities become readily apparent when caries-affected dentin is impregnated with silver nitrate, followed by reduction of the latter to metallic silver grains (Fig 14-3). Although intact collagen fibrils with stainable banding characteristics can be identified in caries-affected dentin, they are sparser than they are in sound dentin and are often separated by wide interfibrillar spaces that contain denatured collagen remnants (Fig 14-4).

The antigenicity of the type I collagen and proteoglycans that are present within caries-affected dentin has been reported to be altered compared with those present in sound dentin.[38] Caries-affected dentin has traditionally been assumed to be remineralizable. Identification of these antigenic alterations raises concern about the potential of the collagen fibrils within this layer to undergo intrafibrillar remineralization, a process that is thought to be crucial to restoring the inherent mechanical properties of dentin.[39]

From a restorative point of view, the increased porosity in caries-affected dentin results in thicker hybrid layers when this substrate is bonded with either etch-and-rinse or self-etching adhesives.[40] However, these hybrid layers are generally less well infiltrated than those that are created in sound dentin,[41] rendering them more

susceptible to water sorption and degradation by host-derived MMPs. Nevertheless, the intrinsic weakness of caries-affected dentin may not be a clinical problem when excavated caries lesions are surrounded by normal dentin and/or enamel. This was probably responsible for the excellent 10-year results of clinical trials of resin-sealed caries lesions.[42]

Although the bacterial etiology of caries is well established, the dynamics of caries progression cannot be explained solely on a chemical basis and is influenced by interaction between the metabolic activities of the bacterial biofilms and the response from odontoblasts. Generation of microbial metabolic products, matrix degradation products, and the release of growth factors from the dentin extracellular matrix will influence disease progression. At the same time, the tubular characteristics of dentin and the extent of tubular sclerosis derived from the reactionary response of the pulpodentin complex affect the permeability of dentin. This in turn slows the diffusion kinetics of the metabolic and degradation products into the pulp.[10,11]

Caries lesions may be slowly or rapidly progressing or may become arrested.[36] Sclerosis of the dentinal tubules is either absent or minimal in rapidly progressing active lesions; within the caries-affected dentin, large bacteria-filled spaces that are produced by the coalescence of adjacent dentinal tubules are frequently observed (Fig 14-5). In the absence of tubular occlusion, rapid diffusion of the metabolic and degradation products is possible, which may overwhelm the pulp's defensive responses and result in pulpal inflammation, absence of tertiary dentinogenesis, and severe pulpal injury.[36]

Conversely, a transparent zone of sclerotic dentin is observed at the base of the caries-affected dentin above the sound dentin in both slowly progressive active lesions and arrested lesions; this zone is considerably thicker in arrested lesions.[43] Within the transparent zone, dentinal tubules are partly or totally occluded by intratubular precipitation of whitlockite (magnesium-substituted tricalcium phosphate) crystals (Fig 14-6) or peritubular dentinlike deposition[15] (Fig 14-7). Tubular occlusion in caries-affected dentin reduces dentin permeability even upon removal of the smear layer. This reduction in permeability may be readily appreciated when dentin adhesives are applied to caries-affected dentin.[44] The occluded tubules prevent water movement from underlying dentin through the polymerized, highly water-permeable adhesives.

Reduction in dentin permeability by tubular occlusion will also impede the diffusion of bacterial products or solubilized matrix components along the tubules,

thereby delaying lesion progression. Thus, defense and healing of pulp tissue are more favorable in response to slowly progressing or arrested lesions.[45]

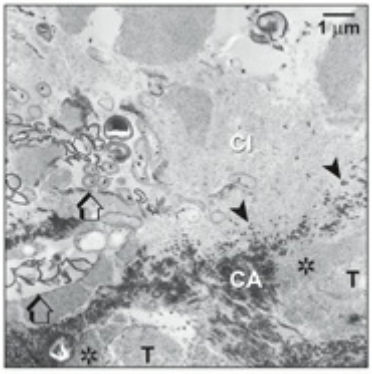

Fig 14-2 Transmission electron micrograph taken from a laboratory-demineralized section of the junction between caries-infected dentin (CI) and caries-affected dentin (CA). The dentin matrix in the caries-infected dentin is grossly denatured, with no stainable collagen fibrils, and contains numerous bacteria *(open arrows)*. Along the surface of the caries-affected dentin, sparsely distributed collagen fibrils are present *(arrowheads)*. Dentinal tubules (T) within the surface of the caries-affected dentin are considerably widened and are continuous with the denatured intertubular dentin *(asterisk)*.

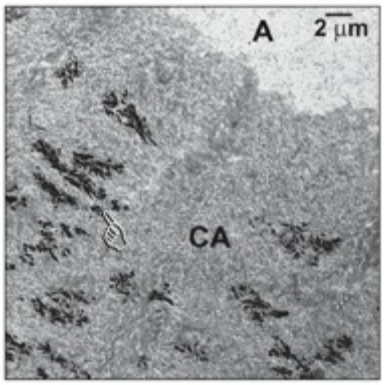

Fig 14-3 Transmission electron micrograph taken from a nondemineralized section of caries-affected dentin (CA) that had been immersed in a silver tracer. The highly porous nature of caries-affected dentin can be seen in the form of islands of silver deposits *(pointer)*. The surface of the caries-affected dentin was bonded with a filled dentin adhesive (A).

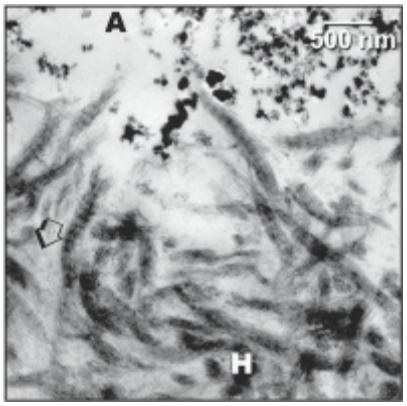

Fig 14-4 Transmission electron micrograph taken from a demineralized, stained section of adhesive-

bonded, caries-affected dentin. Although some of the collagen fibrils were stainable and exhibited cross banding, they were sparsely distributed and were separated by wide interfibrillar spaces *(open arrow)* that contained denatured collagen remnants. H, hybrid layer formed in caries-affected dentin; A, filled dentin adhesive.

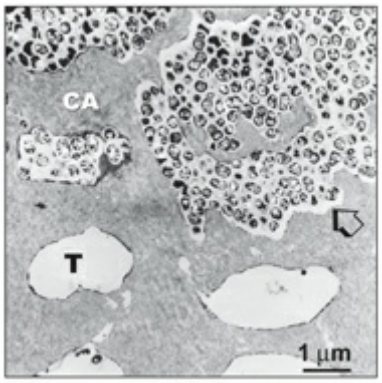

Fig 14-5 Transmission electron micrograph taken from a demineralized, stained section of caries-affected dentin (CA) in a rapidly progressing caries lesion. Dentinal sclerosis is not apparent within the dentinal tubules (T). Some of the tubules appeared coalesced due to the destruction of the adjacent intertubular dentin. These large tubular spaces were filled with numerous bacteria *(arrow)*.

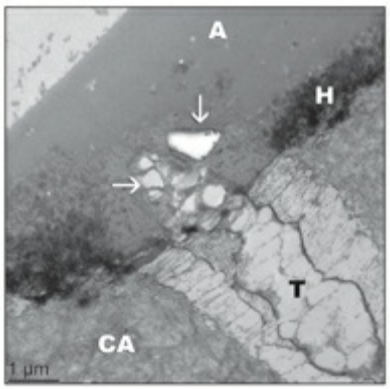

Fig 14-6 Transmission electron micrograph taken from a demineralized, stained section of bonded caries-affected dentin (CA) in a slowly progressing caries lesion. Silhouettes of intratubular caries crystals *(arrows)* can be seen occluding the orifice of a dentinal tubule (T). These caries crystals were partially dissolved during laboratory processing and were trapped by the dentin adhesive (A). A hybrid layer (H) can be identified after the application of a self-etching adhesive to the dentin substrate.

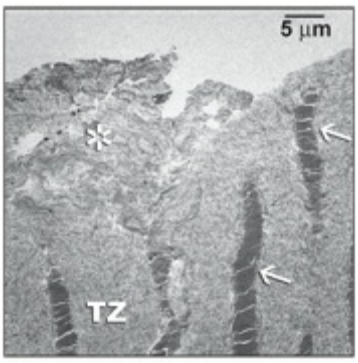

Fig 14-7 Transmission electron micrograph taken from a nondemineralized section of the base of caries-affected dentin in a slowly progressing caries lesion. In this transparent dentin zone (TZ), the dentinal tubules are completely occluded with peritubular dentin *(arrows)*. Tubular occlusion results in a marked reduction in dentin permeability. A thick smear layer *(asterisk)* is formed after caries removal.

Responses of the Pulpodentin Complex to Caries

As a result of the variability in caries progression, there is no single response to caries. Rather, the pulpodentin complex exhibits a broad spectrum of responses that represent a summation of injury, defense, and repair events.[46] The relative contributions and interaction of these interrelated responses are critical in determining the fate of the pulpodentin complex and its ability to survive the caries assault.

Injury responses

Lactic, acetic, and propionic acids account for about 90% of the bacterial acids found in carious dentin.[47] Although these acids have low pH (about 4.9) and are capable of causing direct injury to odontoblasts and adjacent pulpal cells, this is unlikely to happen unless the advancing caries lesion is very close to the odontoblast processes or the pulp. This is because the acids are buffered by dentin and hydrogen ions and do not diffuse effectively across dentin that is more than 0.6 mm thick.[48]

To initiate injury, bacterial acids, soluble plaque metabolic products, and cell wall components have to diffuse pulpward against an outward flow of dentinal fluid.[49] Although outward fluid flow reduces the rates of permeation of exogenous solutes,[50] including lipopolysaccharides from cell walls of gram-negative bacteria, endotoxins derived from the lipopolysaccharides have been observed in vital pulps of human carious teeth but not in noncarious teeth.[51] Other studies have shown that it is possible for fluorescent tracers, applied directly to either intact or acid-etched enamel (ie, in the absence of outward dentinal fluid flow), to reach odontoblasts in vital dental pulps.[52] Communication also exists between odontoblasts and between odontoblasts and subodontoblastic cells via gap junctions.[52]

Taken together, these issues explain why injurious responses to the odontoblasts

and subodontoblastic cells are apparent in active lesions that involve more than a quarter of the thickness of enamel[53] (Fig 14-8). Early caries lesions produce cytoplasmic changes in the odontoblasts that are evident at the ultrastructural level.[55] Before an active lesion reaches the dentinoenamel junction, a significant reduction in the cytoplasm-to-nucleus ratio of odontoblasts and a concomitant reduction in predentin thickness have been observed.[56] Similar changes were not seen in arrested or slowly progressing enamel lesions.

In the absence of an epithelial lining, odontoblasts represent the first cells that come into contact with pathogens that penetrate vital dental tissues. Recent studies indicated that, apart from its well-recognized secretory function and recently proposed function as a sensory receptor,[8,57] the odontoblast serves also as a defense cell that has the ability to recognize a host of bacterial products via cell-surface toll-like receptors (TLRs).[6,58] Lipoteichoic acid derived from the cell membranes of gram-positive bacteria and lipopolysaccharides derived from the cell walls of gram-negative bacteria can be recognized by TLR-2 and TLR-4, respectively, present in odontoblasts.[58,59] Apart from their participation in innate defense responses of the pulp,[60] odontoblasts activated through TLR-2 by lipoteichoic acid exhibit a reduction in their matrix synthesizing and mineralization potential.

Considering that matrix synthesis and mineralization are critical repair responses during reactionary dentinogenesis, it is possible that this reduction in the secretory activities of odontoblasts may be overridden by the release of a soluble pool of growth factors during caries demineralization of dentin. These bioactive molecules are important in signaling the upregulation of the secretory functions of odontoblasts.[61] For example, angiogenic growth factors such as vascular endothelial growth factor (VEGF) are released from the demineralized dentin matrix. Both lipopolysaccharides[62] and lipoteichoic acid[63] are responsible for upregulating the production of VEGF in odontoblasts, macrophages, and other pulpal cells. Release of nerve growth factor from pulpal fibroblasts stimulates nerve sprouting in dental pulps of teeth with moderate to deep caries,[64] with concomitant increases in the expression of sensory and sympathetically and parasympathetically derived neuropeptides in these carious teeth.[65]

The release of both VEGF and vasoactive neuropeptides may increase vascular permeability and edema. They contribute to rises in intrapulpal pressure, increases in outward fluid flow, pain, and irreversible tissue damage. On the other hand, growth factors such as transforming growth factor β1 (TGF-β1), released during dissolution of the carious dentin matrix, inhibit the expression of TLRs in

odontoblasts. This attenuates the response of odontoblasts to oral bacteria, thereby contributing to an anti-inflammatory role in pulpal inflammation induced by caries.[66]

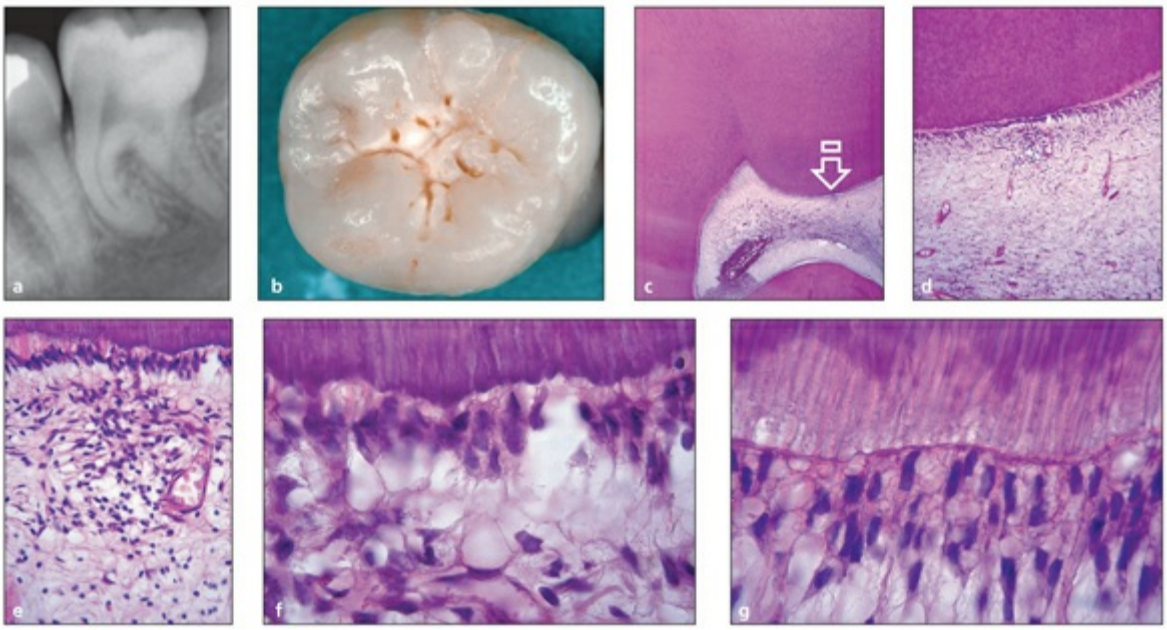

Fig 14-8 *(a)* Mandibular third molar of a 23-year-old woman. The radiograph does not show a caries lesion, but the tooth was extracted after repeated episodes of pericoronitis. *(b)* Fissure discoloration is present on the occlusal surface. The tooth was processed for light microscopy. Sections were cut on a mesiodistal plane. *(c)* Overview of the pulp chamber and the entire dentin thickness (hematoxylin-eosin [H&E] stain; original magnification ×25). *(d and e)* Progressive magnifications of the region indicated by the arrow in *(c)*. Moderate accumulation of chronic inflammatory cells in a localized area of the subodontoblastic space (H&E stain; original magnification ×100 and ×400, respectively). *(f)* Disruption of the odontoblastic layer in that region (H&E stain; original magnification ×1,000). *(g)* Normal odontoblastic layer at a very short distance from the affected region (H&E stain; original magnification ×1,000). (Modified from Ricucci[54] with permission.)

Defense responses

The pulpodentin complex is capable of initiating innate[60] and adaptive immune responses,[67] aiming initially at moderating the caries invasion process. Details of these responses have been covered in excellent reviews,[60,67] as well as in chapters 4, 10, and 11, and will not be reiterated. Innate immunity plays an important role in shallow caries after the initial enamel caries (see Fig 14-8) reaches the dentinoenamel junction. In these shallow dentinal caries lesions, bacteria and tissue degradation products are produced ahead of the bacteria in the lesion front, where bacterial phagocytosis is not yet possible. During this initial stage, pulpal responses

are likely to be low grade and chronic[68] (Fig 14-9).

The transition from innate to adaptive immunity probably occurs in irreversibly inflamed pulps that are separated by less than 2 mm of deep carious dentin.[69] This transition may also be influenced by repair reactions involving dentinal sclerosis and tertiary dentinogenesis, which modify the permeability of the dentin matrix. Nevertheless, bacteria may be trapped within the newly formed reactionary dentin (Fig 14-10). In actively progressing advanced lesions that involve pulpal exposure, the inflammatory reactions may become acute and uncontrolled as bacteria approach and penetrate the pulp (Fig 14-11).

Although inflammation may be regarded as a defense response to injury, severe reactions can result initially as localized microabscesses. Further ingress of bacteria into the pulp produces clinically identifiable abscesses (Fig 14-12) that eventually result in pulpal necrosis and development of periradicular lesions[70] (Fig 14-13). The end result of adaptive immunity is an exaggerated inflammatory response intended to eliminate the infection. However, if the source of caries infection is not eliminated, immune inflammation in pulpitis eventually leads to irreversible destruction of the pulp.

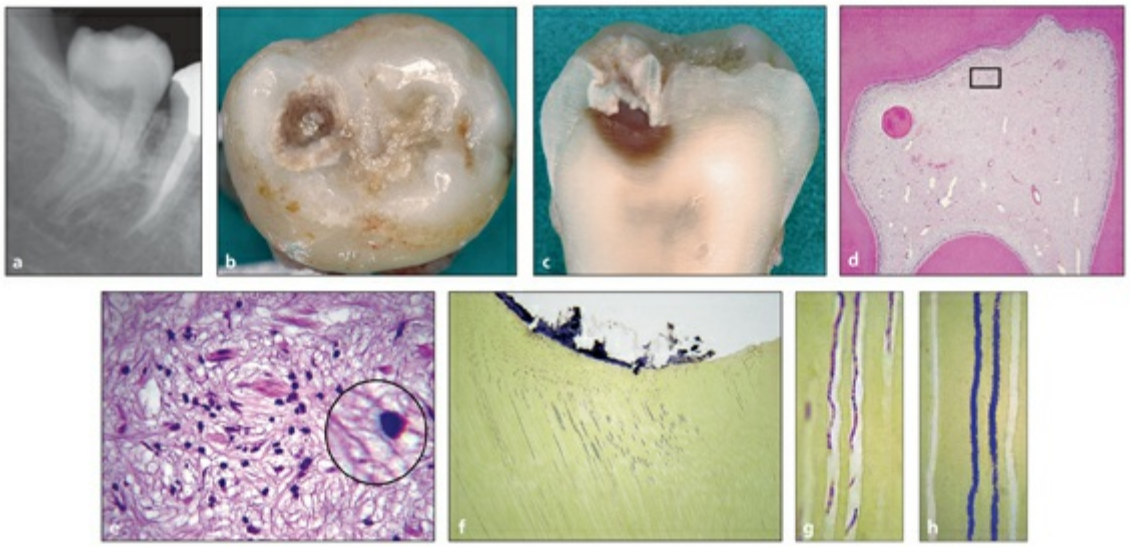

Fig 14-9 *(a)* Occlusal caries is present in this mandibular third molar. The pulp responded normally to sensitivity tests. The tooth was extracted. *(b)* Occlusal view of the extracted tooth. *(c)* The extent of dentinal caries became evident after a surface of the tooth was ground. *(d)* Despite caries penetration to the midcoronal dentin, most of the sections of the pulp exhibited normal histology. A pulp stone could be identified in the distal part of the pulp chamber (H&E stain; original magnification ×25). *(e)* Magnification of the rectangular area in *(d)*. Sparse accumulation of lymphocytes (H&E stain; original magnification ×400). *(f to h)* Dentinal tubules directly beneath the caries lesion were colonized by bacteria (Taylor's modified Brown & Brenn stain; original magnification ×100, ×1,000, and ×1,000, respectively). (Modified from Ricucci[54] with permission.)

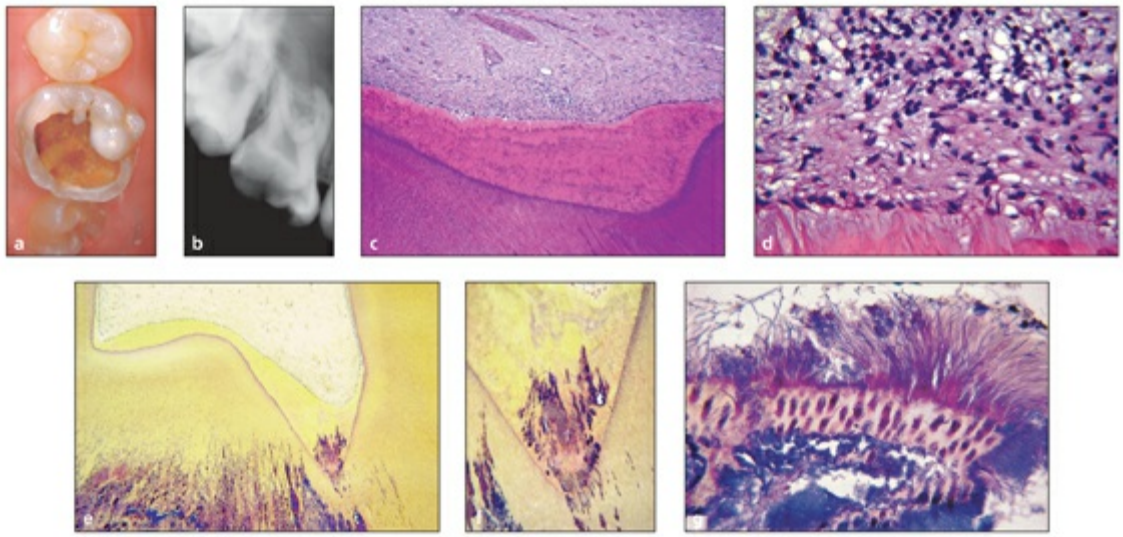

Fig 14-10 *(a)* Maxillary first molar of an 11-year-old girl whose crown has been destroyed by extensive caries. The tooth was painful to cold and sweet stimuli, but there was no spontaneous pain. Sensitivity tests (hot, cold, and electric) elicited exaggerated responses. The young patient and her parents requested extraction. *(b)* The radiograph confirms the severity of the destruction but does not show any periapical changes. *(c)* A considerable amount of reactionary dentin is present on the pulpal side beneath the pulp chamber roof (H&E stain; original magnification ×50). *(d)* The reactionary dentin is bordered by an incomplete layer of flattened odontoblasts. Beneath the odontoblastic layer, a high concentration of chronic inflammatory cells can be seen (H&E stain; original magnification ×400). *(e)* Overview of the caries-infected dentin, the reactionary dentin, and the pulp. In the mesial pulp horn, the reactionary dentin is colonized by bacteria (Taylor's modified Brown & Brenn stain; original magnification ×25). *(f)* Higher magnification of the mesial pulp horn in *(e)* (Taylor's modified Brown & Brenn stain; original magnification ×100). *(g)* Dentin chip at the external surface of the caries lesion. The dentinal tubules are completely filled with bacteria. This spicule provides attachment to a biofilm that is composed primarily of filamentous bacteria (Taylor's modified Brown & Brenn stain; original magnification ×1,000). (Modified from Ricucci[54] with permission.)

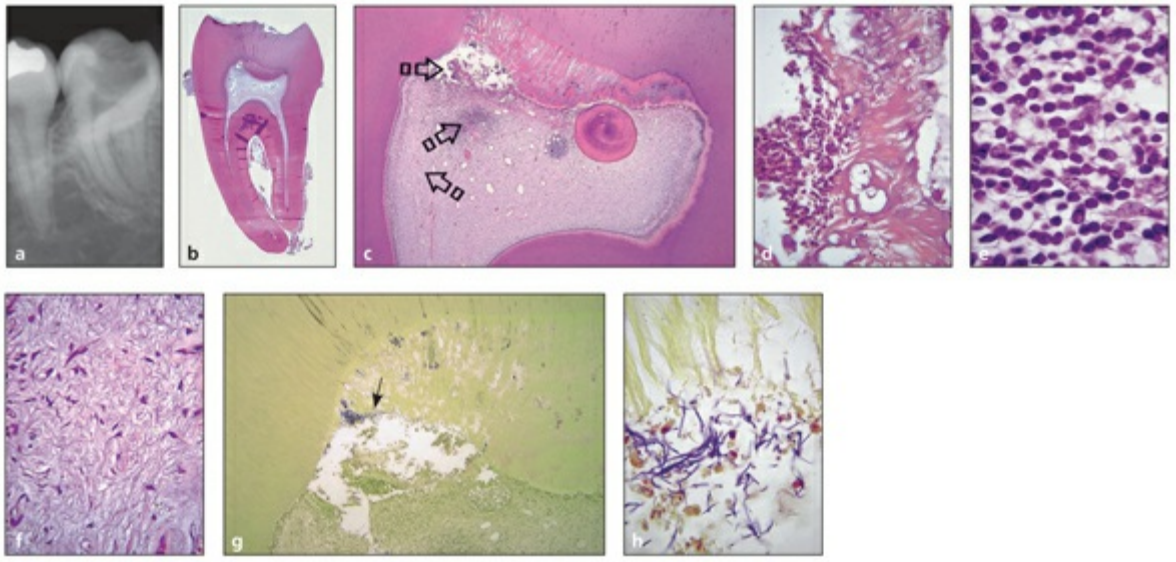

Fig 14-11 *(a)* Mandibular third molar with deep occlusal caries. The tooth was painful to cold stimuli

and to mastication, but there was no spontaneous pain. Sensitivity tests (hot, cold, and electric) elicited normal responses. *(b)* Overview showing a considerable amount of reactionary dentin in the pulp chamber (H&E stain; original magnification ×2). *(c)* Low-magnification view of the pulp chamber, showing a pulp stone in the distal part of the pulp chamber and a microabscess in the mesial pulp horn. Extensive reactionary dentin formation can be seen (H&E stain; original magnification ×25). *(d)* High magnification of the area indicated by the *top arrow* in *(c)*, showing necrotic tissues and acute inflammatory cells (H&E stain; original magnification ×400). *(e)* High magnification of the area indicated by the *middle arrow* in *(c)*. There is severe accumulation of chronic inflammatory cells (H&E stain; original magnification ×1000). *(f)* High magnification of the area indicated by the *bottom arrow* in *(c)*. At a very short distance from the microabscess in the mesial pulp horn, the tissue appears uninflamed, with only fibroblasts and collagen fibrils (H&E stain; original magnification ×1,000). *(g)* Mesial pulp horn. Bacterial masses have reached the pulpal margin of the reactionary dentin. (Taylor's modified Brown & Brenn stain; original magnification ×50). *(h)* High-magnification view of the area indicated by the *arrow* in *(g)*. Filamentous bacteria can be identified (Taylor's modified Brown & Brenn stain; original magnification ×1,000). This case exemplifies the histologic transition from a reversible to an irreversible inflammatory condition. Because there was no spontaneous pain, there was no correlation between clinical symptoms and histologic results. (Modified from Ricucci[54] with permission.)

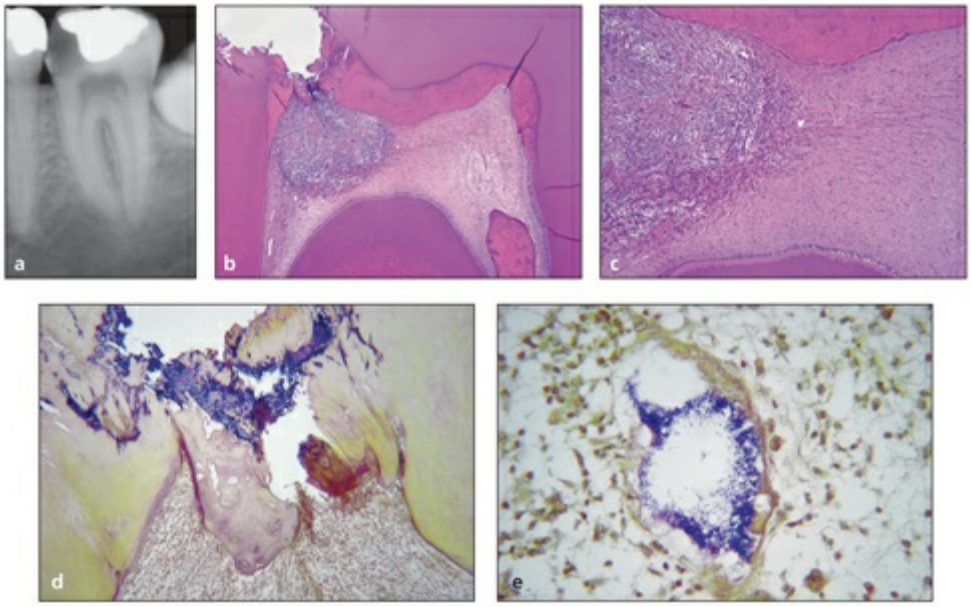

Fig 14-12 *(a)* Mandibular second molar of a 37-year-old man who was seeking treatment for severe spontaneous pain. The diagnostic radiograph shows a deep caries lesion proximal to the mesial pulp horn. The patient did not accept root canal treatment, and the tooth was extracted. *(b)* Overview of the pulp chamber. A considerable part of the mesial pulp appears degenerated. Nevertheless, there is a distinct transition to the surrounding relatively normal pulp tissue. Abundant reactionary and reparative dentin has formed on the pulp chamber roof. A large piece of pulp stone is present at the orifice of the distal canal (H&E stain; original magnification ×25). *(c)* Central part of the pulp. A clear demarcation line can be observed between the tissue disorganized by inflammatory infiltration and the surrounding normal tissue (H&E stain; original magnification ×100). *(d)* Section taken from the area beneath the pulpal exposure shown in *(b)*. Bacterial penetration into the pulp chamber is evident (Taylor's modified Brown &Brenn stain; original magnification ×100). *(e)* Center of the disintegrated tissue under the pulpal perforation, which is not shown in *(d)*. The necrotic tissue is colonized by bacteria and surrounded by inflammatory cells (Taylor's modified Brown & Brenn stain; original magnification

×400). (Modified from Ricucci[54] with permission.)

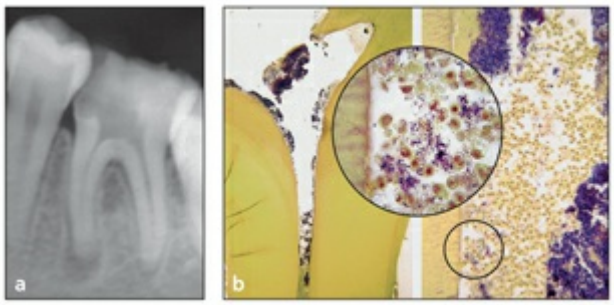

Fig 14-13 *(a)* Mandibular first molar of a 19-year-old woman. There had been several pain episodes in the past, but the tooth was asymptomatic at the time of examination. The patient did not accept any treatment other than extraction. *(b)* Coronal third of the distal canal. Bacterial masses are present and adhered to the root canal walls (Taylor's modified Brown & Brenn stain; original magnification ×400). *(inset)* High magnification from the circled lower part of the canal. Bacterial biofilms are surrounded by a dense concentration of neutrophils engaged in an intense phagocytic activity (Taylor's modified Brown & Brenn stain; original magnification ×1,000). (Modified from Ricucci[54] with permission.)

Repair responses

The pulpodentin complex reacts to stimuli from the bacterial biofilm with dentinal sclerosis and tertiary dentin formation.[71] Dentinal sclerosis has been discussed in previous sections. Unlike primary and secondary dentinogenesis, tertiary dentinogenesis is restricted to the vicinity of the dentin that is directly affected by the caries process. It is not unusual to see partial obliteration of the dental pulp by tertiary dentin in slowly progressing caries lesions that have not undergone restorative treatment.

Tertiary dentinogenesis has been redefined in relation to the nature of the injury (see chapter 2). The term *reactionary dentinogenesis* has been adopted to describe the secretion of a tertiary dentin matrix by primary odontoblasts that have survived injury to the tooth. This is essentially a wound-healing response to produce circumpulpal dentin as a response to injury.[72] Typically, this occurs during injury of milder intensity and represents upregulation of the secretory activities of these odontoblasts. Reactionary dentin formation may be observed as early as in the precavitational stages of active enamel caries and in slowly progressing dentinal caries lesions (see Fig 14-10).

Expression of the fibronectin-binding protein and nestin in the apical areas of

odontoblasts located beneath caries lesions has been associated with cytoskeletal reorganization of surviving odontoblasts after injury.[73] Although reactionary dentin is produced by the same odontoblasts that secrete primary dentin, recent work indicates, at least in rat molars, that primary dentin and reactionary tertiary dentin differ in the distribution of small integrin-binding ligand, N-linked glycoprotein (SIBLING) proteins that are involved in the mineralization of the dentin matrix.[74] This suggests that there are differences in the mechanism of formation between these two forms of dentin. In reactionary dentin, the tubules tend to be continuous with those of overlying primary and secondary dentin.

Reparative dentinogenesis refers to the secretion of tertiary dentin after the death of the primary odontoblasts underlying the injury and requires differentiation of a new generation of odontoblast-like cells.[18,46] Reparative dentin formation occurs after injury of greater intensity and represents a much more complex sequence of biologic events, involving progenitor cell recruitment and differentiation as well as upregulation of cell secretion.[75] In relation to caries, formation of reparative dentin can be observed in deep dentinal lesions (see Fig 14-12). When the pulp is exposed in advanced lesions, reparative dentinogenesis may result in dentin bridge formation, which restores the functional integrity of the pulpodentin complex (see chapter 13).

Recent work indicates that many of the events in primary dentinogenesis during tooth development are recapitulated during pulpal repair, including matrix-mediated cellular processes and the involvement of growth factors and transcription factors as signaling molecules[17] (see chapter 1). Although many of the molecular mediators appear to be common to tooth development and pulp repair, the highly orchestrated events seen in primary dentinogenesis are less ordered during tertiary dentinogenesis.[76] Coupled with the different progenitor cell types that may be involved, this could account for the highly variable reparative dentin morphology observed in tertiary dentinogenesis,[77] including the formation of intrapulpal and intracanal bonelike and cementum-like tissues.[78]

It is noteworthy that the use of low-frequency ultrasound may have the potential to influence the activities of odontoblast and odontoblast-like cells in dentin repair by modulating the production of endogenous growth factors such as VEGF in the pulpodentin complex.[79] Growth factors have recently been employed experimentally for regulating the differentiation of putative odontoblast-like cells from pulpal stem cells.[80,81] However, in the absence of ectomesenchymal interactions after primary dentinogenesis, it appears that the exact molecular signaling mechanisms that enable recapitulation of the odontoblast phenotype, as distinct from the osteoblast

phenotype, have not been completely elucidated.[82] Thus, intracanal bone and cementum may be produced in lieu of reparative dentin.

Pulpal Injury and Healing Responses to Cavity Preparation

Despite the more conservative cavity preparations recommended in contemporary restorative dentistry, cutting of intact dentin is still required in instances such as complete-coverage preparations. The dental literature has shown a 4% to 33% pulpal necrosis rate as a result of preparation of vital teeth for complete-coverage restorations that eventually required root canal treatment. After 15 years of service, the survival rate associated with vital teeth prepared for single-unit ceramometal crowns was higher than that associated with preparations for fixed partial dentures.[83] Pulpal necrosis occurred more often if a tooth served as an abutment for a removable partial denture.[84] As might be expected, the longer the recall period, the greater the occurrence of pulpal necrosis. Karlsson[85] reported that pulpal necrosis occurs at a rate of about 1% per year for single crowns.

Remaining dentinal thickness

Remaining dentinal thickness has been identified as the key determinant of pulpal necrosis after restorative dental treatment.[9] In deep and pulpally exposed cavities in posterior teeth, resin composites were associated with more pulpal breakdown than amalgams.[86] Similar conclusions were reached when odontoblast cell numbers beneath the site of cavity preparation were used to quantify the severity of pulpal injury.[87] The most influential variables in pulpal injury were found to be the RDT, preparation in the absence of a coolant, and the selection of restorative material in exposed dental pulps.[88] These histologic studies in humans were also confirmed by immunohistochemical studies of rat molars using the constitutional heat shock protein 25, produced in odontoblasts and odontoblast-like cells as a marker for odontoblast cell death and differentiation.[89]

Odontoblast survival and reactionary dentin secretion were the two responses most sensitive to cavity RDT.[90] For Class V, vital, nonexposed cavities, odontoblast cell numbers were maintained after cavity preparation in dentin with a 0.50-mm RDT. The percentage of odontoblast survival decreased from 100% in shallow cavities (> 1.00-mm RDT) to 89% in moderately deep cavities (0.5- to 1.00-mm RDT), 83% in deep cavities (0.25- to 0.50-mm RDT), and 68% in cavities with RDT less than 0.25 mm thick.

The greatest areas of reactionary dentin deposition (292% increase) in these cavities were observed with RDTs between 0.25 and 0.50 mm. It was thought that growth factors released from the demineralized dentin matrix by acidogenic bacteria, acid etching, or calcium hydroxide extraction in pulp capping procedures diffused to the odontoblasts more readily with the low RDTs than when the RDT was greater than 0.50 mm. However, little reactionary dentin was identified in cavities when the RDT was less than 0.25 mm. The authors opined that although the shorter distance between the remaining dentin and the pulp would have facilitated the diffusion of the growth factors, the underlying odontoblasts and their processes were injured to a greater extent so that their dentin secretory potential was hampered.[90] The accompanying pulpal inflammation was also found to be the most severe when RDT was less than 0.25 mm, particularly when microleakage was present after the cavities were restored with different materials.[91]

Although the repair capacity of the pulpodentin complex to cavity preparation appears to be age dependent, this factor is considerably less significant, compared with cavity RDT and bacterial leakage, in affecting survival of odontoblasts or the deposition of reactionary dentin.[91] Cavity preparation in deep dentin causes the displacement of some odontoblasts into the dentinal tubules, while others are separated from the predentin by rapid inflammatory exudation.[92] Rapid disruption of the junctional complexes among the odontoblasts results in the loss of integrity of the normal physiologic barrier,[92] allowing leakage of plasma proteins from the pulpal capillaries, between odontoblasts, and out of the tubules to the cut dentin surface.[93] Vascular endothelium of inflamed human dental pulps expresses diverse cell-adhesion molecules that promote leukocyte transmigration from the capillaries into tissue (see chapter 11). Significant alterations in the distribution and staining intensity of cell-adhesion molecules were detected following tooth preparation,[94] suggesting that restorative procedures initiated in deep dentin have the potential to initiate pulpal inflammation. In addition, substance P expression, as well as sensory nerve fibers that are immunoreactive to substance P and calcitonin gene–related

peptide, increased after deep cavity preparation,[95] suggesting the contribution of neurogenic inflammation and nerve sprouting to tooth hypersensitivity after restorative procedures.

Thermal effects

The low thermal conductivity of dentin (see chapter 15) tends to minimize direct elevation of intrapulpal temperature unless the preparation is cut deep in dentin without effective cooling. In addition, the dental pulp responds to irritation stresses by producing *heat shock proteins*. These proteins were originally discovered as a group of molecular chaperones inducible by heat stress but were found subsequently to represent a diverse family of constitutive and inducible proteins. They enhance the ability of cells to withstand stress and signal upregulation of defense and reparative responses during cellular injury.[96] Expression of heat shock proteins in odontoblasts and various cells of the pulp[97] indicates that these proteins are involved in resisting injury during cellular stress.

The thermal tolerance of the dental pulp cells to heat stresses was recently demonstrated by examining the expression of an inducible heat shock protein (HSP70) as well as indicators of cell viability following heat stimulation to 42°C for 30 minutes.[98] Only low levels of HSP70 expression were detected before heat stimulation. However, heat stress markedly induced HSP70 expression with gradual recovery of intercellular communication and alkaline phosphatase activity after the heat stress. These findings provide the rationale that a critical increase of intrapulpal temperature of 5.5°C is required before the occurrence of irreversible pulpal injury and that an increase of 11.0°C would result in pulpal necrosis.[99]

The extent of intrapulpal heat generated during cavity preparation has been shown to be related to the use of coolants; handpiece rotation speed; the size, type, and shape of the cutting instrument; the length of time the instrument is in contact with the dentin; the amount of pressure exerted on the handpiece; and the cutting technique.[88] For example, the use of coarse diamond burs results in more pronounced temperature increases within the pulp chamber during tooth preparation compared to the use of fine diamond or tungsten carbide burs.[100]

In the absence of water coolant, the average temperature rise within the pulp chamber during cavity preparation exceeds 5.5°C. The temperature may increase up

to 20.0°C with the use of high air pressure and a heavy loading technique. When low water cooling is used with high pressure and heavy loading, the average temperature rise is 5.9°C. However, when copious water cooling is employed, the critical 5.5°C value is not reached with any air pressure or loading, and intrapulpal temperature change ranges from –1.8°C to 3.1°C.[101]

Apart from cavity preparations, heat produced during the fabrication of exothermic polymer-based provisional crowns[102] and the use of light-curing units with high-intensity outputs[103] over vital crown preparations with RDT thinner than 1 mm can result in intrapulpal temperature increases that exceed 5.5°C. These procedures may damage the pulp.

Bonding to Dentin

Resin-based materials

A smear layer is formed when dentin is cut or abraded with hand or rotary instruments.[104] The presence of the smear layer affects the strategies that can be employed for dentin bonding. Some strategies call for removal of the smear layer, whereas others penetrate the smear layer and incorporate it into the bonding layer. Both strategies have been employed in contemporary dentin adhesives.

Although enamel bonding has been successfully achieved since the 1950s, the first predictable dentin bonding did not occur until the 1980s.[1] Dentin is a wet substrate, and restorative resins tend to be hydrophobic ("water-hating"). Dentin is comprised of approximately 50% inorganic mineral (apatite) by volume, 30% organic components (primarily type I collagen), and 20% fluid. The wet environment and the lack of a mineralized surface made the development of effective dentin adhesives a challenge.

Nakabayashi and coworkers reported the first successful strategy for dentin adhesion in 1982.1 They showed that resin could be bonded to dentin after the dentin surface was demineralized and a hydrophilic ("water-loving") resin coating was applied. These steps were followed by the application of a hydrophobic layer that was light polymerized. This provided a hydrophobic surface for copolymerization

with resin composite restorative materials. The collagen matrix and the dentinal tubules, to a lesser extent, provide mechanical retention for the resin via the formation of a hybrid layer and resin tags.

Although not as durable and reliable as enamel bonding, dentin bonding forms the foundation for many current procedures in restorative dentistry. Dentin adhesive systems are functionally classified as etch-and-rinse or self-etching adhesives.

Etch-and-rinse adhesives

Most etch-and-rinse adhesive systems utilize a strong acid such as 30% to 40% phosphoric acid for removing smear layers and demineralizing dentin. When phosphoric acid is applied to dentin for 15 seconds, the surface is demineralized to a depth of 5 to 8 μm. The acid removes the smear layer and exposes the collagen matrix and network of dentinal tubules for resin infiltration. After the phosphoric acid is rinsed off, a hydrophilic primer is applied to the surface to infiltrate the collagen matrix and tubules. The primer consists of hydrophilic resin comonomers dissolved in a volatile solvent such as alcohol or acetone, which carries the resinous material into the collagen matrix and dentinal tubules. Most dentin adhesive systems require the surface to be wet (moist) for the primer to penetrate effectively.[105]

After application of the primer, a stream of air is used to evaporate the solvent. A blend of comparatively hydrophobic resin monomers (adhesive) is then applied and polymerized. The latter also copolymerizes with the infiltrated resin from the primer. A hybrid layer or interdiffusion zone that consists of resin, collagen, and partially dissolved apatite crystallites is formed[3] (Fig 14-14). The apatite crystallites are only present when dentin is etched with a mild calcium chelating agent such as ethylenediaminetetraacetic acid (EDTA). The hybrid layer links the hydrophobic restorative materials to the underlying hydrophilic dentin. It also functions as a shock absorber that dissipates stresses created during polymerization shrinkage of direct composite restorations as well as functional stresses created during mastication.[106]

Poor bond strengths and increased microleakage result from excessive etching.[107] The same is true if the residual solvent is not completely removed, which makes the bond more susceptible to hydrolytic breakdown.[105] Nanophase separation of the hydrophobic from the hydrophilic resin phases may also occur because there is no control over the amount of water left on the tooth during bonding with etch-and-rinse adhesives.[108]

Self-etching adhesives

Self-etching adhesives contain increased concentrations of acidic resin monomers. Rather than removing the smear layer, they penetrate and incorporate the smear layer in the hybrid layer (Fig 14-15). The acidic primer is applied to the dentin surface for 15 to 20 seconds and dried with a stream of air. There is no rinsing step. After a resin adhesive is applied and polymerized, the restorative material is placed.

Self-etching systems may be categorized as strong (pH < 1), moderate (pH 1 to 2), or mild (pH > 2).[109] The strong self-etching systems form hybrid layers that are approximately 5 µm thick, similar to those created by phosphoric acid. Mild self-etching systems produce hybrid layers that are less than 1 µm thick. There is no clinical difference with respect to the thickness of hybrid layers created by strong and mild self-etching ahdesives.[110]

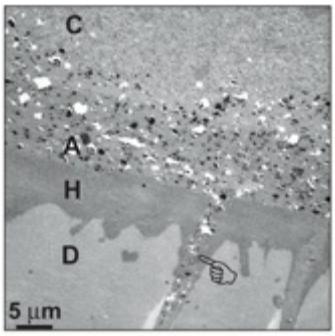

Fig 14-14 *(left)* Transmission electron micrograph of a section through dentin that is bonded with an etch-and-rinse dentin adhesive applied after phosphoric acid etching for 15 seconds, rinsing, and the use of a wet bonding technique. A 5-µm-thick hybrid layer (H) can be seen along the interface between dentin (D) and the filled adhesive (A). Penetration of the filled adhesive into the patent dentinal tubules is evident *(pointer)*. C, resin composite.

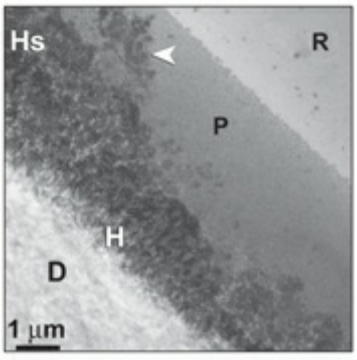

Fig 14-15 *(right)* Transmission electron micrograph of a section through dentin that is bonded with a mild self-etching dentin adhesive. To be effective, the self-etching primer component (P) of the adhesive must be acidic enough to etch through the smear layer to create a hybrid layer (H) in the underlying intact dentin (D). During this process, the smear layer is partially dissolved and any remnant smear layer *(arrowhead)* is incorporated into the resin-dentin interface as a hybridized smear layer (Hs). R, adhesive resin component of the self-etching adhesive.

Chronologic classification of dentin adhesives

Etch-and-rinse adhesives that utilize three steps (etchant, primer, and adhesive) are known as *fourth-generation systems*. Each generation of dentin adhesive is a marketing creation, and naming generally follows the order in which the materials were developed.

Each generation utilizes different bonding procedures. The fifth-generation adhesive systems are etch-and-rinse by functional classification. A combined primer and adhesive solution is applied to the acid-etched dentin. They are sometimes referred to as *single-bottle adhesive systems*. The sixth generation adhesives utilize an acidic (self-etching) primer followed by an adhesive, combining the acid-etching and the priming steps. The fifth and sixth generations are generally two-step procedures, while the seventh generation combines everything (acid, primer, and adhesive) into one step.

In general, self-etching adhesives require fewer steps and less chair time for the bonding procedures than the etch-and-rinse adhesives and are considered to be less technique sensitive.[111] Despite their simplicity and user friendliness, there is a general consensus that both simplified etch-and-rinse and self-etching adhesives behave less favorably and are less durable than their multistep counterparts.[112,113] This is particularly true for the one-step self-etching adhesives.[114]

Self-adhesive resin cements have been introduced recently for bonding of indirect restorations to tooth structure.[115] Mild acid-etching monomers are incorporated in these self-adhesive resin cements so that they do not require the adjunctive use of a dentin adhesive. However, they do not demineralize or dissolve the smear layer completely, and hybrid layer formation is either absent or minimal.[116] Because most of the information available on self-adhesive resin cements comes from in vitro studies, their long-term clinical performance is unknown.

Conventional glass ionomers

Glass ionomers consist primarily of alumina, silica, and polyalkenoic acid and are self-curing materials. They release fluoride for a period of time after initial placement and are the only restorative materials that form a chemical bond to tooth structure.[117] They derive mechanical retention from microporosities created on the

dentin surface by etching with mild acids to create a hybrid layer–like interface (Fig 14-16). Unlike resins, glass-ionomer materials form a "dynamic" bond. As the interface is stressed, bonds are broken and new bonds form. This is one of the factors that enable glass ionomers to succeed clinically.[118] Other factors are low polymerization shrinkage and a coefficient of thermal expansion that is similar to that of tooth structure.

Although resin composites are esthetic and exhibit acceptable physical strength and wear resistance, they are hydrophobic and therefore more difficult to handle in the oral environment. They barely support ion migration. Moreover, the problems of gaining long-term adhesion to dentin have yet to be overcome, as discussed later in this chapter. Conversely, glass ionomers are water based and therefore have the potential for ion migration, both inward to and outward from the restoration, leading to a number of advantages, such as fluoride release and recharging. However, they lack the physical properties required for use in load-bearing areas.[119]

There has also been concern that release of aluminum ions from glass ionomers may increase serum aluminum levels that potentially contribute to renal and neurodegenerative disorders.[120,121] A recent study indicates that even when a glass-ionomer restoration is dissolved completely over a 5-year period, it will only add an extra 0.5% of the recommended maximum intake of aluminum to an adult patient. This led the researchers to conclude that the release of aluminum from glass ionomers in the oral environment poses a negligible health hazard.[122]

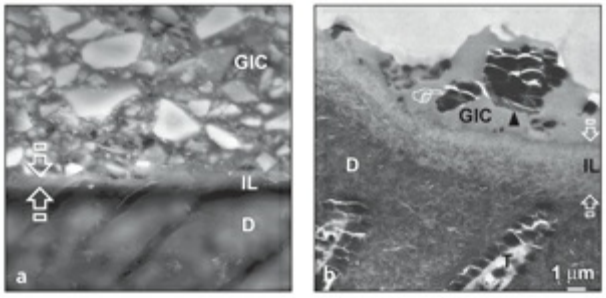

Fig 14-16 *(a)* Environmental scanning electron micrograph, taken at 95% relative humidity, of the interface between a conventional chemically cured glass ionomer (GIC) and dentin (D). Treatment of the dentin surface with a polyacrylic acid conditioner removes the smear layer and partially demineralizes the intact dentin. Chemical interaction of this dentin surface results in the formation of an interaction layer (IL, *between open arrows*) between the glass ionomer and dentin. The use of an environmental scanning electron microscope prevents arti-factual crack formation in a water-based restorative material (original magnification ×3,000). *(b)* Corresponding transmission electron micrograph illustrating the interaction of the glass ionomer (GIC) with dentin (D) to form the interaction layer (IL, *between open arrows*). A hydrogel layer *(arrowhead)* can be seen around the fluoride-releasing leachable glass fillers *(pointer)*.

Resin-modified glass ionomers

Resin-modified glass-ionomer materials (RMGIs) were developed to overcome some of the undesirable properties of the traditional glass ionomers.[123] RMGI materials such as Vitremer (3M ESPE) and Fuji II LC (GC) contain glass-ionomer powder, to which a light-cure resin is added. This allows immediate light polymerization after the material is placed. The resin also protects the glass ionomer from dehydration and improves its physical and mechanical characteristics as well as esthetics. True RMGI materials utilize the same bonding procedures as traditional glass ionomers and do not require a dentin bonding agent (Fig 14-17) because RMGIs contain hydrophilic resin monomers such as 2-hydroxyethyl methacrylate. The latter has a variety of damaging biologic properties, ranging from pulpal inflammation to allergic contact dermatitis. Hence, RMGIs cannot be considered to be as biocompatible as conventional glass ionomers.[124]

Nevertheless, clinical results with these materials are generally positive. A recent review on the clinical use of RMGIs indicates that their retention is generally good, with a reported annual failure rate of less than 3% over 13 years.[125] However, they exhibit margins that are likely to deteriorate over time. They appear to exhibit some wear and loss of anatomical form, particularly in the mid to long term. Large Class II resin composite restorations may be associated with secondary caries[126,127] and postoperative sensitivity,[128] but RMGIs appear to have limited problems associated with these issues.[125]

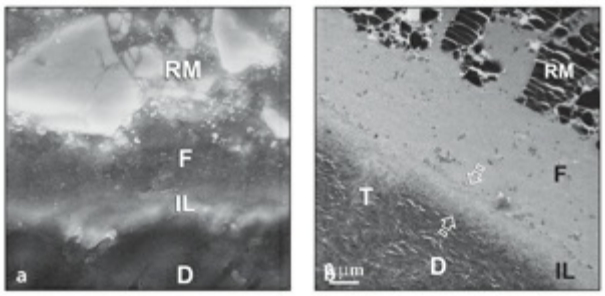

Fig 14-17 *(a)* Environmental scanning electron micrograph illustrating the bonding of a light-cured resin-modified glass-ionomer (RM) to dentin (D). Similar to conventional glass-ionomer cements, an interaction layer (IL) can be seen along the material-dentin interface that is partially infiltrated by resin. Unlike conventional glass ionomers, a filler-free resinous layer (F) can be found directly above the dentin surface, separating the filler-containing cement from the dentin (original magnification ×6,000). *(b)* Corresponding transmission electron micrograph. RM, resin-modified glass ionomer; F, filler-free resinous layer; T, dentinal tubule; D, dentin; IL, interaction layer (*between open arrows*).

Limitations of Dentin Bonding

Dentin bonding has limitations, many of which are related to polymerization shrinkage.[129] When resin-based materials polymerize, monomer molecules join to form chains that contract as the chains grow and intertwine, and the mass undergoes volumetric shrinkage. Resin-based restorative materials shrink from 2% to 6% depending on the volume occupied by filler particles and the test method. The force of polymerization contraction often exceeds the bond strength of dentin adhesives to dentin, resulting in gap formation along the surfaces with the weakest bonds. Separation often occurs within the hybrid layer but can occur in other areas.[130]

The ability of a cavity to dissipate stress induced by polymerization shrinkage of resin composites depends on its cavity geometry.[131] Cavity *configuration factor (C-factor)* refers to the ratio of bonded surface area to unbonded surface area in a cavity. A boxlike Class I cavity has five bonded surfaces and one unbonded surface, giving it a C-factor of 5, assuming all walls have the same surface area. Class II and Class III restorations have C-factors of 1 to 2. Class V wedge-shaped restorations have C-factors of 1.5 to 3, depending on the specific cavity configurations. The lowest C-factors are encountered in Class IV restorations, which have values of 1 or less.

Restorations with higher C-factors have more bonded surfaces and are believed to undergo higher stress. This may cause debonding of the restoration. During the polymerization stage, the maturing resin composite–dentin bond competes with the shrinkage stress of the setting composite. The magnitude of composite shrinkage stress depends on both the C-factor and the composite material. Resins in thin layers generate very high shrinkage stresses from polymerization contraction after bonding. Low-viscosity flowable composites exhibit higher polymerization shrinkage due to their lower filler contents. However, their shrinkage stresses vary according to the type and amount of elastic resin monomers that are included in these composites.

In situations where shrinkage stress exceeds adhesive bonding strength, shrinkage stress affects the composite-dentin bond, causing the composite restoration to debond from tooth structures. Debonding may result in decreased retention of restorations, postoperative sensitivity and pain, micro-leakage, cracked teeth, and secondary caries. Restorations with lower C-factors have more unbonded surfaces, which are free to move and flow during the composite's early gelation stage. This condition relieves shrinkage stresses and helps to preserve the composite-tooth bond. Thus, Class I cavities are the most difficult to bond with direct composite

placement techniques. Conversely, Class IV direct composite restorations create the least shrinkage stresses on enamel and dentin surfaces due to the large surface area available for the relief of shrinkage stresses.

Another important limitation of dentin bonding is deterioration of the resin bond over time.[3,132] This process is well documented in both in vitro and in vivo studies[30,133] (Fig 14-18). One of the most important factors in the stability of the resin-dentin bond is the completeness of resin infiltration within the demineralized dentin. If resin monomers do not completely infiltrate the demineralized collagen matrix, fluid movement between the hybrid layer and unaffected dentin (also known a s *nanoleakage*) expedites bond degradation. Water ingress can also result in hydrolysis and plasticizing of the resin components. Plasticization is a process in which fluids are absorbed by hydrophilic resins. This causes the polymerized resins to swell, resulting in degradation of their mechanical properties.

As discussed earlier in the chapter, MMPs also play an important role in degradation of the incompletely infiltrated collagen fibrils within hybrid layers. MMP inhibitors have been shown to be effective in arresting the degradation of hybrid layers.[134] Chlorhexidine, an antimicrobial agent, possesses anti-MMP activities against MMP-2, -8, and -9. It has been shown to effectively inhibit collagen degradation in vitro[134,135] and in vivo.[30,133] Other MMP inhibitors, such as SB-3CT[135] and GM6001 (galardin),[136] have also shown to be effective in arresting the degradation of hybrid layers created by etch-and-rinse adhesives in vitro.

Although the integrity of hybrid layers may be preserved by application of MMP inhibitors as part of the dentin bonding procedures, several potential issues associated with this technique require further clinical validation. First, although chlorhexidine possesses substantivity, it is water soluble and may eventually leach from hybrid layers. Thus, it remains to be determined whether the MMP-inhibiting activity of chlorhexidine is provisional or permanent (ie, delaying instead of arresting hybrid layer degradation). Second, MMP inhibitors appear to be useful in preventing the degradation of hybrid layers created by etch-and-rinse adhesives only and not those created by self-etching adhesives.[135] Third, chlorhexidine may exert a mild cytotoxic effect if it is applied to acid-etched dentin that is close to the dental pulp (ie, RDT of less than 0.2 mm).[137] Fourth, apart from MMPs, cysteine cathepsins that are capable of collagen degradation are present in mineralized dentin.[28] These proteases are not amenable to inhibition by MMP inhibitors. Finally, even when the hybrid layer may be prevented from degradation by MMPs and cathepsins, a zone of resin-sparse, demineralized dentin invariably remains that is potentially susceptible

to cyclic fatigue after prolonged intraoral function. Thus, the long-term clinical effectiveness of the use of MMP inhibitors in preventing the degradation of hybrid layers has to be further evaluated.

Another approach to preventing the degradation of hybrid layers is to adopt the tissue engineering concept of intrafibrillar remineralization of dentin collagen matrices.[138] In this guided tissue remineralization approach, biomimetic analogs of dentin non-collagenous proteins are used to sequester and template the penetration of amorphous calcium phosphate nanoprecursors into incompletely resin-infiltrated collagen matrices of dentin hybrid layers. These nanoprecursors are subsequently transformed into intrafibrillar apatites within the gap zones of those collagen fibrils.[139] Nevertheless, this biomimetic remineralization strategy is at its proof-of-concept stage of development and awaits translation into a clinically applicable delivery system.

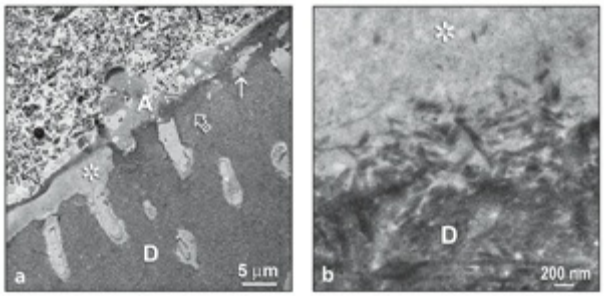

Fig 14-18 *(a)* Low-magnification transmission electron micrograph of the degradation of resin-bonded vital human dentin after 14 months in function in vivo. A zone of complete degradation *(asterisk)* and zones of partial degradation of the collagen matrix *(solid arrow)* are located within the hybrid layer *(between open arrows)*. In the zone of complete degradation, only the surface of the hybrid layer remains *(open arrowhead)*. C, resin composite; A, adhesive; D, dentin. *(b)* Higher magnification of the region indicated by the asterisk in *(a)*. The collagen fibrils within the original hybrid layer have completely degraded into microfibrils (not shown) and further into a stainable, amorphous material *(asterisk)* that probably consists of peptides and amino acids. D, dentin.

Dentin Bonding and the Pulp

Three aspects of dentin bonding may affect the underlying pulp: *(1)* acid etching, which increases dentin permeability; *(2)* cytotoxic effects of resin components diffusing through exposed tubules; and *(3)* ingress of microbes and microbial products (microleakage). Etching with a strong acid removes the smear layer and demineralizes the intertubular dentin to a depth of 5 to 8 µm.[1] In addition, smear plugs are dissolved from within tubules. The latter are enlarged as peritubular dentin

is dissolved to a variable depth. Hence, dentin permeability is increased, at least temporarily, until the resin penetrates and polymerizes.[10] Diffusion of resin components toward the pulp is increased as a result, although the extent of diffusion is strongly influenced by dentin thickness and the outward flow of dentinal fluid in vital teeth.[11,12] The highest bond strength and lowest leakage are achieved when resin tags are bonded to tubule walls so that they form a continuous unit with the hybrid layer present in the intertubular dentin.

Material toxicity to the underlying pulp becomes an issue only when the RDT is less than 0.5 mm thick. The mechanisms of resin cytotoxicity are related first to the short-term release of free monomers occurring during the monomer-polymer conversion. Long-term release of leachable substances subsequently occurs by erosion and degradation of the polymerized resin matrix over time. Molecular mechanisms involve glutathione depletion and reactive oxygen species production as key factors, leading to pulp or gingival cell apoptosis.[140]

Several clinical studies involving human teeth have evaluated the pulpal response to dentin bonding agents applied to acid-etched deep dentin in association with resin composite restorations. Persistent chronic inflammatory responses with only infrequent evidence of hard tissue repair have been reported. Resin globules were frequently observed within the dentinal tubules that were caused by phase separation of the hydrophobic resin components in an environment with a high water-resin molar ratio. These resin globules were often identified within the pulp, provoking a foreign body reaction that was characterized by the presence of macrophages and multinucleated giant cells.[141] In cavity preparations with RDT less than 0.5 mm thick, a comparatively biocompatible cavity liner such as calcium hydroxide or an RMGI-based liner[142] is recommended. Nevertheless, a recent Cochrane systematic review was unable to identify the most effective pulp treatment for deep, nonexposed, asymptomatic teeth with extensive caries because of the lack of sufficient clinical studies that were eligible for inclusion.[143]

Dentin adhesives have also been advocated for direct pulp capping procedures. Most of the studies have yielded negative responses to such treatment, reporting persistent chronic pulpal inflammation and the absence of dentinal bridge formation (see chapter 13). Thus, dentin adhesives should not be used for direct pulp capping procedures. Calcium hydroxide and mineral trioxide aggregate remain the materials of choice when a mechanical exposure occurs.

Polymerization shrinkage of direct resin composite restorations may result in debonding at the tooth-restoration interface and the formation of marginal gaps,

particularly along the cervical margins.[144] Cuspal flexure in large, bonded restorations also increases the possibility for a marginal gap to open with every chewing cycle.[145] Gaps provide avenues for the ingress of bacteria and their by-products. A biofilm model has been used recently to show that mineral loss and lesion depth in secondary caries increased with gap width (50 to 250 μm) for resin composites but not for RMGIs.[146] The authors attributed their findings to the release of fluoride that inhibits dentin demineralization around the marginal gaps of glass-ionomer restorations. Unfortunately, in vitro evaluation of the marginal qualities of restorations is unlikely to predict their clinical outcomes.[147]

Physiologic Responses to Restorative Materials

Apart from the trauma induced in deep cavity preparation, chemicals from restorative materials and bacterial leakage at the tooth-restoration interface are the major causes of adverse pulpal responses.[87]

Effects on odontoblast survival

Resin-based materials have been shown to be cytotoxic to several cell lines in in vitro testing models and cause allergenic, mutagenic, estrogenic, and genotoxic effects.[140] While their potential aggressiveness toward in vitro cell lines is unambiguous, the effects of direct application of resin-based materials to the pulpodentin complex in in vivo usage testing models has generated much controversy. For many years, the toxicity of restorative materials was thought to be of crucial importance in the development of adverse pulpal responses. It is now recognized that bacterial leakage at the restoration-tooth interface is a more important concern with the use of these materials.[22] This does not mean, however, that the cytotoxicity of restorative materials will not adversely affect odontoblast survival and the formation of reactionary dentin. These effects, however, are only apparent when the restorative materials are placed in deep cavities with low RDTs.[9,20,21]

Odontoblast survival has been associated with the extent of pulpal injury. Calcium

hydroxide was used as the baseline for assessing odontoblast survival with different restorative materials.[87] These materials included zinc oxide–eugenol, amalgam lined with polycarboxylate cement, resin composite bonded to dentin, enamel bonding resin, and an RMGI. None of the materials exhibited significant differences in the rate of odontoblast survival when they were placed in cavities with RDTs between 0.5 and 3.0 mm (Fig 14-19). Conversely, different responses were observed when the materials were placed in cavities with an RDT of less than 0.5 mm. Materials had the following rates of odontoblast survival: calcium hydroxide (100.0%), polycarboxylate (81.1%), zinc oxide–eugenol (78.4%), and composite bonded to dentin (74.2%). These values were significantly higher than the odontoblast survival rates of enamel bonding resin (48.3%) and RMGI (43.1%).

The use of etchants for smear layer removal and bonding enhancement of resin composites is now perceived to have a minimal contribution to long-term pulpal injury.[2,22] Nevertheless, bonding of an etch-and-rinse adhesive to acid-etched, deep, vital human dentin with an RDT of less than 0.3 mm resulted in a more intense pulpal response than it did in unetched deep dentin.[141] Removal of the smear layer renders dentinal tubules patent and facilitates the pulpward diffusion of unpolymerized monomers through deep dentin. Because some materials are more cytotoxic to pulp tissues than others,[148] it is prudent to avoid placing comparatively cytotoxic materials in very deep preparations.

Although reduction in odontoblast cell numbers is unavoidable after the trauma of cavity preparation, placement of RMGIs within 0.5 mm of the pulp reduced odontoblast numbers by more than 50% (see Fig 14-19). This is consistent with the observation of moderate to severe inflammation following the use of glass ionomer–based crown luting cement in deep dentin.[149] However, no difference in the severity of inflammation was observed when a calcium hydroxide liner (Dycal, Dentsply Caulk) or an RMGI liner (Vitre-bond, 3M ESPE) was used to protect deep cavities in human dentin prior to the placement of resin composites.[150] A more recent study performed on nonhuman primates also reported that both Vitrebond and Dycal were biocompatible and produced minimal inflammatory responses in deep dentin.[72] Taken together, these results suggest that deep, unexposed dentin should be protected with either a calcium hydroxide liner or an RMGI liner prior to acid etching to avoid unnecessary odontoblast injury.

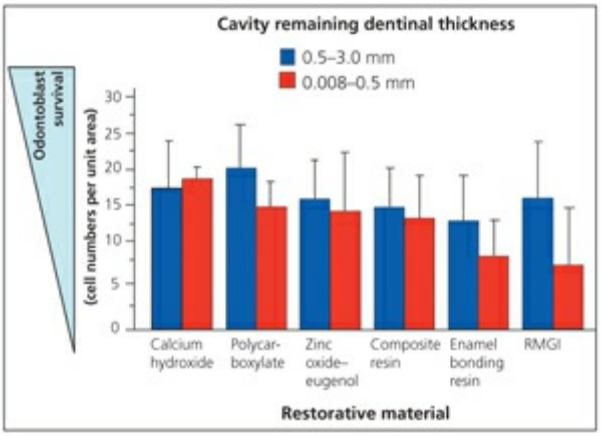

Fig 14-19 Relationship between restorative materials and odontoblast survival. (Reprinted from Murray et al[90] with permission.)

Effects on reactionary dentin formation

The stimulatory role of calcium hydroxide on reactionary dentin deposition has been well reported and forms part of the rationale for its wide use as an indirect pulp capping agent. Others have reported that reactionary dentin deposition is independent of the type of restorative material used. Trauma from cavity preparation and bacterial microleakage were considered to be more important factors in the stimulation of reactionary dentin than material irritation or cytotoxicity.[151] More recent work from Murray and coworkers[9,90] demonstrated that the relationship between reactionary dentin deposition and restorative materials is profoundly influenced by the RDT of the cavity preparations. In cavities with RDTs between 0.5 and 3.0 mm thick, reactionary dentin deposition was minimal for most restorative materials except for calcium hydroxide (Fig 14-20). With a cavity RDT of less than 0.5 mm, the rank order of the areas occupied by reactionary dentin was found to be, in decreasing order: calcium hydroxide, resin composite bonded to dentin, enamel bonding resin, RMGI, and zinc oxide–eugenol. No reactionary dentin formation was formed in cavities that were lined with polycarboxylate cement.

The differences in reactionary dentin deposition by different restorative materials may be caused by a combination of factors. They include the severity of irritational stimuli imposed by the cytotoxicity and chemical activity of these materials on the odontoblasts and adjacent pulpal tissues, as well as the ability of these materials to extract bioactive cell-signaling growth factors from the dentin matrix. Calcium

hydroxide, for example, is able to dissolve bioactive dentin extracellular matrix components.[152]

During restorative procedures, conditioning of the cavity walls with EDTA, phosphoric acid, or polyacrylic acid is frequently employed to improve the adhesion of dentin adhesives and RMGIs to dentin. In vitro dissolution of powdered dentin matrix has demonstrated that most etching agents are capable of releasing TGF-β1 and other growth factors from dentin.[153] The effectiveness of calcium-depleting agents to release TGF-β1 was in the following rank order: EDTA > phosphoric acid . citric acid > polyacrylic acid > nitric acid.[153] Application of EDTA-soluble dentin matrix protein preparation (ESDP), calcium hydroxide (Dycal) and RMGI (Vitrebond) to deep cavities prepared in nonhuman primate teeth showed that the extent of reactionary dentin deposition was in the following rank order: ESDP > calcium hydroxide > RMGI.[72]

Similar to calcium hydroxide, mineral trioxide aggregate also has the ability to release dentin matrix components.[154] This probably accounts for the excellent dentinogenesis and cementogenesis potential of mineral trioxide aggregate when it is used as a root end filling or as a pulp capping material (see chapter 13).

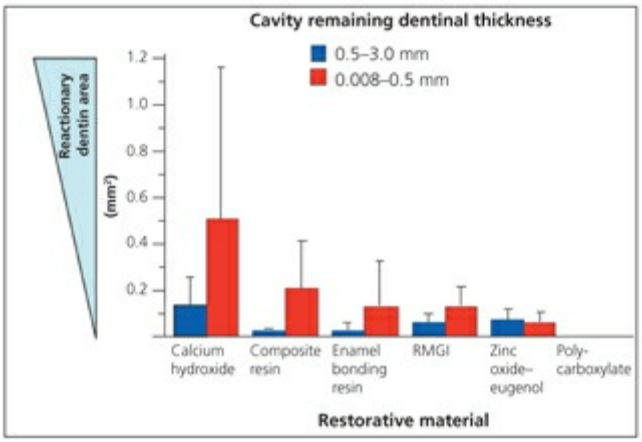

Fig 14-20 Relationship between restorative materials and formation of reactionary dentin. (Reprinted from Murray et al[90] with permission.)

Effects of bacterial leakage

Microleakage that occurs after restorative procedures may result in postoperative sensitivity, marginal discoloration, recurrent caries, and pulpal inflammation.[151]

Studies conducted over the last 40 years have shown that bacterial leakage along the tooth-restoration interface is the predominant cause of adverse pulpal responses.[155,156] Although the severity of inflammatory activity is related to cavity RDT, inflammation gradually subsides in the absence of bacterial leakage. Conversely, in the presence of persistent bacterial leakage, severe inflammation eventually results in pulpal necrosis and the development of periradicular lesions.[70]

In a comprehensive quantitative study, the type of restorative material had an important influence on bacterial leakage in nonexposed Class V restorations.[157] Restorative materials were ranked in the following order for their ability to prevent bacterial leakage: *(1)* RMGI (100%, ie, no leakage), *(2)* bonded amalgam (88%), *(3)* zinc oxide–eugenol (86%), *(4)* bonded resin composite (80%), *(5)* gutta-percha (64%), *(6)* calcium hydroxide (58%), *(7)* compomer (42%), *(8)* silicate (36%), and *(9)* zinc phosphate (0%, ie, all restorations leaked). Pulpal inflammation was also found to be highly correlated with bacterial leakage around the restorations (Fig 14-21).

The detection of bacteria beneath bonded resin composite restorations indicates that contemporary dentin adhesives are not yet able to perfectly seal dentin. Polymerization shrinkage and contraction stresses developed during setting of resin-based materials in cavities with unfavorable cavity geometry.[129] A variety of technical errors encountered during clinical operation[158] may also result in marginal gap formation.

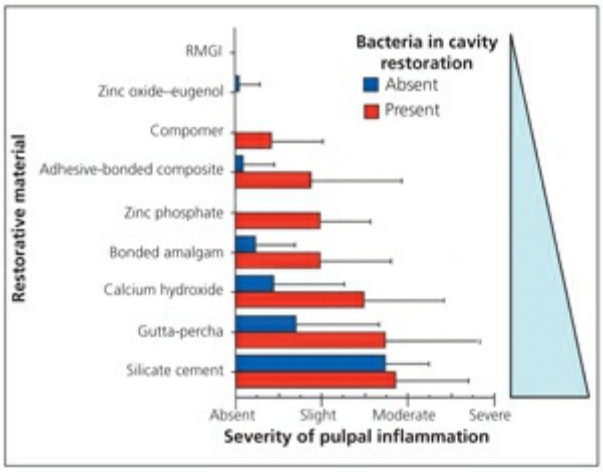

Fig 14-21 Relationship between restorative materials and pulpal inflammation. (Reprinted from Murray et al[90] with permission.)

Conclusion

Caries continues to be the most prevalent problem in dentistry, despite significant advances in prevention over the past few decades. The *NIDCR 2009– 2013 Strategic Plan*159 of the National Institute of Dental and Craniofacial Research lists dental caries as the single most common chronic disease of childhood in the United States:

> *Dental caries begins early in life: 18% of preschoolers in the U.S. have already experienced tooth decay and by age 6–8, more than half have experienced this disease, making it 5–8 times more common than asthma. By age 17, more than 80% of the adolescent population is affected by caries. Dental caries is also a problem among adults; secondary caries and root caries are prevalent among adults and the elderly.*

While strategies such as ozone therapy may have potential cariostatic benefits, the only evidence-based strategy available to date for treatment of dentinal caries is amputation of the grossly decayed tooth structure and the placement of an inert or bio-active restorative material to block further decay.

Restorative dentistry has undergone a paradigm shift from its original intention of restoring function to one that encompasses both functional and esthetic objectives to accommodate the contemporary demands for more natural and imperceptible restorations. Although dental amalgam and gold are effective direct restorative materials and may be considered the materials of choice for some restorations, they are neither tooth colored nor adhesive to remaining tooth structures. Thus, alternative tooth-colored restorative materials that have a greater degree of esthetic appeal have become increasingly popular.

Adhesive restorative dentistry has been progressing at a rapid rate over the last 10 years. A large part of this success is attributed to the significant advances in dentin bonding technology. From the early-generation systems in the 1970s that yielded weak and unpredictable bonds to contemporary glass ionomer–based materials and hydrophilic dentin adhesive systems that produce significant improvements in bond strength to normal dentin, the progress in the development of these synthetic adhesive materials has been nothing short of phenomenal.

It is pertinent that neither dental amalgam nor alternative tooth-colored filling materials have been able to achieve the degree of seal that is accomplished by natural enamel or the shock-absorbing capability that approximates the natural dentinoenamel junction. After all, the creativity of evolution, in which nature

explores all options and produces the best solution, has taken millions of years to arrive at perfection. Restorative materials, in contrast, have been invented for only slightly more than a century.

Systemic accumulation of mercury has the potential to cause a variety of neurologic and psychologic systemic symptoms. There is no scientific evidence, however, to indicate that the use of dental amalgams increases the risks of these adverse systemic effects. Although mercury release during amalgam placement and removal may result in increased mercury exposure to patients and dental personnel, this effect may be minimized with appropriate clinical techniques.

Tooth-colored restorative materials, apart from their esthetic appeal, offer the advantages of minimally invasive cavity preparation as a means to conserve remaining tooth structure. However, they are not without toxicologic hazards and clinical limitations. These materials contain a variety of organic components that are designed to undergo chemical reactions within a tooth cavity and in the vicinity of the dental pulp, gingival tissues, and the periodontium; by contrast, manufacturing of these components is often conducted in fume hoods and under stringent laboratory safety compliance regulations. Some of the monomers and catalysts used in these chemical reactions are cytotoxic and potentially mutagenic to the pulp and gingival cells in vitro. Nevertheless, there is little scientific evidence that they produce clinically significant adverse effects.

On a historical basis, dental amalgam restorations have been found to last longer than restorations performed using tooth-colored restorative materials. Resin composites in particular have been reported to have a higher incidence of secondary caries and require more frequent postinsertion professional interventions than their amalgam counterparts. Resin composite restorations are also susceptible to failure via degradation and fatigue.[160] According to the *NIDCR 2009–2013 Strategic Plan*,[159] resin composites bonded to tooth structure via dentin adhesives have an average replacement time of 5.7 years, and such failures are mainly due to secondary caries and fracture of the restorations.

Both dental amalgams and tooth-colored restorative materials may adequately ensure dental health. However, the relative risks and benefits of these materials should be explained to patients to assist them in making informed decisions.

Indirect restorations produced from precious metal alloys and different types of ceramics may also be used in situations where direct restorative treatments are contraindicated. A discussion of indirect restorative materials is beyond the scope of this chapter.

If nature's creative design of an impervious seal or an impeccable stress breaker in a natural tooth appears overly academic, one simply has to flip through the other chapters of this book to admire the ingenious design of the dental pulp. Indeed, restorative dentistry would not have progressed if the dental pulp did not possess an inherent capacity to cope with many of the injurious challenges to which it may be exposed during dental procedures. The pulpodentin complex is equipped with sensors that are capable of detecting imminent injuries. This notion is fortified by the recent discovery of the odontoblast as a sensory cell. In contrast, contemporary tooth-colored restorative materials lack self-diagnosing capability. The dental pulp is equipped with self-protective mechanisms that can deter or wall off noxious insults via the secretion of neuropeptides, inflammatory mediators, heat shock proteins, and reactionary dentinogenesis. Similar self-protective mechanisms are lacking in contemporary restorative materials. The dental pulp is equipped with the potential to regenerate odontoblast-like cells from dental pulpal stem cells through limited molecular signaling mechanisms after cessation of ectomesenchymal crosstalk that occurs during primary dentinogenesis. Autonomic healing mechanisms are lacking in contemporary restorative materials, with the exception of the limited repair and recharging potential that is present in conventional glass ionomers. Although experimental calcium- and phosphate-releasing remineralizing composites are available, the remineralization strategy involved in those materials has not fully exploited the contribution of SIBLING components of the extracellular matrix (see chapter 1) to biomineralization to optimize intrafibrillar remineralization of collagen fibrils.[161]

As the concept of pulpal regeneration becomes a foreseeable reality, the future of adhesive restorative dentistry should embrace and support this goal by incorporating biomimetic strategies for enhancing the regenerative potential of the dental pulp. This will involve collaboration among polymer chemists, materials scientists, molecular biologists, and tissue engineers. It is at the nanoscopic or molecular scale that the greatest expansion of horizons is anticipated. Advances in different scientific disciplines will enrich the pool of ideas for future developments in restorative materials that possess therapeutic capabilities. The potential depth of this pool is tremendous.

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Effects of Thermal and Mechanical Challenges

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David H. Pashley, DMD, PhD

Clinical dental procedures often generate thermal and mechanical stimuli of sufficient magnitude to injure the dental pulp or surrounding tissues. This chapter reviews the physical properties of these stimuli, the thermal properties of the pulpodentin complex, the ways in which stimuli are transmitted to the dental pulp or surrounding tissues, and the pathophysiologic processes that occur in response to stimuli. It is important for the clinician to understand these factors in order to provide minimally traumatic restorative or endodontic dental care. Other physical properties of the pulpodentin complex are reviewed in chapter 3, and the circulatory responses to thermal and mechanical stimuli are reviewed in chapter 6.

Physical Properties of Dental Materials

For an understanding of the thermal dynamics that occur in the pulpal and

periodontal tissues, it is necessary to review the physical properties of dental materials. Thermal changes occur during dental treatment in response to heat generated by restorative procedures or laser treatment and in the root canal and surrounding tissues during the application of thermoplasticized gutta-percha or polycaprolactonebased products such as Resilon (Resilon Research).

One important thermal property, *thermal conductivity (k)*, is defined as the quantity of heat (in watts [W] or joules [J]) that passes per second through a body 1 m thick, with a cross-sectional area of 1 m^2, when the temperature gradient is 1 Kelvin (K).[1] Thermal conductivity is measured in units of W m^{-1} K^{-1}. Materials with thermal conductivities greater than 10 W m^{-1} K^{-1}, which includes most metals, are considered good thermal conductors. Materials with low thermal conductivities, such as enamel, dentin, porcelain, cements, resins, and gutta-percha, are considered poor thermal conductors or, conversely, excellent insulators. Dentin is an excellent insulator because it is a poor thermal conductor[2–4] (Table 15-1).

A second important thermal property is the *specific heat* of a material. This is the amount of heat, in J, necessary to raise the temperature of 1 kg of substance by 1 K. Water is unique in that it has very high heat capacity. In general, the specific heat of liquids is higher than that of solids, and metals have specific heats that are less than 10% that of water (see Table 15-1).

The higher specific heat of dentin over that of enamel is largely due to its greater water content. The low specific heat of metals, combined with their high thermal conductivity, means that they can be heated and cooled more rapidly than dentin. This property has clear clinical implications when metallic restorations are polished or removed with mechanical friction (ie, handpiece and bur).

Table 15-1 **Thermal properties of teeth and selected materials**

Material	Thermal conductivity (W m^{-1} K^{-1})	Specific heat (J kg^{-1} K^{-1})	Thermal diffusivity (mm^2 s^{-1})	Thermal expansion coefficient (× 10^{-6} mm^{-1} K^{-1})
Gold	297.50	0.13	127.00	14.1
Amalgam	23.01	-	9.6	25.0
Zinc phosphate cement	1.17	-	2.30	-
Acrylic resin	0.21	1.46	1.23	76.0

Enamel	0.92	0.75	4.69	11.4
Dentin	0.63	1.17	1.83	11.0
Water	0.59	4.18	14.00	-
Gutta-percha	-	-	-	55.0
Stainless steel	16.00	0.49	4.20	17.0

-, no data available.

A third important thermal property is the *thermal diffusivity* (D), which determines the transient heat flow.[1] It is defined as the thermal conductivity *(k)* divided by the product of the specific heat of a material times its density *(r)* and is measured in units of $mm^2\ s^{-1}$. The thermal diffusivity of a material describes the rate at which a body of nonuniform temperature approaches equilibrium.[1] The thermal diffusivity of metals is more than 100-fold higher than that of dentin (see Table 15-1).

The flow of heat across dentin obeys fickian diffusion theory. The diffusion coefficient for heat has the same units as thermal diffusivity ($mm^2\ s^{-1}$). In bulk dentin, this value is 1.83 $mm^2\ s^{-1}$.[2,5]

The final important thermal property of materials is the *linear coefficient of thermal expansion* (LCTE), which measures the thermal strain in a material per each 1 K. The units are generally $\times\ 10^{-6}\ mm^{-1}\ K^{-1}$.[5] The LCTE is the change in length per unit length of material for a 1 K change in temperature. Because these values are so small, they are usually expressed in exponential form. Volumetric coefficients of thermal expansion are three times that of LCTE values.

The difference between the LCTE (sometimes referred to simply as α) of dentin and that of a restorative material placed inside or over dentin determines the potential for thermally induced gap formation. The clinical success of gold, glass ionomers, and porcelain restorations is due in part to the fact that their LCTE values are around 11 to $14 \times 10^{-6}\ mm^{-1}\ K^{-1}$, which are very close to that of dentin ($11.0 \times 10^{-6}\ mm^{-1}\ K^{-1}$). Unfortunately, the LCTE of gutta-percha ($55.0 \times 10^{-6}\ mm^{-1}\ K^{-1}$) is much higher than that of dentin[6] (see Table 15-1), making it more likely that gaps will form between root dentin and gutta-percha during thermal compaction unless vertical force is applied during cooling of gutta-percha. That is, as thermoplasticized gutta-percha cools down to body temperature, it undergoes a thermal contraction that

is equivalent to the expansion that occurred when gutta-percha was thermally plasticized.

Coefficients of thermal expansion of dentin (the linear expansion per 1 K) are usually given as a single value that is an average for a large volume of tissue. This assumes that the material is homogenous. However, dentin is a very heterogenous biologic composite material. Hence, it is not surprising that there is a wide range in the coefficients of thermal expansion of dentin rather than a single value.[5]

Using moiré interferometry, the patterns of microstrain in roots were determined at temperatures varying from 30°C to 70°C in steps of 5°C.[5] These authors chose to report their values in $\mu m\ °C^{-1}$, which is equivalent to $\times 10^{-6}\ mm^{-1}\ K^{-1}$. The results indicated that the LCTE of cervical root dentin was not constant with temperature. It was 10.7 $\mu m\ °C^{-1}$ at 35°C, peaked at 25.8 $\mu m\ °C^{-1}$ at 50°C, fell to 10 $\mu m\ °C^{-1}$ at 60°C, and then fell to $^{-30}$ $\mu m\ °C^{-1}$ at 70°C. That is, at temperatures greater than 62.5°C, further heating caused thermal contraction. Similar results were obtained in apical root dentin (Fig 15-1).

When those authors used a thermomechanical analyzer to measure LCTE values in 3 × 3 × 3–mm cubes of cervical or apical root dentin, they obtained values that were twice as high.[5] They concluded that the most accurate measurements of LCTE are made in dentin in situ, where inner dentin is constrained by outer dentin, rather than in the outside dimensions of unrestrained blocks of dentin. Moiré interferometry provides the spatial resolution of thermally induced changes in microstrain that are not possible using resistance-type strain gauges or linear variable differential transformers that are used in thermomechanical analyzers.

There were significant differences in the values of LCTE in cervical and apical dentin locations and differences as a function of temperature.[5] This indicates that computer simulations of changes in stress and strain obtained using finite element analyses may be in error because they use only an average value for the LCTE of dentin. The same authors used moiré interferometry to measure changes in microstrain of dentin subjected to mechanical loads.[6]

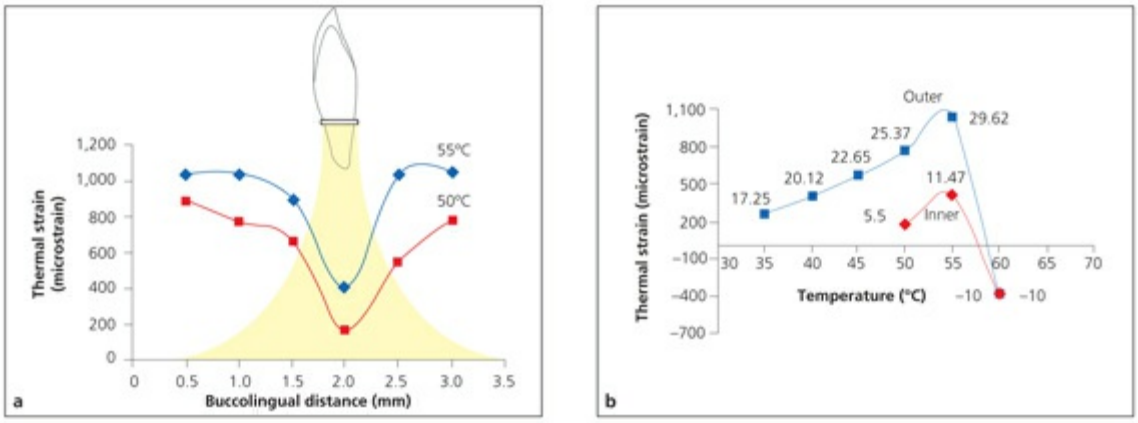

Fig 15-1 *(a)* Thermally induced microstrains across the apical third of root dentin at 50°C and 55°C. *(b)* LCTE of apical root dentin, calculated by dividing the microstrain ($\times 10^{-6}$) by the temperature. The LCTE is higher in outer dentin and is temperature dependent. (Modified from Kishen and Asundi[5] with permission.)

Pulpal Responses to Thermal Stimuli During Cavity Preparation

Early studies on pulpal reactions to thermal challenges were performed by Zach and Cohen,[7] using rather crude instruments. They were concerned about heat generated during cavity preparation or finishing procedures. Their histopathologic assessment of the subsequent pulpal reactions to application of heat to enamel in intact teeth indicated that pulpal temperature increases of 5°C to 17°C would cause progressively more severe pulpal necrosis. A study by Nyborg and Brännström[8] applied heat to the dentinal fl oor of Class V cavities in human volunteers. They applied a 150°C stimulus for 30 seconds to dentin that had a remaining dentinal thickness (RDT) of 0.5 mm. Histologic examination of the pulps of those teeth showed loss of odontoblasts on the side of the pulp that contained the cavity. After 1 month, the pulp beneath the heated dentin exhibited excessive collagen matrix formation that occasionally contained cells and capillaries but did not mineralize. Of 20 test teeth, 14 were free of inflammation. The patients had no painful sensations in the heated teeth over the 30-day period. that produced in pulp tissues. However, the outcome may be influenced by the fact that the blood flow per milligram of tissue is higher in the periodontal ligament (PDL) than in the pulp.[9]

In their classic study of pulpal reactions to cavity preparation, Zach and Cohen[10] demonstrated that pulpal temperature was actually lowered during cavity

preparation with high-speed handpieces and air-water spray because the water spray was cooler than the temperature of the pulp (Fig 15-2) and because of the high heat capacity of water. They recommended what they called the *washed-field technique* of tooth reduction, in which the tooth surface is exposed to the air-water spray for 5 seconds before cutting. After initial cutting, the bur is lifted off the surface for 1 second following every 4 seconds of cutting. When this technique was used, the pulpal temperature never rose above basal temperatures (see Fig 15-2).

Cutting at high speeds with air alone used as a coolant lowered pulpal temperature prior to cavity preparation; however, the pulpal temperature rapidly rose as much as 8°C higher than normal during the procedure.[10] This observation has been confirmed by others.[8,9,11–14] The frictional heat production will depend on rotational speed[15] and torque,[14] the amount of force applied to the bur,[16–18] the cooling efficiency of the irrigant, and the prior wear and design of the bur (eg, cutting blades such as carbide fissure burs or grinding surfaces such as diamond burs).[19] Collectively, these results indicate that pulpal reactions to various restorative procedures[17–19] are not necessarily caused by excessive heat production. However, it is difficult to precisely position temperature sensors to detect heat generated during cutting. In addition, the poor thermal conductivity of dentin can result in thermal burns to surface dentin without much change in pulpal temperature.[20]

Pulpal reactions to restorative procedures may in part be caused indirectly. It is possible that a high surface temperature can thermally expand the dentinal fluid in the tubules immediately beneath poorly irrigated burs. If the rate of expansion of dentinal fluid is high, the fluid flow across odontoblast processes, especially where the odontoblast cell body fills the tubules in predentin, may create shear forces sufficiently large to tear the cell membrane[21] and induce calcium entry into the cell,[22] possibly leading to cell death.[23] This hypothesis suggests that thermally induced fluid shifts across tubules serve as the transduction mechanism for pulp cell injury without causing much change in pulpal temperature.

An additional factor that can cause pulpal irritation is evaporative fluid flow.[24] Blowing air on dentin causes rapid outward fluid flow that can induce the same cell injury as the inward fluid flow caused by heat. For this reason, dry cutting with air is not recommended. Although air blasts lower pulpal temperature,[10–13] they induce very rapid outward fluid flow in dentinal tubules[24–26] that can create shear stress across odontoblasts and subodontoblastic cells and may tear their membranes.

Recent studies have not been able to confirm the earlier reports of pulpal damage

from thermal stress. Because many dental procedures can elevate pulpal temperatures by 9°C to 15°C, Baldissara et al[27] evaluated the pulpal response to these temperature changes in normal young premolars scheduled for extraction for orthodontic purposes. They placed custom-fabricated metal plates on the teeth. Thermoresistors were attached to the plates to produce controlled heat flows. The surface temperature of the test teeth was measured before and during controlled heating in nonanesthetized patients who were able to record both prepain and pain sensations during these procedures.

The rate of heat application in this study[27] was much lower than that used by Zach and Cohen[7] and was selected based on the rate of heating reported in the literature from a variety of restorative procedures (Fig 15-3). In monkeys, Zach and Cohen[7] found that a pulpal temperature of 40.5°C produced pulpal necrosis in 60% of the tested teeth. In the human study conducted by Baldissara et al,[27] heating of teeth to 39.5°C to 50.4°C (average 44.5°C) caused pain. This was perceived first as a "swelling" of the tooth, but as the thermal stimulus continued, the pain became more intense in magnitude, dull in perceptual character, and poorly localized. These symptoms are hallmarks of unmyelinated C-fiber nociceptor pain (see chapters 7 and 8). The occurrence of postoperative symptoms was followed for 63 to 91 days, during which time none of the patients reported any spontaneous tooth pain. Histologic examination of the teeth failed to demonstrate any signs of inflammation or reparative dentin.

Similar in vitro studies[27] were done on extracted human teeth with thermocouples placed at the pulpodentin border, immersed to the cementoenamel junction in 37°C. When the same electric currents were applied to the teeth in vitro, the authors could follow the rate and duration of changes in pulpal temperature (see Fig 15-3). This study concluded that young premolars could withstand increases in pulpal temperature between 8.9°C and 14.7°C without any histologic evidence of pulpal damage. Their rate of heat application was less than that used in the studies by Zach and Cohen.[7,10] Thus, the rate of delivery of heat is probably more important than the absolute rise in pulpal temperature.

This temperature range is similar to that measured in pulp chambers during finishing or polishing of restorations.[28] Even higher increases in pulpal temperature have been measured during self-curing of provisional crowns[28–30] and from visible light–cured crowns.[31] These studies were repeated using the turbo tips that concentrate light on smaller surfaces[32] and new high-intensity light sources,[32,33] with similar results.

Some authors believe that in vitro studies of pulpal temperatures in response to various thermal stimuli should be done while the pulp chamber is perfused by fluid to simulate pulpal microcirculation. A recent study confirmed that perfusion of the pulp chamber significantly lowered the pulpal temperature increases in response to external thermal stimuli[34] as well as to light-curing units.[35]

An in vitro experiment involved the use of 0.5- mm dentin disks covered on their pulp surfaces by odontoblast-like cells. When the investigators irradiated the upper dentin surface with a quartz tungsten halogen or a light-emitting diode (LED) light-curing unit at 553 and 240 mW/cm2, respectively, the "pulp" surface temperature rose 6.4°C and 3.4°C. This resulted in 36.4% and 33.4% reductions, respectively, in cell metabolism as measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The authors concluded that a quartz tungsten halogen curing unit activated for 40 seconds could cause adverse cellular responses in 0.5-mm-thick dentin.[36,37]

The reader is referred to a recent review on heat transfer in human teeth[38] for further details.

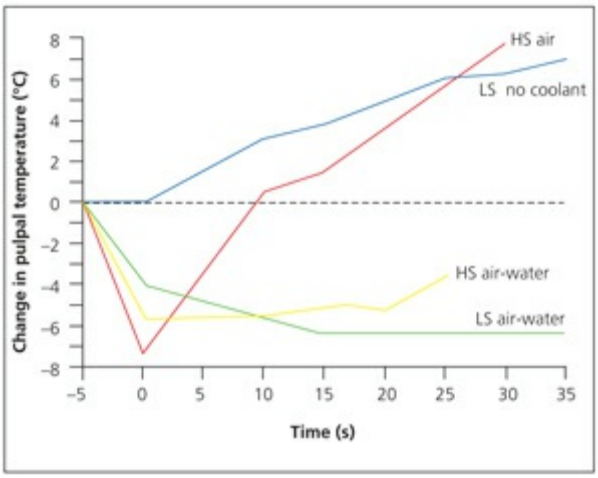

Fig 15-2 Changes in pulpal temperature during low-speed (LS) and high-speed (HS) cavity preparation with and without air-water cooling. (Modified from Zach and Cohen[10] with permission.)

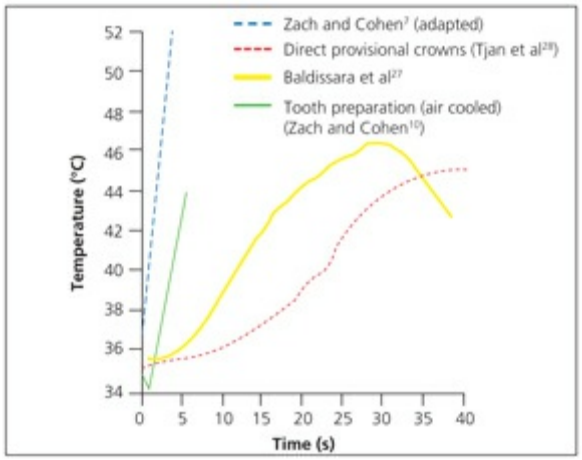

Fig 15-3 Increases in pulpal temperature following a variety of experimental procedures. (Modified from Baldissara et al[27] with permission.)

Pulpal Responses to Mechanical Stimuli During Cavity Preparation

Several studies have reported on the release of enzymes and other immunoreactive substances in the dental pulp during mechanical tooth preparation procedures. The release of these substances may be due to temperature increases, the mechanical stimuli of tooth preparation, or both. Because temperature effects have already been discussed, this section reviews only the mechanical, nonthermal causes of release of these substances.

Many enzymes and other immunoreactive substances are normally present in the pulp under unstimulated conditions. When the pulp is stimulated mechanically, these substances are released by physiologic mechanisms (eg, exocytosis) or by disruption of cell membranes.[39] An early study in monkey teeth examined the effect of cavity preparation on enzyme release (alkaline and acid phosphatases and others) in the pulp.[40] Tooth preparation by air turbine and adequate water cooling did not affect enzyme activity, nor did the application of corticosteroids. When calcium hydroxide was applied to the cavity floor, however, enzyme activity was increased after 24 hours in the odontoblastic and subodontoblastic cell layers adjacent to the calcium hydroxide–covered dentin. Fifteen days later, slight dentin formation was found, possibly indicating a role for these enzymes in stimulating hard tissue formation.[40]

Neuropeptides such as substance P and calcitonin gene–related peptide are

present in dental pulp in relatively high concentrations (see chapters 7 and 8). Rat molar dentin was prepared with a high-speed handpiece and bur to determine injury-related changes in the levels of immunoreactivity of both of these substances.[41] Pulpal exposures caused massive decreases in immunoreactive substance P (10% of baseline levels) and moderate decreases in immunoreactive calcitonin gene–related peptide (45% of baseline levels) because of the destruction of nerves that are the source of these neuropeptides. Preparation and acid etching without exposure caused decreases of 10% to 20% and 60%, respectively, of baseline levels. This study indicated that pulpal neuropeptides undergo dynamic injury-and peptide-specific responses following pulpal trauma (see chapters 7 and 8).

Other changes may occur in the trigeminal ganglion (see chapter 8). For example, dental injuries affect the presence and distribution of neuropeptide Y–like immunoreactivity.[42] In normal trigeminal ganglion, some perivascular nerves displayed neuropeptide Y–like immunoreactivity, but there were no immunoreactive ganglionic cells. After dental injury (extraction and pulpal exposure), neuropeptide Y–like immunoreactive cells appeared in the ganglion, indicating a change in the primary sensory neurons of the ganglion.

When dentin is exposed, plasma proteins such as albumin, immunoglobulin G, and fibrinogen are released by the process of plasma extravasation (see chapter 6). The concentrations of these plasma proteins in dentinal fluid were compared to relative concentrations of these proteins in the dental pulp.[43] Albumin and immunoglobulin G were found in all dentin specimens and were similar to fluid specimens from exposed pulp tissue. Fibrinogen was found in all pulp specimens but in only 25% of dentin specimens. The results indicated varying responses of plasma protein release in reaction to mechanical injuries.

Another study examined changes in the distribution of fibrinogen/fibrin and fibronectin in the pulpodentin complex after Class V cavity preparation in maxillary rat molars.[44] Fibrinogen was detected in the exudate and dentinal tubules at various times after preparation. Fibronectin staining showed a similar pattern in the exudate. At 3 days, the irregularly shaped dentin under the preparation showed strong fibronectin staining. The results indicated that these substances are present during the healing process after mechanical injury.

Mechanically induced injury to the dental pulp elicits a number of responses of immunocompetent Class II major histocompatibility complex (MHC) antigen–expressing cells (see chapters 6, 10, and 11). Cavity preparations in rat maxillary first molars caused an acute edematous reaction between injured odontoblasts and the predentin, and most of the OX6-immunopositive cells normally present in

uninjured teeth shifted away from the pulpodentin junction.[45] At 24 to 72 hours after injury, many of these cells accumulated along this border and newly differentiated odontoblasts appeared, indicating that Class II MHC antigen–expressing cells in the pulp participate in the initial defense reaction and may serve as a biologic sensor for external stimuli. Collectively, these studies indicate that substances and cells normally found in healthy dental pulp play a role in events occurring when the pulp is adversely stimulated.

A later study measured the response of $OX6^+$ and $ED1^+$ cells (Class II MHC cells) and macrophages to mechanical preparation and a resin bonding agent.[46] Preparations were made and immediately restored in maxillary rat molars; unrestored and nontreated teeth served as positive and negative controls. The teeth were evaluated at 3 and 28 days posttreatment using anti–Class II antisera (OX6) and antimacrophage antisera (ED1). At 3 days, densities of both cells were significantly higher in the restored teeth than in the intact group. At 28 days, sound reparative dentin was observed, and the density of immunocompetent cells was comparable to that of the intact teeth. Pulpal abscesses were observed in 14 of the 16 specimens in the teeth without resin, indicating that the resin bonding agent reduced transdentinal antigenic challenges.

A report of the behavior of thick slices of human dentin prepared immediately after extraction attempted to develop a model correlated to tissue healing.[47] This study showed that the damaged pulp beneath the preparation demonstrated cell proliferation, neovascularization, and the presence of functional cuboidal cells close to the injured area. After 30 days of culture, elongated spindle-shaped cells were aligned along the edges of the prepared dentin, which may indicate the formation of odontoblasts and the onset of odontogenesis. This model may be useful for testing factors that regulate pulp repair.

Bone morphogenetic proteins affect the differentiation of pulp cells to odontoblast-like cells after injury during reparative dentinogenesis (see chapter 2). The effect of bone morphogenetic proteins on the expression of nuclear proto-oncogenes (c-Jun and Jun B) was evaluated after injury and during repair in rat molars.[48,49] While both are coexpressed in tooth germs, only c-Jun was expressed in the odontoblastic layer of adult molars, whereas Jun B expression was absent in all pulp cells. After injury, both were coexpressed in cells beneath cavities, and their levels greatly increased during early repair. At 14 days, both were seen only in pulp cells lining the surface of thick reparative dentin. The results indicate a role for active formation of dentin matrix during primary and reparative dentinogenesis. Cavity preparation in rat molars also causes relocalization of cytoplasmic heat

shock protein 70 (HSP70) from the cytoplasm to the nucleus. HSP70 is thought to prevent apoptosis.[50–52] Others have reported a variety of HSPs expressed in human dental pulps.[53]

Several other chemical mediators are released during pulpal injury. Nitric oxide, produced by nitric oxide synthase (NOS; several isozymes have been discovered; see chapter 11), has been implicated in multiple inflammatory processes, and the level of NOS can be used as a marker of tooth pulpal insult. Therefore, relative distributions of NOS in uninflamed and inflamed rat pulps were examined.[54] Tissue levels of both macrophage NOS (macNOS) and neuronal NOS (nNOS) in normal and inflamed rat molar pulp were determined at multiple time points. Deep cavity preparation produced a time-dependent inflammatory response that was acute early, later progressing to a chronic, granulomatous response with necrosis and spreading down the root adjacent to the preparation. Unprepared teeth showed a faint homogenous distribution of nicotinamide adenine dinucleotide phosphate–d and macNOS but no discernible nNOS reactivity. Similar changes were seen around the inflamed areas. The results indicate a role for nitric oxide in mediating pulpal inflammation after an injury.

Odontoblasts are formative cells that are responsible for dentin matrix formation and mineralization. Studies indicate that they display dynamic responses to injurious mechanical stimuli. Recent studies have examined injury to the pulp cells responsible for hard tissue formation. One study measured the changes in odontoblast cell numbers in response to injury with respect to cavity restoration variables and patient factors and the effect those factors had on tertiary dentin repair.[55] Class V cavity preparations and restorations were placed in premolars of patients between the ages of 9 and 17 years. After removal of the teeth (28 to 163 days later), the area of reactionary dentin and the area of the odontoblasts were measured histomorphometrically.

Only the age of the subject appeared to have an effect on odontoblast dentinal secretory capacity; the older subjects demonstrated fewer odontoblasts per unit area.[55] The area of reactionary dentin formation increased in proportion to subject age. Because preparations were made in deep dentin and 0.5 mm of dentin was left over the pulp, the repair capacity of the pulpodentin complex would appear to be age dependent.

A companion study found that RDT was the one variable determining reactive dentin formation.[56] An RDT of less than 0.25 mm caused a 23% decrease in odontoblasts, and minimal reactionary dentin repair was observed. The use of rat

incisor slices maintained in organ culture allows evaluation of operative variables on pulpal viability beneath Class V cavities.[57] The authors of that study confirmed that RDT was of paramount importance in protecting the pulp from operative trauma, followed by bur speed and smear layer removal, but not restorative material[58] (see chapter 14).

It is apparent from these studies that multiple levels of responses and interactions occur in reaction to mechanical injuries of the dental pulp. Responses include inflammatory changes mediated by release of various neuropeptides and changes that are defensive in nature and responsible for genesis of new hard tissue to replace the tissue injured by caries and typically removed by traumatic methods. The pulp is a complex tissue that reacts much as other body tissues react and must be protected in its environment to extend the life of the tooth.

Responses to Thermoplasticized Gutta-Percha Obturation Techniques

The clinical technique of warm vertical compaction for obturation of root canals relies on a combination of fortuitous properties. The high thermal conductivity and low heat capacity of stainless steel (see Table 15-1) means that it can be rapidly heated and can deliver that heat quickly. This permits thermoplasticization of gutta-percha, thereby softening it, lowering its stiffness,[59,60] reducing its viscosity, and allowing it to flow within a cylinder made up of a good thermal insulator (ie, root dentin). The use of metallic carriers at temperatures heated to 321°C[61] would seem extreme, but the mass (and hence the specific heat) of stainless steel times the mass of the metallic heat carrier determines how many calories of heat energy can be transferred to the root canal.

The development of the split-root model containing an array of 16 thermocouples has provided a convenient method for evaluating changes in pulpal and periodontal surface temperatures during various endodontic procedures.[62] The introduction of electrically heated carriers such as the Touch ‘N Heat (SybronEndo) and the System B (SybronEndo) has made it much more convenient to deliver large amounts of heat to gutta-percha in the root canal space. These devices can generate tip temperatures between 250°C and 600°C. There was concern that such high temperatures could cause thermal damage to periodontal[63] and periapical tissues.[64,65] However, when

the temperature at the external surface of the root was measured over an intracanal temperature range of 200°C to 600°C (using a System B heat source), the measured increases in root surface temperature were only 1.04°C to 5.78°C, regardless of the internal root temperature. The authors speculated that this resulted from brief but profound heat loss from the hot gutta-percha back up to the inactivated heat carrier that served as a heat sink.[65]

The use of heat to plasticize gutta-percha during obturation of root canals raises the risk of overheating the PDL[66] or the surrounding bone.[67] The warm vertical condensation technique was shown to increase apical temperature by only 4.0°C and cervical temperatures by only 12.5°C in an in vitro study.[67] These low temperatures were probably due to the low thermal conductivity of gutta-percha and the small size of the heat carriers used with that technique. However, external root temperatures measured by infrared thermogravic cameras were significantly higher than temperatures measured with thermocouples in the same teeth in vitro.[66] In vivo studies of the histologic response of the PDL and surrounding bone to obturation with thermoplasticized gutta-percha found little adverse response.[68] Nevertheless, the 31°C increase reported by Fors et al[69] has made clinicians wary of potential periodontal injury.

Is there any danger that the high internal root temperature produced by thermomechanical compaction can weaken root dentin? The apparent "thermal contraction" reported[5] to occur above 55°C to 60°C should not be interpreted as being due to thermal denaturation of collagen in the mineralized matrix. The denaturation temperatures of mineralized dentin (hydrated versus dehydrated) are shown in Fig 15-4. In these experiments, small pieces of dentin were sealed in small pans that were placed in a calorimeter within an oven. As the oven temperature increased from 25°C to 200°C, the heat flow between an empty reference pan and the pan containing dentin was measured. When collagen denatured (ie, when highly structured linear collagen was converted to random coils of gelatin), heat was absorbed and produced a peak in the differential scanning calorimeter tracing.[70]

Hydrated mineralized dentin does not denature until the temperature of dentin reaches 170.4°C (see Fig 15-4). Dehydrated dentin requires even higher temperatures to denature (186.5°C).[71] Completely demineralized, fully hydrated dentin matrix denatures at 65.5°C. While this is similar to the thermal contraction reported in roots by Kishen and Asundi,[5] their dentin was fully mineralized. The apparent contraction that they reported is due to the unique structure of dentin and the manner in which those measurements were made.

The differential scanning calorimeter tracing measurements of thermal denaturation have potential implications in endodontics. Although heat sources such as System B produce tip temperatures of 200°C to 300°C, the presence of gutta-percha and sealer between the heat source and the dentin may protect the dentin from exceeding the thermal denaturation temperature (177°C) of mineralized, hydrated, old root dentin (see Fig 15-4). As soon as the heat source is turned off, the heat flow reverses and travels back up the metal tip into the heating device. Only if the activated hot tip touched the dentin wall of the root canal could it deliver enough heat to have the potential to denature dentin collagen.

However, if the root canal treatment involves the use of acidic agents such as BioPure MTAD irrigant (Dentsply Tulsa Dental), the mineral phase of the root dentin is removed to a depth of 10 μm.[72] This completely demineralized dentin can denature at a temperature of 66°C if the demineralized matrix is hydrated. Although endodontists attempt to dry root canals prior to filling, one study indicated that use of one or two paper points is not sufficient to really dry the apical third of the canal.[73] An alternative approach is to remove the water with ethanol.[74] Because the denaturation temperature of demineralized dentin is highly dependent on its state of hydration, much more research is needed to determine the optimum conditions and techniques for adhesive endodontics if acidic irrigants are to be used.

Numerous studies of the internal temperature of the root canal's dentin surface revealed temperature increases of 45°C to 85°C when thermal obturation techniques were used.[75] If the initial temperature is 34°C, the intracanal dentin temperatures could be between 82°C and 122°C. The frictional heat generated by thermomechanical compaction techniques produced temperatures of 55°C to 75°C[76] and 65°C to 100°C within the root canal. These intracanal temperatures are enough to denature any exposed collagen in predentin or any root canal dentin that has been acid etched (ie, treated with BioPure MTAD).[72] However, more recent work indicates that demineralized dentin that has been infiltrated with adhesive resins (and perhaps resin-based endodontic sealers) is protected from thermal denaturation.[71,72] Denatured insoluble collagen becomes soluble gelatin and may slowly dissolve, leaving a 10-μm-wide gap between the underlying mineralized dentin and the root canal filling material.

Although these temperatures would appear to be sufficient to cause irreversible damage to the PDL and bone, endodontists report anecdotally few adverse incidents occurring with the use of gutta-percha heating devices or gutta-percha placed by obturators or plasticizing guns. It appears that a temperature rise of 10°C or less can

be tolerated for short periods of time (less than 1 minute) without irreversible damage to the PDL or bone. The introduction of heat delivery devices and low-temperature thermoplasticized gutta-percha delivered by the gutta-percha delivery guns seems to ensure that temperature rises will be low enough to avoid damage to adjacent soft and hard tissues (PDL and bone).

Temperatures recorded at the midroot of canines during thermomechanical compaction of gutta-percha revealed no statistically significant differences between the elevation of temperatures in vitro and in vivo. However, temperature elevations dissipated more rapidly in vivo, which was thought to result from the cooling effect of the microcirculation. The PDL temperatures that were recorded were lower than the critical 10°C level.[77] This is an important study because several other studies have reported inconsistent findings about in vitro and in vivo temperature differences measured on the outer root surface. When a System B device was tested in vitro with temperatures recorded using a fine Buchanan plugger,[78] temperatures were elevated 2°C 5 mm from the apex but only 1°C at the apex. Extracted molars were mounted in an artificial PDL and alveolar socket, and thermocouples were placed at the outer surface of the roots. Similar results, with temperatures recorded through thermocouples, found increased temperatures of 0.5°C to 4.1°C.[78]

Questions as to the accuracy of thermocouples in comparison to thermographic assessment of root surface temperatures have led to testing of obturation techniques. An infrared thermography camera was used to measure external root surface temperatures during placement of Thermafil (Dentsply) gutta-percha on a carrier in extracted molars.[79] Mean temperature rises differed according to the root tested and ranged from 4.3°C to 4.9°C. When four different gutta-percha obturation techniques were evaluated (carrier or injection application) with a thermal imaging camera,[80] temperature rises externally were 2.0°C for injected- and 3.7°C to 3.9°C for carrier-applied gutta-percha. Both applications were described as low-temperature gutta-percha.

A later study[81] used a high-temperature injectable technique to test external root temperatures in vivo and found temperature increases ranging from 8.5°C to 22.0°C; the lower temperatures were found in thinner maxillary central incisors and the higher elevations in the mandibular incisors. Certainly, the thickness of hard tooth structure is important, as is the microvasculature present around intact, treated teeth.

A more recent study utilized finite element analysis to determine the distribution and temperature rise in a model of a maxillary canine, surrounding PDL, and bone during a System B obturation simulation.[82] Heat applications of 100.0°C and

200.0°C were considered. The maximum predicted temperature at the PDL was 43.5°C, which would have created no harmful temperature effects.

Lipski and Woźniak,[83] using an infrared thermal imaging camera, recorded temperatures when System B was used to retrieve Thermafil gutta-percha and obturators from treated, extracted teeth. They recorded temperatures that ranged from near 28°C to 46°C. This may be of some concern in retreatment, when obturation material is to be retrieved. It would appear that these types of cases require and generate higher temperatures, which may result in injury to the PDL and bone.

Temperature changes during application of gutta-percha, thermoplastic gutta-percha, and Resilon cones with a thermomechanical compactor were measured. Significant differences between mean initial temperature increases (5°C) and maximum temperature increases (8°C) occurred with all materials. Changes in temperatures were higher in the apical third than in the cervical third of root canals.[84]

Ultrasonic compaction of gutta-percha has been suggested as an alternative to use of heat-generating devices that can be used to soften materials such as the newer thermoplastic resins (eg, Resilon). When different power settings (1, 3, or 5) and durations of activation (4, 10, or 15 seconds) were used to test temperature rise at the root surface, the combination of a setting of 5 and a 15-second activation caused a temperature rise greater than 10°C, above the critical temperatures necessary to maintain healthy attachment tissues.[85] Furthermore, a case report described severe damage to alveolar bone, gingiva, and nasal mucosa.[86] Overheating caused necrosis of soft tissue and bone on two surfaces of a maxillary central incisor and an inflammatory response in the adjacent cavity after the use of an ultrasonic packing regimen. This event reinforces the need to reassess ultrasonic use in root canal obturation procedures.[86]

Some endodontic techniques aim to pack root canal systems in a process that results in a three-dimensional fill through the entire length of the system. It is important that the operator understand that materials requiring use of high temperatures may cause PDL and bone damage. Awareness of the research findings in this area should decrease the potential for thermal damage.

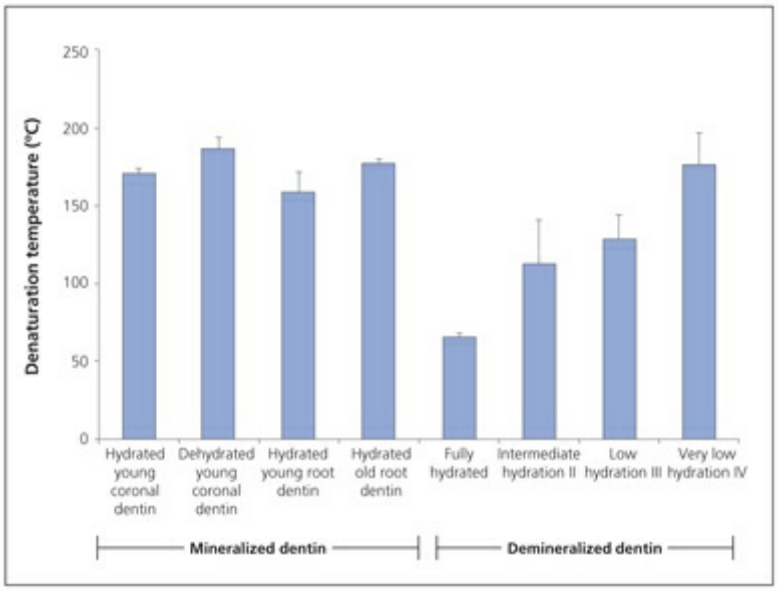

Fig 15-4 Denaturation temperatures (°C) of mineralized and demineralized dentin. Bars identified by different lowercase letters are significantly different ($P < .05$). (Modified from Armstrong et al[70] with permission.)

Thermal Responses to Laser Treatment

The search for alternative methods to remove enamel and dentin has led to the development of techniques such as lasers and air abrasion devices. The word *laser* is an acronym for *light amplification by the stimulated emission of radiation*. Dental lasers used today for clinical procedures and research operate at the infrared, visible, or ultraviolet (UV) range of the electromagnetic spectrum (Figs 15-5 and 15-6). While *l* stands for light, the actual physical process that occurs within a laser device is amplification by stimulated emission of radiation. The laser beam (restimulated emission of radiation) differs from conventional light sources in three ways: *(1)* It is a single wavelength (monochromatic); *(2)* it is collimated (very low divergence); and *(3)* the photons are in phase (referred to as *coherence*).

The medium producing the beam is what identifies the laser and distinguishes one from another. Different types of lasers used in dentistry, such as carbon dioxide (CO_2), erbium (Er), and neodymium (Nd), various other substances used in the medium (eg, yttrium-aluminum-garnet [YAG] and yttriumscandium-gallium-garnet [YSGG]), and argon, diode, and excimer types all produce light of a specific wavelength. The CO_2, Er:YAG, and Nd:YAG lasers emit invisible beams in the

infrared range. These lasers are coupled with a nonabsorbing light source (often red, green, or white) that serves as a pointer for the working laser. The argon laser emits a visible light beam at either 488 or 514 nm, while the excimer lasers emit invisible UV light beams at various predetermined wavelengths (see Fig 15-5).

Laser photons interact with tissue in one of four general ways: they are *(1)* transmitted through tissue, *(2)* reflected from tissue, *(3)* scattered within tissue, or *(4)* absorbed by tissue (Fig 15-7). Transmission of light passes energy through the tissue without interaction and thus causes no effect or injury. Reflection of laser photons allows little interaction between the laser energy and the substrate and hence causes no effect or injury. When scattered, light travels in different directions and energy is absorbed over a greater surface area, producing a less intense and less precise thermal effect. When absorbed, light energy is converted into thermal energy. In general, a single laser device cannot perform all possible functions because the beam is absorbed or reflected according to its wavelength and the color of the object impacted.

Different laser wavelengths affect biologic tissue in different ways. In other words, there is no one laser wavelength that can be utilized to deliver various modalities of dental care. Some wavelengths are useful and efficient in creating tooth preparations (hard tissue applications in enamel and dentin), while other wavelengths are more effective when used for soft tissue applications. Therefore, it is difficult to understand the role of dental laser devices in contemporary dentistry. Yet lasers have the potential to aid dentists in a manner that would be much more acceptable to dental patients than some current devices.

The ability to effect a change in the enamel and/ or dentin in a manner that will not cause adverse temperature rises in the dental pulp is an important consideration in the various methods used to prepare tooth structure to receive restorations. The particular properties of each type of laser and the specific target tissue make them suitable for different procedures. The CO_2 laser is most effective on tissues with high water content and is highly absorbed by all biologic hard and soft tissues. This results in high thermal absorption and may damage pulp. Argon lasers are more effective on pigmented or highly vascular tissues. The photons of the Nd:YAG laser are transmitted through tissues by water and interact well with vascularized tissues such as the dental pulp. The excimer lasers generate light in the UV range of the electromagnetic spectrum and function by breaking molecular bonds. The Nd:YAG, argon, Er:YAG, Er:YSGG, and excimer lasers may have utility for cleaning and shaping of root canal systems, mainly because of their use in a contact mode.

The extent of the interaction of laser energy with tissue will generally be

determined by two dependent variables: *(1)* the specific wavelength of the laser emission and *(2)* the optical characteristics of the particular target tissue.[87] These variables dictate absorption (eg, ability to effect tissue changes and generation of heat) and are important for pulp tissue safety. The clinician controls four parameters when operating the laser: *(1)* the level of applied power (*power density*), *(2)* the total energy delivered over a given surface area (*energy density*), *(3)* the rate and duration of the exposure (*pulse repetition*), and *(4)* the mode of delivery of the energy to the target tissue (ie, continuous versus pulsed energy and direct contact or no contact with target tissue).

The pulpal responses to laser application have been adequately described.[88] Depending on the experimental conditions, pulpal responses to lasers include altered presence and position of odontoblast nuclei, destruction of odontoblasts, and changes in the consistency and composition of the extracellular matrices. The threshold response for adverse pulpal reactions through intact enamel and dentin is thought to occur at energy densities somewhat less than 60 J/ cm^2, although RDT is an important variable.[88]

Several studies have measured laser-induced increases in pulpal temperature,[89] although results from in vitro studies may overestimate pulpal temperature responses because blood flow is not available to moderate temperature changes.[90] White et al[89] published the only available report that compared the increase in pulpal temperature induced by pulsed Nd:YAG laser irradiation with that of a high-speed bur operated with only air spray for 20 seconds (Fig 15-8). They obtained a 4.7°C rise in pulpal temperature using a high-speed bur across 1.0 mm of RDT, similar to that induced by the 0.7 W (10 Hz) of Nd:YAG laser irradiation.

At least four approaches have been developed to reduce laser-induced increases in pulpal temperature. First, the use of an air-water spray provides pulpal protection equivalent to that of the common dental drill.[10,91–93] Second, the development of extremely brief pulsed laser systems (ie, nanosecond, picosecond, or femtosecond pulses)[94] permits heat to dissipate from the site of irradiation before a second pulse impacts the tissue. Third, the development of excimer lasers that operate in the UV range with short (15-nanosecond) pulses minimizes the transfer of heat compared to earlier lasers while still forming plasma at high enough temperatures for hard tissue destruction.[95] Fourth, patent dentinal tubules have been recognized as potential pathways for direct transference of light energy to the pulp.[96] This has led to the suggestion that dentinal tubules should be closed or occluded by lasers or conventional methods.[97] Both the Nd:YAG laser and the excimer laser have been

shown to reduce dentinal permeability or sensitivity.[98]

Numerous studies have evaluated the effects of lasers on enamel and dentin. Under certain conditions, use of lasers increases the acid resistance of enamel or dentin. Thus, it is not surprising to find that laser-treated dentin may produce lower resin-dentin bond strengths than untreated acid-etched dentin.[99,100]

Several lasers have been used inside root canals to clean and shape the canal, to remove smear layers, or to sterilize the canal.[101–103] There are several limitations to intracanal use of lasers: The light is emitted at the end of the light guide instead of the side; the light guide must be very small (approximately 0.2 mm), and it must be stiff but not brittle to permit easy manipulation within the canal. It is almost impossible to obtain uniform coverage of the canal surface using a laser. Further, bacteria often invade the tubules and remain viable below the surface, where they can multiply back into the canal.

The potential for thermal damage to the periapical tissues remains a concern with lasers operated in the nanosecond to millisecond pulse widths. The intracanal use of a potassium-titanyl-phosphate (KTP)–532 laser (an Nd:YAG beam passed through a potassium titanyl phosphate crystal to change the wavelength from invisible infrared to visible green light) increased the permeability of ethylenediamine-tetraacetic acid–etched root dentin by removing organic material, as was definitively shown by the elegant Fourier transform infrared photoacoustic spectroscopy studies of Spencer et al.[104] The authors used pulse widths of 0.2 to 1.0 second. When pulse widths were decreased to 100 picoseconds, Serafetinides et al[105] obtained very different results with the same wavelength laser; thermal damage was minimized. Even less thermal damage was obtained at 1,064 nm for 100-picosecond pulses, confirming the earlier work of Willms et al.[106] Similar impressive results were reported by Niemz et al,[94] who used a 1,030-nm laser with pulse durations of less than 500 femtoseconds to lower heat transfer to dentin.

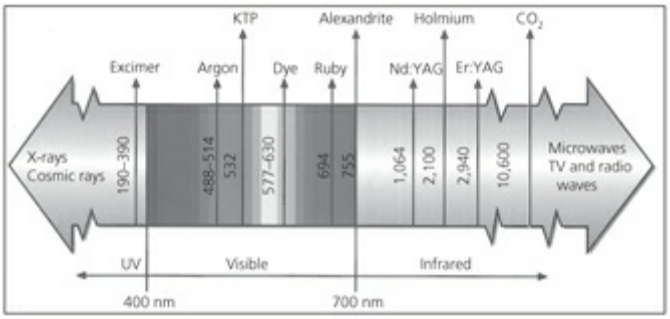

Fig 15-5 Wavelengths of different types of laser according to their emission spectrum. Laser types differ in wavelength, beam characteristics, and available energies. KTP, potassium-titanyl-phosphate. (Courtesy of Opus Dent.)

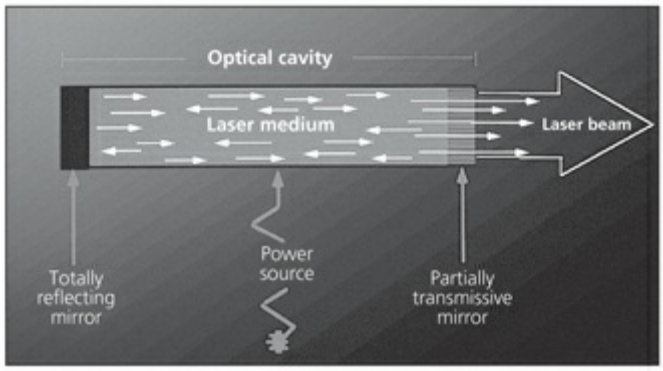

Fig 15-6 Diagram of a laser.

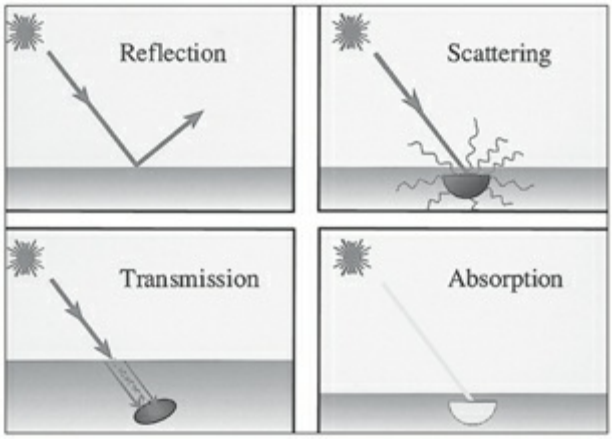

Fig 15-7 Four basic types of laser interaction that occur when light hits matter or tissue: reflection, scattering, transmission, and absorption. (Courtesy of Opus Dent.)

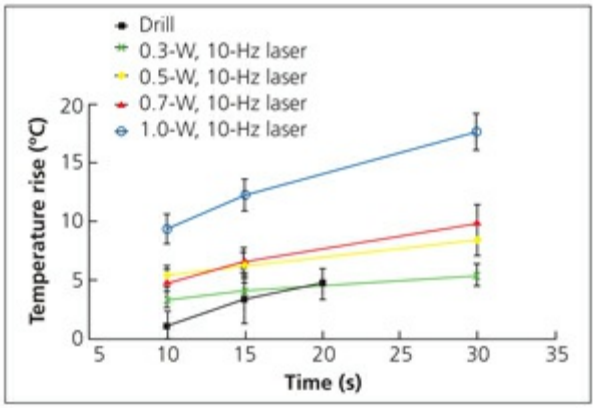

Fig 15-8 Comparison of the effects of a high-speed drill and Nd:YAG laser irradiation on pulpal temperature. No statistically significant differences were found between the drill and the 0.3-, 0.5-, and 0.7-W (10-Hz) lasers ($P < .05$). (Reprinted from White et al[90] with permission.)

Laser types

Ruby lasers

Ruby lasers, one of the first types of laser used in dentistry, produced cratering in enamel, particularly at higher energy densities or when applied to dark or carious enamel.[107] In one study of the use of ruby lasers on hamster teeth, application of 55 J of energy produced complete pulpal necrosis at 3 days, while 35 J produced pulpal inflammation that was reversible in some cases.[108] In the first report of a laser application in humans, two 1-millisecond pulses of 17 J produced no pain sensation in spite of the destruction of some enamel.[109] In a comprehensive study on ruby lasers applied to dog incisors, it was concluded that the amount of energy required for hard tissue removal caused pulpal necrosis, leading the investigators to consider alternative therapies.[110]

CO_2 lasers

CO_2 lasers represent an alternative to ruby lasers because the infrared wavelength produces significantly different thermal effects, permitting fusion of pits and fissures, conversion of carbonated apatite to the more insoluble calcium orthophosphate, stimulation of new dentin formation, and reduced pulpal responses.[111,112] In a study measuring hydraulic conductance across dentin disks, CO_2 lasers increased dentin permeability 1.4- to 24.0-fold by such mechanisms as removal of smear layer and smear plugs, cratering, and cracking in the glazed surface of the crater[113,114] (Figs 15-9 and 15-10). However, there is a major limitation to the CO_2 laser: When used to prepare cavities, it creates severe thermal damage to dentin due to the strong absorption of the emitted photons by water[114] (Fig 15-11). In a clinical trial with CO_2 lasers, the increase in pulpal temperature never exceeded 10.5°C, although the odontoblast destruction was inversely related to remaining dentinal thickness.[115] Others have demonstrated that as little as 3.5 J of CO_2 laser irradiation may produce pulpal damage in vivo, and concern has been raised about using levels as low as 1.0 J.[116]

To adapt CO_2 lasers to the needs of dentists, CO_2 wavelengths have been tested in an effort to find a wavelength that would allow its use without causing damage to the soft tissue core of a tooth. A high–pulse rate CO_2 laser designed for soft tissue surgery was tested for its effects on dental hard tissue.[117] Pulpal temperatures ranged from 0.5°C to 19.0°C. At cumulative fluences of 40 J/cm2, 200 pulses/s and higher caused measurable hard tissue loss, indicating that there were threshold conditions

above which a pulsed CO_2 laser would cause hard tissue damage. Two 9.6-µm wavelength CO_2 lasers caused no pulpal damage at 4 days and 4 weeks postirradiation in dog canines.[118] Using healthy human molars in situ, Nair et al[119] found no pulpal damage at 7 days or 3 months after laser application. The use of a transversely excited atmospheric pressure, 9.6-µm wavelength CO_2 laser produced no permanent pulpal sequelae at 1 week to 1 month after the procedure.[120] This same wavelength was also used to produce an enamel surface that was resistant to acid dissolution,[121] to remove carious dental hard tissue and prepare cavities, and to prevent caries by sealing the surface pits and tissues of partially erupted third molars.[122]

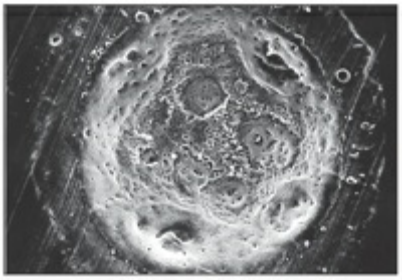

Fig 15-9 Scanning electron micrograph (SEM) of CO_2-irradiated dentin at an energy density of 113 J/ cm^2. Much of the crater wall appears to be glazed, but the floor is covered with pits (original magnification ×100). (Reprinted from Pashley et al[114] with permission.)

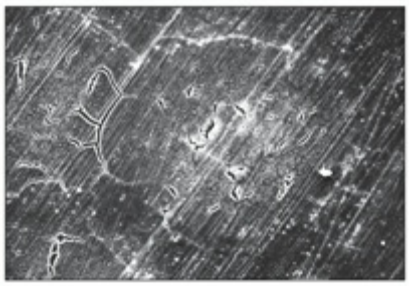

Fig 15-10 SEM of CO_2-irradiated dentin at an energy density of 566 J/cm2. Most of the crater surface is glazed, although numerous pits remain. The inner halo is devoid of smear layer or smear plugs; the outer halo shows more tubule occlusion (original magnification ×120). (Reprinted from Pashley et al[114] with permission.)

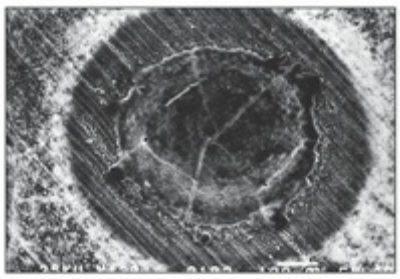

Fig 15-11 SEM of CO_2-irradiated dentin at an energy density of 11 J/cm^2 showing a single-impact crater. Large cracks were not present when the hydraulic conductance of the specimen was measured (original magnification ×100). (Reprinted from Pashley et al[114] with permission.)

Er:YAG lasers

Several studies have evaluated the properties of the Er:YAG laser. The depth and diameter of Er:YAG laser–drilled holes are a function of pulse number and the amount of exposure to energy parameters.[123] Using various wavelengths, Wigdor et al[124] compared the histologic response of the dental pulp to dentinal Er:YAG laser ablation in dog teeth and suggested that the thermal effect might be less than that of the other lasers tested. Goodis et al found that, when used in cavity preparation, the Er:YAG produced no detectable pulpal damage at energy levels of 3W and 10 to 30 Hz (unpublished data, 2003) (Fig 15-12). Even when Oelgiesser et al[125] used the maximum laser energy of 12 W at 12 Hz with a pulse width of 350 microseconds, water cooling limited increases in pulpal temperatures to less than 3.5°C when Class I and Class V cavities were prepared.

However, when Er:YAG–treated dentin (180 mJ, 2 Hz, 250-microsecond pulse duration with water cooling) was examined by transmission electron microscopy, the hybrid layer created by bonding with a dentin adhesive appeared badly damaged[126] (Fig 15-13). Collagen fibrils located at the surface of the hybrid layer were partially vaporized, and the remaining fibrils were fused together to produce crustlike features (Fig 15-14). Because peritubular dentin contains no collagen and is more highly mineralized, the fibrils appeared as a peripheral mineral ring around resin tags, with a free space that was left after vaporization of the adjacent collagen-rich intertubular dentin (Fig 15-15). Even the collagen fibrils beneath the hybrid later exhibited a variable degree of damage, ranging from fusion of collagen fibrils to a disintegrated mass that was devoid of interfibrillar spaces to unraveling of the microfibrillar elements of the grossly denatured collagen fibrils to the loss of cross banding on structurally intact fibrils (Fig 15-16). Resin-dentin bonds in such dentin had very low bond strengths compared to bonds in control acid-etched dentin. Based

on these ultrastructural observations, the damage created by laser ablation contraindicates its use as an adjunctive treatment in dentin bonding.

Too often, investigators rely only on scanning electron microscopy to evaluate the effect of lasers on dental hard tissues. Although this technique has served well, it provides little subsurface detail of potential damage. More transmission electron micrographic studies should be done in the future.

There are many procedures used in the practice of dentistry, and it is not surprising that laser energy has been suggested for some of those procedures. However, because of the cost of laser devices, dentists would prefer to be able to use the device for several different procedures. As previously stated, a single wavelength may be effective for a particular procedure but not for other procedures. There are several different applications for which lasers have been advocated.

In 1997, the US Food and Drug Administration approved the use of the Er:YAG laser for caries removal, tooth preparations, and modification of dentin and enamel. As to pulp tissue effects, the Er:YAG laser and the turbine handpiece were judged to be equivalent.[127] However, a general impression among clinicians is that the speed of preparation in dentin and especially in enamel is much slower with the Er:YAG laser than with the conventional high-speed drill.

Maintenance of pulpal health during cavity preparation is a critical requirement to be able to utilize laser energy in hard tissue removal in vital teeth. During tooth preparation procedures, both lasers and high-speed handpieces generate heat, which, without proper cooling, can cause pulpal temperatures to rise to a level where irreversible pulpal damage occurs. To this end, several studies have compared the use of the Er:YAG wavelength to handpieces. Class V cavities were placed in 28 teeth of a female baboon.[128] Fourteen teeth in two quadrants were prepared conventionally (handpiece), while 14 other teeth in the remaining two quadrants were prepared with the Er:YAG laser delivering 500 mJ at 10 Hz and at a wavelength of 2.94 μm. At remaining dentinal thicknesses of 0.77 mm for the handpiece and 0.81 mm for the laser, most pulps appeared normal; one pulp in each group was judged to be irreversibly damaged.

A similar study in bovine mandibular incisors found similar results between handpiece and laser when water cooling was used during the cutting preparations.[129] The test group treated without water cooling developed increases in pulpal temperature of 11.6°C. When pulpal temperatures were measured in freshly extracted teeth during Class I and Class V cavity preparations,[124] the measured temperature increases were well below the threshold of 45°C.[7] Nair and

coworkers,[130] using healthy human third molars, concluded that the Er:YAG laser at a 2.94-μm wavelength would cause little, if any, pulpal damage when specific energy settings were used. Other studies have concluded that the Er:YAG wavelength is a reasonable alternative to turbine handpieces when used with water cooling.[131,132] Theodoro et al[133] compared the erbium to a diode laser in generation of heat on root surfaces when the devices were used for scaling. Recorded pulpal temperatures were well below the level (10°C) that would cause irreversible pulpal problems.

Many of the studies cited in the previous paragraphs tested Er:YAG lasers in extracted teeth and only measured pulpal temperatures. Complete evaluation of these devices requires the use of in vivo studies to compare laser energy to conventional tooth preparation methods using histopathologic and immunohistochemical studies of the effects on pulpal nerves. When cavity preparations were placed in rat molars in vivo, repair of nerve fibers was observed.[134] Laser-treated molars demonstrated a marked fibroblast cell proliferation and formation of more reparative dentin than was seen when compared to molars prepared with a high-speed handpiece. Increased levels of calcitonin gene–related peptide fibers were also observed earlier than in conventional high-speed handpiece preparations, but levels returned to normal at 7 days. The result suggests that use of Er:YAG laser energy leads to pulpal repair earlier than that which occurs following the use of a high-speed turbine handpiece.

Similar results were found when maxillary molars were used to evaluate the role of immunocompetent cells and expression of HSP25.135 Rat molars were examined after tooth preparation or replantation.[136] They showed degeneration of odontoblasts with loss of HSP25 immunoreactions. Numerous Class II MHC–positive cells appeared along the pulp-dentin borders but disappeared by postoperative days 3 to 5. Suzuki et al[137] found that cavity preparation caused destruction of odontoblasts and a shift of Class II MHC–positive cells from the pulp-dentin border to the middle of the pulp. However, no effort was made to close the exposed dentinal tubules, and subsequent inflammatory events occurred due to invasion of microorganisms, which delayed pulpal regeneration.

Neural elements examined in rat pulps after Er:YAG laser irradiation demonstrated disruption of nerve terminals in dentinal tubules, degeneration of nerve terminals between odontoblasts, and disruption of the myelin sheaths within the central pulp.[138] This effect may lead to pain reduction with laser use.

Other uses have been suggested for Er:YAG lasers, including laser treatment of

margins of Class V preparations to decrease microleakage.[139] Others found that laser intervention did not lessen micro-leakage.[140] Delme et al[141] found that cavity seals were dependent on the type of glass ionomer used.

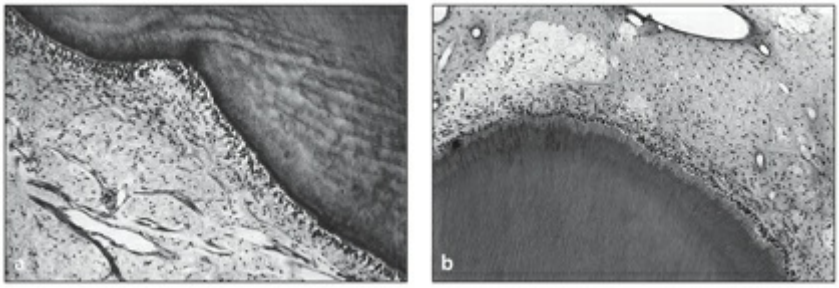

Fig 15-12 *(a)* Er:YAG laser cavity preparation in dentin in monkey teeth (hematoxylin-eosin stain; original magnification ×40). *(b)* CO_2 laser cavity preparation in dentin in monkey teeth (hematoxylin-eosin stain; original magnification ×25).

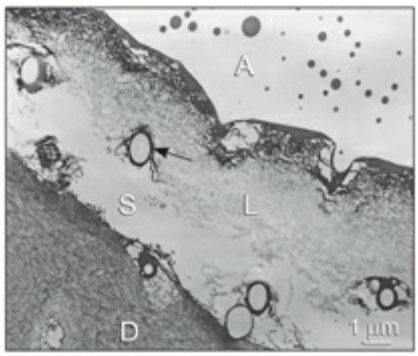

Fig 15-13 Transmission electron micrograph of Er:YAG laser–treated dentin that was acid-etched after laser application and bonded with Single Bond adhesive (A). This low-magnification image of the resin-dentin interface shows a 3- to 5-µm-thick laser-modified layer (L) that has separated from the underlying intertubular dentin (D). There was incomplete infiltration of resin (dark staining material on the surface) into the laser-modified layer because the collagen fibrils had become fused together, thereby eliminating the interfibrillar spaces that normally serve as diffusion channels for resin infiltration. The resultant space (S) has been infiltrated by the laboratory embedding resin. Remnant resin tags *(arrow)* can be seen within the laser-modified layer. (Courtesy of Dr Franklin Tay, Augusta, GA.)

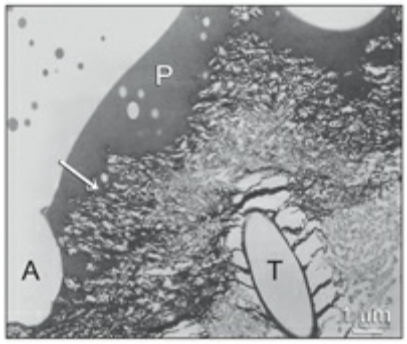

Fig 15-14 Higher-magnification image of the surface of the structurally degraded hybrid layer after the application of Single Bond adhesive to acid-etched, laser-ablated coronal dentin. The laser has melted

the collagen fibrils, which have fused together to produce platelike elements *(arrow)* that are partially infiltrated by the electron-dense, polyalkenoic acid copolymer (P) component of the dentin adhesive (A). An electron-lucent resin tag (T) within a dentinal tubule is surrounded by a peripheral layer of peritubular dentin (see Fig 15-15). (Courtesy of Dr Franklin Tay, Augusta, GA.)

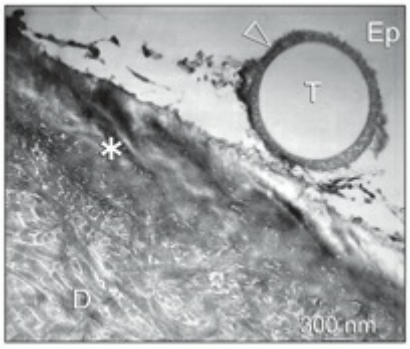

Fig 15-15 High-magnification view of the basal portion of the laser-modified hybrid layer. Collagen fibrils from the base of the hybrid layer have completely disappeared and are replaced by the laboratory epoxy resin (Ep). Because peritubular dentin is devoid of collagen and is more highly mineralized, it is not vaporized completely after laser treatment. Infiltration of the adhesive through a dentinal tubule (T) has resulted in the retention of a peripheral ring of resinencapsulated peritubular dentin *(open arrowhead)* around the resin tag. The mineralized dentin base beneath the hybrid layer also exhibits extensive damage after laser ablation. This mineralized layer had been demineralized during laboratory specimen preparation, enabling the structural components of the stained collagen matrix to be identified. Collagen fibrils from the most superficial part of the mineralized dentin base have completely melted and fused together, producing a layer that is completely devoid of interfibrillar spaces *(asterisk)*. Beneath this fused layer, the intertubular dentin (D) is also denatured. No cross banding can be observed from the collagen fibrils. (Courtesy of Dr Franklin Tay, Augusta, GA.)

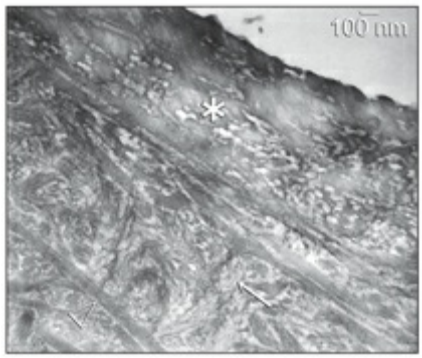

Fig 15-16 Stained, laboratory-demineralized intertubular dentin beneath the hybrid layer of laser-ablated resin-bonded dentin. Laser ablation has created structural damage to the mineralized intertubular dentin that is far deeper than the depth of the hybrid layer (usually 3 to 5 μm thick), although this badly damaged collagen matrix was still protected by minerals. When the apatite minerals are removed by laboratory demineralization agents, the surface of the mineralized dentin base (ie, adjacent to the base of the hybrid layer) appears amorphous, with the degraded gelatin melted into a fused, structureless mass *(asterisk)*. A few remnant intact collagen fibrils are visible *(open arrowhead)*, although these fibrils do not exhibit any cross banding. This means that those collagen fibrils had already been denatured by the heat generated during laser ablation. The rest of the collagen fibrils have been broken down into their microfibrillar components *(arrow)*, producing regions that contain only gelatin. (Courtesy of Dr Franklin

Tay, Augusta, GA.)

Nd:YAG lasers

The lack of thermal damage but improved cutting efficiency of picosecond pulses of Nd:YAG laser irradiation is thought to be due to the fact that the energy per single photon emission is only 1.18 electronvolts (eV), which is insufficient to break molecular bonds or destroy ionic crystalline lattices. Nd:YAG lasers were initially investigated using a contact mode. Other laser wavelengths (holmium, CO_2, and erbium) could not be used in this mode. Manufacturers also developed a 100-µm-diameter light guide (equivalent in size to a No. 10 endodontic file). The studies above were representative of the early investigations into the neodymium wavelength. Interest lessened as other manufacturers developed other wavelengths using "hot tips" to mimic tooth content when used. Recently, clinical interest in the 1.06-µm Nd:YAG laser has grown.

Although the photon absorption of 1,064-nm energy by dentin is low,[142] Nd:YAG energy absorption by water is relatively high, allowing vaporization to occur so rapidly as to create microexplosions that cause mechanical ablation.[117,142–144] According to Niemz,[145] mechanical disruption can be of two types. Plasma-mediated ablation results when the laser energy ionizes enough tissue components and heats it to a plasma state, whereas a photodisruption type of ablation is due largely to acoustical shockwaves from rapid vaporization of water.

Scan irradiation (movement of the laser contact probe across a tooth to cause an effect) was used to determine if the laser had any deleterious effects on pulpal nerve activity and pulpal blood flow in cats.[146] The use of this 1,064-nm wavelength caused pulpal damage and decreases in compound acting potentials to external stimuli and decreased the activity of both single Aδ and C fibers during irradiation. In a human study, pulpal blood flow, responsiveness to electrical pulp testing, systemic blood pressure, and pulse rate were recorded during Nd:YAG irradiation of an isolated tooth in 13 patients.[147] While pulpal blood flow increased during laser irradiation, electrical pulp testing results increased in six patients and decreased in six others; one patient showed no change. One month after laser treatment, all values returned to pretesting levels. Systemic blood pressure and pulse rate were not affected.

In a pulpal study, laser-irradiated rat mandibular incisors showed a slight delay in eruption.[148] Histologically, reparative dentin formation was accompanied by the formation of a layer of odontoblast-like cells in the damaged area.

Immunohistochemistry studies revealed pulpal areas expressing transforming growth factor β, which was judged to play a role in dentin regeneration.

Excimer lasers

Excimer lasers, which emit photons in the UV range, offer the potential advantage of reduced heat absorption and reduced dentin cracking. Both the argon-fluoride excimer (193 nm) and the xenon-chloride excimer (308 nm) laser melted dentin but did not occlude dentinal tubules.[149] Although permeability of the dentin was not measured, subsurface deposits that may have reduced dentinal permeability were unlikely.

The use of excimer lasers as a method for cutting enamel and dentin without generating excess thermal stress looks very promising. Operating at 248 nm, the excimer laser can preferentially remove intertubular dentin without creating a smear layer; the dentinal surfaces are so clean that they appear as if they were fractured[150] (Fig 15-17). The photon energy in excimer lasers operating at 193 and 248 nm is higher than the molecular bond energies holding collagen together. This results in photoacoustic destruction of dentin without melting.

Reductions in the pulse length from millisecond to picosecond have been shown to reduce thermal damage caused by excimer, Nd:YAG, [151] and neodymium:yttrium-lithium-fluoride lasers.[152] It is thought that picosecond pulse durations are less than the thermal relaxation times of dentin, thus minimizing any thermal effects.[153–155]

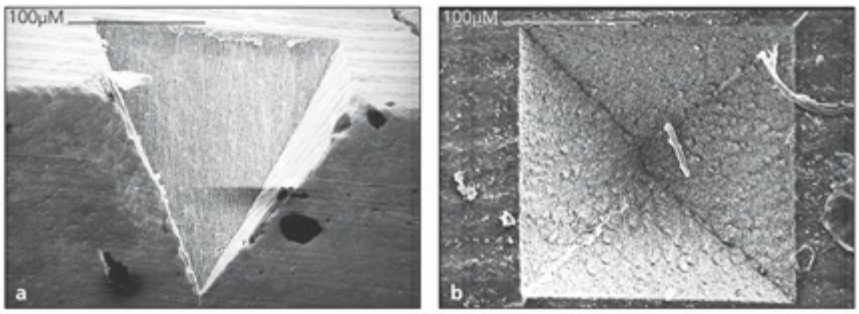

Fig 15-17 *(a)* SEM of enamel irradiated with an excimer laser, revealing the precise removal of hard tissue at 248 nm with 15-nanosecond pulses. *(b)* SEM of dentin irradiated at 248 nm with an excimer laser used at 15-nanosecond pulse widths. (Reprinted from Pearson and McDonald[151] with permission.) advocated to reduce or eliminate cervical sensitivity, including placement of resins, glass ionomers, varnishes, and other chemical solutions to close the dentinal tubules and halt movement of dentinal fluid through the tubules. It is this fluid movement that activates primary afferent nociceptors coursing in and around odontoblast processes and cell bodies. Two mechanisms have been proposed for laser-induced reduction in dentinal hypersensitivity. First, lasers may occlude dentinal tubules by melting and fusing dentin or the smear layer or by coagulating proteins in dentinal tubules.[153] Second, lasers may directly reduce neuronal activity.[142]

Treatment of dentinal sensitivity

Dentinal sensitivity continues to present problems for patients who have undergone periodontal surgery that may result in cervical exposure of root dentinal tubules. Various treatment regimens have been

The use of laser energy to occlude tubule orifices by melting and resolidification of dentin alone or in combination with materials to achieve the same result has been investigated to evaluate possible irreversible pulpal reactions. The Nd:YAG laser appears to be the device most studied for use in treating dentinal sensitivity. A YAG laser was used with an energy output of 30 mJ, 10 Hz for 2 minutes in 30 patients suffering from cervical hypersensitivity.[156] The patients were followed for 3 months, and pulpal effects were assessed with mechanical and thermal stimuli. Results showed a 65% reduction in sensitivity to air and a 72% reduction in sensitivity to probing and no other adverse reactions. The same group later compared sodium fluoride varnish and the same laser both alone and in combination.[157] When the fluoride varnish and laser treatment were combined, more than 90% of tubule orifices were occluded.

In a double-blind, controlled, split-mouth designed clinical trial, the effect of a single Nd:YAG laser application on dentinal sensitivity was assessed in 17 patients, each of whom had two sensitive teeth. One tooth was treated with the laser and the other with a nonactivated laser probe (placebo). Although there were no statistically significant differences between test and control teeth at 1, 4, and 16 weeks, both groups improved significantly from baseline to 16 weeks. When the morphologic changes of sensitive cervical dentin were studied, impressions taken before laser treatment displayed protrusive rods (an indication that the impression material had penetrated open tubules), while impressions taken after laser treatment showed no protrusive rods.[158]

Other studies have demonstrated the efficacy of the Nd:YAG laser combined with 5% sodium fluoride.[159] Those authors reported that the combined treatment occluded tubule orifices, and the same effect was noted by Hsu et al.[160] The latter study found that the sodium fluoride crystals were “burned into” the tubules when tubular orifices melted and resolidified and could not be removed with normal brushing.

Other studies have focused on the use of Nd:YAG lasers for desensitization of hypersensitive teeth. In a 2-week clinical trial, hypersensitive teeth treated with

Nd:YAG lasers were significantly less sensitive to air blasts than were untreated control teeth.[97] During treatment, the power was increased either until the patient detected the laser energy or until a maximum of 100 mJ was reached. While it is unlikely that this relatively low energy would have sealed exposed dentinal tubules, it might have altered Aδ nerve thresholds.[141] Although more research is indicated, there seems to be some potential for use of the Nd:YAG laser as a method for obtaining temporary analgesia.[142]

When Nd:YAG lasers were compared to Er:YAG lasers for treatment of dentinal hypersensitivity, the Nd:YAG laser was found to be more effective.[161] Aranha et al[162] found that both neodymium and erbium wavelengths could be effective in treating dentinal sensitivity. A safety study evaluated the effects of the Nd:YAG laser on oxygen saturation of pulpal blood in 65 patients with sensitive incisors and found little change before or after laser application.[161] The Er:YAG laser was found to be more effective for dentinal sensitivity treatment than a dentin desensitizer (Systemp Desensitizer, Vivadent).[163] Both treatment modalities were successful, but after 2 months, discomfort in the dentin desensitizer group increased to up to 65% of the baseline score.

Tooth bleaching

Another dental application that has potential to cause adverse pulpal reactions is the use of laser irradiation of vital teeth in an attempt to change their color.[164–167] Bleaching of endodontically treated teeth was once considered a normal part of posttreatment endodontics. With the advent of resin composites and porcelain facings, which are used to improve the esthetics of vital teeth (especially incisors), and because of the problems sometimes associated with bleaching procedures (such as cervical resorption; see chapter 17), bleaching is seldom performed now.

Several investigations have examined the use of lasers for this procedure. The effects of the presence and absence of heat-enhancing colorant added to bleaching gels was determined by comparing various heat-generating lamps to an argon laser.[163] Temperatures rose in response to all lights and the laser, and the increased surface and intrapulpal temperatures could adversely impact patient sensitivity and health. Other studies have compared diode lasers with LED irradiation. The use of bleaching gels with light-curing units and diode lasers has been compared with mixed results.[165–167] Some believe that such tooth bleaching is the result of dehydration of teeth and that much of the lightening effect is lost on slow rehydration.[168–170]

Other procedures

While the use of lasers for hard tissue procedures is increasing, they have been approved for use in soft tissue procedures, such as periodontal pocket elimination, closure of oral surgical wounds, frenectomy, and operculum excisions, for approximately 10 years. Their use for contouring of bone or removal of bone lesions is questionable at best, but they have been examined for root canal cleaning and shaping procedures and for use in obturation. Further study must be carried out before their use can be fully recommended. As their use in dentistry increases, there will be a continuing need to evaluate pulpal responses following laser application to teeth.

Pulpal Responses to Airborne Particle Abrasion

Another technology, airborne particle abrasion (kinetic cavity preparation), has recently been reintroduced for caries removal and cavity preparation.[171] Airborne particle abrasion had fallen into disuse because the stream of particles used in tooth preparation procedures could not be controlled, resulting in pitting and abrasion of adjacent teeth and injury to gingival tissues.[172] It was easily replaced with high-speed, air-driven turbine handpieces, which are more efficient and can construct a more precisely defined tooth preparation.[160]

Airborne particle abrasion technology has recently been refined and used as a method to produce “kinetic” cavity preparations.[171] Its potential for pulpal damage has not been fully investigated, but its use has been suggested for newer restorative materials and their direct placement in altered preparations, sometimes referred to a s *micropreparations*. Advances in microabrasion technology allow for more precise removal of enamel and dentin compared to the older systems.

Laurell et al[173] examined the pulpal responses to an air abrasion system in 120 molars and premolars of dogs. Two pressures (80 and 160 psi) and two aluminum oxide particle sizes (27 and 50 μm) were used. Class V cavity preparations were made and restored with an intermediate restorative material, and the teeth were removed in 72 hours. Sections were examined for odontoblast displacement, disruption of cell layers, inflammatory infiltrate, and necrosis and were compared to preparations made with a high-speed turbine handpiece. The researchers found that higher pressures and smaller particles caused significantly fewer pulpal effects than

the high-speed handpiece–treated teeth. This study represents the only controlled pulpal injury study to date in the literature. While other articles in proprietary (commercial) journals report similar results, they are anecdotal. The ever-increasing popularity of such technology indicates that more studies are needed to be certain of the safety of these devices.

Some air abrasion techniques advocate large particles and more pressure. This has been tested on areas of tooth structure considered softer than noncarious tooth structure.[174] Alumina powders, glass beads, crushed glass powders, and crushed powders of polycarbonate resin were applied to intact human enamel and dentin and artificially demineralized dentin. The particle size of the abrasives and the air pressure on abraded depths were also examined. Only crushed powders of polycarbonate resin abraded the caries-model dentin without reducing intact enamel and dentin. The results with the other materials were size and pressure dependent, but the materials were generally considered to be difficult to control.

Direct pulp capping of pulp exposures made by air abrasion may force abrasive particles into the pulp, causing delayed healing.[175] Clearly, more research is required before this technique can be recommended without qualification.

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Interrelationship of Pulpal and Periodontal Diseases

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Endodontic-periodontal diseases often present challenges to the clinician in their diagnosis, treatment, and prognosis assessment. Etiologic factors such as microorganisms as well as contributing factors such as trauma, root resorptions, perforations, and dental malformations play a role in the development and progression of such diseases. The treatment and prognosis of endodontic-periodontal diseases vary and depend on the etiology, pathogenesis, and correct recognition of each specific condition. Therefore, understanding the interrelationship between endodontic and periodontal diseases will enhance the clinician's ability to establish a correct diagnosis, select a treatment plan based on biologic and clinical evidence, and assess the prognosis of the teeth involved.

Anatomical Relationships

The dental pulp and the periodontium are connected via three main avenues of communication: *(1)* exposed dentinal tubules, *(2)* smaller portals of exit, and *(3)* the apical foramen.

Exposed dentinal tubules

Exposed dentinal tubules in areas devoid of cementum may serve as viable communication pathways between the dental pulp and the periodontal ligament (Fig 16-1). Exposure of dentinal tubules may result from developmental defects, disease, or periodontal or surgical procedures. Radicular dentinal tubules extend from the pulp to the cementodentinal junction. They run a relatively straight course and range in size from 1 to 3 μm in diameter.[1] The diameter of the tubules decreases with age or as a response to chronic low-grade stimuli that cause apposition of highly mineralized peritubular dentin. The number of dentinal tubules varies from approximately 8,000/mm^2 at the cementodentinal junction to 57,000/mm^2 at the pulpal end. In the cervical area of the root, the number of dentinal tubules is about 15,000/mm^2.

When the cementum and enamel do not meet at the cementoenamel junction, these tubules remain exposed, thus creating pathways of communication between the pulp and the periodontal ligament. Depending on their location, these pathways can contribute to conditions such as cervical dentinal hypersensitivity. Fluid and irritants may flow through patent dentinal tubules, and, in the absence of an intact enamel or cementum covering, the pulp may be considered exposed to the oral environment via the gingival sulcus or periodontal pocket. Experimental studies have demonstrated that application of soluble material from bacterial plaque to exposed dentin could cause pulpal inflammation, indicating that dentinal tubules may provide ready access between the periodontium and the pulp.[2] Additional details on the parameters affecting diffusion of substances through the dentinal tubules can be found in chapter 3.

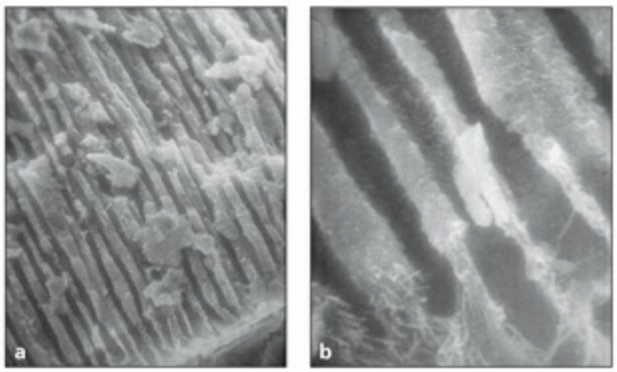

Fig 16-1 *(a)* Scanning electron micrograph of open dentinal tubules (original magnification ×500). *(b)* Higher magnification demonstrates the absence of the odontoblastic process (original magnification ×2,000).

Scanning electron microscopic studies have demonstrated that dentinal exposure at the cemen-toenamel junction occurs in about 18% of teeth in general and in 25% of anterior teeth in particular.[3] In addition, the same tooth may have different cementoenamel junction characteristics, presenting dentinal exposure on one surface while the other surfaces are covered with cementum.[4] This area becomes important in assessing the progression of endodontic pathogens as well as the effect of root scaling and planing on cementum integrity, trauma, and bleaching-induced pathosis.[5–7] Other areas of dentinal communication may be through developmental grooves, both palatogingivally and apically.[8]

Other portals of exit

Lateral and accessory canals can be present anywhere along the length of the root (Fig 16-2). Their incidence and location have been well documented in both animal and human teeth using a variety of methods. Such methods include dye perfusion, injection of impression materials, microradiography, light microscopy, and scanning electron microscopy.[9–15]

It is estimated that 30% to 40% of all teeth have such ancillary canal systems, and the majority of them are found in the apical third of the root. De Deus[12] reported that 17% of teeth presented multiple canal systems in the apical third of the root, about 9% in the middle third, and less than 2% in the coronal third. However, it seems that the incidence of periodontal disease associated with these types of canals is

relatively low. Kirkham,[13] studying 1,000 human teeth with extensive periodontal disease, found that only 2% of such canals were associated with the involved periodontal pocket.

Other canal systems in the furcation of molars may also be a direct pathway of communication between the pulp and the periodontium.[10,14] The incidence of accessory canals may vary from 23% to 76%.[11,12,16] These accessory canals contain connective tissue and blood vessels that connect the circulatory system of the pulp with that of the periodontium. However, not all of these canals extend the full length from the pulp chamber to the floor of the furcation.[16]

Seltzer et al[17] reported that pulpal inflammation may cause an inflammatory reaction in the interradicular periodontal tissues. The presence of these patent smaller canals is a potential pathway for the spread of microorganisms and their toxic by-products from the pulp to the periodontal ligament and vice versa, resulting in an inflammatory process in the involved tissues (Fig 16-3).

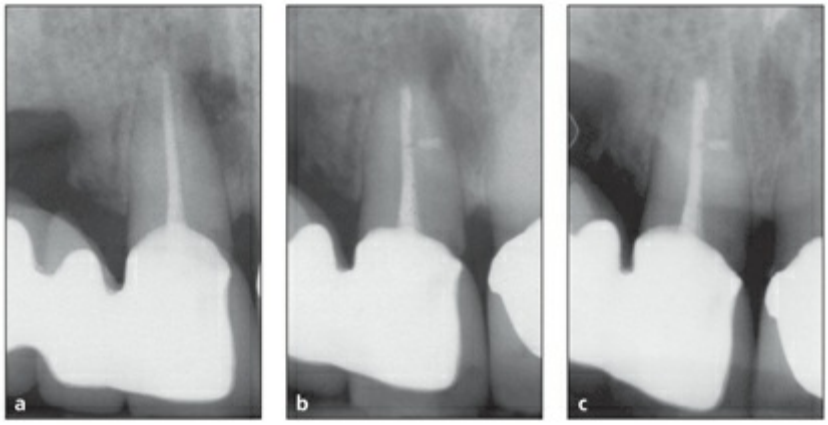

Fig 16-2 Nonsurgical endodontic treatment of a maxillary central incisor with a lateral radiolucency. *(a)* Preoperative radiograph showing previously treated canal with a mesiolateral lesion. *(b)* Tooth after retreatment and the restoration of the root canal with thermoplas-ticized gutta-percha. Note the lateral canal extending toward the lesion. *(c)* One-year recall radiograph revealing evidence of active healing.

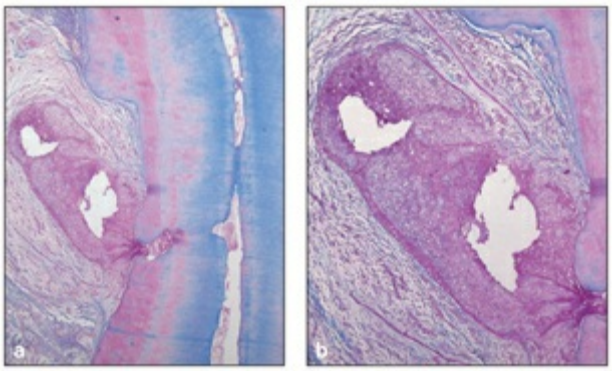

Fig 16-3 Micrograph of a maxillary lateral incisor with a necrotic pulp associated with a lateral

inflammatory process in the periodontal ligament. *(a)* Main canal, accessory canal, and the resultant inflammatory response in the periodontal ligament are evident (Masson trichrome stain; original magnification ×100). *(b)* Higher magnification of the area shows chronic inflammation with proliferating epithelium (Masson trichrome stain; original magnification ×200).

Apical foramen

The apical foramen is the principal route of communication between the pulp and periodontium. Microbial and inflammatory by-products may exit readily through the apical foramen to cause periradicular pathosis. The apex is also a potential portal of entry of inflammatory by-products from deep periodontal pockets to the pulp. Pulpal inflammation or pulpal necrosis extends to the periradicular tissues, causing a local inflammatory response often associated with bone and root resorptions.[17] Endodontic treatment aims to eliminate the intraradicular etiologic factors, thereby leading to healing of the affected periradicular tissues.

Disease Relationships

When the pulp becomes inflamed, it elicits an inflammatory response in the periodontal ligament at the apical foramen and/or adjacent to openings of the smaller canal systems.[18] Inflammatory by-products of pulpal origin may permeate the apex, smaller canals in the apical third of the root canal system, and exposed dentinal tubules and trigger an inflammatory vascular response in the periodontium. Among those are living pathogens such as certain bacterial strains (including spirochetes), fungi, and viruses,[19–28] as well as other noxious substances.[28–32] Many of these are similar to pathogens encountered in periodontal inflammatory disease. In certain cases, pulpal disease will stimulate epithelial growth that affects the integrity of the periradicular tissues.[33,34]

The effect of periodontal inflammation on the pulp is controversial, and conflicting studies abound.[17,35–42] It has been suggested that periodontal disease has no effect on the pulp at least until it involves the apex.[37] On the other hand, results of several studies have suggested that the effect of periodontal disease on the pulp is degenerative, including an increase in calcifications, fibrosis, and collagen

resorption as well as a direct inflammatory effect.[43,44] It appears that the pulp is usually not severely affected by periodontal disease until recession has opened an accessory canal to the oral environment. At this stage, pathogens leaking from the oral cavity through the accessory canal into the pulp may cause a chronic inflammatory reaction followed by pulpal necrosis. However, as long as the accessory canals are protected by sound cementum, necrosis usually does not occur. Additionally, if the microvasculature of the apical foramen remains intact, the pulp will maintain its vitality.[43]

The effect of periodontal treatment on the pulp is similar during scaling, curettage, or periodontal surgery if accessory canals are severed and/or opened to the oral environment. In such cases, pathogenic invasion and secondary inflammation and necrosis of the pulp can occur.

Etiology

Live pathogens and infectious biofilms

Among the live pathogens encountered in a diseased pulp and periradicular tissues are bacteria, fungi, and viruses (Figs 16-4 to 16-6). These pathogens and their by-products may affect the periodontium in a variety of ways and must be eliminated during root canal treatment.

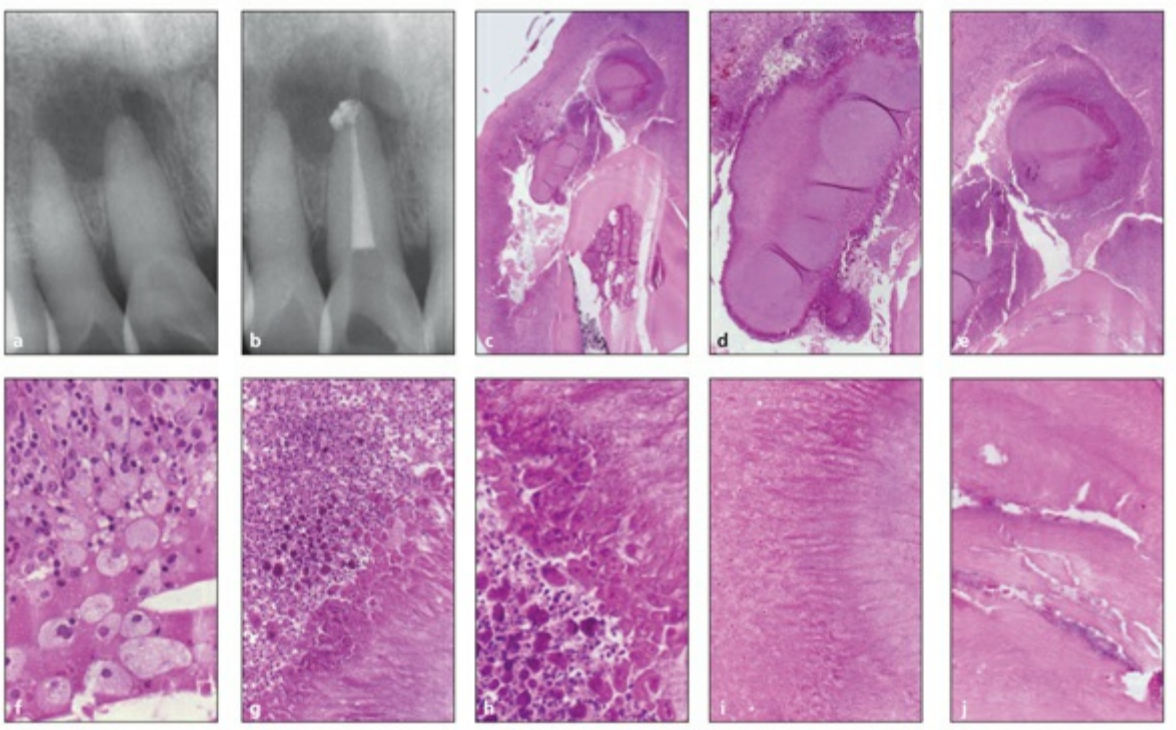

Fig 16-4 Periapical *Actinomyces* infection. This case demonstrates the growth of bacteria past the apical foramen and its invasion of apical cementum and periapical tissues. *(a)* Radiograph of a maxillary central incisor with a necrotic pulp showing a large periapical lesion. *(b)* Radiograph after nonsurgical endodontic therapy. Symptoms persisted after treatment, so apical surgery was performed subsequently. *(c)* Photomicrograph showing part of the root with the attached lesion (hematoxylin-eosin [H&E] stain; original magnification ×20). *(d)* Colonies of *Actinomyces* in the lumen of the lesion (H&E stain; original magnification ×100). *(e)* A large colony of *Actinomyces* (H&E stain; original magnification ×100). *(f)* Foamy macrophages attacking the bacteria (H&E stain; original magnification ×400). *(g)* Edge of the bacterial megacolony, revealing the absence of inflammatory cells, which are unable to penetrate the colony (H&E stain; original magnification ×200). *(h)* Detail of the bacterial colony (H&E stain; original magnification ×200). *(i)* Center of the colony, devoid of inflammatory cells (H&E stain; original magnification ×200). *(j)* Viable bacteria within the apical cementum (H&E stain; original magnification ×40).

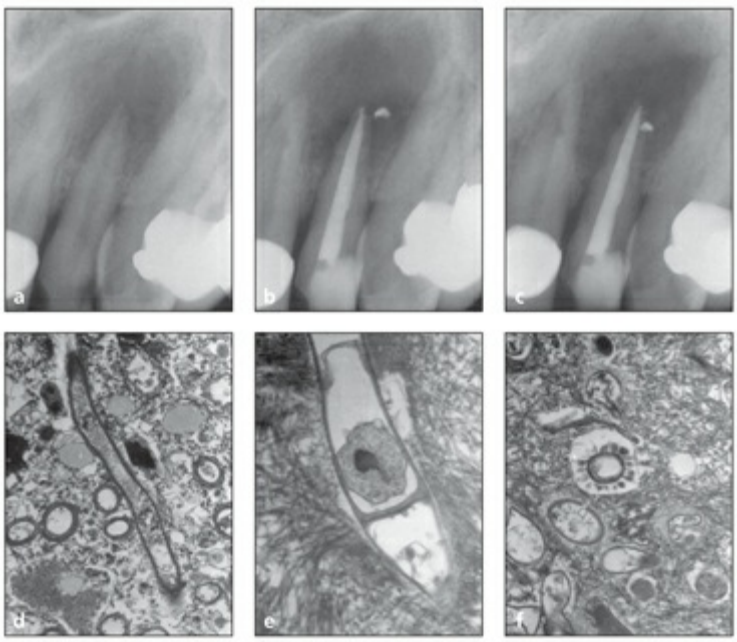

Fig 16-5 Fungi in a persistent periapical lesion. *(a)* Radiograph of a maxillary lateral incisor with a necrotic pulp and a periapical radiolucency. *(b)* Radiograph taken immediately after nonsurgical treatment. *(c)* Radiograph taken at the 3-month recall. The patient is still symptomatic, and the periapical radiolucency is larger. *(d)* Transmission electron micrograph revealing the growing hyphae of a fungus (original magnification ×2,000). *(e)* Higher magnification of the hyphae, showing the cell wall (original magnification ×4,000). *(f)* Reproductive fungal spores (original magnification ×4,000).

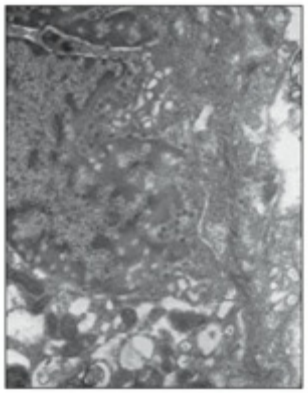

Fig 16-6 Transmission electron micrograph of the nucleus of a macrophage in a periapical lesion, suggesting a possible viral infection (original magnification ×5,000).

Bacteria

Bacteria play a crucial role in the formation and progression of both endodontic and periodontal diseases.[26,45–52] The periradicular tissues become involved when bacteria invade the pulp, causing either partial or total necrosis. In a classic study, Kakehashi et al[45] demonstrated the relationship between the presence of bacteria in the pulp and periradicular diseases. In this study, pulps of normal rats were exposed

and left open to the oral environment. Consequently, pulpal necrosis ensued, followed by periradicular inflammation and lesion formation. However, when the same procedure was performed on germ-free rats, not only did the pulps remain vital and relatively uninflamed but the exposure sites showed evidence of dentin repair.

Möller et al[46] confirmed these findings in monkeys and reported that uninfected necrotic pulp tissue did not induce periradicular lesions or inflammatory reactions. Nonetheless, once the pulp became infected, periradicular lesions and inflammation in the apical tissues occurred. Korzen et al[47] reported similar results and suggested that pulpal infections were usually mixed infections. Collectively, these studies provided early key evidence regarding the role of microorganisms in pulpal and periradicular diseases.

Blomlöf et al[53] created defects on the root surfaces of intentionally extracted monkey teeth with either open or mature apices. The canals were either infected or filled with calcium hydroxide and reimplanted back in their sockets. After 20 weeks, marginal epithelial downgrowth was found on the denuded dentin surfaces of the infected teeth, a finding indicative of the association between infected pulp tissue and periodontal pathoses.

Jansson et al[54] assessed the effect of endodontic pathogens on marginal periodontal wound healing of denuded dentin surfaces surrounded by healthy periodontal ligament. Their results showed that, in infected teeth, the defects were covered by 20% more epithelium, while the uninfected teeth showed only 10% more connective tissue coverage. The authors concluded that pathogens in necrotic root canals may stimulate epithelial downgrowth along denuded dentin surfaces with marginal communication and thus augment periodontal disease.

Jansson et al,[55] in a retrospective 3-year study, evaluated radiographs of 175 endodontically treated single-rooted teeth from 133 patients. Patients who were more prone to periodontitis and exhibited evidence of endodontic treatment failures showed approximately three times greater marginal bone loss than did patients without endodontic infection. Additionally, the effect of endodontic infection on periodontal probing depth and the presence of furcation involvement in mandibular molars was investigated.[56] Endodontic infection in mandibular molars was associated with more attachment loss in the furcal area. It was therefore suggested that endodontic infection in molars associated with periodontal disease might enhance the progression of periodontitis by spreading pathogens through accessory canals and dentinal tubules.[56] However, when the endodontic infection was treated successfully, the furcal lesion healed, indicating that there was only one infective

vector present.

Proteolytic bacteria predominate in the root canal flora, which changes over time to a more anaerobic microbiota.[57,58] Rupf et al[59] studied the profiles of periodontal pathogens in pulpal and periodontal diseases associated with the same tooth. Specific polymerase chain reaction methods were used to detect *Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia*, and *Treponema denticola*. These pathogens were found in all endodontic samples, and the same pathogens were found in teeth with chronic apical periodontitis and chronic adult periodontitis. Therefore, it appears that periodontal pathogens accompany endodontic infections and that endodontic-periodontal interrelationships are a critical pathway for both diseases.

Spirochetes are another class of microorganism associated with both endodontic and periodontal diseases. Spirochetes are usually found more frequently in subgingival plaque than in root canals. Several studies have shown a large diversity of oral treponemes present in subgingival biofilms of periodontal pockets.[60–62] It has been previously proposed that the presence or absence of oral spirochetes can be used to differentiate between endodontic and periodontal abscesses.[20] Currently, however, the presence of spirochetes in the root canal system is well documented and has been demonstrated by different identification techniques, including darkfield microscopy, electron microscopy, and biochemical identification.[23,24,63–67]

The differences in the reported incidences of spirochetes associated with endodontic infection may be attributed to the different detection methods used. It has been demonstrated that the spirochete species most frequently found in root canals are *T denticola*[65,67] and *Treponema maltophilum*.[66] The main virulence factor of *T denticola* includes surface-expressed proteins with cytotoxic activities, such as the major surface protein and the chymotrypsin-like protease complex, extracellular or membrane-associated proteolytic and hydrolytic enzymes, and metabolites.[68] This microorganism possesses an array of virulence factors associated with periodontal disease and may also participate in the pathogenesis of periradicular disease.[67]

The virulence factors of *T maltophilum* have not yet been fully elucidated. It has been proposed that the motility of *T maltophilum*, caused by the rotation of its periplasmic flagella, might contribute to its pathogenicity.[69] This microorganism was also frequently isolated from patients with rapidly progressive periodontitis.[70] However, the exact role of this microorganism in combined endodontic-periodontal diseases requires further investigation.

L-form bacteria (ie, bacteria without cell walls) also have been suggested to have a role in periradicular disease.[71] Some bacterial strains can undergo morphologic transition to their L-form after exposure to certain agents, particularly penicillin.[72] The L-form and the bacterium may appear individually or together and may transform from one variant to another with numerous intermediate L-form transitional stages. This may occur either spontaneously or by induction in a cyclic manner. Under certain conditions, depending on host resistance factors and bacterial virulence, the L-forms revert to their original pathogenic bacterial form and may then be responsible for acute exacerbation of chronic periradicular lesions.[71]

Fungi (yeasts)

The presence and prevalence of fungi associated with endodontic disease are well documented.[27] Yeast colonization associated with radicular pathosis has been demonstrated in untreated root caries,[73,74] dentinal tubules,[75–77] failing root canal treatments,[78–81] apices of teeth with asymptomatic apical periodontitis,[82] and periradicular tissues.[83] Most studies have reported that the prevalence of fungi in cultured root canal systems varies and may reach up to 26% in untreated root canals[73,84–87] and 33% in previously treated canals.[73,79,80,83,88] A few studies, however, have demonstrated an even higher incidence of up to 55%.[77,89]

The predominant fungi recovered were *Candida albicans*.[88,90] *C albicans* has been detected in 21% of infected root canals using 18S rRNA–directedspecies-specific primers[87] and also has shown an ability to colonize canal walls and invade dentinal tubules.[77] Other species of fungi, such as *Candida glabrata*, *Candida guilliermondii*, *Candida incospicua*,[88] and *Rhodotorula mucilaginosa*, [25] were also detected.

Factors affecting the colonization of the root canal by fungi are not completely understood. It appears, however, that among the predisposing factors of this process are immunocompromising diseases such as cancer,[76] certain intracanal medicaments,73 local and systemic antibiotics,[74,91] and previous unsuccessful endodontic therapy.[80,92,93] Reduction of specific strains of bacteria in the root canal during endodontic treatment may allow fungi overgrowth in the remaining low-nutrient environment.[80,93] Another possibility is that fungi may gain access to the root canal from the oral cavity as a result of poor asepsis during endodontic treatment or post-preparation procedures. It has been reported that approximately 20% of patients with adult periodontitis also harbor subgingival fungi,[94,95] and *C albicans* was the most common species isolated.[96] In addition, it has been demonstrated that

the presence of fungi in root canals is directly associated with their presence in saliva.[25] These findings further confirm the importance of using aseptic endodontic and periodontal techniques, maintaining the integrity of dental hard tissues, and covering the tooth crown as soon as practical with a well-sealed permanent restoration to prevent reinfection.

Viruses

There is increasing evidence to suggest that viruses may be associated with both endodontic and periodontal diseases. Herpes simplex virus was frequently detected in gingival crevicular fluid and gingival biopsies of periodontal lesions from patients with periodontal disease.[97,98] Human cytomegalovirus was observed in about 65% of periodontal pocket samples and about 85% of gingival tissue samples.[97] Epstein-Barr virus type I was observed in more than 40% of periodontal pocket samples and in about 80% of gingival tissue samples.[97] Gingival herpes-viruses were found to be associated with increased occurrence of subgingival *P gingivalis, B forsythus, P intermedia, Prevotella nigrescens, T denticola*, and *A actinomycetemcomitans*, thus suggesting their role in the overgrowth of periodontal pathogenic bacteria.[99]

The presence of viruses in the dental pulp was first reported in a patient with AIDS.[100] The DNA of human immunodeficiency virus (HIV) was also detected in periradicular lesions.[101] However, it has not been established that HIV can directly cause pulpal disease. Herpes simplex virus was also studied in relation to endodontic disease. It seems that, unlike its role in periodontal disease, herpes simplex virus does not play a significant role in endodontic disease.[102,103]

On the other hand, other common types of human viruses may be involved in pulpal and periradicular diseases. It has been suggested that human cytomegalo-virus and Epstein-Barr virus play a role in the pathogenesis of symptomatic periradicular lesions.[104,105] It seems that active infection may give rise to production of an array of cytokines and chemokines with the potential to induce immunosuppression or tissue destruction.[106] Herpesvirus activation in periradicular inflammatory cells may impair the host defense mechanisms and give rise to overgrowth of bacteria, as seen in periodontal lesions. Herpesvirus-mediated immune suppression may be detrimental in periradicular infections because host responses in the granulomatous tissue are already compromised.[107] Alterations between prolonged periods of herpesvirus latency interrupted by periods of activation may explain some burstlike symptomatic episodes of periradicular disease. Frequent reactivation of

periradicular herpesvirus may support rapid periradicular breakdown. The absence of herpesvirus infection or viral reactivation may be the reason that some periradicular lesions remain clinically stable for extended periods of time.[104]

Infectious biofilms

The majority of bacteria in virtually all natural ecosystems grow in biofilms, and their growth in affected tissues is characterized by matrix-enclosed communities.[108,109] Biofilm microcolonies are composed of approximately 15% cells (by volume) embedded in 85% matrix material.[110] They are bisected by ramifying water channels that carry bulk fluid into the community by convective flow.[111] The structural composition of biofilms indicates that these communities are regulated by signals analogous to the hormones and pheromones that regulate many cellular eukaryotic communities.[110]

Biofilm formation has a developmental sequence that results in the formation of a mature community of tower-shaped and mushroom-shaped micro-colonies, with some variation between species. The sequence of events usually is microbial surface attachment, cell proliferation, matrix production, and detachment.[112] Biofilm formation and detachment are under the control of chemical signals that regulate and guide the formation of slime-enclosed microcolonies and water channels.[110] It has been stated that microbial biofilms constitute the most “defensive” life strategy that can be adopted by prokaryotic cells.[113] In very hostile environments, such as extreme heat, acidity, or dryness, this stationary mode of growth is inherently defensive because bacterial cells are not swept into areas where they can be killed.[110]

Infectious biofilms are difficult to detect through routine diagnostic methods and confer great resistance to host defenses and antibiotic therapies.[112] In addition, biofilms facilitate the spread of antibiotic resistance by promoting horizontal gene transfer. They are also actively adapted to environmental stresses, such as alteration in nutritional quality, cell density, temperature, pH, and osmolarity.[114] Prolonged starvation induces loss of cultivability under standard conditions while the microorganism remains metabolically active and structurally intact.[115] This is considered the main reason for the low detection rate of biofilm infections by routine culture methods. To date, however, the exact role of biofilms in the interrelationship between endodontic and periodontal diseases has not been fully elucidated.

Nonliving pathogens

Nonliving pathogens can be either extrinsic or intrinsic, depending on their origin and nature.

Extrinsic (foreign bodies)

Foreign bodies are often found in association with the inflammatory process of the periradicular tissues (Figs 16-7 and 16-8). Although endodontic and periodontal diseases are primarily associated with the presence of microorganisms, the presence of certain foreign substances in situ may explain some treatment failures. Substances such as dentin and cementum chips,[116–118] amalgam,[118,119] root canal filling materials,[116,118–120] cellulose fibers from absorbent paper points,[119,121,122] gingival retraction cords,[123] leguminous foods,[124] and calculus-like deposits[125] have all been reported to trigger periradicular inflammatory reactions.

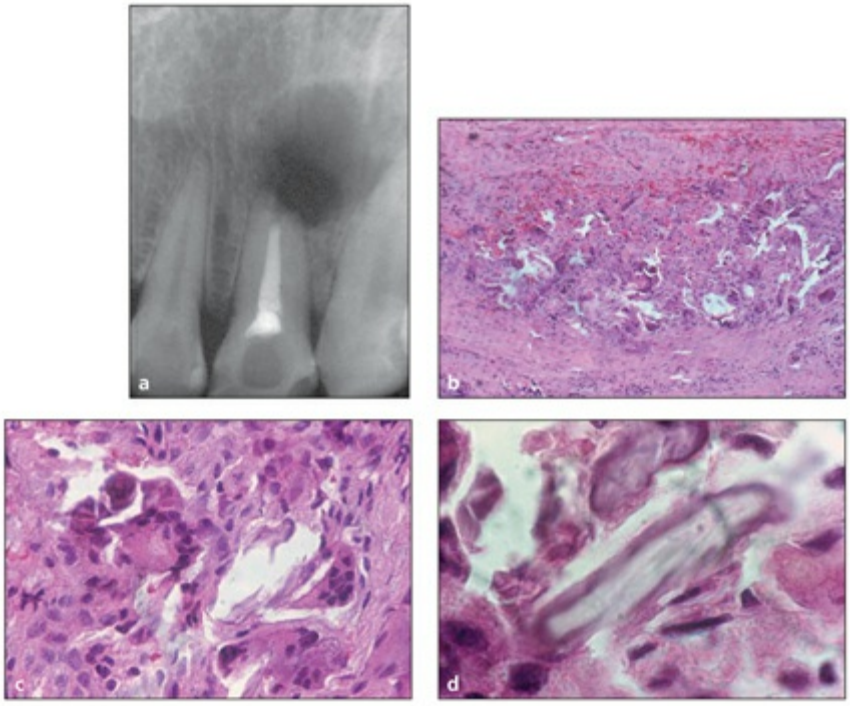

Fig 16-7 Foreign body particles in a periapical lesion. *(a)* Radiograph of a symptomatic maxillary central incisor with a large periapical lesion. Endodontic treatment had been performed 27 years previously. *(b)* Photomicrograph of apical tissue, showing foreign body particles in the presence of giant cells (H&E stain; original magnification ×40). *(c)* Higher magnification of the foreign body particles and giant cells (H&E stain; original magnification ×200). *(d)* Part of the foreign body. When put under polarized light, the substance responds as vegetable matter (H&E stain; original magnification ×400). The diagnosis is that part of a paper point had extended past the apical foramen.

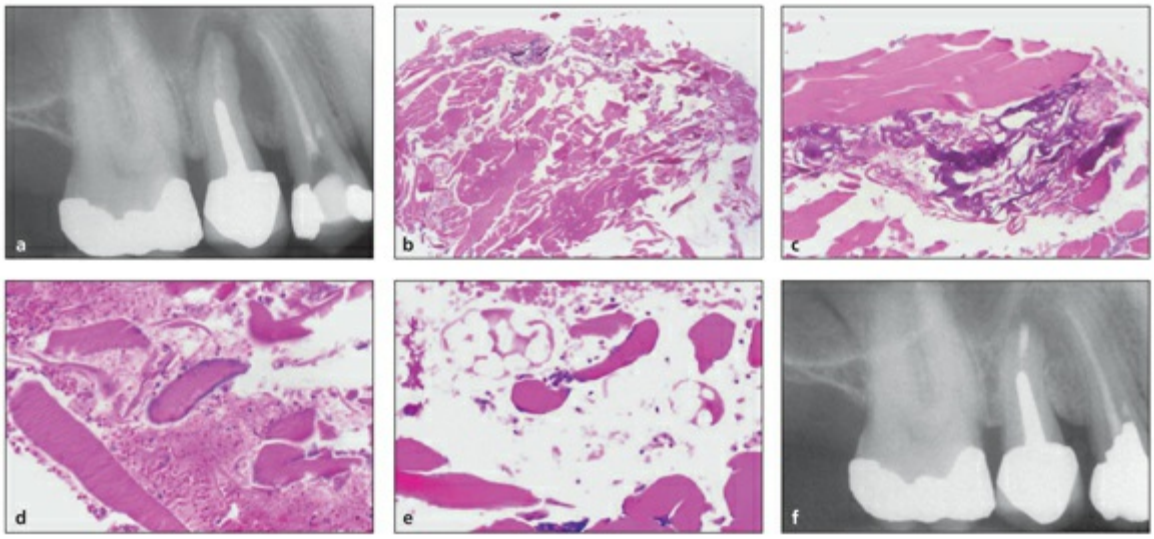

Fig 16-8 Multiple etiologic factors past the apical foramen associated with failing treatment. *(a)* Radiograph showing treatment failure in a maxillary second premolar. The tooth had been treated by intentional reimplantation, during which the apical lesion was removed. *(b)* Photomicrograph of the lesion, revealing presence of foreign material (H&E stain; original magnification ×20). *(c)* Higher magnification showing unidentified purple foreign material and necrotic muscle tissue (H&E stain; original magnification ×100). *(d)* Different area of the lesion, showing necrotic muscle with viable bacterial colonies (H&E stain; original magnification ×100). *(e)* Necrotic muscle tissue infected by bacteria and the presence of lentil beans (pulse granuloma) (H&E stain; original magnification ×100). *(f)* One-year follow-up radiograph. The tooth is asymptomatic and nonmobile, and bony healing is evident.

A foreign body response may occur to any of these substances; clinically, such conditions may be either symptomatic or asymptomatic. Microscopically, these lesions demonstrate the presence of multinucleated giant cells surrounding the foreign material in a chronic inflammatory infiltrate. Mechanical or surgical removal of the foreign bodies is usually the treatment of choice.

Intrinsic

Epithelium. Among the normal components of the lateral and apical periodontal ligament are the epithelial rests of Malassez. The term *rests* is misleading in that it evokes a vision of discrete islands of epithelial cells. These rests are actually a fishnet-like, three-dimensional, interconnected network of epithelial cells. In many periradicular lesions, epithelium is not present and therefore is presumed to have been destroyed.[126] If the rests remain, they may respond to stimuli by proliferating in an attempt to wall off the irritants coming through the apical foramen. Epithelium can be surrounded by chronic inflammatory tissue. This type of lesion is termed an *epitheliated granuloma,* and, if not treated, the epithelium will continue to proliferate in an attempt to wall off the source of irritation communicating from the

apical foramen.

The term *bay cyst* has been introduced for the microscopic representation of this situation.[34] This is a chronic inflammatory lesion in which an epithelial lining surrounds the lumen but the lumen has a direct communication with the root canal system through the apical foramen (Fig 16-9). On the other hand, a *true cyst* is the completion of the epithelial proliferative lesion. It is a three-dimensional, epithelium-lined cavity with no communication between the lumen and the canal system (Fig 16-10). When periapical lesions are studied in relation to the root canal, a clear distinction should be made between these two entities.[33–34]

There has been some confusion regarding the diagnosis when lesions are studied only on curetted biopsy material. Because the tooth is not attached to the lesion, the orientation to the apex is lost. Therefore, the criterion used for diagnosis of a cyst is a strip of epithelium that appears to be lining a cavity. Thus, curettage of a bay cyst and a true cyst could lead to the same microscopic diagnosis. A bay cyst could be sectioned in such a way that it could resemble or give the appearance of a true cyst. This distinction between a bay cyst and a true cyst is important from the standpoint of healing. True cysts must be surgically removed, but bay cysts that communicated with the root canal may heal with nonsurgical root canal treatment. Because root canal treatment can directly affect the lumen of the bay cyst, the environmental change may bring about resolution of the lesion. The true cyst is independent of the root canal system, so conventional root canal treatment may not have an effect on this entity.

The formation of a cyst and its progression from a bay cyst to a true cyst occurs over time. Valderhaug,[127] in a study performed in monkeys, found no cyst formation until at least 6 months after the canal contents became necrotic. Thus, the longer a lesion was present, the greater the probability that it would become a true cyst. However, the incidence of true cysts is probably less than 10%.[34] This may explain the relatively high success rate of nonsurgical root canal treatment in teeth associated with periradicular lesions.

Cholesterol. The presence of cholesterol crystals in apical periodontitis is a common histopathologic finding.[128–132] With time, the cholesterol crystals are dissolved and washed away, leaving behind spaces as clefts. The reported incidence of cholesterol clefts in periradicular disease varies from 18% to 44%.[128,130,131] It has been suggested that the crystals could be formed from cholesterol released by disintegrating erythrocytes of stagnant blood vessels within the periradicular

lesion[130]; by lymphocytes, plasma cells, and macrophages that die in great numbers and disintegrate in chronic periradicular lesions[131]; or by the circulating plasma lipids.[128] It is possible, however, that all of these factors may contribute to the accumulation, concentration, and crystallization of cholesterol in a periradicular lesion (Fig 16-11).

It has been suggested that accumulation of cholesterol crystals in inflamed periradicular tissues in some cases might cause failure of endodontic treatment.[30,132] The macrophages and the multinucleated giant cells that congregate around cholesterol crystals are not efficient enough to destroy the crystals completely. In addition, the accumulation of macrophages and giant cells around the cholesterol clefts in the absence of other inflammatory cells, such as neutrophils, lymphocytes, and plasma cells, suggests that the cholesterol crystals induce a typical foreign body reaction.[30]

Russell bodies. Russell bodies can be found in most inflamed tissues throughout the body, including the periradicular tissues (Fig 16-12). These are small, spherical accumulations of an eosinophilic substance found within or near plasma cells and other lymphoid cells. The presence and occurrence of Russell bodies in oral tissues and periradicular lesions is well documented.[133,134]

Studies have indicated the presence of Russell bodies in about 80% of periradicular lesions. Recently, large intracellular and extracellular Russell bodies were also found in inflammatory pulp tissue of carious primary teeth.[31] It is hypothesized that Russell bodies are caused by synthesis of excessive amounts of normal secretory protein in certain plasma cells engaged in active synthesis of immunoglobulins. The endoplasmic reticulum becomes greatly distended, thus producing large homogenous eosinophilic inclusions.[135] However, the incidence of Russell bodies, their production mechanism, and their exact role in pulpal inflammation has not yet been fully elucidated.

Rushton hyaline bodies. The presence of Rushton hyaline bodies is a feature unique to some odontogenic cysts. Their frequency varies from 2.6% to 9.5%.[136] Rushton hyaline bodies usually appear within the epithelial lining or the cyst lumen (Fig 16-13). They have a variety of morphologic forms, including linear (straight or curved), irregular, rounded, and polycyclic structures, or they may appear granular.[29,136]

The exact nature of Rushton hyaline bodies is not fully understood. It has been

suggested that they are keratinous,[128] of hematogenous origin,[137] a specialized secretory product of odontogenic epithelium,[138] or degenerated red blood cells.[29] Some authors have suggested that Rushton hyaline bodies are material left behind at the time of a previous surgical operation.[139] It is not clear yet why the Rushton hyaline bodies form mostly within the epithelium.

Charcot-Leyden crystals. Charcot-Leyden crystals are naturally occurring hexagonal bipyramidal crystals derived from the intracellular granules of eosinophils and basophils.[140–142] Their presence is most often associated with increased numbers of peripheral blood or tissue eosinophils in parasitic, allergic, neoplastic, and inflammatory diseases.[140,141,143] Activated macrophages are reported to have an important role in the formation of Charcot-Leyden crystals in several disease processes.[144] Charcot-Leyden crystals and damaged eosinophils, along with their granules, have been observed within macrophages.[143–145]

It has been proposed that, after the degranulation of eosinophils, Charcot-Leyden crystal protein could be phagocytized into acidified membrane-bound lysosomes.[143] At some point, Charcot-Leyden crystal protein would begin to crystallize, forming discrete particles that increase in volume and density over time. Ultimately, these crystals would be released via phagosomal exocytosis or by piercing through the membrane of the phagosome and macrophage cytoplasm, becoming free in the stromal tissue.

Recent findings support the theory that activated macrophages have a role in the formation of Charcot-Leyden crystals.[32] In addition, the presence of Charcot-Leyden crystals can be detected within a periradicular lesion that failed to resolve after conventional endodontic treatment (Fig 16-14). Although the biologic and pathologic roles of Charcot-Leyden crystals in endodontic and periodontal disease are still unknown, some cases of treatment failure may be attributed to them.

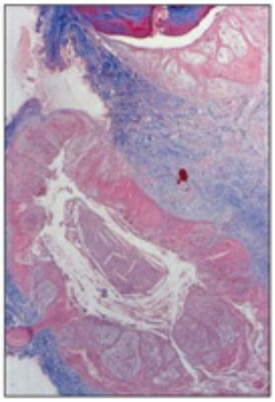

Fig 16-9 Photomicrograph of a bay cyst associated with a root canal that opens directly into the lumen

of the lesion (H&E stain; original magnification ×20).

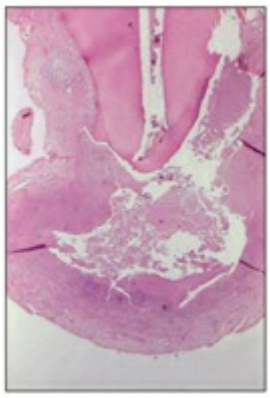

Fig 16-10 Photomicrograph of a true inflammatory cyst. It is a three-dimensional epithelial-lined lesion with no connection to the root canal system and apical foramen (Masson trichrome stain; original magnification ×20).

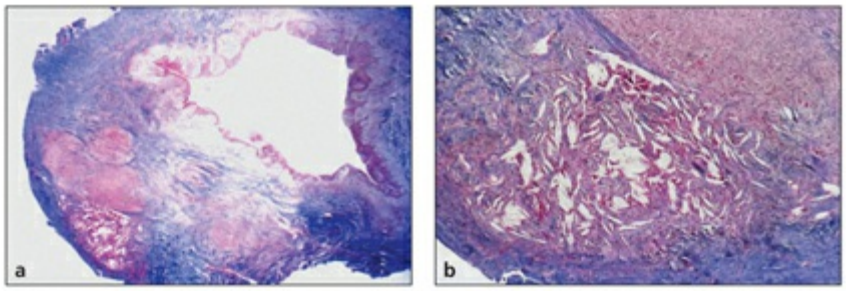

Fig 16-11 Cholesterol clefts in a periapical lesion. *(a)* Photomicrograph of a cyst with a thick fibrous wall. Embedded in the wall is a large collection of cholesterol clefts (Masson trichrome stain; original magnification ×20). *(b)* Higher magnification showing empty clefts where cholesterol has been dissolved during the histologic preparation (Masson trichrome stain; original magnification ×100).

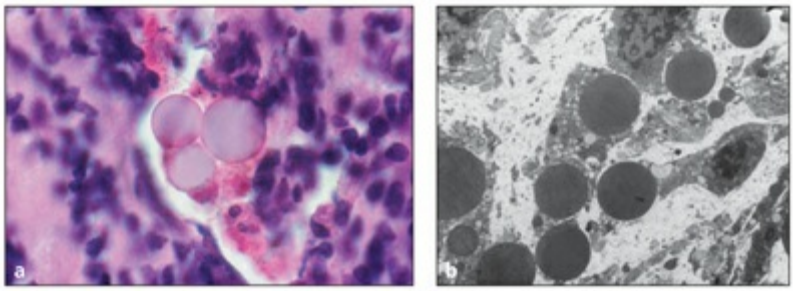

Fig 16-12 *(a)* Photomicrograph of a periapical lesion showing the presence of Russell bodies (H&E stain; original magnification ×200). *(b)* Transmission electron micrograph demonstrating the round, amorphous shape of these structures (H&E stain; original magnification ×500).

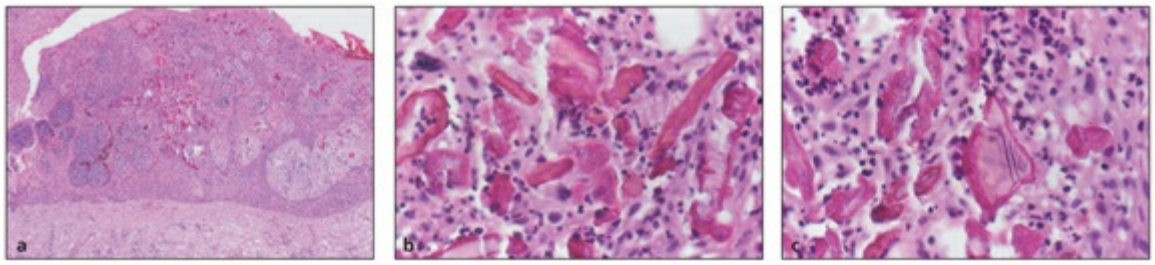

Fig 16-13 *(a)* Photomicrograph showing Rushton hyaline bodies in the epithelial lining of a periapical cyst (H&E stain; original magnification ×20). *(b and c)* Higher-magnification photomicrographs demonstrating the pleomorphism of these bodies (H&E stain; original magnification ×100).

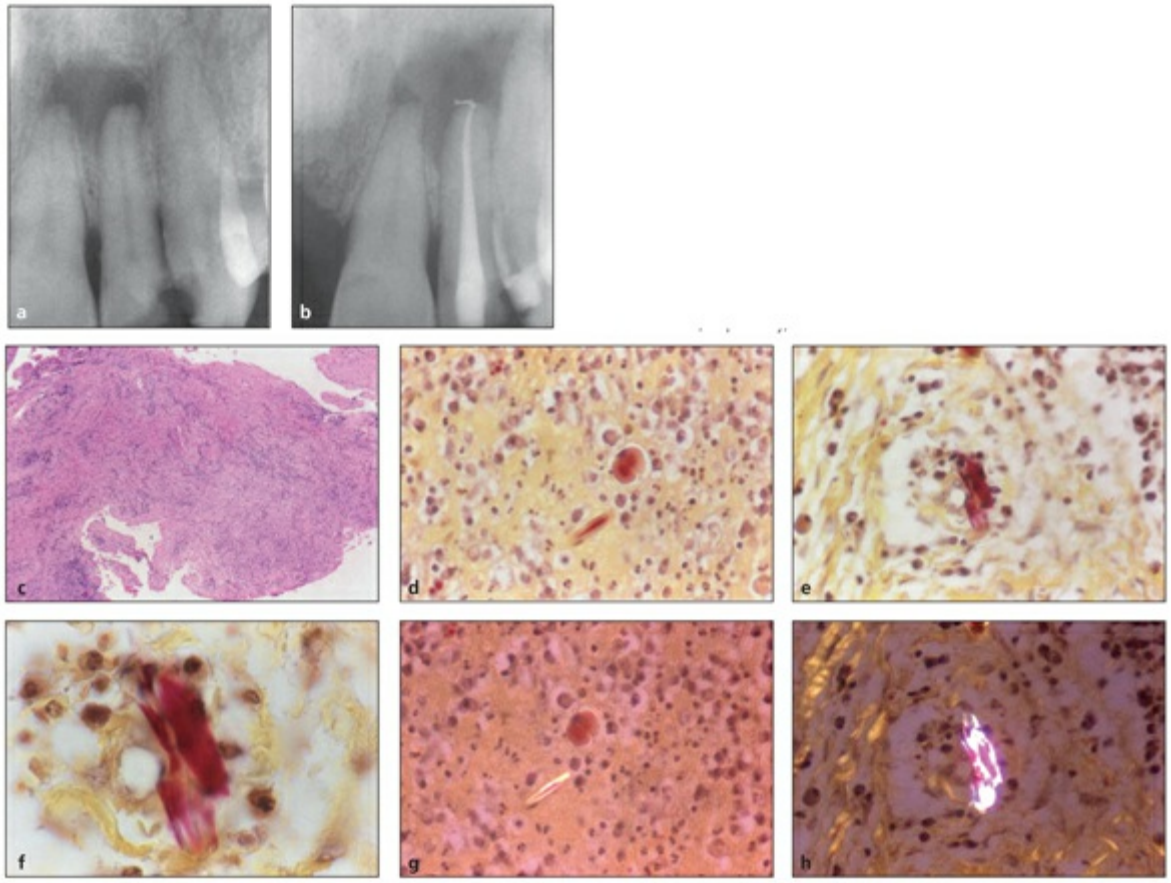

Fig 16-14 Charcot-Leyden crystals in a periapical lesion. *(a)* Maxillary lateral incisor with necrotic pulp and a periapical lesion. *(b)* Radiograph taken 9 months after endodontic treatment. The tooth is still symptomatic, and the lesion is larger. *(c)* Photomicrograph of the lesion reveals only acute and chronic inflammatory infiltrate (H&E stain; original magnification ×40). *(d to f)* May-Grünwald-Giemsa staining reveals the presence of Charcot-Leyden crystals (original magnification ×100). *(g and h)* Polarized light demonstrates refraction of the Charcot-Leyden crystals (original magnification ×200 and ×400, respectively).

Contributing factors

Poor endodontic treatment

Correct endodontic procedures and techniques are key factors for treatment success. Assessments of the retention rate of endodontically treated teeth have found that nonsurgical endodontic treatment is a predictable procedure with excellent long-term prognosis.[146–148] It is imperative that the canal system be completely cleaned and shaped and well obturated to enhance successful outcomes. Poor endodontic treatment allows reinfection of the canal, which may often lead to treatment failure.[149]

Endodontic failures can be treated either by orthograde or retrograde retreatment with good success rates. The success rate for retreatment is similar to that of initial conventional endodontic treatment if the cause of failure is properly diagnosed and corrected.[150] In recent years, retreatment techniques have improved dramatically because of the use of the operating microscope and development of a new armamentarium.

Poor restoration

Coronal leakage is an important cause of endodontic treatment failure. Root canals may become recon-taminated by microorganisms if there is a delay in placement of a coronal restoration or fracture of the coronal restoration and/or the tooth.[151] Madison and Wilcox[152] found that exposure of root canals to the oral environment allowed coronal leakage to occur, in some cases along the entire length of the root canal. Ray and Trope[153] reported that teeth with defective restorations and adequate root canal fillings had a higher incidence of failure than did teeth with inadequate root canal fillings and adequate restorations. Teeth in which both the root canal fillings and restorations were adequate had only 9% failure, whereas teeth in which both root canal fillings and restorations were defective had a failure rate of about 82%.[153]

Saunders and Saunders[154] showed that coronal leakage was a significant clinical problem in endodontically treated molars. In an in vitro study, they found that packing excess gutta-percha and sealer over the floor of the pulp chamber after completion of root canal filling did not provide a better seal of the root canals. It is therefore recommended that excess gutta-percha restorative material be removed to the level of the canal orifices and that the floor of the pulp chamber be protected with a well-sealed restorative material.[154]

Coronal restoration is the primary barrier against coronal leakage and bacterial

contamination of the root canal. Therefore, lack of coronal coverage following endodontic treatment can significantly compromise the prognosis of the tooth.[147] For this reason, it is essential that the root canal system be protected by good endodontic obturation and a well-sealed coronal restoration. Nevertheless, even popular permanent restorative materials may not always prevent coronal leakage.[155] Cemented complete crowns[156,157] as well as dentin-bonded crowns[158] have also shown leakage.

A review of the literature[159] examined the factors associated with the long-term prognosis of enodontically treated teeth. These results indicated that *(1)* post space preparation and cementation should be performed with rubber dam isolation; *(2)* the post space should be prepared with a heated plugger; *(3)* a minimum of 3 mm of root canal filling should remain in the preparation; *(4)* the post space should be irrigated and dressed as during root canal treatment; *(5)* leak-proof restorations should be placed as soon as possible after endodontic treatment; and *(6)* endodontic retreatment should be considered for teeth with a coronal seal that has been compromised for longer than 3 months.[159] If these recommendations are followed, many enodontic and periodontal complications can and should be prevented.

Trauma

Trauma to teeth and alveolar bone may involve the pulp and the periodontal ligament. Both tissues can be affected either directly or indirectly. Dental injuries may take many shapes but generally can be classified as enamel fracture, crown fracture without pulpal involvement, crown fracture with pulpal involvement, crown-root fracture, root fracture, luxation, and avulsion.[160] Treatment of traumatic dental injuries varies depending on the type of injury, and it will determine pulpal and periodontal ligament healing prognosis.[161]

Enamel fracture

This type of injury involves the enamel only and includes enamel chipping and incomplete fractures or enamel cracks. Treatment usually includes grinding and smoothing of the rough edges or restoration of the missing enamel structure. When only the enamel is involved, the pulp usually maintains its vitality and the prognosis for both pulp and periodontium is good.

Crown fracture without pulpal involvement

This is an uncomplicated fracture that involves enamel and dentin without pulpal

exposure. Treatment may include conservative restoration with resin composite or reattachment of the separated fragments. It has been reported that reattachment of dentin-enamel crown fragments is a conservative possibility for crown restoration.[162] The pulpal and periodontal prognosis is good.

Crown fracture with pulpal involvement

This is a complicated fracture involving enamel and dentin and exposure of the pulp. The extent of the fracture helps to determine the necessary pulpal and restorative treatments.[160] A small fracture may indicate the need for vital pulp therapy followed by placement of an acid-etched resin composite restoration. A more extensive fracture may require partial pulpectomy or complete root canal treatment.

The stage of tooth maturation is an important factor in choosing between partial and full pulpotomy.[160] The amount of time elapsed since the injury often affects pulpal prognosis. The sooner the tooth is treated, the better the prognosis.

Crown-root fracture

This type of fracture is usually oblique and involves both crown and root. It includes enamel, dentin, and cementum and may or may not include the pulp. Crown-root fractures can affect molars and premolars as well as anterior teeth. Cusp fracture extending subgingivally is a common finding that often presents a diagnostic and clinical challenge.[160]

Treatment depends on the severity of the fracture and may vary from removal of the fractured tooth fragment and restoration to endodontic treatment to periodontal treatment and/or surgical procedures. Sometimes the prognosis is poor and the tooth has to be extracted. Because of the complexity of this injury, a team approach involving endodontists, periodontists, orthodontists, and prosthodontists is highly recommended.[160]

Root fracture

This type of fracture typically involves cementum, dentin, and pulp. It may be horizontal or transverse. Clinically, root fracture may often cause mobility of the segment coronal to the fracture as well as pain on biting. Often, a periodontal defect or a sinus tract is associated with the fractured root. Radiographically, a root fracture can only be visualized if the x-ray beam passes through the fracture line. Horizontal and oblique root fractures are easier to detect radiographically, while the diagnosis of vertical root fractures is more challenging. Advanced imaging technology may prove beneficial for diagnostic purposes.[163]

Treatment, when feasible, usually includes repositioning of the coronal segment and stabilization by splinting.[160] A flexible splint using orthodontic or nylon wire and acid-etched resin for periods of up to 12 weeks will enhance pulpal and periodontal repair.[164] Teeth with fractured roots do not necessarily require root canal treatment if healing takes place with no evidence of pulpal disease.[165]

Luxation

This category involves different types of tooth displacement injuries. It includes concussion, subluxation, extrusive luxation, lateral luxation, and intrusive luxation. Generally, the more severe the luxation injury, the greater the damage to the periodontium and to the dental pulp.[160]

In a concussion injury, the tooth is only sensitive to percussion. There is no increase in mobility, and no radiographic changes are found. The pulp may respond normally to vitality tests, and no immediate treatment is usually necessary.[160]

In a subluxation injury, the tooth is sensitive to percussion and also has increased mobility. Often sulcular bleeding is observed, indicating damage to the periodontal ligament. Radiographic findings are unremarkable, and the pulp may respond normally to vitality tests.[160] No treatment is usually required for minor subluxations. If mobility is severe, stabilization of the tooth is necessary.

In extrusive luxation, the tooth has been partially displaced from the socket and increased mobility is found. Radiographs also reveal displacement. The pulp usually does not respond to vitality tests, and root canal treatment is required once irreversible pulpitis is diagnosed.[160] The tooth requires repositioning and splinting, usually for a 2- to 3-week period.

In lateral luxation, the tooth has been displaced away from its long axis. Percussion sensitivity may or may not be present. A metallic sound on percussion indicates that the root has been forced into the alveolar bone.[160] Treatment includes repositioning and splinting. Lateral luxations that involve bony fractures usually require up to 8 weeks of splinting. Endodontic treatment should be performed only when a definite diagnosis of irreversible pulpitis or pulpal necrosis is established.

During intrusive luxation, the tooth is forced into the sockets in an axial direction. The tooth has decreased mobility resembling ankylosis.[160] Treatment depends on root maturity. If the root is not completely formed and presents with an open apex, it may reerupt. In such cases, root canal treatment is not necessary because the pulp may revascularize.[166] If the tooth is fully developed, active extrusion is indicated. In such cases, root canal treatment is indicated because pulpal necrosis develops in

almost all cases.[166]

Avulsion

In avulsion, the tooth is totally displaced from its alveolar socket. If the tooth is reimplanted soon after avulsion, the periodontal ligament has a good chance of healing.[160] Extraalveolar time and the storage media used to transport the tooth are critical factors for successful reimplantation. Root canal treatment within 10 days of injury and the degree of recovery of the periodontal ligament cells will determine long-term success.

Perforation

When root perforation occurs, communications between the root canal system and either periradicular tissues or the oral cavity may often lead to treatment failure. Root perforations may result from extensive caries lesions, resorption, or operator error during root canal instrumentation or post preparation.[167,168]

The prognosis for a tooth with a perforated root depends on the size and location of the perforation, time of diagnosis and treatment, degree of periodontal damage, and the sealing ability and biocompat-ibility of the repair material.[169] Treatment success depends mainly on immediate sealing of the perforation and appropriate infection control. Several materials have been recommended for sealing root perforations, including mineral trioxide aggregate, SuperEBA (Bosworth), Cavit (3M ESPE), IRM (Caulk Dentsply), glass ionomers, composite resins, and amalgam.[170–174] Today, mineral trioxide aggregate is the most widely used material.

An excellent and conservative treatment modality for perforations, root resorptions, and certain root fractures is controlled root extrusion.[175] The procedure has a good prognosis and a low risk of relapse, and its versatility has been demonstrated in multiple clinical situations.[176–178] It can be performed either immediately or over a few weeks, depending on each individual case. The goal of controlled root extrusion is to modify the soft tissues and bone; therefore, the technique is used to correct gingival discrepancies and osseous defects at periodontally involved teeth.[176] It is also used in the management of unrestorable teeth.

The objective of forced eruption in prosthetically compromised endodontically treated teeth is to allow the restoration of a subcrestal defect by elevating the defect to a point where access is no longer a problem.[179] In all cases, the epithelial attachment remains at the level of the cementoenamel junction. Forced eruption also

presents a good alternative to crown lengthening because it prevents esthetic alterations and unnecessary reduction of bony support of adjacent teeth.

Developmental malformation

Teeth with developmental malformations tend to fail to respond to periodontal and/or endodontic treatment when they are directly associated with an invagination or a vertical developmental radicular groove. Such conditions can lead to an untreatable periodontal condition. These grooves usually begin in the central fossa of maxillary central and lateral incisors, crossing over the cingulum and continuing apically down the root for varying distances. Such a groove is probably the result of an attempt of the tooth germ to form another root.

As long as the epithelial attachment remains intact, the periodontium remains healthy. However, once this attachment is breached and the groove becomes contaminated, a self-sustaining infrabony pocket can be formed along its entire length. This fissurelike channel provides a nidus for accumulation of bacterial biofilm and an avenue for the progression of periodontal disease that may also affect the pulp. Radiographically, the area of bone destruction follows the course of the groove.[180]

From a diagnostic standpoint, the patient may present with symptoms of a periodontal abscess or a variety of asymptomatic endodontic conditions. If the condition is purely periodontal, it can be diagnosed by visually following the groove to the gingival margin and by probing the depth of the pocket, which is usually tubular and localized to this one area, as opposed to a more generalized periodontal problem. The tooth will respond to pulpal vitality testing. Bone destruction that follows the groove vertically may be apparent radiographically. If this condition is also associated with endodontic disease, the patient may present clinically with any of the spectrum of endodontic symptoms.

The prognosis of root canal treatment in such cases is guarded, depending on the apical extent of the groove. The clinician must look for the groove because it may have been altered by a previous access opening or restoration placed in the access cavity. The appearance of a teardrop-shaped area on the radiograph should immediately arouse suspicion. The developmental groove may actually be visible on the radiograph. If so, it will appear as a dark vertical line. This condition must be differentiated from a vertical fracture, which may have a similar radiographic appearance.

Treatment consists of flattening the groove with a bur, placing bone substitutes,

and surgically managing the soft tissues and underlying bone. A clinical case using Emdogain (Straumann) as a treatment adjunct was recently described.[180] Radicular grooves are self-sustaining infrabony pockets, and therefore scaling and root planing will not suffice. Although the acute nature of the problem may be alleviated initially, the source of the chronic or acute inflammation must be eradicated by a surgical approach. Occasionally, the tooth has to be extracted because of its poor prognosis.

Differential Diagnosis

For differential diagnostic and treatment purposes, so-called endo-perio lesions are best classified as endodontic, periodontal, or combined diseases.[181] These include primary endodontic diseases, primary periodontal diseases, and combined diseases. The combined diseases include primary endodontic disease with secondary periodontal involvement, primary periodontal disease with secondary endodontic involvement, and true combined diseases.

This classification is based on the theoretic pathways explaining how these radiographic lesions are formed. By understanding the pathogenesis, the clinician can then suggest an appropriate course of treatment and assess the prognosis. Once the lesions progress to their final involvement, they present a similar radiographic picture and the differential diagnosis becomes more challenging.

Primary endodontic disease

An acute exacerbation of a chronic apical lesion in a tooth with a necrotic pulp may drain coronally through the periodontal ligament into the gingival sulcus. This condition may simulate clinically the presence of a periodontal abscess. In reality, it is a sinus tract of pulpal origin that opens through the periodontal ligament area. For diagnostic purposes, it is essential that the clinician insert a gutta-percha cone, or another tracking instrument, in the sinus tract and take one or more radiographs to determine the origin of the lesion. When the pocket is probed, it is narrow and lacks width. A similar situation occurs when drainage from the apex of a molar tooth extends coronally into the furcation area. This may also occur in the presence of

lateral canals extending from a necrotic pulp into the furcation area.[181]

Primary endodontic diseases usually heal following root canal treatment (Fig 16-15). The sinus tract extending to the gingival sulcus or furcation area disappears at an early stage once the affected necrotic pulp has been removed and the root canals have been well cleaned, shaped, and obturated.[181]

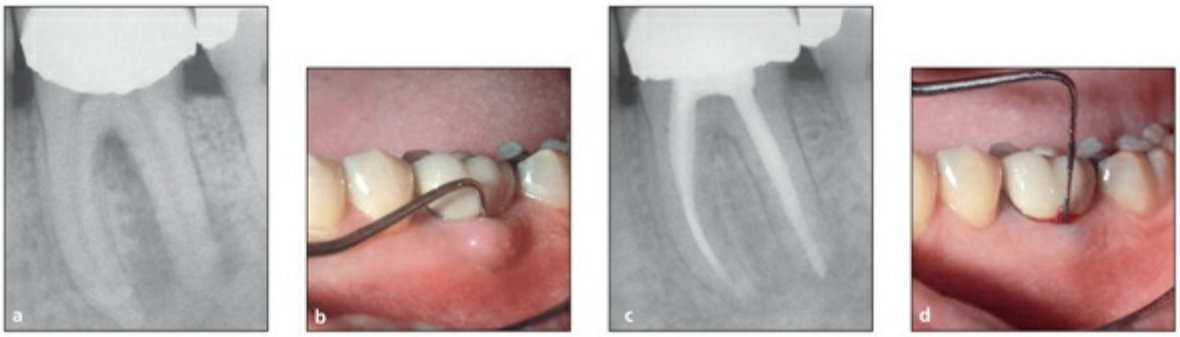

Fig 16-15 Primary endodontic disease in a mandibular first molar with a necrotic pulp. *(a)* Preoperative radiograph showing a periradicular radiolucency associated with the distal root. *(b)* Clinically, a deep, narrow buccal periodontal defect can be probed. *(c)* Radiograph taken 1 year after root canal therapy. Resolution of the periradicular bony radiolucency is evident. *(d)* Clinical appearance 1 year posttreatment. The buccal defect has healed and probing is normal.

Primary periodontal disease

These lesions are caused primarily by periodontal pathogens. In this process, chronic marginal periodontitis progresses apically along the root surface. In most cases, pulpal vitality tests indicate a clinically normal pulpal reaction (Figs 16-16 and 16-17). There is frequently an accumulation of plaque and calculus, and the pockets are wider.

The prognosis depends on the stage of periodontal disease and the efficacy of periodontal treatment. The clinician must also be aware of the radiographic appearance of periodontal disease associated with developmental radicular anomalies (Fig 16-18).

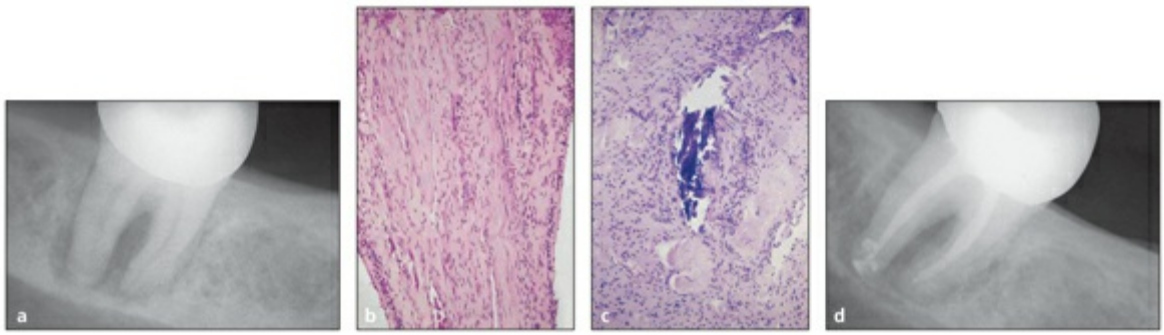

Fig 16-16 Primary periodontal disease in a mandibular second molar. The patient has been referred for endodontic therapy. *(a)* Preoperative radiograph revealing periradicular radiolucency. The tooth responds normally to pulpal sensitivity tests. The referring dentist, however, insisted that endodontic therapy be done. *(b)* Photomicrograph of pulp tissue removed during treatment. Note the normal appearance of the pulp (H&E stain; original magnification ×40). *(c)* Higher-magnification photomicrograph showing normal cellular components as well as blood microvasculature (H&E stain; original magnification ×100). *(d)* Postoperative radiograph. The tooth was subsequently lost to periodontal disease.

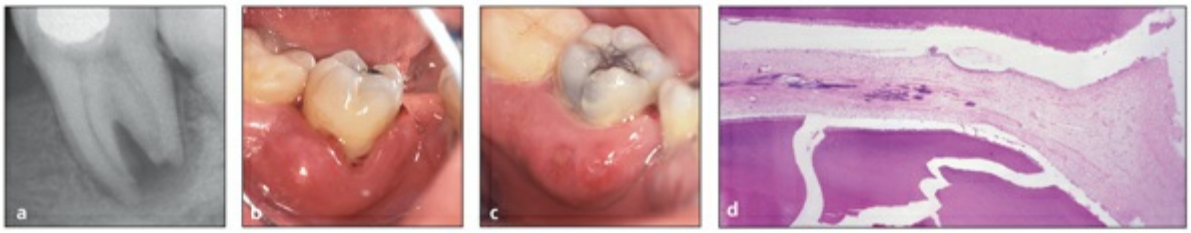

Fig 16-17 Primary periodontal lesion simulating an endodontic lesion. *(a)* Radiograph of a mandibular first molar revealing a periradicular radiolucency and periapical resorption. *(b and c)* Buccal and lingual views, respectively, of the affected tooth. Note the gingival swelling and evidence of periodontal disease. In addition, an occlusal restoration is present close to the pulp chamber. Despite the clinical and radiographic appearances, the pulp responds normally to vitality testing procedures, indicating that the radiolucency, resorption, and gingival swelling are of periodontal origin. *(d)* Microphotograph showing the floor of the pulp chamber and entrance to the mesial canal, which contain normal pulp tissue (H&E stain; original magnification ×40).

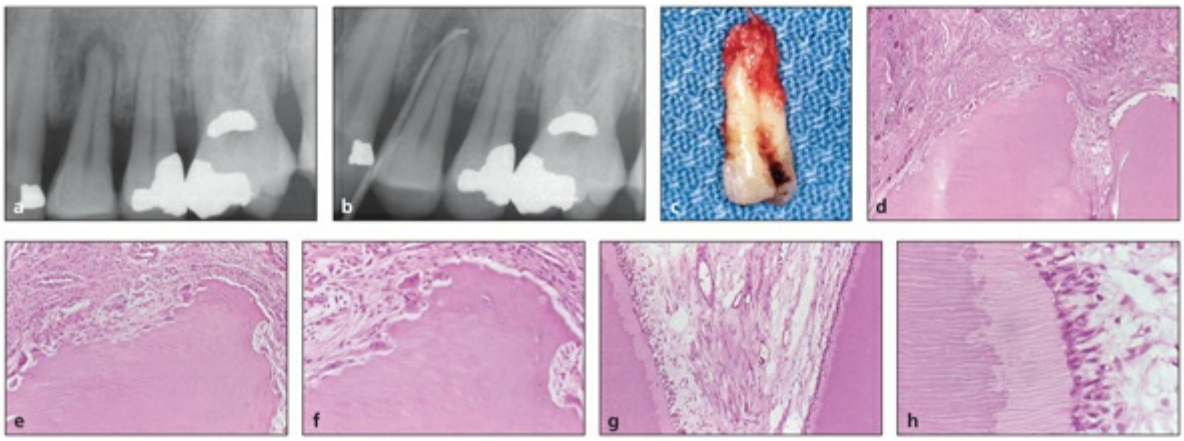

Fig 16-18 Primary periodontal disease in a maxillary second premolar *(a)* Radiograph showing alveolar

bone loss and a periapical lesion. Clinically, a deep, narrow pocket is present on the mesial aspect of the root. There is no evidence of caries, and the tooth responds normally to pulpal sensitivity tests. *(b)* Radiograph showing pocket tracking with a gutta-percha cone to the apical area. The tooth will be extracted. *(c)* Clinical view of the extracted tooth with the attached lesion. Note the presence of a deep mesial radicular developmental groove. *(d)* Photomicrograph of the apex of the tooth with the attached lesion (H&E stain; original magnification ×40). *(e and f)* Higher-magnification photomicrographs showing the inflammatory lesion, cementum and dentin resorption, and osteoclasts (H&E stain; original magnification ×100). *(g and h)* Histologic sections of the pulp chamber, revealing uninflamed pulp, the odontoblastic layer, and intact predentin (H&E stain; original magnification ×40 and ×200, respectively).

Combined disease

Primary endodontic disease with secondary periodontal involvement

If suppurating primary endodontic disease remains untreated, it may become secondarily involved with marginal periodontal breakdown (Fig 16-19). Plaque forms at the gingival margin of the sinus tract and leads to marginal periodontitis. The treatment and prognosis of the tooth are different when plaque or calculus is present compared to when the tooth exhibits only primary endodontic disease. The tooth now requires both endodontic and periodontal treatments. If the endodontic treatment is adequate, the prognosis depends on the severity of the marginal periodontal damage and the efficacy of periodontal treatment. With endodontic treatment alone, only part of the lesion will heal to the level of the secondary periodontal lesion. In general, healing of the tissues damaged by suppuration from the pulp can be anticipated.[181]

Primary endodontic lesions with secondary periodontal involvement may also occur as a result of root perforation during root canal treatment or where pins or posts have been misplaced during coronal restoration. Symptoms may be acute, with periodontal abscess formation associated with pain, swelling, purulent exudate, pocket formation, and tooth mobility. A more chronic response may sometimes occur without pain; this involves the sudden appearance of a pocket with bleeding on probing or purulent exudate.

When the root perforation is situated close to the alveolar crest, it may be possible to raise a flap and repair the defect with an appropriate restorative material. In deeper perforations, or in the roof of the furcation, immediate repair of the perforation has a better prognosis than management of an infected one. The use of

mineral trioxide aggregate in such cases may enhance cemental healing following immediate perforation repair.[182]

Root fractures may also present as primary endodontic lesions with secondary periodontal involvement. These typically occur on endodontically treated teeth, often those with posts and crowns. The signs may range from a local deepening of a periodontal pocket to more acute periodontal abscess formation. Root fractures have also become an increasing problem with molar teeth that have been treated by root resection.[183–186]

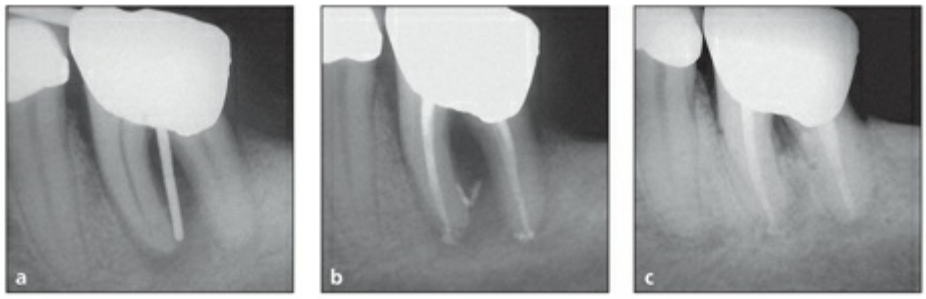

Fig 16-19 Primary endodontic disease with secondary periodontal involvement in a mandibular first molar. *(a)* Preoperative radiograph demonstrating an interradicular defect extending to the apical region of the mesial root. *(b)* Radiograph taken at completion of root canal therapy. *(c)* One-year follow-up radiograph showing resolution of most of the periradicular lesion; a bony defect remains at the furcal area. Endodontic treatment alone has not yielded complete healing of the defect. Periodontal treatment is necessary for further healing of the furcal area and inflamed gingival tissues.

Primary periodontal disease with secondary endodontic involvement

The apical progression of a periodontal pocket may continue until the apical tissues are involved. In this case, the pulp may become necrotic as a result of infection entering via lateral canals or the apical foramen (Fig 16-20). In single-rooted teeth, the prognosis is usually poor. In molar teeth, the prognosis may be better: Because not all the roots may suffer the same loss of supporting tissues, root resection can be considered as a treatment alternative.

The effect of progressive periodontitis on the vitality of the pulp is controversial.[40–41,43] If the blood supply circulating through the apex is intact, the pulp has good prospects for survival. It was reported that pulpal changes resulting from periodontal disease are more likely to occur when the apical foramen is involved.[43] In these cases, bacteria originating from the periodontal pocket are the source of root canal infection. A strong correlation between the presence of microorganisms in root canals and their presence in periodontal pockets of advanced

periodontitis has been demonstrated.[187,188] Support for this concept has come from research in which cultured samples obtained from the pulp tissue and radicular dentin of periodontally involved human teeth showed bacterial growth in 87% of the teeth.[40–41]

The treatment of periodontal disease can also lead to secondary endodontic involvement. Lateral canals and dentinal tubules may be opened to the oral environment by curettage, scaling, or surgical flap procedures. It is possible for a blood vessel within a lateral canal to be severed by a curette and for microorganisms to be pushed into the area during treatment, resulting in pulpal inflammation and necrosis.

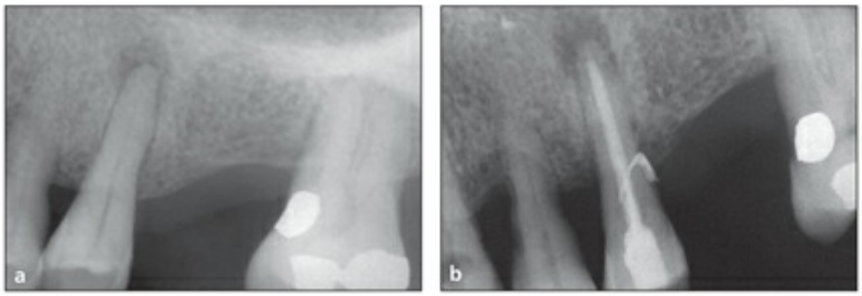

Fig 16-20 Primary periodontal disease with secondary endodontic involvement in a maxillary premolar. *(a)* Radiograph showing bone loss in one-third of the root and a separate periapical radiolu-cency. The crown is intact, but pulpal sensitivity tests are negative. *(b)* Radiograph taken immediately after root canal therapy, showing sealer in a lateral canal that had been exposed because of bone loss.

True combined disease

True combined endodontic-periodontal disease occurs with less frequency. It is formed when an endodontic disease progressing coronally joins with an infected periodontal pocket progressing apically.[17,189] The degree of attachment loss in this type of lesion is invariably large and the prognosis guarded (Fig 16-21). This is particularly true in single-rooted teeth (Fig 16-22). In molar teeth, root resection can be considered as a treatment alternative if not all roots are severely involved. Sometimes, supplementary surgical procedures are necessary (Fig 16-23). In most cases, periradicular healing may be anticipated following successful endodontic treatment. The periodontal tissues, however, may not respond well to treatment, and tooth retention will depend on the severity of the combined disease.

The radiographic appearance of combined endodontic-periodontal disease may be similar to that of a vertically fractured tooth. A fracture that has invaded the pulp space, with resultant necrosis, may also be labeled a true combined lesion and yet not be amenable to successful treatment. If a sinus tract is present, it may be

necessary to raise a flap to determine the etiology of the lesion.

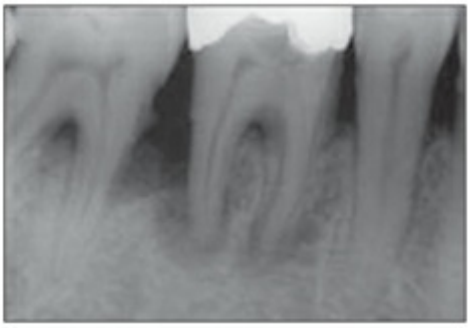

Fig 16-21 True combined endodontic-periodontal disease in a mandibular first molar. Radiograph showing separate progression of endodontic disease and periodontal disease. The tooth had remained untreated, and, consequently, the two lesions joined together.

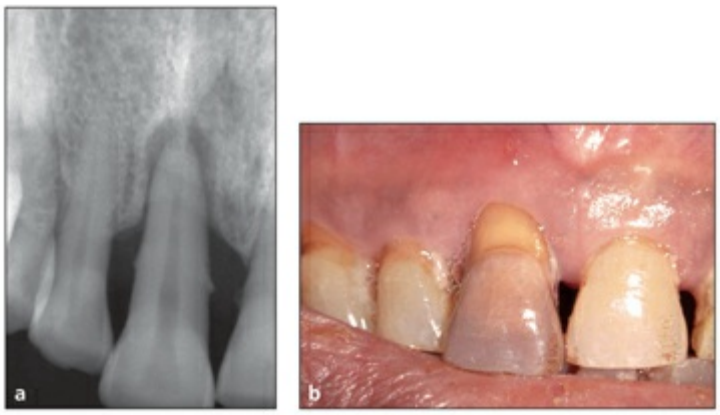

Fig 16-22 True combined endodontic-periodontal disease. *(a)* Radiograph showing bone loss in two-thirds of the root. Calculus and a separate periapical radiolucency are present. *(b)* Clinical examination revealing the coronal color change of the involved tooth and pus exuding from the gingival crevice. The results of pulpal sensitivity tests were negative.

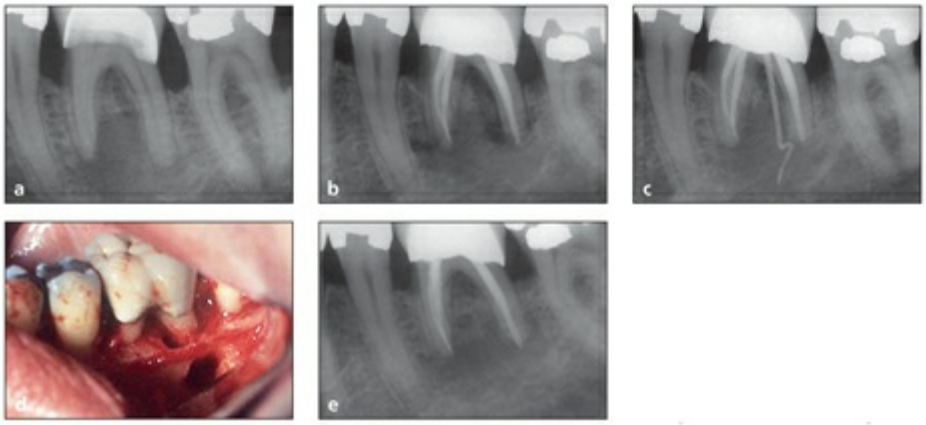

Fig 16-23 True combined endodontic-periodontal disease in a mandibular first molar. *(a)* Preoperative radiograph showing periradicular radiolucencies. The results of pulpal sensitivity tests were negative. *(b)* Immediate postoperative radiograph of the molar after nonsurgical endodontic treatment. *(c)* Six-

month follow-up radiograph, revealing no evidence of healing. A gutta-percha cone is inserted in the buccal gingival sulcus. *(d)* Treatment of the root surfaces and removal of the periradicular lesion. *(e)* One-year follow-up radiograph, demon-**d**strating evidence of active healing.

Prognosis

Treatment prognosis depends primarily on the diagnosis of the specific endodontic and/or periodontal disease. The main factors to consider for treatment planning are pulpal vitality and the type and extent of the periodontal defect. Diagnoses of primary endodontic disease and primary periodontal disease usually present little or no clinical difficulty. In primary endodontic disease, the pulp is infected and nonvital. On the other hand, in a tooth with primary periodontal disease, the pulp is vital and responsive to testing. However, primary endodontic disease with secondary periodontal involvement, primary periodontal disease with secondary endodontic involvement, and true combined disease are clinically and radiographically very similar.

If a lesion is diagnosed and treated as primary endodontic disease because of a lack of evidence of marginal periodontitis, and if there is soft tissue healing on clinical probing and bony healing on a recall radiograph, a valid retrospective diagnosis can then be made. The degree of healing that has taken place following root canal treatment will determine the retrospective classification. In the absence of adequate healing, further periodontal treatment is indicated.

The prognosis and treatment of each endodontic-periodontal disease type varies. Primary endodontic disease should only be treated by endodontic treatment and has a good prognosis. Primary periodontal disease should only be treated by periodontal therapy. In this case, the prognosis depends on the severity of the periodontal disease and the patient's response.

Primary endodontic disease with secondary periodontal involvement should first be treated with endodontic treatment. Treatment results should be evaluated in 2 to 3 months, and only then should periodontal treatment be considered. This sequence of treatment allows sufficient time for initial tissue healing and better assessment of the periodontal condition.[15,190] It also reduces the potential risk of introducing bacteria and their by-products during the initial healing phase. In this regard, it has been suggested that aggressive removal of the periodontal ligament and underlying cementum during interim endodontic therapy can adversely affect periodontal

healing.[191] Areas of the roots that are not aggressively treated show unremarkable healing.[191]

The prognosis of primary endodontic disease with secondary periodontal involvement depends primarily on the severity of periodontal involvement, the periodontal treatment, and the patient's response. Primary periodontal disease with secondary endodontic involvement and true combined endodontic-periodontal disease require both endodontic and periodontal therapies. It has been demonstrated that intrapulpal infection tends to promote marginal epithelial downgrowth along a denuded dentin surface.[53] Additionally, experimentally induced periodontal defects in infected teeth were associated with 20% more epithelium than uninfected teeth.[22] Uninfected teeth showed 10% more connective tissue coverage than infected teeth.[22] The prognosis of primary periodontal disease with secondary endodontic involvement and true combined disease depends primarily on the severity of the periodontal disease and the response of periodontal tissues to treatment.

True combined diseases usually have a more guarded prognosis. In general, assuming the endodontic therapy is adequate, what is of endodontic origin will heal. Thus, the prognosis of combined disease rests with the efficacy of periodontal therapy.

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Root Resorption

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Root resorption is a rare occurrence in permanent teeth. Unlike bone, which undergoes resorption and apposition as part of a continual remodeling process, the roots of permanent teeth are not normally resorbed.[1] Only resorption of primary teeth during exfoliation is considered physiologic.[2,3]

Mechanisms of Resorption

Even under conditions that would normally result in bone resorption, such as alterations in oxygen tension, hormonal fluctuations, locally produced chemical mediators, or electrical currents, the root is resistant to resorption on both its external and internal surfaces.[4] Although several hypotheses have been proposed, the exact mechanism by which the resorptive process is inhibited is unknown. One hypothesis maintains that the remnants of Hertwig's epithelial root sheath surround the root like a net and impart resistance to resorption and subsequent ankylosis.[5,6]

While a protective role for Hertwig's epithelial root sheath has not been established, there is evidence that these cells are involved in cemental repair subsequent to resorption by sequential elaboration of specific matrix proteins, namely osteopontin, bone morphogenetic protein 2, and ameloblastin.[7]

A second hypothesis is based on the premise that the covering of cementum and predentin on dentin is essential for the resistance of the dental root to resorption. There is some support for this hypothesis in that it has long been noted that osteoclasts will not adhere to unmineralized matrix.[8] Recent studies indicated that the polarity of osteoclasts is regulated by their actin cytoskeleton. Non-resorbing osteoclasts form podosomes at the cell periphery when they come into contact with unmineralized substrates. Conversely, on contact with mineralized extracellular matrices, the actin cytoskeleton of an actively resorbing osteoclast is reorganized into a sealing zone. Formation of this sealing zone is mediated by the presence of apatite.[9–11]

Osteoclasts bind to extracellular proteins containing the arginine-glycine–aspartic acid (RGD) sequence of amino acids[12] via specific membrane receptors known as *integrins*. Integrins are a family of cell-surface adhesion glycoproteins containing different α and β subunits (see chapter 11). It is generally accepted that the $\alpha_v\beta_3$ integrin plays a central role in osteoclast polarization[13] and adhesion.[14] Extracellular proteins containing the RGD peptide sequence are bound to calcium salt crystals on mineralized surfaces and serve as osteoclast binding sites. In its normal state, the most external aspect of cementum is covered by a layer of cementoblasts over a zone of unmineralized cementoid and therefore does not present a surface satisfactory for osteoclast binding. Internally, the dentin is covered by predentin matrix, a similarly organic surface (Fig 17-1). Thus, the lack of RGD proteins in both cementum and predentin reduces osteoclast binding and confers resistance to resorption. Numerous studies lend support to this theory.[15–18] It has also been demonstrated in cases of extensive external root resorption that the circumpulpal dentin in close proximity to the predentin is spared, even though most of the peripheral dentin may have been resorbed (Fig 17-2). Conversely, damage to cementum or predentin increases the probability of osteoclast-induced resorption, which can occur in teeth damaged during avulsions or other traumatic injuries. Localized replacement resorption and mild to severe external inflammatory root resorption with pulpal involvement have been demonstrated when the cementum was damaged by inadvertent contact of root surfaces with miniscrew implants employed for anchorage during orthodontic treatment.[19–21] Although this hypothesis has both

experimental support and clinical implications, other hypotheses have also been advanced.

Another possible explanation for the relative resistance of teeth to resorption is that intrinsic factors found in predentin and cementum, such as amelogenin or osteoprotegerin (OPG), act as inhibitors of resorptive cells.[22,23] OPG, a member of the tumor necrosis factor (TNF) superfamily, has the ability to inhibit osteoclast-mediated bone loss. OPG acts as a decoy receptor by binding to the receptor activator of nuclear factor κB ligand (RANKL). Binding of OPG reduces RANKL concentration and thereby inhibits its ability to bind to receptor activator of nuclear factor κB (RANK) receptors on the surface of osteoclast precursors (circulating monocytes) and stimulate osteoclast production (osteoclastogenesis).[24–28]

Cementoblasts have been shown to constitutively express OPG in vitro. Furthermore, co-cultures of cementoblasts with mononuclear precursor cells diminished the effects of RANKL on osteoclastogenesis.[29] Human gingival fibroblasts, human periodontal ligament cells, and human pulp cells also produce OPG.[29–32]

Finally, another hypothesis applicable to certain types of external root resorption involves the barrier formed by the less highly calcified intermediate cementum or the cementodentin junction[33–35] (Fig 17-3). The intermediate cementum, the innermost layer of cementum, creates a barrier between the dentinal tubules and the periodontal ligament.[36–38] Under normal circumstances, this barrier does not allow irritants such as bacterial by-products to pass from an infected pulp space to stimulate an inflammatory response in the adjacent periodontal ligament. However, if the intermediate cementum is lost or damaged, then proinflammatory mediators may diffuse from an infected pulp space into the periodontal ligament, setting up an inflammatory response and subsequent external inflammatory root resorption. In addition, the collagen-rich barrier provided by intermediate cementum could prevent the attachment of osteoclasts to mineralized dentin even when external resorption has occurred in the peripheral, more highly mineralized cementum. When the intermediate cementum is lost or damaged, the osteoclasts may continue to induce resorption in the underlying dentin in the presence of inflammatory stimulators, resulting in the progression of external inflammatory root resorption to the radicular dentin. These different hypotheses are not mutually exclusive, and their relative contributions to various clinical conditions may also vary depending on the circumstances of each case.

It is now well established that a strong interplay among factors involved in

hematopoiesis and immune function are responsible for osteoclastogenesis. The myeloid and B-cell transcription factor PU.1 is the earliest identifiable determinant of the osteoclast lineage[39] and is actively involved in the regulation of β_3 integrin expression during osteo-clast differentiation.[40] Other factors involved include the lipopolysaccharide receptor CD14 and the early monocytic integrins CD11b and CD11c. CD11b is one of the recognized markers of osteoclast precursors in humans.[41]

Once recruited, a variety of mediators such as hormones, integrins, transcription factors, and cytokines affect osteoclast differentiation, maturation, and function[41–43] (see chapter 16). The primary factors involved in pathologic root resorption are fewer, however, and are primarily or secondarily associated with the inflammatory response to infection. It is known that inflammatory mediators are potent stimulators of osteoclast and odontoclast recruitment and function. TNF, interleukins 1 (IL-1), 6, (IL-6), 11 (IL-11), and 17 (IL-17), and prostaglandin E_2 influence stromal cells to produce pro-osteoclastogenic molecules (Fig 17-4). Receptor-ligand interactions perpetuate the process; these include RANK and RANKL, previously mentioned RGD-containing extracellular matrix molecules and $\alpha_V\beta_3$ integrin, and macrophage colony-stimulating factor and cFms. Receptor-ligand binding initiates a cascade of signaling intermediates (Src, TNF receptor–associated factor 6, and P13K), cytoplasmic phosphokinases (protein kinase B [Akt], c-Jun N-terminal kinases, p38, and extracellular signal–regulated kinase), transcription factors (c-Fos, c-Jun, nuclear factor κB, and nuclear factor of activated T cells, cytoplasmic 1), and effector molecules necessary for osteoclastic activity (matrix metalloproteinase 9 [MMP-9], vacuolar H^+-adenosine triphosphatase [V-ATPase], and cathepsin K).

Osteoclastic activity involves removal of both the inorganic and organic components from the mineralized tissue.[44] Dissolution of the mineralized component of bone and cementum by osteoclasts is largely dependent on acidification of the extracellular resorption lacuna by V-ATPases within the ruffled border membranes of the osteoclasts.[45,46] After dissolution of the mineral phase, specific members of two specific classes of proteolytic enzymes, cysteine proteinases (cathepsin K) and matrix metalloproteinases (MMP-1, -2, and -9), secreted by the osteoclasts, contribute differentially to the degradation of the extracellular demineralized type I collagen matrix.[47–50]

Some of the mediators of inflammation-induced clastic function belong to the glycoprotein 130 (gp130) cytokine family that has been shown to play a key role in bone remodeling.[51] This family is a pleomorphic group that shares a common signal

transducer (gp130) and includes cardiotrophin 1, oncostatin M, ciliary neurotropic factor, IL-6, IL-11, and leukemia inhibitory factor. Of these factors, IL-6 and IL-11 appear to have the most profound effect on hard tissue resorption, and IL-6 has been shown to stimulate osteoclastogenesis in the presence of IL-1 but not in the presence of anti–IL-1.52 This effect is dependent on the expression of the IL-6 receptor on osteoblastic cells and occurs in a dose-dependent manner.[53] IL-6 also appears to reverse the inhibitory effects of extracellular $Ca2^{+}$ by reducing the ability of osteoclasts to detect extracellular $Ca2^{+}$ concentrations and is expressed by cells of the stromal/osteoblastic lineage.[54]

IL-11 is an osteoblast-derived mediator that induces prostaglandin synthesis and thereby triggers differentiation of clastic cells. Cells of the osteoclast phenotype have been shown to express the IL-11 receptor gene, which in turn seems to be related sequentially to expression of the calcitonin receptor gene, a recognized osteoclast differentiation marker.[54] Furthermore, gp130 signal induction by IL-11 appears to be necessary for IL-1–induced osteoclast formation.[55–57]

The singular defining feature of the mature osteoclast is functional, namely the ability to resorb calcified tissues. Morphologically, the osteoclast is described as a multinucleated giant cell formed by the fusion of monocytic precursors.[42,58] Odontoclasts differ slightly from their bone-resorbing counterparts in that they are usually smaller, have fewer nuclei, and have very small (if any) sealing zones.[59] Nonetheless, these cells are thought to represent a variant of osteoclasts with morphologic differences that reflect the nature of the resorption substrate as well as the resorption kinetics of the cells.[42]

Dendritic cells, the mononuclear cells that initiate the adaptive immune response, have previously been regarded as having limited phagocytic activity, although they share a common hematopoietic lineage with the multinucleated bone-resorbing osteoclasts. Recent studies, however, have indicated that immature dendritic cells have the potential to transdifferentiate into osteoclasts, in a process regulated by innate and adaptive cytokines.[60,61] Both human and murine dendritic cells have been shown to be novel osteoclast precursors that are able to generate osteoclasts more efficiently than monocytes.[62] Because dendritic cells are present in the dental pulp (see chapter 4), it is not known if they may function also as precursors of clastic cells that are responsible for internal resorption of the canal space. On a cellular level, osteoclasts are usually large and have multiple nuclei. They possess a well-defined Golgi apparatus, numerous lysosomal vesicles, and high polarity.[63] Characteristic morphologic features of the osteoclast are the ruffled border, formed

by membrane and cytoplasmic undulations, and the sealing zone, consisting of the ventral surface of the osteoclast membrane in contact with the targeted bone or root surface. The mature osteoclast is further defined by a series of molecular markers, each representing genes integral to clastic function.[64] These include transcripts coding for tartrate-resistant acid phosphatases, calcitonin receptors, vitronectin receptors, carbonic anhydrase II, cathepsin K, and V-ATPase.[65]

Within the last few years, the dynamic interactions between osteoclasts and the innate immune system have increasingly been recognized, resulting in a rapidly developing field of research that is known as *osteoimmunology*.[66–71] There is increasing evidence that osteoclast precursors and osteoclasts modulate the differentiation of osteoblastic cells, regulate hematopoietic stem cell movement from the bone marrow to the bloodstream, and function as secretory cells that participate in immune responses. Osteoclasts also function as highly regulated innate immune cells of the bone that respond to stress and inflammatory changes in their microenvironment. Their interactions with the immune system are mediated not only by the release of cytokines and chemokines but also by direct cell-cell contact.

An understanding of the complex interactions between osteoclasts and the immune system will provide a scientific basis for future therapeutic approaches to diseases related to both the bone and immune systems. In the context of root resorption, elucidating the interaction of osteoclasts with the immune system may, for example, improve the understanding of the pathogenesis and may provide solutions to the treatment of patients with idiopathic multiple subepithelial inflammatory root resorption lesions. These were purported to be caused by transmission of feline viral infections from cats to humans.[72,73]

A model for the process of root resorption can be depicted based on what is known about odonto-clasts combined with data extrapolated from studies of osteoclasts (see Fig 17-4). As previously mentioned, initiation of root resorption has two requirements: *(1)* removal of the protective layer (predentin internally and precementum externally) of the root and *(2)* the presence of a noxious stimulus that results in an inflammatory response adjacent to the damaged external root surface. Given these conditions, the first step in root resorption is binding of the odontoclast to its substrate.

Various RGD peptide–containing proteins (eg, osteopontin, bone sialoprotein, fibronectin, and vitronectin) have been shown to be involved in osteo-clast binding. Most notably, osteopontin has been shown to play an important role in regulating osteo-clast recruitment and activation by binding to the osteoclast integrin receptor

$\alpha_V\beta_3$.[13,14] Osteopontin deficiency resulted in osteoclast suppression and reduction in tooth resorption in a murine model.[74] Osteopontin serves as a linker molecule so that one domain is bound to apatite crystallites in exposed dentin and another domain is bound to the integrin protein ($\alpha_V\beta_3$) extending from the osteoclast's plasma membrane.

The binding of osteopontin to $\alpha_V\beta_3$ integrin facilitates clastic cell adhesion and subsequent establishment of the clear zone or extracellular resorbing compartment. Osteopontin-$\alpha_V\beta_3$ interaction with odontoclasts stimulates a second messenger pathway (ie, gelsolin association with phosphatidylinositol-3-hydroxyl kinase via a pp60src-and RhoA-dependent pathway) that mediates cytoskeletal rearrangement, ruffled border formation, and substrate adhesion.[75] Once the ruffled border has been formed, the odontoclast secretes an acidic solution containing proteolytic enzymes, specifically carbonic anhydrase and V-ATPase, into the extracellular resorbing compartment to effect decalcification.[63]

During root resorption, organic matrix proteins and cytokines from the surrounding bone and dentin are released locally into the gingival crevicular fluid. The concentrations of dentin phosphophoryn and dentin sialoprotein were found to be higher in the gingival crevicular fluid in human subjects with severe root resorption. These two dentin-specific organic matrix proteins have been suggested as biologic markers for monitoring root resorption during orthodontic treatment.[76] Osteopontin, OPG, and RANKL could also be detected in the gingival crevicular fluid derived from teeth with severe root resorption. An increased RANKL-OPG ratio was found to be correlated with an increase in bone resorption activity during orthodontic tooth movement.[77]

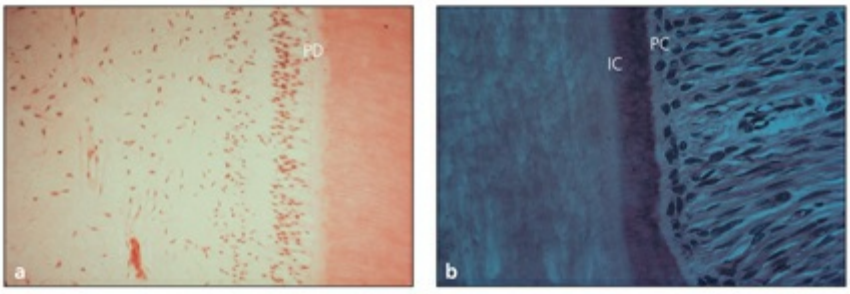

Fig 17-1 *(a and b)* Histologic appearance of pulp, periodontal ligament, and adjacent dentin. The precementum (PC) and predentin (PD) have antiresorptive properties. If the intermediate cementum (IC) is penetrated, any pulpal toxins that may be present will cause periodontal inflammation with bone and root resorption (hematoxylin-eosin [H&E] stain; original magnification ×25).

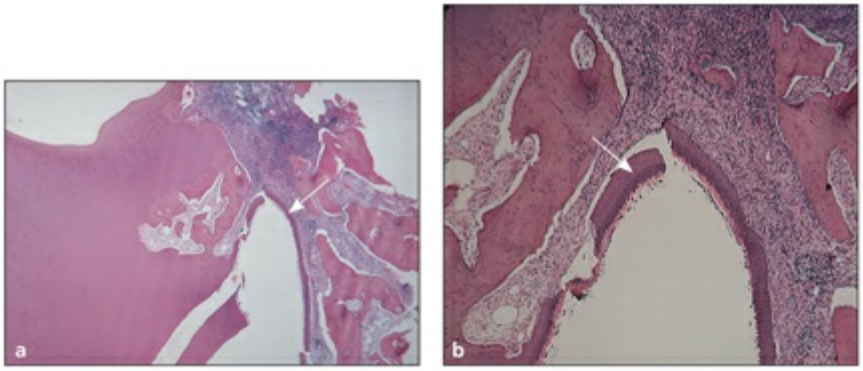

Fig 17-2 *(a and b)* Histologic appearance of a molar with extensive external root resorption. Although the dentinal resorption is extensive, the odontoblast-predentin layer *(arrows)* is intact (H&E stain; original magnification ×50 and ×100, respectively).

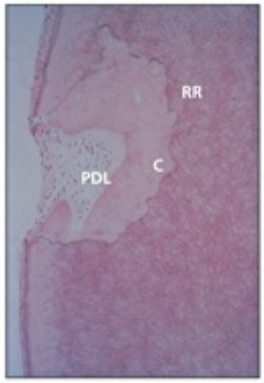

Fig 17-3 Histologic appearance of a localized area of root resorption (RR) that has healed with new cementum (C) and periodontal ligament (PDL). The initial damage was caused by a mild localized luxation injury (H&E stain; original magnification ×100).

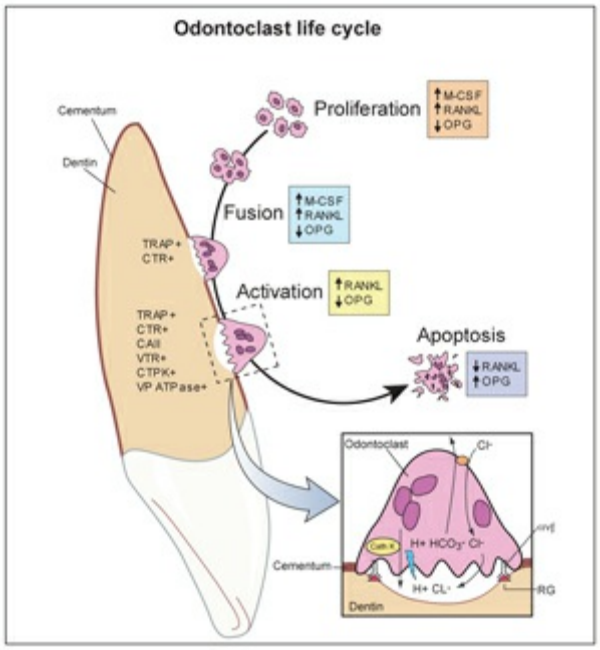

Fig 17-4 Odontoclast life cycle. Shaded boxes list molecules involved in osteoclast maturation, function, and demise. Tartrate-resistant acid phosphatase (TRAP), calcitonin receptors (CTRs), vitronectin receptors (VTRs), carbonic anhydrase II (CAII), cathepsin K (CTPK), and vacuolar H^+-adenosine triphosphatase (V-ATPase) are molecular markers for the mature clastic phenotype. M-CSF, macrophage colony-stimulating factor.

Role of Dental Pulp in Resorption

Because the focus of this book is the dental pulp and its interaction with other tissues, those types of resorption in which the pulp plays a major role are discussed in detail. However, it is important to discuss briefly additional common dental root resorptions because this knowledge allows the practitioner to accurately differentiate these resorption phenomena and support correct diagnosis before treatment is rendered.

The pulp plays an essential role in two types of resorption. The first type of resorption is external inflammatory root resorption. In external inflammatory root resorption, the necrotic infected pulp provides the stimulus for periodontal inflammation. If the cementum has been damaged and the intermediate cementum is penetrated, as when the tooth undergoes a traumatic injury, the inflammatory stimuli in the pulp space are able to diffuse through the dentinal tubules and induce an inflammatory response over large areas of the periodontal ligament. Without cemental protection, this will result in resorption of both tooth and bone.

The second type of resorption in which pulp tissue plays an important role is internal inflammatory root resorption. In internal inflammatory root resorption, the inflamed pulp is the tissue involved in resorption of the root structure. The pathogenesis of internal root resorption is not completely understood. One theory maintains that coronal necrotic infected pulp provides a stimulus for inflammation in the more apical parts of the pulp. An alternative theory is based on the knowledge that apoptotic osteocytes induce the secretion of osteoclastogenic cytokines that enhance bone resorption.[78,79] It is known that odontoblasts undergo apoptosis both in tooth development as well as in response to certain types of trauma.[80–82] In view of the parallels between dentin and bone physiology, it is probable that odontoblasts or pulp fibroblasts undergoing apoptosis in response to trauma induce cytokines that initiate and perpetuate the internal resorptive response. In rare cases in which the inflamed pulp is adjacent to a root surface that has lost its precemental protection, internal root resorption will result. Thus, both the necrotic infected pulp and the

inflamed pulp can contribute to this type of root resorption.

External Root Resorption

External root resorption caused by an injury restricted to the external root surface

Injury to the root surface can cause a loss of the protective cemental layer, providing a stimulus for inflammation. This type of resorption is self-limiting. The inflammatory mediators evoked by the mechanical damage stimulate the cellular inflammatory response. The character of the healing response is dependent on the extent of the initial damage and can therefore take several forms.

Localized injury: Healing with cementum

When the injury is localized (eg, after concussion or subluxation injury), mechanical damage to the cementum occurs, resulting in a local inflammatory response and a localized area of root resorption. If no further inflammatory stimulus is present, periodontal healing and root surface repair will occur within 14 days[1] (see Fig 17-3). The resorption is localized to the area of mechanical damage, and treatment is not required because the resorption is free of symptoms and in most cases undetectable with routine radiography. However, in a minority of cases, small radiolucencies can be seen on the root surface if the radiograph is taken at a specific angle (Fig 17-5).

It is important not to misinterpret these cases as progressive. It is equally important to understand that the pulp is not involved in these cases. When no potential inflammatory stimulus can be identified (ie, when a positive sensitivity test indicates a vital pulp), no endodontic treatment should be performed. Treatment is generally limited to clinical monitoring to verify that spontaneous healing is taking place.

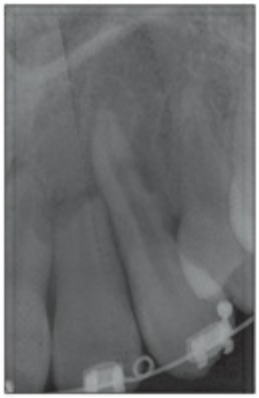

Fig 17-5 Active transient external inflammatory root resorption. Note the lucent areas on the root surface. Because the pulp is vital, a wait-and-watch strategy is followed.

Diffuse injury: Healing by osseous replacement

When the traumatic injury is severe (eg, intrusive luxation or avulsion with extended dry time) and involves diffuse damage on more than 20% of the root surface, an abnormal attachment may occur after healing takes place.[83] The initial reaction is an inflammatory response to the severe mechanical damage to the root surface. After the initial inflammatory response, a diffuse area of root surface will be devoid of cementum. Cells in the vicinity of the denuded root compete to repopulate it, and often cells that are precursors of bone, rather than periodontal ligament cells, will move across from the socket wall and populate the damaged root (Fig 17-6). The result is that bone comes in direct contact with the root, without the intermediate cementum to serve as an attachment apparatus. This phenomenon is termed *dentoalveolar ankylo-sis*[84] (Fig 17-7).

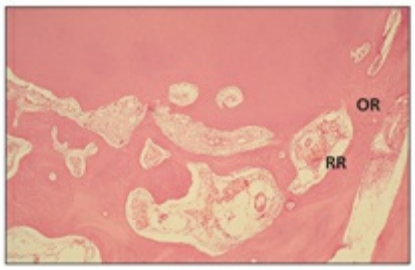

Fig 17-6 Histologic appearance of active osseous replacement (OR). Without an intermediate periodontal ligament, bone attaches directly to the root. Areas of active root resorption (RR) are seen in the bone and root (H&E stain; original magnification ×100).

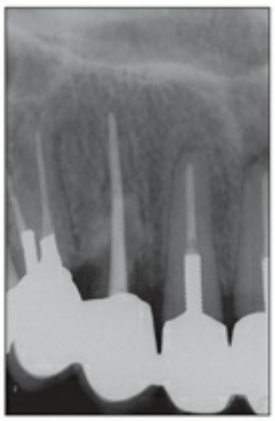

Fig 17-7 Radiographic appearance of osseous replacement. The bone that is replacing root has a mottled appearance. Radiolucencies are not apparentin the adjacent bone.

Bone resorbs and forms physiologically throughout life. Once in direct contact with bone, dentin becomes part of the bone-remodeling process. Osteoclasts resorb root structure, and the resorptive lacunae are repopulated by osteoblasts, not odontoblasts or cementoblasts.[85] The osteoblasts then synthesize and deposit bone matrix directly on the denuded dentin, and mineralization ensues. The resultant hard tissue is bone, not dentin or cementum. This process can continue until the entire root is replaced by bone.

Treatment strategies for osseous replacement resorption are directed at avoiding or minimizing the initial inflammatory response. Five specific strategies should be considered: *(1)* Prevention of the initial injury should be emphasized by counseling or advocating use of mouthguards for athletic endeavors; *(2)* treatment should minimize additional damage after the initial injury; *(3)* pharmacologic interventions that inhibit the initial inflammatory response should be considered; *(4)* the possibility of stimulating cemental (rather than osseous) healing should be considered; and *(5)* interventions that reduce the rate of osseous replacement, when inevitable, should be considered. Space limitations preclude a more thorough review of these current areas of therapy and research; the interested reader is encouraged to seek reviews on this aspect of care.[86,87]

External root resorption caused by an injury to the external root surface with an inflammatory component

There are four general types of inflammatory stimulus that cause external root resorption: *(1)* pressure-induced damage to the root surface, *(2)* microbial infection of the root canal system or periodontal sulcus, *(3)* sulcular infection, and *(4)* chemical damage secondary to bleaching.[88–90]

Pressure

Pressure both damages the cementum and provides a continuous stimulus for the resorbing cells. The most common example of this type of pressure resorption is root resorption caused by excessive forces of orthodontic tooth movement. Other examples are resorption caused by impacted teeth and tumors.

Pressure resorption has been assumed to be external, but this is not necessarily true. In orthodontics, for example, the process takes place at the apex of the tooth near the cementodentin junction. Because the force affects the apical root, either the cementum or predentin may be damaged. Because the predentin may be affected also, it is not unusual to see radiographic evidence of internal apical resorption in the active stage of the process (Fig 17-8).

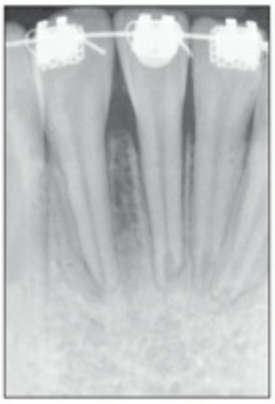

Fig 17-8 Root resorption during active orthodontic tooth movement. Man-dibular central incisors show apical external and internal resorption.

In 1997, Bender and colleagues[91] labeled this type of resorption *periapical replacement resorption* and offered a hypothetical explanation for it, suggesting that pulp neuropeptides may be involved in periapical replacement resorption in both vital and endodontically treated incisors. Their review indicated that apical resorption is significantly less frequent and less severe in endodontically treated incisors than in untreated teeth. These findings are consistent with other studies that found endodontically treated teeth were less likely to undergo resorption during orthodontic therapy.[92,93] The use of a calcium hydroxide interappointment dressing has recently been shown in a canine model to produce a favorable outcome on the

repair of orthodontic root resorption in endodontically treated teeth.[94]

Orthodontically induced inflammatory root resorption (OIRR) has been attributed to both treatment-and patient-related factors.[92,95–98] Treatment-related factors include the duration of treatment, the magnitude of force exerted, the direction of tooth movement, and the method of movement.[99] Prolonged orthodontic treatment may result in an increased incidence and severity of apical root resorption.[100] The use of intermittent forces was found to produce less OIRR than the use of continuous forces.[101] Root resorption continued for 4 weeks after the cessation of orthodontic treatment, although the use of light forces produced less root resorption and better repair than the use of heavy forces.[102,103]

The distance of movement also has an impact; movement over greater distances increases the probability of OIRR.[104] Intrusive movements and lingual root torque tend to be associated with higher levels of root resorption because they concentrate forces at the apex.[105,106] Bodily tooth movement, extrusion, and lingual tipping movement, by contrast, result in a lower incidence, presumably because the stress is evenly distributed over a larger area of the root during these movements.[96]

In addition to factors relating to the mechanics of tooth movement, there are specific patient-related risk factors for OIRR. Patients who have short roots prior to orthodontic treatment or who show signs of root resorption before treatment are at higher risk for OIRR.[97] In addition, there is mounting evidence for a genetic predisposition to OIRR.[107–110] A link has been made between a polymorphism in the IL-1 gene (*IL1B* allele 1) and the occurrence of OIRR. Patients who are homozygous for *IL1B* allele 1 have a 5.6-fold greater risk for OIRR.[111] The *TNFRS-F11A* gene that encodes for a TNF receptor that is involved in osteoclastogenesis represents another candidate gene for OIRR.[112]

If OIRR is detected during active orthodontic treatment, it is recommended that treatment be halted for 2 to 3 months with passive archwires and bite. Severe OIRR may require cessation of orthodontic treatment indefinitely.[113]

Pulp space infection

Pulp space infection represents the second general type of inflammatory stimuli that can cause external root resorption. Pulp space infection can lead to external root resorption in either the apical or lateral regions of the root. The classic example of pulp space infection causing apical external root resorption is apical periodontitis with apical root resorption.

The etiology for this form of apical external root resorption is infection of the root canal system. The pulp of the tooth may become necrotic for many reasons, but the predominant cause is a bacterial challenge through caries (see chapters 10 and 14). When the pulp defenses are overcome, the pulp becomes necrotic and infected, and subsequently there is diffusion of microbes and their by-products into the surrounding periodontium. In most routine cases, the root surface is protected by its intact cementum, and communication will be primarily through apical foramina or occasionally through accessory canals.

Invariably, the periodontal inflammation is accompanied by slight resorption of the root at the cementodentin junction. This resorption is usually not apparent radiographically but is routinely visible upon histologic evaluation (Fig 17-9). It is not obvious why the apex of the root is not as well protected from the resorbing factors produced during the inflammatory response as are the other areas of the root. A simple explanation might be that inflammation is confined to a small area at the apex of the root so that the concentration of resorbing factors is so high that the resistance of the root to resorption is overcome. Another speculation is that the cementodentin junction at the apical foramen provides an extremely thin protective layer compared with the other areas of the root. It is also possible that the cementum and dentin could fail to meet in a certain percentage of the cases, as with the cementoenamel junction. Thus, the mineralized tissue would be exposed, permitting attachment of the clastic resorbing cells via the $\alpha_V\beta_3$ integrins to RGD-containing peptides such as osteopontin.

There are no clinical manifestations because apical root resorption is asymptomatic. Symptoms that might lead to its diagnosis are associated with periapical inflammation and not root resorption per se. The radiographic appearance, as mentioned earlier, is not detectable in most cases; however, when apparent, the radiolucencies are evident at the root tip and adjacent bone. Irregular shortening, stumping, or thinning of the root tip is sometimes observed (Fig 17-10).

The histologic appearance of a periapical lesion may be either granulomatous or cystic.[114,115] On the root surface, resorption of the cementum and dentin results in a scalloped appearance of the root end (see Fig 17-9). Attempts at repair are often evidenced by the presence of the resorption lacunae, resulting in resorptive and mineralization processes observed adjacent to each other.[4]

The treatment protocol for external root resorption with apical periodontitis should be directed at removing the stimulus for the underlying inflammatory process, the microbes in the root canal system.[116,117] If a thorough disinfection protocol is

followed, an extremely high success rate can be expected.[118,119] At present, the treatment protocol of choice is complete debridement of the root canal systems with sodium hypochlorite irrigation at the first visit. A creamy mix of calcium hydroxide is then introduced with a lentulo instrument. After the intracanal medicament has been in place for at least 7 days, the root canal system is sealed. Recent research indicates that the canal may be obturated after the first visit if larger than currently accepted instrumentation is used.[120,121] When extensive resorption is present apically, the clinician should account for canal-altering pathologic processes when establishing a working length for canal debridement.

Attempts should be made to instrument the full length of the remaining canal while a dentin shelf is created to serve as a stop for the gutta-percha obturation. Apical closure techniques with long-term calcium hydroxide treatment or short-term barriers (eg, mineral trioxide aggregate and collagen plugs) may also be used to ensure a better prognosis for nonsurgical endodontic therapy[122,123] (see Fig 17-10). If nonsurgical therapy has been unsuccessful in arresting the resorption, apical surgery should be attempted, provided that a sufficient crown-root ratio will be present after the surgery.

The second type of external root resorption caused by pulp space infection is lateral external inflammatory resorption. Lateral periodontitis with external root resorption may result when the root loses its cemental protection (Fig 17-11). For pulp space infection to develop, the pulp must first become necrotic. Necrosis occurs after a serious injury in which displacement of the tooth results in severing or compression of the apical blood vessels. In mature teeth, the dental pulp has little regeneration potential, and the necrotic pulp will usually become infected within 3 weeks. The microbial profile of a traumatized necrotic pulp should resemble that of a primary endodontic infection with upward of 107 colony-forming units and more than 80 strains[124] (see chapter 10).

Because a serious injury is required for pulpal necrosis, the protective cemental covering of the root is usually damaged or lost as well. As described in chapter 3, studies have shown that the loss of the cemental layer leads to a large increase in permeability. Bacterial toxins are free to pass through the dentinal tubules and stimulate an inflammatory response in the corresponding periodontal ligament.[125] The result is resorption of the root and bone.

The periodontal infiltrate consists of granulation tissue with lymphocytes, plasma cells, and polymor-phonuclear leukocytes. Multinucleated giant cells may bind to exposed RGD peptides and resorb the denuded root surface. This process continues

until the stimulus (microbes within the pulp space) is removed (Fig 17-12). Radiographically, the resorption is observed as progressive radiolucent areas of the root and adjacent bone (see Fig 17-11).

The treatment protocol for external root resorption caused by pulp space infection is to first recognize the attachment damage resulting from the traumatic injury and minimize the subsequent inflammation. The clinician should ideally evaluate pulp space infection 7 to 10 days after the injury.[86,87] Disinfection of the root canal systems removes the stimulus to the periradicular inflammation, and the resorption will stop. In most cases, a new attachment will form; however, if a large area of root is affected, osseous replacement may result by the mechanism already described.

The two major treatment principles are prevention of pulp space infection and elimination of any bacteria present in the pulp space. An effective way to prevent pulp space infection is, of course, to maintain the vitality of the pulp. If the pulp remains vital, the canal will be free of bacteria, and thus this type of external inflammatory root resorption will not occur.

After severe injuries where vitality has been lost, it is possible under some circumstances to promote revascularization of the pulp. Such revascularization is possible in young teeth with incompletely formed apices if the teeth are replaced in their original position within 60 minutes of the injury.[126] If the tooth has been avulsed, soaking it in doxycycline for 5 minutes before reimplantation has been shown to double the revascularization rate. However, even under the best conditions, revascularization will occur only about 50% of the time, which poses a diagnostic dilemma. If the pulp revascularizes, external root resorption will not occur and the root will continue to develop and strengthen. However, if the pulp becomes necrotic and infected, the subsequent external inflammatory root resorption that develops could result in the loss of the tooth in a very short time.

At present, the diagnostic tools available cannot detect a vital pulp in this situation until approximately 6 months following successful revascularization. This period of time is unacceptable because by that time the teeth that have not revascularized would be lost to the resorptive process. Recently, however, the laser Doppler flowmeter has been shown to be an excellent diagnostic tool for the detection of revascularization in immature teeth. This device appears to accurately detect the presence of vital tissue in the pulp space by 4 weeks after the traumatic injury.[127]

The second major treatment principle is the elimination of any microbes present in the pulp space. Revascularization cannot occur in teeth with closed apices. These

teeth must be endodontically treated before the ischemically necrotic pulp becomes infected, that is, within 7 to 10 days of the injury.[128] From a theoretic point of view, timely treatment of these teeth may be considered equivalent to the treatment of teeth with vital pulp. Therefore, endodontic treatment may be completed in one visit. However, efficient treatment is extremely difficult so soon after a serious traumatic injury; therefore, it is beneficial to start the endodontic treatment with chemomechanical preparation, after which an intracanal dressing of creamy calcium hydroxide is placed.[129] The practitioner can then obturate the canal when periodontal healing of the injury is complete, approximately 1 month after the instrumentation visit. There appears to be no need for long-term calcium hydroxide treatment in cases where endodontic treatment is started within 10 days of the injury. However, calcium hydroxide can be applied in a compliant patient for up to 6 months to ensure periodontal health prior to root canal filling with gutta-percha.

When root canal treatment is initiated later than 10 days after the accident or when active external inflammatory resorption is observed, the preferred antibacterial protocol consists of chemomechanical preparation followed by long-term dressing with densely packed calcium hydroxide. The latter creates an alkaline pH in the surrounding dentinal tubules (Fig 17-13), kills bacteria, and neutralizes endotoxin, a potent inflammatory stimulator.[130]

The patient's first visit consists of thorough chemomechanical instrumentation of the root canal systems and placement of a creamy mix of calcium hydroxide and an intracanal antibacterial agent with a lentulo instrument. The patient is then seen in approximately 1 month, at which time the canal systems are filled with a dense mix of calcium hydroxide. Once filled, the canals appear radiographically to be calcified because the radiodensity of calcium hydroxide in the canal is usually similar to that of the surrounding dentin.

Additional radiographs are taken at 3-month intervals. At each visit, the tooth is tested for symptoms of periodontitis. In addition to tracking the resorptive process, the clinician should assess the presence or absence of the calcium hydroxide (ie, calcium hydroxide washout). Because the root surface is so radiodense as to make the assessment of healing difficult, healing of the adjacent bone is also assessed. If the adjacent bone has healed, the resorptive process can be presumed to have stopped in the root as well, and the canal systems may be obturated with gutta-percha (Fig 17-14).

If the practitioner believes that additional healing before obturation would be beneficial, the condition of the calcium hydroxide in the canal should be assessed. If the canal still appears radiographically to be calcified, there is no need to replace

the calcium hydroxide. If, on the other hand, the canal has regained its radiolucent appearance, then the calcium hydroxide should be repacked and reassessed in another 3 months.[86,87]

Fig 17-9 Histologic appearance of apical resorption caused by an infected root canal. Chronic inflammation is present apically. Resorption of the external and internal aspects of the root can be seen (H&E stain; original magnification ×100).

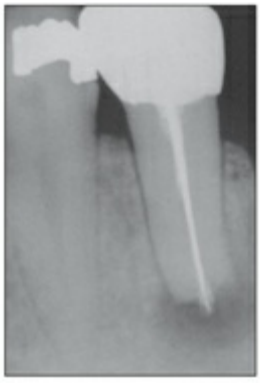

Fig 17-10 Tooth with external apical resorption caused by apical periodonti-tis. The pulp is necrotic and infected.

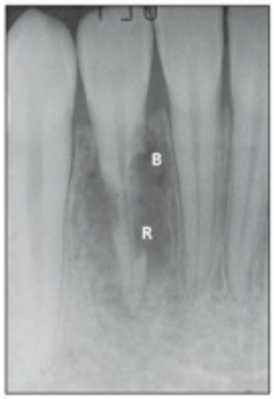

Fig 17-11 Radiographic appearance of external inflammatory resorption caused by pulpal infection. Note the radiolucencies in the root (R) and adjacent bone (B).

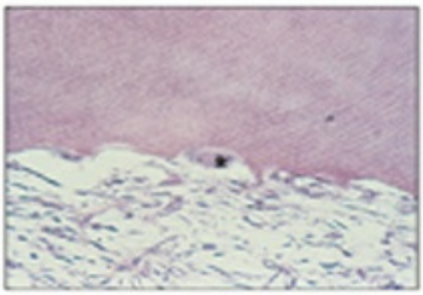

Fig 17-12 Histologic appearance of inflammatory resorption revealing chronically inflamed tissue in relation to the resorbed root surface. Multinucleated giant cells *(asterisk)* are present in the areas of active resorption on the root surface (H&E stain; original magnification ×100).

Fig 17-13 Cross section of a root filled with calcium hydroxide. The pH indicator shows that the canal and surrounding dentin are basic, while the surrounding tissue is slightly acidic.

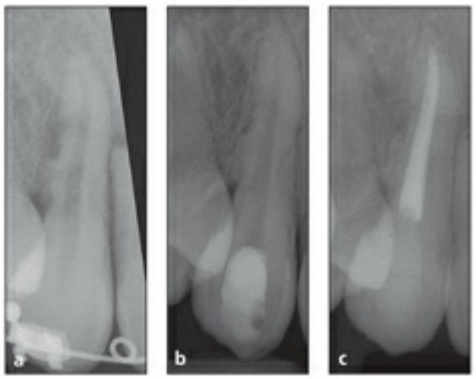

Fig 17-14 Tooth treated for external inflammatory resorption with long-term calcium hydroxide medication. *(a)* At the start of treatment, radiolucencies are present in the root and adjacent bone, indicating active resorption. *(b)* At the 9-month follow-up, the radiolucencies have disappeared on the adjacent bone, and a lamina dura has re-formed, indicating that the process has stopped. *(c)* The canal is obturated with gutta-percha and sealer.

Sulcular infection

Sulcular infection represents another inflammatory stimulus that can cause external root resorption. This progressive external root resorption of inflammatory origin occurs immediately below the epithelial attachment of the tooth, usually (but not exclusively) at the cervical area of the tooth. Because of this location, it has been

referred to as *subepithelial inflammatory root resorption*. Although this process is often called *cervical root resorption*, the perio-dontal attachment of teeth is not always at the cervical margin; the same process can occur more apically on the root surface.

In fact, the anatomical connotation of "cervical" root resorption has led to confusion and misdiagnosis of this condition, inspiring attempts to rename this type of external resorption.[89,131–133] The pathogenesis of subepithelial inflammatory root resorption is not fully understood.[89] However, because its histologic appearance and progressive nature are identical to other forms of progressive inflammatory root resorption, it seems logical that the pathogenesis would be the same (ie, an unprotected or altered root surface that attracts resorbing cells and an inflammatory response maintained by infection). Other theories propose that the process could be a type of benign proliferative fibrovascular or fibro-osseous disorder in which bacteria are only involved secondarily.

Although an exact etiology has not been identified, several predisposing factors have been associated with subepithelial inflammatory root resorption. These include trauma, orthodontics, intracoronal bleaching, dentoalveolar surgery, and periodontal disease or treatment. The dental pulp does not appear to play a direct role and is usually normal in these cases, unless exposure results from the resorptive process.[132] Because the source of stimulation (infection) is not the pulp, it has been postulated that bacteria in the sulcus of the tooth stimulate and sustain an inflammatory response in the periodontium at the attachment level of the root.[134]

Subepithelial inflammatory root resorption is asymptomatic and is usually detected only through routine radiographs. Occasionally, symptoms of pulpitis will develop if the resorption is extensive. When the resorption is long-standing, granulation tissue may undermine the enamel of the crown of the tooth, resulting in a pinkish appearance. This "pink spot" has traditionally been used to describe the pathognomonic clinical picture of internal root resorption, leading to the misdiagnosis and treatment of many cases of subepithelial inflammatory root resorption as internal root resorption (Fig 17-15).

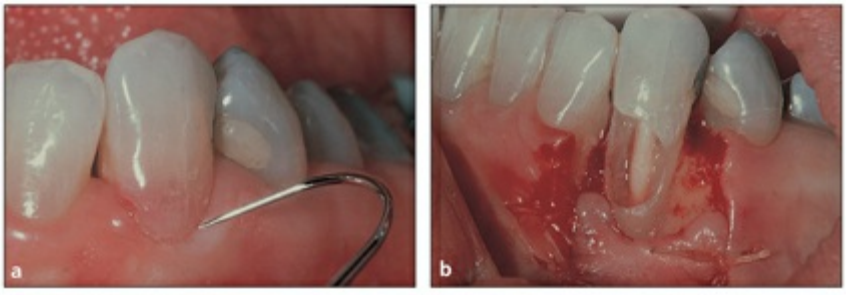

Fig 17-15 *(a)* Pink spot of external inflammatory resorption. The granulomatous tissue has spread coronally and undermined the enamel, causing the pink color in the crown. *(b)* Careful removal of the granulomatous tissue shows the canal to be almost entirely encircled but not penetrated by the resorptive defect. (Courtesy of Dr Henry Rankow, Harrisburg, PA.)

The radiographic appearance of subepithelial inflammatory root resorption can be quite variable. If the resorptive process occurs mesially or distally on the root surface, small radiolucent openings into the root are common. The radiolucency expands coronally and apically in the dentin and reaches, but usually does not perforate, the root canal (Fig 17-16). If the resorptive process is buccal or palatolingual, the radiographic picture is dependent on the extent to which the resorptive process has spread in the dentin. It is seen as a radiolucency at the attachment level or as mottled if it has spread coronally or apically to any considerable degree. Because the pulp is not involved, its outline can usually be distinguished through the resorptive defect (Fig 17-17). Cone beam computed tomographic (CBCT) technology has been utilized to allow better judgment of the location and extent of invasive cervical root resorption.[135–137]

A useful classification scheme has been proposed for the evaluation of and treatment planning for subepithelial inflammatory root resorption lesions[89] (Fig 17-18). It is based on the extent of invasion and has been correlated with outcomes in one clinical study.[88]

Treatment modalities rely on the removal of the resorptive tissue and restoration of the defect with a suitable material. The outcome of treatment appears to be directly related to the extent of the resorptive process at the time of intervention. Therefore, early intervention improves the prognosis. Space limitations preclude a complete review of the treatment strategies for subepithelial inflammatory root resorption. Recent reviews discuss strategies for treating this form of external root resorption.[133,139]

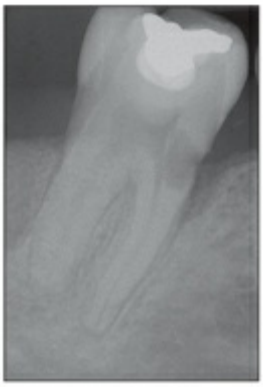

Fig 17-16 Radiographic appearance of external subepithelial root resorption. The resorptive defect on

the mesial side of the molar shows a small opening into the root. The apical and coronal expansion reaches but does not penetrate the pulp canal. Note the adjacent bone resorption.

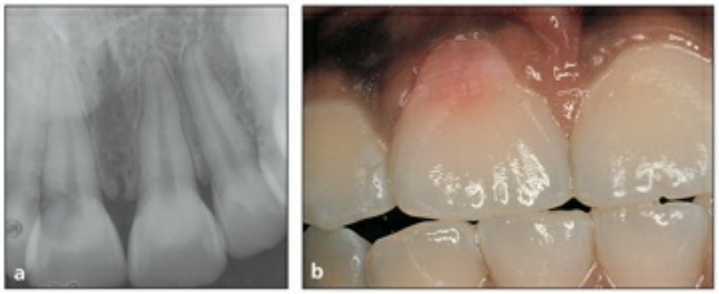

Fig 17-17 *(a)* Radiographic appearance of a maxillary incisor with external root resorption extending coronally. Note the outline of the root canal through the resorptive radiolucency. *(b)* The clinical appearance shows a pink spot close to the gingival margin on the labial surface of the tooth.

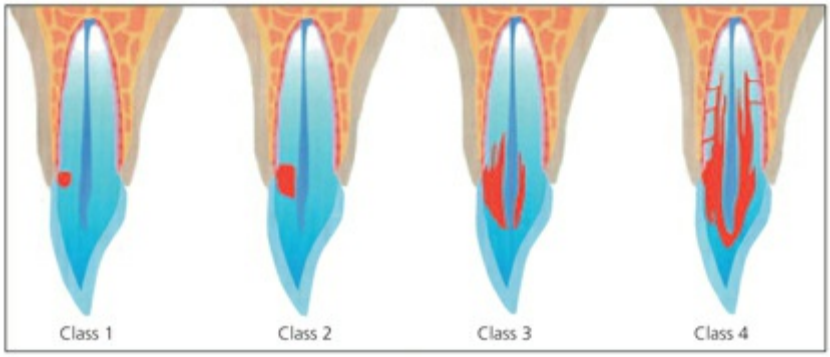

Fig 17-18 Clinical classification of invasive cervical resorption. Class 1: small, invasive resorptive lesion near the cervical area with shallow penetration into dentin. Class 2: well-defined, invasive resorptive lesion that has penetrated close to the coronal pulp chamber but with little or no extension into the root dentin. Class 3: deeper invasion of the root dentin by resorbing tissues that extend into the coronal third of the root. Class 4: large, invasive resorptive process that has extended beyond the coronal third of the root. (Reprinted from Heithersay[138] with permission.)

Bleaching

Intracoronal bleaching of discolored endodontically treated teeth (ie, the walking bleaching technique) has been cited as a cause of external resorption.[140–143] Case reports published between 1979 and 1991 indicated that cervical resorption of endodontically treated teeth was mostly associated with trauma and the use of hydrogen peroxide as the sole intracoronal bleaching agent or with the use of sodium perborate as an adjunctive bleaching agent. Root resorption was considerably more severe when the oxidizing effect of hydrogen peroxide was catalyzed by the use of a heat source.

Four major follow-up studies between 1988 and 1998 examined the occurrence of

cervical root resorption in endodontically treated teeth that had undergone intracoronal bleaching from 1 to 15 years. Of the 58 teeth that were bleached using 30% hydrogen peroxide and heat, approximately 7% (ie, four patients) exhibited evidence of cervical root resorption after 1 to 8 years.[144] Another follow-up study that involved the recall of 95 teeth that had histories of traumatic injury over a period of 3 years did not reveal any evidence of cervical root resorption with the use of sodium perborate in water.[145] Similarly, two other follow-up studies, in which the walking bleaching technique had been used to bleach nontraumatically involved, tetracyclinestained, endodontically treated teeth with sodium perborate and oxygen-water[146] or sodium perborate with 30% hydrogen peroxide[147] did not reveal any sign of cervical resorption. Approximately 4% of invasive cervical resorption (subepithelial inflammatory root resorption, discussed previously) was attributed to intracoronal bleaching.[88]

To date, the exact mechanism for the sporadic occurrence of cervical root resorption after intracoronal bleaching of endodontically treated teeth has not been fully elucidated. It appears that a history of traumatic injury, the application of heat, and the acidity of the external root environment adjacent to the application of the bleaching agent all contribute to the resorption phenomenon. Resorption does not occur immediately but appears a few years after the bleaching procedure. Thus, follow-up of intracoronally bleached teeth is mandatory. Manifestations of cervical resorption could be caused by bacterial penetration into dentinal tubules after traumatic damage to the cementum. Also, increased dentin permeability associated with the use of hydrogen peroxide has been surmised as a predisposing factor in the initiation of such a resorptive process.

A patient who requests tooth whitening of endodontically treated teeth should be duly informed of the potential consequence of cervical resorption before such a procedure is administered. In view of the potential complications associated with the use of hydrogen peroxide and occasionally sodium perborate as intracoronal bleaching agents, 10% to 37% carbamide peroxide has been suggested as an alternative intracoronal bleaching agent.[148–150] Although carbamide peroxide has been shown to be as effective as sodium perborate for lightening tooth color under in vitro conditions, there are no clinical data available on its efficacy in bleaching discolored endodontically treated teeth.

Internal Inflammatory Root Resorption

Internal inflammatory root resorption occurs in response to infection and in general responds favorably to conventional treatment. It is classified, based on location, as apical or intraradicular. Apical internal inflammatory root resorption has been shown to be a common occurrence in teeth with periapical pathosis.[151] The majority of teeth with periapical pathosis demonstrate some degree of apical internal resorption when analyzed with scanning electron microscopy. These lesions may not be apparent clinically; nevertheless, the clinician should presuppose their presence. Apical internal inflammatory resorptive lesions most likely contribute to the observed inaccuracies of electronic length determination in teeth with large apical lesions. Intraradicular internal resorption is identified as a round or oval lesion contained within the root canal.

Conventional endodontic therapy will stop progression of the lesion. Early intervention can prevent perforation of the periodontium, which can complicate clinical management.

Etiology

Internal root resorption is marked by resorption of the internal aspect of the root via multinucleated giant cells adjacent to granulation tissue in the pulp (Fig 17-19). Chronic inflammatory tissue is common in the pulp, but only rarely does it result in the conditions necessary for recruitment and activation of the clastic cells that mediate resorption.

There are different hypotheses on the origin of the pulpal granulation tissue involved in internal resorption. The first and most widely accepted hypothesis is that infected coronal pulp tissue leads to the formation of adjacent apical granulation pulp tissue (see chapter 10). A second hypothesis proposes that the granulation tissue is of nonpulpal origin, possibly originating from cells circulating in the vascular compartment or from cells originating in the periodontium. It has been shown that communication between the coronal necrotic tissue and the vital pulp is through appropriately oriented dentinal tubules[152] (see Fig 17-19a). One investigation reported that resorption of the dentin is frequently associated with deposition of hard tissue resembling bone or cementum, not dentin.[126] The investigators postulated that the resorbing tissue is not of pulpal origin but is actually "metaplastic" tissue derived from pulpal invasion by macrophage-like cells

circulating in the vascular compartment. Still others have proposed that the pulp tissue is replaced by periodontium-like connective tissue when internal resorption is present.[153] These hypotheses are not mutually exclusive, and it is possible that all contribute to the formation of granulation tissue in pulp in various clinical cases.

In addition to the presence of granulation tissue in pulp, internal root resorption takes place only when the odontoblastic layer and predentin are lost or altered.[132] Causes of predentin loss adjacent to granulation tissue are not obvious, but trauma has been frequently suggested,[154] perhaps even as an initiating factor in internal resorption.[153] Traumatic episodes are divided into two types: transient and progressive, the latter of which requires continuous stimulation by infection.

Another possible cause of predentin loss may be extreme heat produced by cutting of dentin without an adequate water spray (see chapters 14 and 15). The heat presumably destroys the predentin layer, predisposing the tooth to internal resorption if there is continued inflammatory stimulation of the coronal pulp. Under these conditions, bacteria and their by-products initiate inflammation, thereby stimulating resorbing giant cells in the vital pulp adjacent to the denuded root surface. In support of this possible scenario, internal root resorption has been produced experimentally by the application of diathermy.[155]

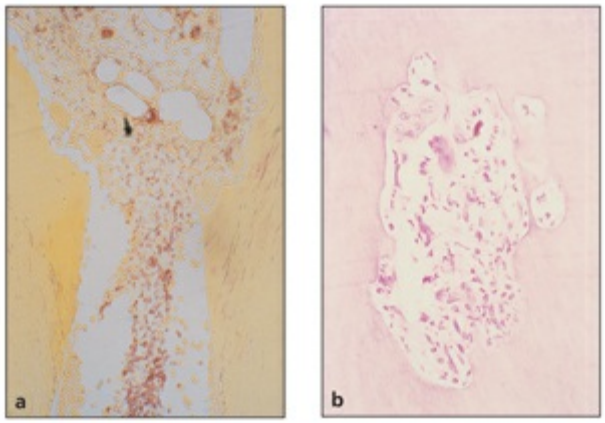

Fig 17-19 Histologic sections of internal root. *(a)* Bacteria are seen in the dentinal tubules communicating between the necrotic coronal segment and the apical granulation tissue and resorbing cells. *(b)* Apical dentin is resorbed (Brown & Brenn stain; original magnification ×100). (Courtesy of Dr Leif Tronstad, Oslo, Norway.)

Clinical manifestations

Internal root resorption is usually asymptomatic and is first recognized clinically

through routine radiographs. Pain may be a presenting symptom if perforation of the crown occurs and the granulation tissue is exposed to oral fluids. For internal resorption to be active, at least part of the pulp must be vital so that a positive response to pulpal sensitivity testing is possible. The coronal portion of the pulp is often necrotic, whereas the apical pulp, which includes the internal resorptive defect, may remain vital. Therefore, a negative sensitivity test result does not rule out active internal resorption. It is also possible that the pulp will become nonvital after a period of active resorption, resulting in a negative sensitivity test, radiographic signs of internal resorption, and radiographic signs of apical inflammation.

Traditionally, the pink tooth, resulting from the granulation tissue in the coronal dentin that undermines the coronal enamel, has been thought to be pathognomonic of internal root resorption (Fig 17-20). However, as already discussed, a pink tooth can also be a feature of subepithelial external inflammatory root resorption, which must be ruled out before a diagnosis of internal root resorption is made.

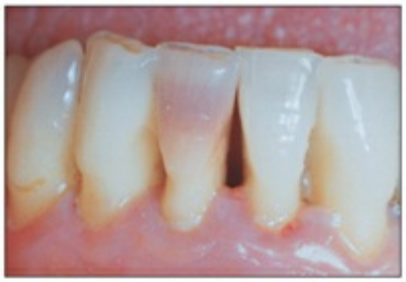

Fig 17-20 Mandibular central incisor with a pink spot indicating internal root resorption. The pink discoloration is due to the undermining of the enamel by granulomatous tissue. Because the pink spot is so far from the periodontal attachment level, this example is unlikely to be external in nature.

Radiographic appearance

The usual radiographic presentation of internal root resorption is a fairly uniform radiolucent enlargement of the pulp canal (Fig 17-21). Because the resorption is initiated in the root canal, the resorptive defect includes some part of the root canal space. Therefore, the original outline of the root canal is distorted. Only on rare occasions when the internal resorptive defect penetrates the root and impacts the periodontal ligament does the adjacent bone show radiographic changes. Angled conventional radiographs (Fig 17-22) as well as newer three-dimensional imaging methods can help to discriminate between internal and external resorptive

processes.

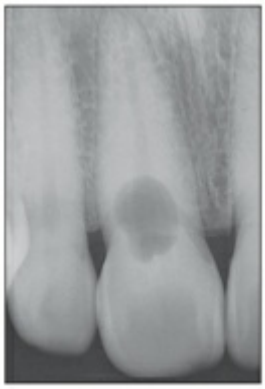

Fig 17-21 Incisor with internal root resorption. Uniform enlargement of the pulp space is apparent. The outline of the canal is not visible in the resorptive defect. Adjacent bone is intact.

Histologic appearance

Like that of other inflammatory resorptive defects, the histologic presentation of internal resorption is granulation tissue with multinucleated giant cells. An area of necrotic pulp is present coronal to the granulation tissue. Dentinal tubules that contain microorganisms and communicate between the necrotic zone and the granulation tissue are sometimes visible (see Fig 17-19a). Unlike the bone in external root resorption, the adjacent bone is not affected by internal root resorption.

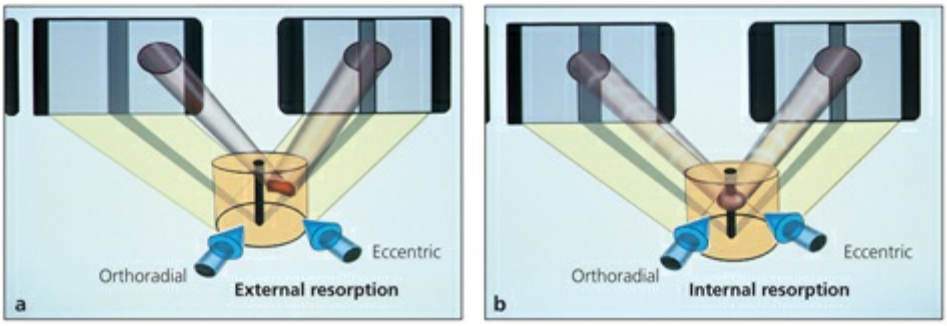

Fig 17-22 *(a and b)* Difference between appearances of external *(a)* and internal *(b)* root resorption when the radiographic angle is changed. (Courtesy of Dr Claudia Barthel, Düsseldorf, Germany.)

Treatment

Treatment of internal root resorption is conceptually very easy. Because the resorptive defect is the result of the inflamed pulp and the blood supply to the tissue is through the apical foramina, endodontic treatment that effectively removes the blood supply to the resorbing cells is the treatment approach. After adequate anesthesia is obtained, the canal apical to the internal defect is explored and a working length determined. The apical canal is thoroughly instrumented to ensure that the blood supply to the tissue resorbing the root is eliminated.

When the root canal instrumentation is completed, paper points should be able to maintain a blood-free, dry canal. Calcium hydroxide is administered to the canal to facilitate removal of the tissue in the irregular defect at a subsequent visit, when the tooth and defect are sealed. Ultrasonic instrumentation and irrigation may also be of help in removing tissue in irregularities and undercuts that conventional instrumentation cannot remove. Barrier techniques utilizing mineral trioxide aggregate may be necessary in large apical resorptive defects.[156]

Diagnostic Features of External and Internal Root Resorption

It is often very difficult to distinguish external from internal root resorption, so misdiagnosis and incorrect treatment can result. This section discusses typical diagnostic features of each type of resorption. Box 17-1 provides an overview of the key elements of these diagnostic features and may serve as a quick reference for clinical practice.

Box 17-1	Typical diagnostic features of root resorption
Apical external inflammatory root resorption due to pulpal infection • Negative pulpal sensitivity test, with or without a history of trauma	
Lateral external inflammatory root resorption due to pulpal infection • History of trauma • Negative pulpal sensitivity test • Lesion repositioned on angled radiographs • Root canal visible radiographically overlying the defect • Apparent bony radiolucency	

Subepithelial external inflammatory root resorption due to sulcular infection
• History of trauma (often forgotten or not understood by the patient) • Positive pulpal sensitivity test • Lesion at the attachment level of the tooth • Lesion repositioned on angled radiographs • Undistorted and radiographically visible root canal outline • Crestal bony defect associated with the lesion • Pink spot possible
Internal root resorption
• History of trauma, crown preparation, or pulpotomy • Positive pulpal sensitivity test likely • Lesion at any location along the root canal (not only at attachment level) • Lesion associated with the root canal on angled radiographs • Radiolucency contained in the root without an adjacent bony defect • Pink spot possible

Radiographic features

A change of x-ray angle should give a fairly good indication of whether a resorptive defect is internal or external. A lesion of internal origin appears close to the canal whatever the angle of the x-ray (Figs 17-22 and 17-23). On the other hand, a defect on the external aspect of the root seems to move away from the canal as the x-ray angle changes (Figs 17-22 and 17-24). In addition, it is usually possible to distinguish whether the external root defect is buccal or palatolingual by using the buccal object rule. In internal resorption, the outline of the root canal is usually distorted, and the root canal and the radiolucent resorptive defect appear contiguous. When the defect is external, the root canal outline appears normal and can usually be seen "running through" the radiolucent defect (see Fig 17-21).

Although intraoral radiography provides an acceptable level of accuracy in the diagnosis of root resorption, the use of CBCT enables three-dimensional imaging of root resorption defects from the coronal, sagittal, and axial aspects (Fig 17-25). In addition, the associated use of volumetric construction enhances visualization of the portals of entrance in the case of external resorption and also the location and extent of the resorption defect as well as the degree of osseous involvement[135–137] (Fig 17-26). Improvements in diagnostic accuracy with the use of CBCT also increase the likelihood of correct management of resorptive lesions.[157]

External inflammatory root resorption is always accompanied by resorption of the bone in addition to the root (see Figs 17-11 and 17-16). Therefore, radiolucencies are apparent in the root as well as the adjacent bone. Internal root resorption does not involve the bone; as a rule, the radiolucency is confined to the root (see Figs 17-21 and 17-23). On rare occasions, if the internal defect perforates the root, the adjacent bone is resorbed and appears radiolucent on the radiograph.

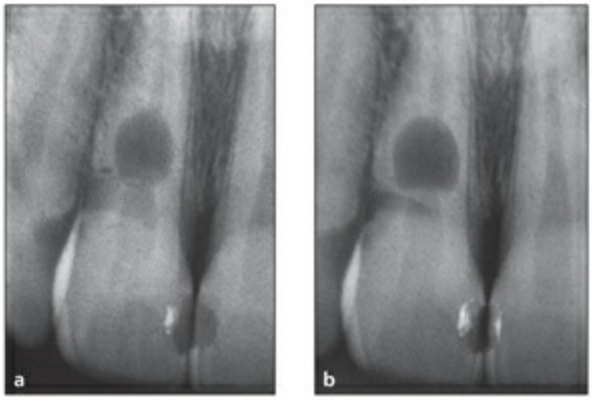

Fig 17-23 *(a and b)* Internal resorption. Radiographs from two different horizontal projections depict the lesion within the confines of the root canal.

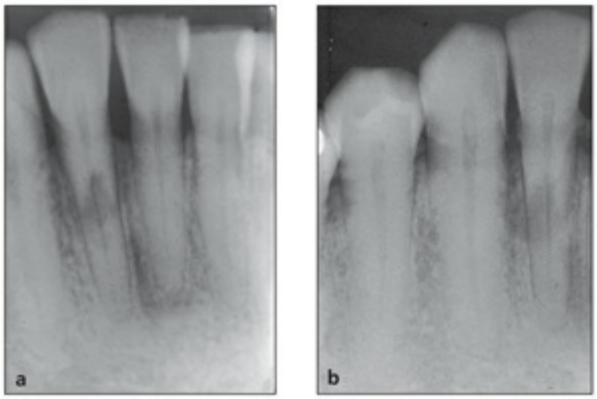

Fig 17-24 *(a and b)* External resorption. Radiographs from two different horizontal projections depict movement of the lesion to outside the confines of the root canal.

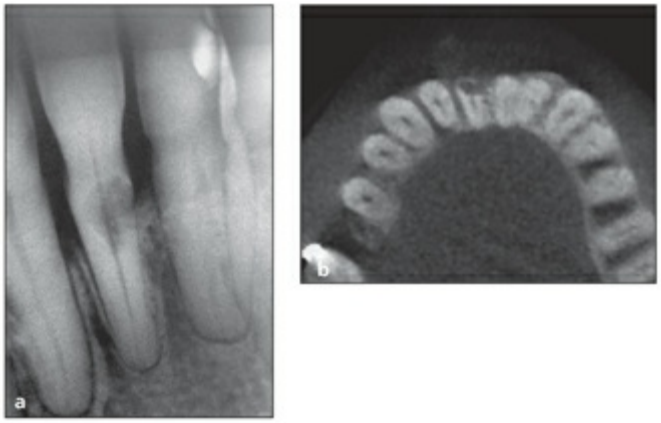

Fig 17-25 *(a)* Periapical view of the mandibular right central incisor showing subepithelial external resorption. *(b)* Axial cut from a volumetric reconstruction shows the location, extent, and proximity of external resorption to the root canal. (Reprinted from Cohenca et al[135] with permission.)

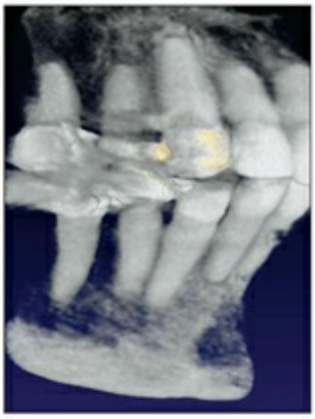

Fig 17-26 Proximal volumetric reconstruction shows the presence of root resorption on the facial surface of a mandibular right central incisor. (Reprinted from Cohenca et al[135] with permission.)

Vitality testing

External inflammatory resorption on the apical and lateral aspects of the root involves an infected pulp space, indicated by a negative response to sensitivity tests. On the other hand, because subepithelial external root resorption does not involve the pulp (the bacteria are thought to originate in the sulcus of the tooth), this type of resorption is often associated with a normal response to sensitivity testing. Internal root resorption usually occurs in teeth with vital pulps and elicits a positive response to sensitivity testing. However, teeth that exhibit internal root resorption

sometimes register a negative response to sensitivity testing, often in cases where the coronal pulp is removed or necrotic and the active resorbing cells are located more apical in the canal. In addition, the pulp may become necrotic after active resorption has taken place.

Pink spot

Because the pulp is nonvital in both apical and lateral external root resorption, the granulation tissue that produces a pink spot is not present in such cases. However, a pink spot caused by granulation tissue that undermines enamel is a possible sign of both subepithelial external root resorption (see Fig 17-15) and internal root resorption (see Fig 17-20).

Common misdiagnoses

The majority of misdiagnoses of resorptive defects are made in distinguishing between subepithelial external root resorption and internal root resorption. A diagnosis should always be confirmed while treatment is proceeding. When root canal therapy is the treatment of choice for an apparent internal root resorption that has not perforated the periodontium, bleeding within the canal should cease quickly after pulp extirpation if the blood supply to the granulation tissue is the apical blood vessels. However, if bleeding continues during treatment, particularly if it is still present at the second visit, the source of the blood supply is external and treatment for external resorption should be initiated. Also, upon obturation in cases of internal resorption, it should be possible to fill the entire canal from within. Failure to fill the canal suggests an external lesion. Finally, if the blood supply of an internal resorption defect is removed upon pulp extirpation, any continuation of the resorptive process on recall radiographs should alert the dentist to the possibility that an external resorptive defect was misdiagnosed.

Systemic Causes of Root Resorption

The roots of teeth show a remarkable resistance to detectable resorption, even with systemic diseases that can cause significant bone resorption (see chapter 20). With hyperparathyroidism osteitis deformans (Paget disease), for example, radiographically apparent bone resorption is not accompanied by resorption of the roots.[158] However, hormonal disturbances and genetic factors have been shown sometimes to cause resorption of the roots.[96,107,159]

The preponderance of research on the genetic propensity to develop root resorption has been conducted on populations of orthodontic patients. An IL-1β polymorphism has been positively correlated with an increased risk for external apical root resorption secondary to orthodontic tooth movement. Individuals homozygous for the *IL1B* (+3953) allele 1 have a 5.6-fold greater risk of root resorption greater than 2 mm than do control populations.[110,111] Whether this same genetic variation plays a role in other types of dental resorption is not presently known.

Renal dystrophy results in an increased oxylate concentration in the blood and precipitation in the hard tissues, which can cause root resorption.[160,161] Genetic linkage is implicated because idiopathic external root resorption has been observed in members of the same family.[107] As knowledge and test procedures advance, resorption presently diagnosed as idiopathic will probably be increasingly found to be of systemic or genetic origin.

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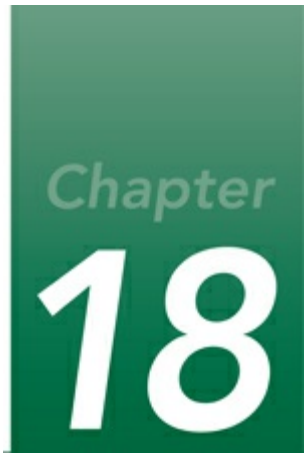

Aging and the Pulp

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Advances in living standards, including medical and dental care, have contributed to increased life spans and a growing proportion of elderly people in the population. The elderly often need more medical and dental services compared to the average citizen, including root canal treatment.[1] Results of a survey[1] of Diplomates of the American Board of Endodontists indicated that Diplomates examine patients covering a wide spectrum of ages but most fall into the age range of 45 to 64 years. Respondents indicated that about 26% of their patients are at least 65 years old. A substantial majority (59%) of respondents (n = 334) indicated that the number of patients aged 65 years or older is increasing in their practices.

The increased need for endodontic treatment among older individuals is due partly to naturally occurring anatomical and physiologic senescent changes that are associated with the aging process and partly to diseases that occur more commonly in older adults[2] (see chapter 20). Oral health is important because oral diseases affect more than the mouth.[3] Normal aging processes in healthy individuals often

have few adverse effects in the oral cavity, but tooth loss, caries, periodontal diseases, and pulpal and periradicular diseases will have deleterious consequences.[4]

The increased need for dental services for older individuals is also a reflection of the greater retention of teeth into old age.[5] Utilization of dental care services increases with increasing age; clinical findings suggest that individuals 65 years old or older have more caries than young children,[6,7] although the caries attacks cervical rather than occlusal surfaces. Further, the number of teeth with carious or restored root surfaces increases the longer a person lives; more than half the retained teeth in individuals 75 years of age or older are affected.[8] The changes occurring in the dental pulp of elderly patients may explain the increase in requests for endodontic treatment.[9] This chapter reviews age-dependent and age-independent processes in the dental pulp and evaluates their impact on the quality of oral health care in the elderly patient.

Process of Aging

A discussion of aging in a particular tissue or area of the body is based on biologic theories of replicative senescence, together with the roles of oxidative stress and telomeres on the aging process. *Organismal senescence* is the aging of whole organisms. The term *aging* has commonly been equated with *senescence* such that the terms can be used interchangeably. The role of telomeres, structures found at the ends of chromosomes in the cells of eukaryotes (single-celled or multicelled organisms whose nucleus is surrounded by a membrane), is an important consideration in the aging process. *Cellular senescence* is the combination of processes of deterioration that follow the period of the development of an organism.[10]

Aging is generally characterized by the declining ability of cells or organismal systems to respond positively to stress.[11] Therefore, the ultimate consequence of aging is death. There are differences in maximum life span between species, which correspond to differences in the "rate of aging." The inherited rate of aging makes a mouse elderly at 3 years old and a human elderly at 90 years old. These genetic differences affect the efficiency of DNA repair and oxidative enzymes as well as the rates of free radical production.

Evidence that cultured normal human and animal cells undergo a finite number of population doublings in vitro has provided new insights into age-related changes at the cellular level.[12] The number of mitotic events that cultured, normal animal cells undergo seems to be inversely related to the age of the donor.[13,14] Limits on cell division and function also occur in vivo when normal cells are transplanted serially; as cell doublings reach their limit, hundreds of variables change from the molecular level to the whole cell, and many of these changes are identical to those seen in intact humans and animals as they age.[15,16]

Aging can be distinguished from disease by several differences. Aging occurs as a result of either a purposeful program or random (stochastic) accidental events.[17] Evidence indicates that genes do not drive the aging process but instead seem to be involved in the loss of molecular form and function. The molecular fidelity of all molecules produced either before or after reproductive maturity is the determinant of longevity, which is governed by the genome. Distinction between the aging process and age-associated diseases is based on the molecular definition of age. Unlike disease, age changes occur in every multicellular animal that reaches a certain size when reproductively mature. Further, age changes cross all species barriers and occur in all members of a species only after the age of reproductive maturation. Aging also occurs in all animals removed from the wild, occurring in all animated matter and having the same universal molecular etiology—that is, thermodynamic instability. No disease shares these distinctions.[18]

Theories of senescence

The process of senescence is complex, a manifestation of many different mechanisms.[19,20] Aging involves genetic, molecular, cellular, systemic, and organismal processes. However, despite being an intensely active area of research, aging remains one of the most poorly understood of all biologic processes. This lack of understanding is mostly due to the complexity of aging and its integrated nature as well as the difficulty of dissociating the effects of normal aging from those manifested as a consequence of age-associated disease conditions.[19]

Theories of senescence generally have been divided between the programmed and stochastic theories of aging. Programmed theories imply that aging is regulated by biologic clocks operating throughout human lifetimes. This regulation depends on

changes in gene expression that affect cellular maintenance, repair, and defense responses. Stochastic theories purport that environmental conditions impact living organisms, causing cumulative damage to DNA, tissues, and cells by free radicals and oxidative stress.[17,20]

Although the aforementioned theories still linger in recent literature, most gerontologists today believe that aging is a process of progressive failure of homeodynamics of genes that maintain and repair, stochastic events that cause molecular damage, and change events that determine the probability of death. The interaction of maintenance and repair constitutes the homeodynamic dimension of a biologic system, and aging can be considered a reduction of homeodynamic space, mainly resulting from increased heterogeneity.

Evolutionary theory was advanced because of the smaller probability that an organism will remain alive at an older age. The decreasing probability is due to disease and accidents (random events not dependent on age). This results in a higher reproductive rate at a young age and a shorter life span. This theory has not been supported by research carried out in cell cultures.

Gene regulation has been identified using model organisms such as budding yeast, worms, or fruit flies. Studies involving these organisms have demonstrated the presence of at least two conserved aging pathways. Although gene expression is imperfectly controlled, it is possible that random fluctuations in the expression levels of many genes contribute to aging.[21]

While senescence is not universal, evidence suggests that cellular senescence evolved in certain species as a mechanism to prevent cancer. In a few simple species, senescence cannot be detected. These species have no postmitotic cells; they reduce the effects of damaging free radicals by cell division that results in cytoplasmic dilution of noxious substances. Eventually they succumb to trauma or disease.

The mitochondrial respiratory system is the major cellular source of reactive oxygen species (ROS) and free radicals. Age-dependent imbalances in the fraction of toxic by-products that may escape the defense mechanisms of human cells can induce a broad spectrum of oxidative damage to the molecules in the mitochondria and the cells as a whole.[22]

Accumulation of ROS inside of cells over time and as a function of cellular activity is a major determinant of aging. Cellular damage caused by ROS impairs physiologic functions, increases the risk of tissue disease, and reduces life span. The role that ROS play in mitochondrial DNA (mtDNA) function and apoptosis in aging

was reviewed by Lee and Wei.[23] They suggested that enhanced oxidative stress, accumulation of mtDNA mutations, altered expression of a few clusters of mitochondrial genes, mitochondrial dysfunction, and apoptosis are major contributors to human aging.[24]

This oxidative stress theory of aging issued from Harman's free radical theory,[25] which postulates that production and accumulation of free radicals in aerobic organisms is an important determinant of cellular life span. Today, the oxidative stress theory is well accepted, but the direct cause-and-effect relationship between the accumulation of oxidative damage and aging remains poorly understood.[19]*Oxidative stress* is defined as an excessive accumulation of ROS caused by an imbalance between production and destruction of ROS, the latter by antioxidant defenses.

Building further on the oxidative stress theory, new theories of aging have been advanced in recent years.[26] The mitochondrial theory of aging hypothesizes that mitochondria play a key role in the control of aging: The production of ROS by electrons leaving the electron transport chain can damage components of the electron transport chain itself as well as mtDNA, leading to a reduction of mitochondrial function.[27] The evidence of increasing mtDNA damage with age may support this theory.[28]

The cellular senescence theory of aging emphasizes the importance of cellular signaling responses to damage. Accumulation of ROS in the cell modulates various signals, resulting in accelerated mito-genesis and premature cellular senescence.[29]

The molecular inflammatory theory of aging, proposed more recently, stresses that activation of redox-sensitive transcriptional factors by age-related oxidative damage induces the upregulation of proinflammatory gene expression. Accumulation of ROS during aging might be responsible for aging-related pathologic conditions such as cancer, arthritis, and neurodegenerative diseases.[30]

Oxidative stress

Cellular senescence occurs when normal diploid differentiated cells lose the ability to divide, a phenomenon now known as *replicative senescence* or the *Hayflick phenomenon*.[12] In response to DNA damage, cells with shortened telomeres either senesce (age) or self-destruct (apoptosis) if repair cannot occur. As noted earlier,

senescence is not universal, and senescence is not seen in single-cell organisms that reproduce through cellular mitosis. In some species (sponges, coral, lobsters), highly differentiated cells become postmitotic and can no longer replicate, thus experiencing replicative senescence. Because human cellular senescence evolved as a way to prevent the onset and spread of cancers if cell division continued, such cells accumulate DNA mutations and therefore would be in increased danger of developing disease.

Replicative senescence and oxidative stress, as discussed previously, play an important role in cellular aging of epithelial cells and increase the risk of aging in several systems in humans.[31–34] Oxidative stress causes a large increase (becoming less negative) in cellular oxidation reduction potential, or a large decrease in the reducing capacity of cellular redox couples such as glutathiones.[35] The effects of oxidative stress depend on the size of these changes because cells are able to overcome small changes and regain an optimal redox state. However, more severe oxidative stress can cause cell death via apoptosis in moderate oxidation[36]; more intense stresses cause cell necrosis.

A particularly destructive aspect of oxidative stress is the production of ROS, which include free radicals and peroxides. Some less reactive species (superoxide) are converted by oxide-reduction reactions or other redoxcycling compounds (quinines) into more aggressive species that cause extensive cellular damage.[37] Most of these oxygen-derived species are produced at a low level by aerobic metabolism, and the cellular damage caused is constantly repaired. However, under severe levels of oxidative stress that cause necrosis, the resulting damage causes adenosine triphosphate depletion, preventing controlled apoptotic death and resulting in increased membrane permeability, loss of cellular enzymes, and osmotic lysis.[37]

One of the most widely accepted theories of aging concerns the idea that oxygen-derived free radicals cause age-related impairment through oxidation to biomolecules, and mitochondria are the main target of this free radical attack.[38] Many metabolic and physiologic processes need oxygen radicals, so an equilibrium between their production and their antioxidant-linked inactivation is required to preserve health. Thus, senescence is the result of an imbalance between free radical production and antioxidant defenses. This process is evident in immune cells, which use free radicals in their protective functions, resulting in a senescent deterioration linked to oxygen stress.[38]

The most direct test of the free radical–oxidative stress theory is to specifically alter the age-related increase in oxidative damage and determine how this alteration

affects aging. New investigations in the use of genetically altered laboratory animals test the role of oxidative damage in aging.[39] In mammalian model systems, evidence of oxidative stress effects indicates that antioxidant treatment protects against age-related dysfunction. Linkage between aging and oxidative stress is believed to occur because ROS generated under various conditions are able to oxidize nucleic acids, proteins, and lipids, and aging is associated with the accumulation of oxidized forms of cellular constituents.[40] The accumulation of oxidative damage is one of the most widely accepted causes of aging. Mitochondrial dysfunction, in particular damage to mtDNA, appears to be responsible for increased production of ROS, thus acting as a causal factor for aging.[41]

Mitochondria are a cell's single greatest source of both adenosine triphosphate and ROS. ROS are important for many life-sustaining processes of cells and tissues, but they can also induce cell damage and death. If their production and levels within cells are not effectively controlled, the detrimental effects of oxidative stress can accumulate.[31,40,42] Mitochondrial dysfunction in cells leads to replicative senescence, where the irreversible loss of division potential of somatic cells occurs after a more or less constant number of cell divisions.[42]

Telomeres

Telomeres have an important role in replicative senescence. Telomeres are extensions of the linear, double-stranded DNA molecules that compose chromosomes. They are located at each end of both chromosomal strands. Therefore, 1 chromosome will have 4 telomeric tips, and the 46 chromosomes found in human cells have 92 telomeric ends. In 1960, it was found that cells have a built-in counting mechanism that limits their capacity to replicate (the Hayflick limit).[11] The only cells capable of continued division appear to be cancer cells. This suggests that normal cells do not have the potential to divide and function indefinitely.

Hayflick and Moorhead[11] suggested that the limited capacity of normal cells to divide is an expression of aging, leading to the determination of the longevity of the organism. Therefore, cells require a mechanism, located in the cell nucleus, to count the number of times cellular replication occurs. A group of researchers at the University of California[43,44] found that telomeres in cultured fibroblasts shorten each time a cell divides. At a certain shorter length, telomeres signal a cell to stop

dividing, indicating that cellular aging is not based on the passage of time but on the telomeric measurement of rounds of DNA replication (Fig 18-1).

Von Zglinicki[45] showed that replicative senescence is tied to organismal aging processes and that telomeres appear to be a major trigger. He developed a model of telomere shortening and signaling by determining telomere structure, function of telomere-binding proteins, and sensitivity of telomeres to oxidative damage. He later suggested that oxidative stress is an important modulator of telomere loss, leading to telomere-driven replicative senescense.[46] Additional details are available in articles on the physiology and pathophysiology of aging.[18,47–50]

Others have found that shortening of telomeres in immortal eukaryotic cells (cells with a nucleus that contains the cell's chromosomes and that are larger and more structurally and functionally complex) leads to loss of telomeres with age and hence the loss of the ability to grow. Others have found that an enzyme that elongates telomeres overcomes age-related shortening[51,52]; however, this enzyme is not found in normal somatic cells.[52] In contrast, telomeres from sperm DNA did not decrease in length as donor age increased, suggesting that a mechanism for maintaining telomere length, such as telomerase expression, may be active in germ-line tissue.[53]

Telomeres' DNA sequences cap the ends of all eukaryotic chromosomes and shorten during repli-cative aging of normal cells. Variations in the initial length of terminal restriction fragments account for much of the variation in fibroblast replicative capacities, and it has been found that the existence of a critical telomere length in senescing cells occurs in aging.[54] A more detailed discussion of the telomere's role in replicative senescence can be found in other publications.[55–63]

Taken together, studies of replicative senescence, oxidative stress, and telomere shortening indicate that cells and tissue age in a manner that appears to be well controlled. Similar studies should be done using pulp tissues and processes to define the changes that occur with aging of the teeth.

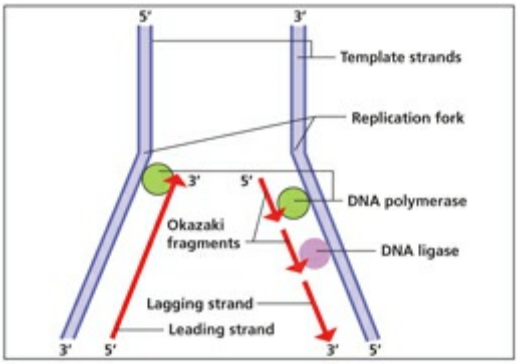

Fig 18-1 Telomere shortening. Telomeres shorten because of the leading-strand phenomenon that is

exhibited during DNA replication in eukaryotes only. Because DNA replication does not begin at either end of the DNA strand but starts in the center, and because all DNA polymerases that have been discovered move in the 5' to 3' direction, the DNA molecule being replicated contains a leading strand and a lagging strand.

Age-Related Changes in the Pulp

Numerous changes in the dental pulp occur as a consequence of the aging process. These changes have been observed in animal experiments and human studies through examination of extirpated dental pulp tissue or tissue harvested from extracted teeth, especially third molars. Age-related changes have been viewed as either functional, occurring through use (ie, "wear and tear"), or geriatric (ie, due to factors intrinsic to the normal aging process).[64,65]

Characteristic changes with aging in fully formed human teeth were first described by Lacasagne in 1889 (cited by Johanson[66]) and scientifically confirmed in 1950.67 In human teeth, aging alters the cell density of the pulp tissue and the dentinogenic activity of the surviving odontoblasts, leading to an increase in dentinal thickness and progressive reduction of space in the pulp canal and pulp chamber. In general, the number of cells in the pulp diminishes with age; proliferative activity peaks early in life. However, the capacity of cells to proliferate is probably maintained well into adulthood, if not old age. This capacity reflects a continuing ability of the pulp to provide odontoblasts for secondary dentin formation. The predominant cell in the pulp, the fibroblast, shows and perhaps dictates the pattern seen in the pulp cell population as a whole. Immune cells and macrophages show a varied pattern of presence and activity within the aging pulp. For example, immunoglobulin G levels seem to diminish with age (in animals), likely resulting from fewer lymphocytes, as does the number of Class II major histocompatibility complex–expressing cells.

Connective tissue changes are also observed in aging pulps. The amount and type of collagen present in the pulp change with advancing age, leading to an overall increase in fibrosis and levels of calcification. This fibrosis occurs even though collagen synthesis appears to diminish in older teeth. This finding is consistent with a reduction in the level of matrix metalloproteinases (ie, MMP-2, -8, -9) with collagenase activity or possibly an increase in tissue inhibitors of MMPs, leading to a reduced turnover of existing collagen. In addition, the fibrous sheaths associated with blood vessels and nerves persist even as the structures diminish in number with age. Finally, there is a relative (apparent) increase in the amount of fibrous

connective tissue due to the reduction in volume of the pulp chamber in older individuals. As later described in more detail, the occlusion of the canal is due principally to the accumulation of secondary dentin (see also chapter 2).

Histologic and histomorphologic analyses

Several well-controlled animal studies have focused on age-related changes in cell proliferation and collagen turnover. Early studies on the cell kinetics of aging in rat molar pulp cells found detectable DNA synthesis up to 400 days of age, indicative of continued cell division and cell turnover in older animals. The proliferative activity of the pulp was greater in younger rats, while the volume of pulp tissue was reduced by the continuing dentin apposition in aging animals.[68] Using stains and silver impregnation for collagen and reticular fibers, histologic studies found changes in connective tissue collagen.[69] Examination of the cellular dynamics of the incisor pulps of different-aged rats showed no consistent change in odontoblast number with advancing age. However, a slight but continuing reduction of basal pulp cells occurred throughout life.[70] Thymidine-labeling studies indicate that proliferative activity of fibroblasts contained in the pulp and periodontal ligament is reduced with age.[71] The pattern of decreased activity seems to follow that of human pulp, with DNA synthesis showing a significant decrease over time. Increases in fibrosis may be due to increases in the age-related accumulation of insoluble collagen that accompanies decreased cellularity. In bovine studies, collagen concentrations increased from about 9% to 25% as pulp matured, and the ratio of type III to type I collagen also increased, suggesting a substantial increase in type III synthesis during aging.[72,73]

Animal studies have also found age-related changes in the immune system in dental pulp. Examination of immunoglobulins in bovine dental pulp found that immunoglobulin G levels decreased as the animal aged.[74] Alterations of the levels of immunoglobulins may indicate maturation of the animals and may reflect physiologic changes rather than the presence of localized pathoses. This finding is consistent with other evidence that the overall immunodefense potential of the pulp decreases in older animals[75] (Figs 18-2 and 18-3). The distribution of pulp cells expressing reactivity to monoclonal antibody ED1 was examined immunohistochemically in the mandibular first molars of developing (newborn to 10-week-old), adult (14- to 24-

week-old), and aged (1- to 5-year-old) Wistar rats. Results indicated that ED1-labeled cells were maintained throughout life, but a significant decrease of OX6-positive (Class II major histocompatibility complex–expressing) cells was observed. The density and composition of pulp cells expressing macrophage-associated antigens decreased with increasing age, most probably related to changes in the immunologic defense potential of the pulp against infection.

A later study used rats in two age groups. The dental pulp monocyte/macrophage system response to cavity preparation in animals 12 and 18 months old was compared to that in younger animals 3 and 6 months old. No differences were found in the monocyte/macrophage defense mechanism to injury in either group, indicating the possibility that the dental pulp retains the ability to defend itself against injury during the aging process.[76]

In addition to changes in cell number, there are also age-related alterations in the levels and distributions of the cytoskeletal proteins actin, cytokeratin, and vimentin. The levels of these proteins diminish with age, and the decreases are most striking in odontoblasts.[72,73]

There are also marked reductions in odontoblast processes.[75] Although it is currently impossible to determine what initiates age-related changes in odontoblasts, one explanation might be alteration in the blood supply.[76] Capillaries form a dense plexus about 10 μm beneath the predentin. As animals age, odontoblasts lose their cytoplasmic processes; the loss of fenestrations in the capillaries coincides with a reduction of odontoblast function. Subsequently, odontoblastic capillaries are lost and there is evidence of degenerating vessels, cells, and extracellular debris near the incisal edge of the tooth. Thus, the changes in odontoblast function may be linked to the loss of pulpal vascularity that occurs with age[77] (see also chapter 6).

Histologic analysis on human teeth confirms that with age comes a reduction in cell numbers and a narrowing of the pulpal space.[78] Similarly, when the cellularity of the outer cell-rich zone of pulp was compared to the less cellular middle zone, it was found that the cell number decreases in all three zones starting at 20 years of age; by the age of 70 years, the total cell number is reduced by 50%.[79]

In addition to these quantitative studies, Murray et al[9] confirmed and quantified more precisely the reduction in cell density and compared the pulp in the crown with the pulp in the root. In comparison with younger patients aged 10 to 30 years, the number of crown odontoblasts decreased by 15.6% for all teeth and the density of root odontoblasts decreased by 40.6% in patients 50 to 59 years old. Similarly, the density of fibroblasts decreased by 26.9% in the crown and by 41.3% in the root

with aging.[9]

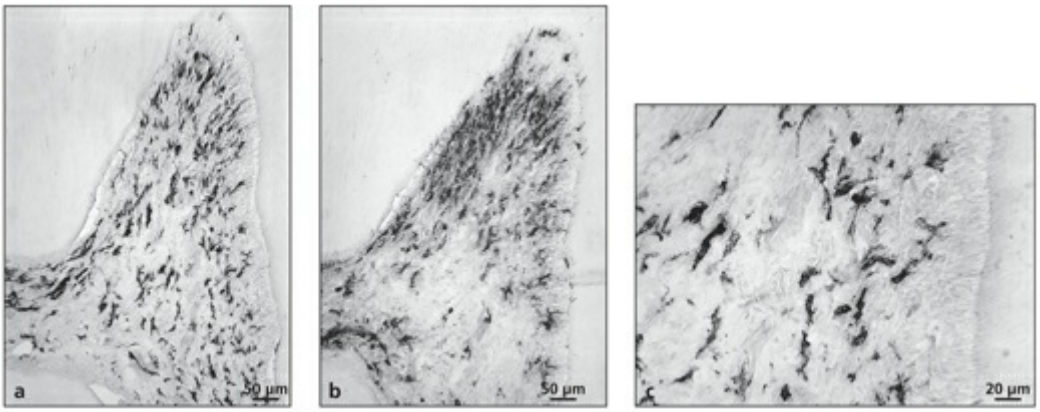

Fig 18-2 ED2-positive cells in the coronal pulp of the mandibular first molar of a 24-week-old rat. Positively stained cells are predominantly irregular or dendritic (OX6 stain; original magnification ×130). (Reprinted from Okiji et al[75] with permission.)

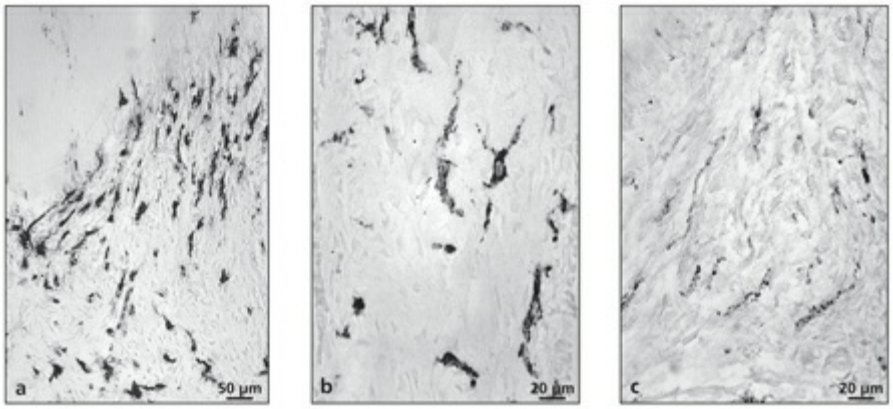

Fig 18-3 Distribution of macrophages in the coronal pulp of the mandibular first molar of a 1½-year-old rat. Positively stained cells are predominantly elongated (OX6 stain; original magnification ×130). (Reprinted from Okiji et al[75] with permission.)

A review of age-related changes in the human pulpodentin complex divided the aging process into four groups, distinguished by changes resulting from physiologic aging rather than irritant-based pathology or trauma.[80,81] The measured cell density of the pulp decreases by about half as a person ages from 20 to 70 years,[82] as odontoblasts decrease in number and become smaller and flattened. An apparent increase in the number of collagen fibers results from a reduction in space in the pulp caused by secondary dentin formation, which gives the appearance that there are more fibers in the remaining space.[83]

In another histologic analysis of 100 noncarious molars from males and females aged 15 to 75 years, Bernick and Nedelman[84] found that aging was associated with

increased calcification of the extracellular matrix, decreased numbers of blood vessels and nerves, increased fibrosis (collagen sheaths), increased collagen fiber thickness, changes in the chemical properties of collagen fibers, and fusion of von Korff fibers in the odontoblastic area. The prominence of collagen fiber bundles was attributed to the persistence of the connective tissue sheaths in the narrowed pulp chamber after loss of the vascular and nerve supply and not to continued formation and reorientation of collagen fibers during the aging process. This finding is consonant with the conclusions of other investigators.[80,85]

Clinical studies have also documented age-related changes in collagen in dental pulp. In human third molar pulps from patients aged 16 to 40 years, there was a substantial increase in calcium and decrease in levels of dihydroxylysinonorleucine (DHLNL)[81](Fig 18-4a). This modified amino acid is a major cross-linker that binds collagen fibers together and can be detected by the addition of reducing agents. Because collagen maturation is characterized by the presence of reducible cross-links, this study demonstrated that collagen synthesis in the coronal pulp decreased with age; this change was accompanied by a decrease in collagen concentration in terms of both dry weight and total protein (Fig 18-4b).

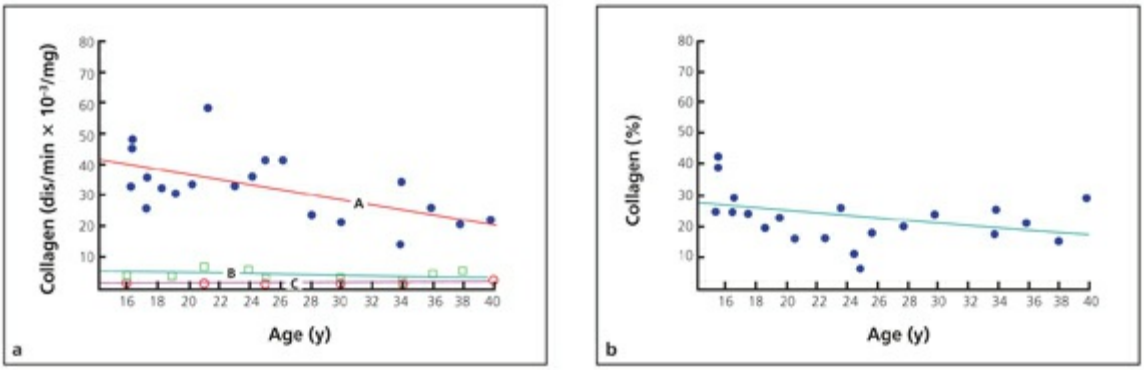

Fig 18-4 *(a)* Concentrations of reducible collagen cross-links in pulp from teeth removed from patients at different ages. Closed circles and line A represent dihydroxylysi-nonorleucine (DHLNL). Open squares and line B represent hydroxylysinonorleucine (HLNL). Open circles and line C represent lysinonorleucine (LNL). Slopes for A, B, and C were calculated using best-fit regression analysis. *(b)* Collagen content of human dental pulp at different ages. The slope of the line, calculated using best-fit regression analysis, is not significantly different from 0. (Reprinted from Nielsen et al[81] with permission.)

Another study evaluated 239 noncarious human teeth in individuals from 10 to 78 years of age.[82] The older dental pulps were characterized by a relative compression of collagen fibers that resulted from accumulated dystrophic or degenerative calcifications, giving an appearance of fibrosis. Although increased fibrosis was not actually observed in this study, others have reported increased fibrosis and

calcification radicularly that is more severe than that found coronally.[83]

Clinical trials have noted distinct age-related patterns in cellular changes in dental pulp. Numerous star-shaped fibroblasts are seen in the pulp of young individuals, yet they are diminished in size and number in teeth from older individuals. The number of odontoblasts and amount of predentin are also diminished as teeth age, indicating that fibrosis and cell depletion are a normal aspect of aging.

More recently, a transcriptome analysis was conducted to clarify the genetic changes that underlie the histologic modifications in the dental pulp with aging.[86] Microarray analysis of third molars of 18- to 20-year-old and 57- to 60-year-old patients revealed several differentially expressed genes that were categorized as encoding growth factors, transcriptional regulators, apoptosis regulators, and components of the extracellular matrix. In young dental pulp, high expression levels were detected for genes involved in cell proliferation, cell and tissue differentiation, and development, as well as in the immune, lymphatic, and hematologic systems; in older dental pulp, genes involved in apoptosis were more highly expressed. Higher expression of genes for synthesis products (collagenous and noncollagenous) was noticeable in the younger dental pulp, whereas genes encoding specific markers such as dentin sialophosphoprotein (DSPP), ameloblastin, or osteonectin were highly upregulated in the older dental pulp. Although proteomic experiments are needed to confirm the differential gene expression at the protein level, these results are interesting because they may be related to the intrapulpal calcification and mineralization observed during aging.

Other studies have evaluated whether tissue matrix components change in aging dental pulp. One study compared third molar pulps of 17- to 25-year-olds with that of patients aged 50 years or older.[87] Using osteocalcin as an index of matrix production, the researchers found that osteocalcin expression (presumably of odontoblast cell lineage) does not diminish relative to the explant cell population. The authors concluded that, despite a reduction in tissue volume and cell numbers, the pulps of aging teeth retain a capacity for dentin deposition.

Another study evaluated matrix components in 332 human teeth in three age groups (10 to 30 years, 31 to 51 years, and 52 to 72 years).[88] The results indicated that collagen types I, II, V, and VI are present at all ages. Staining of type I collagen is weak, and staining of types III, V, and VI collagen is strong (Figs 18-5 to 18-8). With advancing age, fiber bundles of type I collagen increase in frequency and thickness while the fine fibrils of types III, V, and VI collagen disappear or are

replaced by thick fiber bundles (see Fig 18-7). The connective tissue matrix appears condensed and stained homogenously in older individuals.

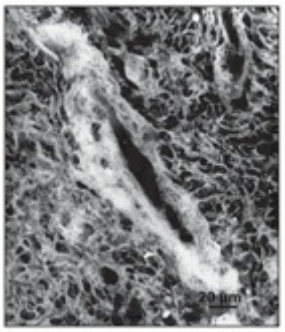

Fig 18-5 In the coronal and radicular pulp, blood vessels are surrounded by type III collagen. Fibers and bundles of fibers that are strongly stained extend between the blood vessels and the connective tissue matrix (paraffin section, immunofluorescence microscopy; original magnification ×375). (Reprinted from Hillmann and Geurtsen[89] with permission.)

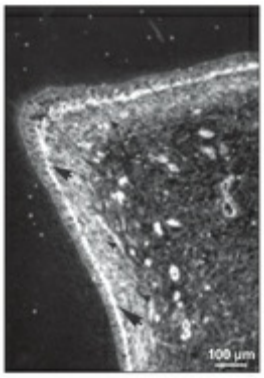

Fig 18-6 In the pulp horn, two layers of type VI collagen are adjacent to the odontoblasts. A thin line of very intense fluorescence labeling is located directly adjacent to the odontoblastic layer *(large arrowheads)*. A second layer, consisting of fine fibrils *(small arrowheads)*, lies adjacent to this line (paraffin section, immunofluorescence microscopy; original magnification ×100). (Reprinted from Hillmann and Geurtsen[89] with permission.)

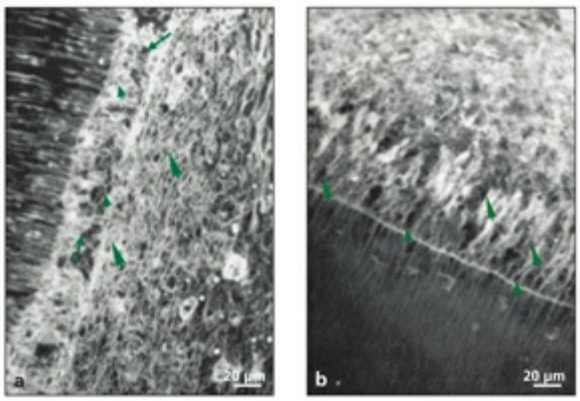

Fig 18-7 *(a)* Longitudinally arranged fibers of type V collagen *(large arrowheads)* are adjacent to the layer of odontoblasts *(arrows)*. Some thin fibers extend between the odontoblasts *(small arrowheads)*

(paraffin section, immunofluorescence microscopy; original magnification ×375). *(b)* In the odontoblastic layer of the coronal pulp, immunolocalization of collagen type VI reveals corkscrew fibers passing from the pulp between the odontoblasts *(arrowheads)* (paraffin section, immunofluoresence microscopy; original magnification ×500). (Reprinted from Hillmann and Geurtsen[89] with permission.)

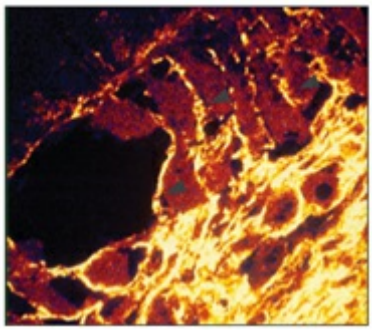

Fig 18-8 Confocal laser scanning microscopy. Fibers of type VI collagen with corkscrew pattern pass from pulp into predentin parallel to the long axis of the odontoblasts *(arrowheads)* of the coronal and radicular pulp (paraffin section; original magnification ×500). (Reprinted from Hillmann and Geurtsen[89] with permission.)

Odontoblasts and aging

Several studies have focused on age-related changes in odontoblasts and report a consistent reduction in numbers of cells in the odontoblastic layer. An early scanning electron microscopic study examined cell processes in caries-free and attrition-free premolars in 14- to 16-year-old patients and compared them to those of 59- to 72-year-old patients.[90] In the younger pulps, the odontoblast processes extended into the dentinal tubules, often reaching the dentinoenamel junction. However, in the older pulps, fewer processes were found in sclerotic dentin and they never extended close to the dentinoenamel junction.

Dentinogenesis is a continuous process of matrix deposition throughout the life of a tooth (see also chapter 2). Primary dentinogenesis occurs during development, leading to formation of the crown and root of the tooth, whereas secondary dentin is secreted throughout the life of the tooth and is responsible for the reduction in the size of the pulp chamber and root canals and the deposition of peritubular dentin.[91] So far, no histologic difference has been described between primary and secondary dentin other than at the interface between the two tissues, which is delimited by a calciotraumatic line.[92] The two types of dentin are secreted by the same cells, albeit at different times and rates. Odontoblasts are actively secreting during primary

dentinogenesis but become significantly less active during secondary dentinogenesis.

The actual time of switching from the primary stage to the secondary stage is still not well defined. The odontoblast transcriptome has recently been investigated by microarray analysis.[93] The results showed that differential dentin secretion is associated with changes in transcriptional activity within the cell. Although the two types of dentinogenesis are reported to differ in their rates of matrix deposition, expression of dentin matrix protein 1 (DMP-1) and osteocalcin genes was upregulated in the mature odontoblasts, while expression of type I collagen, DSPP, transforming growth factor β1 (TGF-β1), and TGF-β1 receptor genes was downregulated. Microarray analysis highlighted 574 differentially regulated genes involved in the p38 mitogen-activated protein kinase (MAPK) pathway, in which *PTPRR*, *NTRKK2*, *MAPK13*, *MAP2K6*, and *MKK3* were strongly implicated.

It has already been shown that the morphology of the odontoblast changes over time (cylindrical in younger cells and flattened in older cells) and is linked to changes in biologic activity of the cells. Simon et al[93] showed that the phenotype also evolves with time and that these modifications must be taken into account in the investigation on aging. In the future, these animal experiments (on bovine teeth) should be reproduced on human cells to analyze the genomic evolution of the odontoblast with aging. This study also supported further existing similarities between pulp cells and bone cells. DMP-1, which is expressed in osteocytes but not in osteoblasts, is associated with the maturation of the odontoblast. Based on these results, it is tempting to consider young and old odontoblasts as two different cell types, and some authors have even proposed renaming mature odontoblasts as *odontocytes*.[94]

Odontoblasts may exhibit reduced function over time, even when their numbers remain stable. Odon-toblast function, as measured by reactionary dentin formation, was determined after dental injury (Class V cavity preparations and restorations) to intact first or second premolars of patients between 9 and 17 years of age.[87] Although odontoblast cell numbers were maintained in this relatively young population, the older subjects had less reactionary dentin formation. This intriguing study should be extended to evaluate odontoblast function in the elderly, in whom greater changes may be anticipated, with consequences for endodontic treatment or tooth loss.

Odontoblast function is often considered as unalterable during the life of the dental pulp. Nevertheless, the survival mechanisms that preserve cell viability are still poorly understood. Recently, the autophagic-lysosomal system of human

odontoblasts has been investigated[95] to analyze the mechanisms that maintain the functional viability of these secreting cells. Odontoblasts were found to develop an autophagic-lysosomal system with large autophagic vacuoles that expressed the autophagosomal (LC3) and lysosomal (LAMP2) markers in an age-related pattern, indicating organization of a dynamic autophagic machinery. In this study, the authors suggested that autophagic activity in odontoblasts is a fundamental mechanism to ensure turnover and degradation of subcellular components. A reduction in the efficacy of this system—during aging, for example—might compromise cell viability and dentinogenic secretory activity at the "old odontoblast" stage.

Dentinogenesis and aging

Aging studies related to changes in dimension of the pulp chamber and root canal spaces have been undertaken with the aid of radiographs, microcomputed tomography (ìCT) scans, and scanning electron microscopy (SEM). Some of these devices are research tools and cannot be used on a regular basis clinically, although they are now appearing in legal cases. The focus of this work has been to describe age-related changes in terms of apposition of dentin in various areas of the coronal and radicular pulp canal space. The use of SEM and radiographic devices demonstrates these age changes; these studies have been carried out by a disparate group of investigators, including clinicians, anthropologists, and forensic scientists.

SEM and radiographs were used to measure pulp chamber and root canal spaces in subjects aged 40 to 70 years and 40 to 97 years; both the pulp chamber and root canal spaces were found to narrow and become restricted with age.[96] The changes occurred mostly in a mesiodistal direction rather than coronoapically.

Raman spectroscopy characterized the chemical composition and the stages of increased dentin deposition.[97] Thirty teeth were analyzed, and the results led to a good age prediction. Other studies suggested the use of CT to determine age-related changes. These studies were carried out, for the most part, by forensic scientists using material available through examination of skeletal remains.[98] All of these studies were able to estimate the ages of the deceased by examination of dental pulp spaces.

The use of ìCT is a more recent development. The devices are very expensive and generally not available except at a few dental schools. They are used primarily in

orthodontic programs and are considered research tools rather than devices for diagnostic or treatment-outcome studies.[99–101] Studies with ìCT and cone-beam CT can determine age changes when patients are scanned.

Normal dental radiographic studies have also been used to determine age-related changes but with varying results. This type of study can be done using radiographs of patients of record or in schools and private practices and are relatively routine in their application. Internal review boards regulate radiographic studies, which are usually conducted in orthodontic programs. Igbigbi and Nyirenda[102] measured the height of the tooth crown and decreases in the height of the coronal pulp cavity in 134 adult men and women 20 to 80 years of age. They reported correlation coefficients that ranged from –0.650 to –0.799 and were statistically significant for both genders in both premolars and molars. The results also allowed for the estimation of subject age with an error of ±5 years.

A similar study used 197 panoramic radiographs of patients 19 to 75 years of age.[103] Six teeth were selected for measurement: a maxillary central incisor, lateral incisor, and second premolar and a mandibular lateral incisor, canine, and first premolar. Multiple regression analyses were used with measurements of the relationship between chronologic age and the two-dimensional dental pulp size. The data showed that this approach is successful in correlating age from this type of film.

Biologic considerations

Aging induces several degenerative processes, including fibrosis, atrophy, loss of cellularity, decalcification, and degeneration of odontoblasts.[84,104] These phenomena depend on a dysfunctional homeostatic mechanism. It is also known that mesenchymal cells in pulp have the potential for mineralization, based on the formation of dentin induced by a variety of stimuli.

Quantification of various biologic substances from dental pulp and dentin have been used to determine the age of subjects. In one study, small pieces of dentin were used to assess mtDNA removed from third molars of 21 individuals aged 15 to 85 years.[105] Amplification and agarose gel electrophoresis of the amount of mtDNA were semi-quantified from the intensity of stained bands in the gel. The results showed that the amount of mtDNA declines with age. The method could be used to determine tooth age.

Age can be estimated from human dental pulp DNA based on telomere shortening. One study found that the terminal restriction fragment length tends to shorten with aging.[106] Several studies attempted to estimate chronologic age in cadavers, human remains, and living human beings by examining changes in certain biologic substances in the dental pulp. One study found that impaired repair of pulp and dentin in aged patients is partly due to a decrease in the proliferative ability of human pulp cells from aged donors.[107] The in vitro proliferative life span of human pulp cells from young donors was longer than that of cells from older donors. Growth rates and alkaline phosphatase activity decreased with increasing donor age.

Characterization of aging can be measured by expression of other substances in the dental pulp. Connexin 43 is a protein for gap junctions; reverse transcription polymerase chain reaction detected its expression in all pulp samples.[108] However, connexin 43 was abundantly expressed in young adult dental pulps, while its expression was dramatically decreased in aged dental pulps. The same researchers used real-time reverse transcription polymerase chain reaction to investigate expression of osteo-calcin messenger RNA (mRNA) in third molar dental pulps from healthy 17- to 23-year-olds and subjects older than 50 years.[109] Osteocalcin RNA was expressed in all samples, but expression decreased in aged pulp. This change may be associated with loss of viability and may be a characteristic of aging.

In another study, a Japanese group highlighted differential expression of core-binding factor α1 (Cbfa1), vascular endothelial growth factor (VEGF), and heat shock protein (HSP-27) mRNAs in young and old odontoblasts in rat teeth. Cbfa1 is a transcription factor required for osteoblast maturation and is also expressed in odontoblasts when they differentiate from preodontoblasts. Expression of Cbfa1 mRNA was higher in young rats than in adult rats, while expression of VEGF and HSP-27 mRNAs was higher in the adults. The authors concluded that a self-defense system in odontoblasts operates differently depending on the age of the tissue: It promotes calcification in young teeth but expresses self-defense proteins and stimulates regeneration of blood vessels in adult dental pulp cells.[110]

Various signaling pathways are involved in all biologic processes, and senescence is no exception. For example, the Notch signaling pathway has been implicated in odontoblast differentiation and is activated during reactionary dentinogenesis,[111] but it is also known to be involved in the maintenance of dental stem cell plasticity by regulating odontoblast differentiation.[112] Recently, it has been demonstrated that the Notch signaling pathway is also involved in the senescence process: When this pathway is blocked by experimental methods, cell proliferation

was found to be decreased, and a marker of senescence (SA-β-Gal in the model used) was over-expressed.[112]

While cell behavior during aging has begun to be documented, the effect of aging (microenvironment) on cells is less investigated. In a recent study, a Chinese team investigated the potential effect of aging of the extracellular matrix on cells themselves. They exposed aged pulp cells to juvenile-conditioned medium and juvenile cells to adult-conditioned medium. Adult pulp cells cultured with juvenile-conditioned medium showed enhanced proliferation but a reduced ability to differentiate. In contrast, young cells cultured with adult-conditioned medium changed their behavior and proliferated and differentiated as adult cells.[113] Such a rejuvenation process has already been described for stem cells in the course of nuclear reprogramming.[114]

While methodologies necessarily differ according to the biologic substance being studied, it is apparent that many pulpal substances can serve as markers of age-related changes in pulp tissue. Markers that decrease with age may impact the retention of viable pulp tissue, leading to situations that increase the need for root canal treatment as a person ages. It also seems evident that if aging has direct effects on cellular behavior, the cellular microenvironment, and extracellular matrix components, then microen-vironmental effects related to aging must also have an effect on a cell's biology.

Blood vessels

The pulpal vascular system is extensively reviewed in chapter 6. In general, the dental pulp receives blood supply via arterioles that enter the apical formina and branch to give rise to a capillary network or plexus, some of which is juxtaposed to the predentin, and odontoblastic layer. Drainage of blood occurs via venules that exit the tooth through the apical and lateral foramina. There is some evidence for the existence of arteriole-to-arteriole, arteriole-to-vein, and vein-to-vein anastomoses, suggesting that there is extensive regional control of blood flow to the pulp.[115]

The microvascular supply to the pulp is small relative to the volume of tissue that is supports. The number of vessels entering the tooth diminishes with age and the vascular plexus becomes reduced, sometimes appearing entirely absent. Additional age-related changes include intimal hyperplasia of arterioles, narrowed vessel lumens, and calcification of the vessel wall. In a study of 239 pulps from subjects 10 to 78 years of age, researchers observed reduced vascularity in coronal pulp with increasing age, although radicular vascularity was observed, indicating the presence

of an effective, anabolically active tissue.

In addition to the changes within the vessels per se, age-related diminution in the dimensions of the pulp space also occur, caused by secondary dentin formation (also dystrophic calcification and tertiary dentin formation). This process of space reduction also results in a marked narrowing, and sometimes obliteration, of the medium-sized vessels and capillaries.

Odontoblast activity and viability are intimately linked to the vascular supply, particularly the supply from the capillary plexus located proximate to the layer of preodontoblasts and predentin. With the age-related reduction (compression) of the feeder arteriolar system to the pulp, there is an associated diminution of the capillary plexus, including vessel degeneration near the incisal end of the pulp. There is also a loss of fenestration in the capillaries.

It is highly likely that these marked age-related changes in the vascular supply to the pulp contribute to the diminution in the capacity of odontoblasts to survive and produce new dentin matrix. To examine this question, one study of pulp vascularity collected single-rooted, permanent human teeth from individuals in three age groups: (10 to 20 years, 20 to 40 years, and 40 to 70 years).[116] With increasing age, the teeth showed reduced terminal branching of vascular structures and reduced numbers of vessels entering and exiting the apical foramen. Teeth in the 40- to 70-year group demonstrated the fewest number of vessels, often having only one primary arterial vessel entering the tooth compared to three or four vessels in the 10- to 20-year age group (Fig 18-9). The peripheral capillary plexus was also reduced or entirely absent in most pulps of the older age group.

An additional study compared teeth from individuals younger than 20 years and individuals aged 40 to 70 years and demonstrated a highly vascularized tissue in the younger group; vessels of arteriole size were found in the coronal portion of the tissue of younger teeth.[117] The pulps from older patients were characterized by a gradual narrowing of the circumference of the pulp and vessels that became more prominent centrally. As secondary dentin formation continued, the pulpal area and circumference decreased, leaving only a thread of pulp tissue and causing narrowing and elimination of medium-sized vessels and capillaries.

Another study evaluated changes in alkaline phosphatase and adenosine triphosphatase activity in three groups of patients (10 to 30 years, 31 to 50 years, and 51 to 70 years). Results demonstrated decreases in both enzymes with aging.[118] The most notable change in the expression of these phospha-tases occurred in the endothelial cells of the capillaries. The study indicated that reduction of these

substances would be accompanied by a reduction in metabolic activity of the pulp with aging, which certainly may indicate a finite ability of odontoblasts to form dentin.

A different study[119] described the ultrastructural changes that occur in pulpal capillaries as a result of aging. Samples of dental pulps were obtained from functional human teeth, and tissue from 10- to 17-year-olds and tissue from individuals more than 60 years of age was compared. The pulp tissue was processed using transmission electron microscopic techniques. Results demonstrated an endothelial cell layer characterized by the presence of numerous pinocy-totic vesicles and microvesicles, rough endoplasmic reticulum, cisternae, free ribosomes, a small Golgi complex, centrioles, microtubules, microfilaments, and mitochondria. In the endothelial cell cytoplasm of older pulpal vessels, pinocytotic vesicles, microves-icles, and microfilaments were more numerous. Lipid-like vacuoles, monogranular glycogen granules, and extensive Golgi complexes with dilated cisterns were also present, suggesting that capillary endothelium experiences morphologic changes that could be associated with advancing age.

Collectively, these studies of the aging dental pulp demonstrate that vessels decrease in number and the pulp chamber narrows with deposition of secondary dentin. Even within the context of a smaller tissue volume, a diminished vasculature appears incapable of supplying adequate nutrients to the tissue, resulting in reduced metabolic activity. This combination of senescent changes probably affects the ability of the remaining tissue to react appropriately to an adverse stimulus, resulting in reduced capacity of older individuals to retain their teeth, even in a healthy environment. The expectation is that elderly patients may require an increasing number of restorative or endodontic procedures to retain their teeth. The alternative is for these patients to lose their natural teeth, diminishing the capacity to maintain a normal diet and level of health.[120] More studies are needed to investigate this clinical problem.

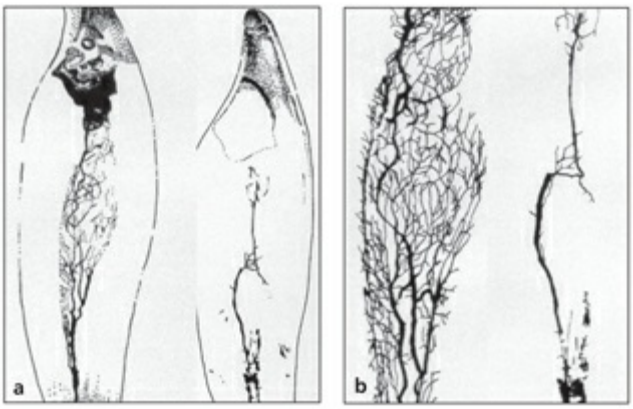

Fig 18-9 *(a)* High-magnification view of the central portion of the pulp chamber of an incisor from a 30-year-old patient. The interior vascular structures are reduced in number, and the peripheral arcade is less intense (original magnification ×100). *(b)* High-magnification view of the pulpal structures of an incisor from the older age group (40 to 70 years). Centrally located major vessels are visible, although interrupted, and the subodontoblastic capillary plexus is absent (original magnification ×100). (Reprinted from Bennett et al[116] with permission.)

Sensory nerves

The innervation patterns of pulpal neurons and their responses to injury are described in chapter 7. In general, it should be appreciated that nociceptor responses to tissue injury protect the individual from harm. When that response is diminished or absent, damage to the tissue may occur, necessitating clinical intervention to prevent permanent damage and loss of function.

The nerve supply to the dental pulp is a unique tissue in humans in that its afferent innervation is almost entirely nociceptors, including Aδ and C fibers. A reduction in the nerve supply to teeth is well documented to occur with age. This loss appears to parallel the age-related loss observed in vascular supply described earlier. Indeed, the afferent blood supply and the nerves constitute a neuro-vascular bundle that generally terminates together peripherally.

Prior studies have demonstrated age-related mineralization (calcification) of the endoneurium and perineurium and ultimately the nerve proper and the loss of the plexus of nerve fibers (plexus of Rasch-kow) that is located in the subodontoblastic layer. In addition, the number of myelinated fibers diminishes with age, and both myelinated and unmyelinated fibers show an increased threshold of response to stimulation. This increased threshold for stimulation probably is due to both a loss of the total number of fibers and a reduced terminal arborization of remaining fibers.

Detailed studies of age-related changes in pulpal sensory neurons have been conducted in cats. In the incisor, the number of fibers (axons) increases with age until about the third year and then diminishes thereafter. The diameters of axons and nerve fibers become reduced in size, as does the length of the internode segments. Part of the reduction in pulpal nerve supply may be the result of reduced numbers of receptors to nerve growth factor (NGF), which serves as a trophic factor for a substantial proportion of nociceptors.

Two effector substances (neuropeptides) important in mediating the inflammatory response— calcitonin gene–related peptide (CGRP) and substance P—also diminish with age. This may weaken the potential for neurogenic inflammatory and healing responses in aged pulp tissue (see chapters 7 and 8). Collectively, these

observations show a clear loss of pulpal nerve supply with advancing age. These changes may result from some underlying mechanism of aging that acts on neurons in a fundamental manner. More likely, however, they result from a combination of age-related events including compression (as the pulp space becomes smaller due to secondary dentin formation), fibrosis and calcification, the loss of blood supply, and the diminution in the fibroblast and odontoblast population (sources of neurotrophic factors).

One study examined pulpal neurons from 150 teeth in individuals aged 40 to 70 years.[121] Calcification occurred in 90% of all pulps. This was first seen in isolated regions in the endoneurium and perineurium of nerve fibers, although older specimens were characterized by mineralization of the entire endoneural or perineural connective tissue and often appeared to have a calcified ring around the nerve fibers. The younger control group had no calcifications and demonstrated evidence of pulpal nerve branching in the subodontoblastic plexus near the pulp horn region (Fig 18-10a). In the sample of older pulps, there was a decreased overall number of nerves, with few fibers seen in the subodontoblastic plexus areas (Fig 18-10b). The nerves that persisted in the pulps of older teeth were often characterized by degenerative changes including fragmentation, beading, and reticulation. This decrease in nerve quality and quantity may explain the reduction in pulpal sensitivity anecdotally reported in aging individuals.

Fig 18-10 *(a)* Thick section of the coronal portion of the pulp of a premolar from a teenage individual. There is extensive branching of the pulpal nerves and a rich subodontoblastic network. B, blood vessels; PN, pulpal nerve; N, nerve fascicularis (Verhoeff's iron hematoxylin stain; original magnification ×50). *(b)* Thick section of the coronal portion of the pulp of a molar tooth from a 50-year-old individual. Only the pulpal nerve is demonstrable; the cuspal branches and subodontoblastic network are absent. PN, pulpal nerve; B, blood vessels (Verhoeff's iron hematoxylin stain; original magnification ×50). (Reprinted from Bernick[121] with permission.)

A quantitative assessment of sensory nerve development in human premolars was made in teeth from subjects in four age groups (11 to 15 years, 20 to 35 years, 35 to

50 years, and 50 to 71 years).[122,123] The teeth were tested with an electrical pulp tester prior to removal and then examined for numbers of axons present in the apical third of the pulp. The results showed that myelinated axons were greatest in number in subjects 15 years of age or older, with a trend to fewer myelinated axons in the older age groups (Fig 18-11). In older teeth, small, unmyelinated axons predominated. Younger teeth, with larger numbers of myelinated and unmyelinated fibers, had a lower threshold of response when tested with an electrical pulp tester than did older teeth with fewer fibers.

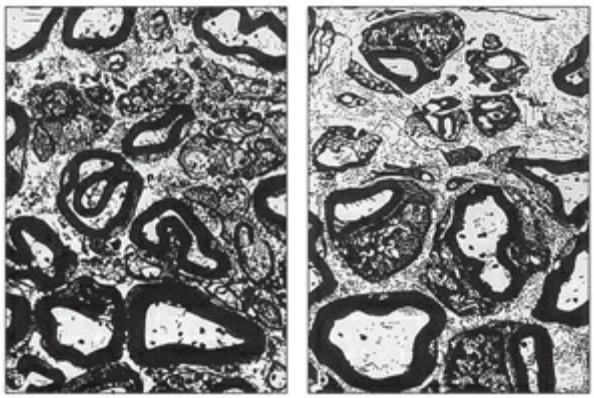

Fig 18-11 *(a)* Electron micrograph of a cross section of axons in a premolar from a 15-year-old subject. Unmyelinated axons form groups of varying sizes (original magnification ×6,000). *(b)* Electron micrograph of a cross section of axons in a premolar from a 34-year-old subject. Unmyelinated axons are usually in small groupings (original magnification ×6,000). (Reprinted from Johnsen et al[123] with permission.)

A study in cat incisors showed an increase in the number of axons in the apical region up to the third year and reduced numbers of myelinated axons from 3 to 11 years of age.[124] These axons were also smaller in relation to myelin sheath thickness. The inferior alveolar nerve underwent retrograde changes, probably due to pulpal axon degeneration, and the axons appeared to be of a rather transient nature. In a comparison study, the same results were seen in feline primary incisors but with the added aspect of resorption.[125] One possible explanation for the pulpal axon degeneration seen in these teeth could be the early disappearance of odontoblasts, as the axons are closely associated with these cells.

In another study, pulps from five healthy cats (3 to 10 years of age) displayed an age-related decrease in the number of pulpal axons, nerve fiber diameters, and intermodal lengths.[126] The decrease in intermodal lengths may be due to demyelination and remyelination, as aging fibers exhibited qualitative changes in their myelin sheaths. Age changes in the peripheral nervous system may be related to neuron aging, giving rise to distal axon degeneration, but may also be due, as noted,

to age-related changes of the target tissue.

There is an age-related change in the ratio of myelinated to unmyelinated neurons in dental pulp. One study evaluated the variation in numbers of pulpal myelinated nerves in subjects 10 to 72 years of age and reported that the total numbers decreased with age, particularly at the expense of myelinated fibers.[127] The decrease in sensory A fibers may be related to reduced sensitivity to the perception of dental pain in aging patients. Another related study measured myelinated and unmyelinated axon populations in the dental pulp and reported that the pulps of older individuals showed a loss of small Aδ fibers and unmyelinated C fibers, with a proportionately greater decrease in the number of C fibers.[128]

Fried[129] has reviewed these findings and reiterated that aging involves both structural and neuro-chemical regressive changes in pulpal innervation. He notes marked age-related decrease in pulpal CGRP- and substance P–like immunoreactivity together with a reduction in NGF receptor–like immunoreactivity. These changes in neuropeptide expression are not entirely due to loss of nerve fibers because most aging pulps contain NGF receptor– positive fibers but lack neuropeptide expression. It is important to understand that these changes appear as a physiologic response to aging.

However, an important question remains: What is the functional significance of an age-related reduction in peripheral neuropeptide levels? It is well recognized that CGRP and substance P modulate neurogenic inflammation and healing (see chapters 7, 8, and 11). Comparatively few studies have evaluated age-related decreases in neuropeptide response and modulation of inflammation caused by operative cavity preparations. One study compared pulpal neuropeptide responses to tooth preparations in young (3- to 4-month-old) and old (1- to 2-year-old) rats.[130] Examination of the control pulp revealed that older animals had a substantial reduction in levels of the low-affinity nerve growth factor receptor (p75-NGFR). The authors concluded that this might be a biochemical marker of pulpal aging. After injury, the neuropeptide levels were increased in both young and old rats in pulp adjacent to the Class V preparation site.

The older animals displayed a greater proportional neuropeptide response near the injury than did the younger animals. This enhanced response could be due to reduced pulpal volume or to lower basal levels. In either case, the results indicated that older rats exhibit appropriate neuropeptide responses to tissue injury despite several age-related tissue changes.[130]

A related study analyzed innervation of pulp in young (6- to 8-week-old and 3-

month-old) and older (5- to 12-month-old) mice with a null mutation in the p75 gene and compared the results with age-matched, wild-type controls.[131] The results showed intense CGRP immunoreactivity in pulpal nerve endings of mutant mice but a gradual decrease in CGRP intensity in controls during aging. Collectively, these studies indicate that there is an age-related reduction in peripheral neurons and neuropeptide expression but that the aged pulp can mount an appropriate neuronal response to tissue injury.

Response to pulp testing

Although an age-related reduction in the density of pulpal afferent fibers may mediate reduced sensitivity, pulpal injury may still cause pain in the elderly. Indeed, the assessment of orofacial symptoms and pain intensity was found to be the best predictor of whether elderly individuals utilize dental care, suggesting that some level of nociceptor activation may still exist in the elderly and prompt the seeking of such care.[132]

In contrast to the prevalence of age-related anatomical studies, there are relatively few studies evaluating pulpal response to testing in the elderly. Anecdotal evidence indicates that dental procedures evoke less discomfort in older individuals and that the requirement for local anesthesia is often reduced.

Maturational status of the tooth is important from the standpoint of responsiveness to pulp testing. Pulp testing is often ineffective in older patients, especially if they are extremely aged and their teeth have a history of caries and restorations (ie, due to tertiary dentin formation and loss of innervated dentin).[133,134] Others have replicated this finding of reduced responsiveness to thermal testing in the elderly.[135,136]

However, this age-related reduction in pulpal responsiveness may be modality specific; in most (but not all) studies, no differences in pain threshold to electrical stimulation are found between young and elderly patients.[137,138] A stimulus-dependent difference in pulpal pain perception may be due to aging-related changes in pulp anatomy. Secondary dentin deposition, narrowing of the pulp chamber, and closing of tubules could decrease thermal conductivity, leading to a longer latency or the need for a greater stimulus intensity to reach a temperature level sufficient to activate primary afferent nerves. Changes in the structure of secondary dentin that

limit fluid movement in dentinal tubules may also explain why older subjects are less responsive to cold stimulation.

Most recent studies concerning dental pulpal response to various stimuli report the use of various devices (pulse oximetery, laser Doppler flowmetry, temperature change) to detect and provide a response or nonresponse ending with a definitive diagnosis. However, because limited data have been gathered on the correlation between pulpal nerve activity and sensation from intact human teeth, a study used microneurography to examine this relationship to determine changes in response as a function of age. Three groups of individuals were tested: 18 years, 38 years, and 64 years of age.[139] Ratings of perceived pain intensity to thermal stimulation were made using a visual analog scale. Findings showed that mean conduction velocities correlated closely with age. With advancing age, the percentage of subjects whose teeth were not sensitive to thermal stimulation increased. In older tooth pulps, the decrease in number of fast-conducting afferents and mineral apposition of tubules impaired nerve activation, especially by heat, as per the hydrodynamic mechanisms (Fig 18-12).

Finally, the decreased vascularity and nerve content of the pulp itself must also contribute to decreased response to stimulation in older individuals.

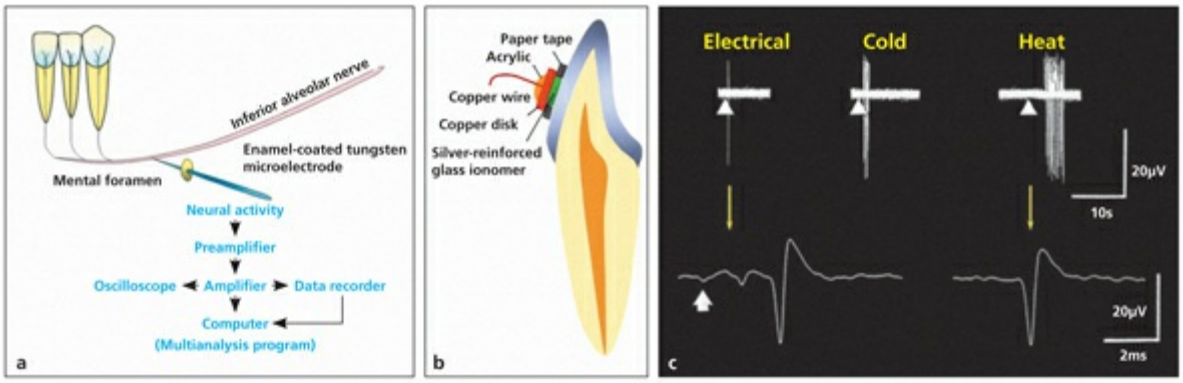

Fig 18-12 *(a)* Experimental setup for microsurgery. Neural activity is recorded with an enamel-coated, needlelike tungsten microelectrode inserted precutaneously through the mental foramen into the inferior alveolar nerve. (Reprinted from Ikeda and Suda[139] with permission.) *(b)* Setup for pulpal testing with electrical stimulation and thermal testing (heat and cold). The stimulating electrodes were copper disks soldered to copper wires placed on the center of the labial surface of the dried tooth and cemented with silver-reinforced glass ionomer (which has impedance significantly lower than that of other materials). (Courtesy of Dr H. Ikeda and Dr H. Suda.) *(c)* Electrical readout of results from electrical and temperature stimulation. (Courtesy of Dr H. Ikeda and Dr H. Suda.)

Pulp stones

Pulp mineralization is commonly observed on bite-wing radiographs. Several factors can induce this pulpal response, and aging is one of them.

Two types of calcified bodies in the dental pulp have been described[140]: *(1)* a full mass filling the pulp chamber with epithelial remnants surrounded peripherally by odontoblasts and *(2)* several pulp stones, compact degenerative masses of calcified tissue. Many studies confirm the influence of aging on pulp stone formation. In a study of 519 patients (with an age range of 18 to 54 years) and 13,474 teeth, Gulsahi and coworkers[141] confirmed that the prevalence of pulp stones increases with age and is significantly higher in molars than in premolars and incisors. They could not find any significant relationship between pulp stones and gender, systemic diseases, impacted third molars, condition of the crown, or dental anomalies. These results were confirmed in another study by observation of panoramic radiographs of 247 patients.[142] Although pulp stones can be related to aging, no relationship has been found between pulp calcification and renal disease or carotid artery calcification.[142–144]

Dentinogenesis

As noted earlier, secondary dentin formation is a major contributing factor to the volumetric loss (compression) of the soft tissue space of the pulp (see chapters 2 and 3). In secondary dentin formation, odontoblasts deposit new dentin in a process that appears to occur continuously throughout adult life, even in the absence of dental trauma and infection. This distinguishes secondary dentin from tertiary dentin, which forms only in response to injury or infection.

Studies of secondary dentin formation in erupted teeth indicate that the process begins in the coronal portion of the tooth and extends apically over time. In contrast, impacted but otherwise apparently healthy teeth show the reverse pattern, with most of the new dentin formation occurring apically.[145] The constriction of the pulp space as a result of secondary dentin formation does not occur uniformly around the interior root surface. For example, in subjects covering an age range of 10 to 97 years, it was found that the pulp chambers and root canal spaces became constricted in the mesiodistal but not the faciolingual direction.[96,146]

The effect of aging on the patency of dentinal tubules depends on the cumulative effect of age-dependent (eg, secondary dentin) and age-independent (eg, tertiary

dentin) processes. One study reported no difference in tubule diameter and tubule density between younger or older subjects (8 to 25 years old versus 40 to 60 years old).[147] A reduction in dentinal tubules may occur in still older individuals (older than 60 years of age) or may occur in relation to deposition of tertiary dentin (see chapters 2 and 3).

Age is the principal determinant (risk factor) for the diminution of pulp size over time. The rate of pulp size reduction (secondary dentin formation) may become slower in the elderly and may be slower in women than in men.[148] Although a reduction in secondary dentin formation may be related to a smaller or less active pool of odontoblasts, gender-related differences have been attributed to endocrine factors.[96]

Various techniques have been utilized to study physiologic deposition of secondary dentin throughout life. One study evaluated ground sections from 273 maxillary central incisors divided into 14 5-year age groups and demonstrated an age-related increase in cumulative secondary dentin formation.[149] Young teeth (6 to 11 years old) demonstrated metamorphosed (transparent) tracts at the incisal and cervical areas. Teeth from teenagers (11 to 15 years old) showed initial signs of irregular dentin formation (fewer tubules) (Fig 18-13a), and the prevalence of irregular dentin increased in older individuals as dentin was deposited on the lingual walls of the coronal samples. The oldest teeth had sufficient amounts of secondary dentin to virtually obliterate the coronal space (Figs 18-13b and 18-14). Two types of secondary dentin apparently were formed slowly: *(1)* transparent dentin increasing from the periphery of the crown and root inward toward the pulp with increased age and *(2)* secondary dentin increasing circumferentially in the remainder of the coronal pulp space.

Another study used light microscopy to measure the formation of secondary dentin in 240 central incisors.[151] Teeth from subjects older than 30 years demonstrated increasing deposits of secondary dentin.

Several studies have attempted to use these structural features to develop a method to identify the age of a tooth.[152,153] Of the various indices assessed, the transparency of radicular dentin and the magnitude of secondary dentin formation had the highest direct correlation with age. Given the apparent steady closure of the pulp chamber with age, others have suggested that measurements of pulp chamber or root canal dimensions can be used either as a biomarker for the aging process or to establish, from a forensic perspective, the age at death of cadavers or skeletal specimens.[153] However, the reliability of this measurement has been questioned

because the teeth of certain aging individuals show no detectable change in canal size even when followed for a period of more than 10 years.[146]

Other investigators have focused on using the accumulation of mineralized peritubular dentin as an index of aging. Peritubular dentin forms centripetally in dentinal tubules as an individual ages, reducing tubular diameters in older people.[145,154] Studies in human teeth indicate that the thickness of peritubular dentin, but not tubular diameter, could be used as an indicator of age. Others have suggested that the relationship between the two factors may be a parameter for age estimation.[155]

Fig 18-13 *(a)* Low-power photomicrograph of an unstained central section of a maxillary central incisor ground in a labiolingual direction. The appearance is typical of that of the 11- to 15-year age group. The primary dentin and the apex are completed. There is no attrition, but the earliest stage of irregular secondary dentin formation is visible, mostly on the lingual wall of the pulp chamber. The transparency of the primary dentin of the root is unusual (original magnification ×20). *(b)* Low-power photomicrograph of a maxillary central incisor ground in a labiolingual direction. The central incisor is from a subject in the 71-year and older age group. The irregular secondary dentin fills the entire pulp chamber of the crown and extends down to the apical third of the root canal. This figure shows the culmination of the trend toward increase of irregular secondary dentin formation and metamorphosis of primary dentin with age (original magnification ×20). (Reprinted from Philippas and Applebaum[149] with permission.)

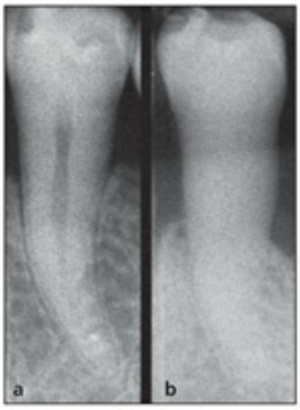

Fig 18-14 Radiographs of the same individual at 58 years old *(a)* and 77 years old *(b)*. Note the almost

complete obliteration of the root canal space coronoapically. No caries, restorations, or extreme occlusal wear is present. (Reprinted from Woo[150] with permission.)

Senescent changes in teeth are not the consequences of trauma or infection but everyday usage and appear to be unavoidable. This point was stressed by Ketterl[104] in a comprehensive review of age-related changes in the mineralized and soft tissues of the tooth, including the adjacent periodontium. For example, older pulps have increased mineral content in teeth with both intact and worn coronal dentin.[156] In the pulp, the number, nature, properties, and capabilities of the cells change over time.[76] Deposition of secondary dentin causes narrowing of the root canal space,[157] reducing the amounts of dentinal fluid and dentinal sensitivity. Taken together, the changes occurring in the pulp and dentin, while physiologic in nature, may increase the need for clinical interventions to preserve the teeth.

The rate of formation of secondary regular and irregular dentin may differ after dental restorative procedures. In a study with monkeys, restorative procedures evoked a fourfold greater rate of tertiary dentin formation than secondary dentin formation[158]; other studies, however, have confirmed the physiologic deposition of secondary dentin in relation to aging.[139,159] In a study of impacted third molars, the rate of secondary dentin formation was not linear.[160] While there were increases in the thickness of secondary dentin over time, the increases did not approach those seen in erupted teeth, indicating that the formation of secondary dentin may be a cumulative process occurring in functioning teeth.[161]

Several studies examined pulp cell population in rats, demonstrating reductions in odontoblasts and pulpal fibroblasts over time. Histomorphometric analysis found decreases in cell density in the incisors of Wistar rats.[162] Irrespective of age, odontoblast and subodontoblast cell density decreased significantly with increasing age, whereas fibroblasts showed small but significant increases with age.

Animals can also be used to determine morphogenic changes in the size of pulp cells over time. Rat molars were perfused with gluteraldehyde at 19 days to 24 months, and sections were processed for light and transmission microscopy. Odontoblasts changed from a tall, columnar morphology in the coronal pulp chamber to a more cuboidal or flattened shape near the apex.[163] Using fluorogold to examine tooth permeability, a study was designed to test the effects of aging on vital rat teeth.[164] Cavity preparations were placed in rat molars, and changes in cellular content and structure were found to be age related and regulated by odontoblasts.[165] Other changes found in neural activity were determined to be injury produced from

cavity preparation.

Lastly, analysis of the proteins and proteinases involved in dentin formation may aid in understanding the mechanisms involved in aging processes. In dentin, synthesis of complex specialized extracellular matrices precedes mineralization (see chapters 2 and 3). While dentin, unlike bone, generally is not remodeled, the unmineralized predentin is remodeled in response to a stimulus (eg, caries, trauma). MMPs have been implicated in the physiologic remodeling of the dentin extracellular matrix.[166] A recent study examined dentin proteins extracted from the permanent molars of patients aged 15 to 73 years for analysis of the MMP gelatinase A. This enzyme has been implicated in the mineralization of predentin to form dentin.[167] The data demonstrated that MMP gelatinase A was present in most teeth of individuals 20 years of age or younger but only in one tooth of one patient 30 years old and in no teeth in subjects 40 years of age or older. The results suggest that the capacity to mineralize predentin may decrease with age, rendering the teeth of elderly individuals less protected from external stimuli.[168]

Intratubular and intertubular dentin formation

In addition to secondary dentinogenesis and reduction of the pulp space, a further process of dentin secretion occurs throughout tooth life. There is gradual growth of peritubular dentin along the entire length of the tubules together with intratubular mineralization in the most pulpal part of the dentin. Intratubular dentin formation reduces the size of the tubule, sometimes leading to full occlusion or total sclerosis of the dentinal tubules. The mineral deposit is composed of rhombohedral crystals of whitlockite. This process progresses with age from the apical third toward the cervical area, obviously in conjunction with retraction of the odontoblast process. Intertubular and intratubular dentin secretion is under the control of odontoblasts, whereas the other mechanism for occlusion of tubules is mineral precipitation.

Differences in the peritubular organization between coronal and radicular dentin are noticeable, and these have clinical implications. The density of tubules and their diameter varies considerably. The variation in size occurs because peritubular dentin increases as the distance from the pulp increases. At the inner surface of coronal dentin, no significant difference is noticeable between young and aged teeth, either in the number or the diameter of tubules. The change in size of the tubules is

directly related to the permeability of the dentin, which has several clinical implications.

In the root portion, a reduction in the size of the tubules as a function of patient age was demonstrated by Pouëzat in 1975.169 The average size decreased from 3.75 µm in 18-year-olds to 1.50 µm in 65-year-olds. The mean increase in the volume of intertubular dentin from the younger group to the older group was 18%.

Another age-related process is the absence of the odontoblast process in the tubules 0.7 mm beyond the pulp, which Osborn and Ten Cate considered to be an essential requirement of advanced histology.[170]

Dentin maturation is governed by increasing intertubular dentin thickness, by the formation of peritubular dentin, and by intratubular sclerosis by apatite or whitlockite precipitation. Progressively with age, the peripheral tubules are replaced by peritubular and intratubular dentin formations.[91]

Clinical Implications of the Aging Pulpodentin Complex

Dentin is a permeable tissue, permitting the passage of fluid, molecules, and bacterial toxins in pathologic conditions. This permeability is extremely important to support the physiology and homeostasis of the pulpodentin complex because the presence of dentinal fluid inside the tubules maintains the vitality of this tissue. Any change of permeability under physiologic (such as aging) or pathologic (such as caries or infection) conditions perturbs the homeostasis and can lead to pulpal disease, notably inflammation. Maintaining the patency of the tubules is a key factor in maintaining the permeability, and conditions that modify this patency can have clinical repercussions.

As detailed earlier, tooth aging involves a gradual increase in dentin mineralization and a reduction in tubule diameter. When tubule occlusion is complete, the structure is described as being transparent under a light microscope and is associated with reduced permeability of the tissue. The typical clinical manifestation is diminution of pulpal expression in old teeth compared to newly formed ones because of the reduction in permeability.[171] Although this diminution of permeability can be considered an advantage in preventing the movement of bacteria and toxins into the tubules, the reduction in size of the tubules is also a limiting

factor for restorative considerations. Because most bonding systems require the penetration of the resin to create tags inside the dentin thickness, the partial sclerosis of the tissue considerably reduces the retention value of these products.

With regard to endodontic considerations, because bacteria are the cause of apical periodontitis, all potential niches of bacterial retention are an obstacle to bone healing. Reduction of size of the tubules is a limiting factor in bacterial penetration to the depth of the dentin and can be considered an advantage. In a recent study of 56 teeth of two different age groups (18 to 25 years versus older than 60 years) with 20 days of bacterial incubation in the canals, the results suggested that bacterial penetration inside the tubules occurred to a lesser extent in the older patients.[172]

These results are in accordance with the findings of another study of teeth from four different age groups (younger than 30 years, 30 to 45 years, 46 to 60 years, and older than 60 years) involving dye penetration. After 1 and 30 days of dye penetration, significant differences were found among teeth of the different age groups, but only in the apical third. The dye-penetration areas systematically decreased with increasing age and from coronal to apical. Although dye-penetration techniques are controversial, these results tend to confirm the age dependence of dentin permeability.[173]

However, if the reduction in size of the tubules is a limiting factor to bacterial penetration, it also appears that the penetration and efficiency of disinfectant solutions are more limited in teeth of older patients than in teeth of younger ones and that biofilm is more difficult to eliminate from mature teeth.[174]

Radiographic signs of aging

Radiographs have been used in the diagnosis of pulpal and periradicular diseases. They are equally important in defining the anatomy of root canal systems and the presence of calcification that may influence treatment options (see Fig 18-11). Although radiographs do not reveal the actual types of dentin present in calcified root canal systems, they provide a measure of age-related changes occurring in root canal systems due to secondary or tertiary dentin formation. Cross-sectional radiographic surveys indicate a consistent reduction in metrics used to measure pulp chamber and root canal dimensions.[156–158,165,175,176] One study compared tooth types

to determine which type provides the most reliable data in age estimation.[175] Canines showed the highest correlation with patient age, and the radiolucency of the root was the variable most clearly related to the patient's age.[177]

More recent studies have demonstrated that teeth provide several useful reference points that may indicate an individual's age. Secondary dentin is deposited along the walls of the pulp chamber and roots, leading to a reduction in the size of the pulp chamber. The deposition of this form of dentin is a physiologic process, and it can be measured indirectly as a function of chamber size using various radiographic methods and films.

These studies confirm that radiographs can be utilized to detect changes occurring in root canal spaces in aging individuals. The importance of this effort lies in the clinical application of the findings to tooth retention. It appears that treatment modalities currently in place were developed for use in generally young patient populations using approaches that may not work for the elderly individual. Thus, without interventions at an earlier time, the elderly may require complicated, lengthy endodontic surgical procedures or risk losing teeth. In addition, elderly patients may have serious health problems that make such procedures risky.

Effects of pulp capping procedures in aging tissue

The success of pulp capping procedures depends on many factors, including the size of the exposure, extent of bacterial infection, materials and techniques employed, and preparation of a clean and disinfected surface (see chapter 13).

Most clinical investigations on pulp capping are conducted in young to early middle-aged patients; comparatively few studies include older patients who qualify as aging to elderly individuals. One study evaluated the success of pulp capping in 149 patients (aged 8 to 74 years) with a minimum 5-year follow-up.[177] The authors found an overall success rate of 87.3% and reported that patient age at the time of pulp capping was not a factor in success or failure of the treatment. Another study evaluated the success of pulp capping in 148 patients (aged 16 to 67 years) with a 3-year follow-up.[178] These authors found a similar rate of clinical success (88%) and reported that older patients also had similar success after pulp capping. Similar results have been reported by most, but not all, clinical investigations evaluating the efficacy of pulp capping in a broad range of patient age groups (10 to 70 years).[179]

Thus, age as a prognostic factor does not appear to play a decisive role in success or failure of pulp capping procedures. This is probably because pulp capping procedures are only performed on teeth with a patent, radiographically visible pulp chamber.

Future Directions

Dramatic demographic changes have led to a recognition of the importance of the aging process as it relates to oral health. More information is essential if the dental profession is to develop the strategies necessary to diagnose and treat an increasingly older patient population. As humans live longer, tissues undergo age-dependent and age-independent changes. This necessitates an understanding of the aging process and its impact on local conditions (caries) and systemic conditions such as diabetes or coronary artery disease (see chapter 20).

Too often we face situations that remind us of the time when teeth were removed for no better reason than no one knew that they could be retained. Not too many years ago, the dental pulp under "stress" signaled the demise of the tooth, leading to its removal and replacement (or not) with a prosthetic appliance. More recently, improved instrumentation and clinical procedures have allowed treatment of such clinically compromised teeth, leading to their retention as useful and pain-free contributors to the masticatory process. The continued development of basic and clinical science procedures and products based on aging mechanisms and their impact on the dental pulp are likely to lead to more effective oral care of the aging patient.

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Differential Diagnosis of Toothache:Odontogenic Versus Nonodontogenic Pain

Jeffrey P. Okeson, DMD

Epidemiologic studies indicate that toothache represents the most prevalent form of orofacial pain, with about 12% to 14% of the population reporting a history of a toothache over a 6-month period.[1–3] Although these studies indicate that dental pain is the most commonly reported form of orofacial pain, it should be recognized that perception of a toothache may not always have its origin in the dental structures. Thus, pain management begins with an accurate differential diagnosis of the origin of the pain. This is a critical first step in pain management because effective treatment must be directed toward removing or controlling the underlying cause.

Understanding the basic science of pain mechanisms and the therapeutics of pain management is a major theme in pulpal biology. Accordingly, this text includes detailed reviews of common etiologic factors (chapter 10), inflammatory responses in pulp (chapters 7, 8, and 11) and periradicular tissue (chapter 12), the

neuroanatomy and neurophysiology of dental pulp (chapter 7), pain mechanisms in pulp tissue and the brain (chapter 8), and strategies for pain management (chapter 9). This chapter contributes to that foundation of knowledge by focusing on the differential diagnosis of odontogenic and nonodontogenic dental pain.

In recent years, there has been significant interest in pain research, leading to the development of new theories, diagnostic tests, and treatment strategies. Major references on this topic are available to the interested reader.[4–6] The primary purpose of this chapter is not to provide a broad overview of this topic but instead to focus on strategies for developing a differential diagnosis of odontogenic and nonodontogenic dental pain. Other sources should be reviewed for an extensive discussion of etiology, diagnosis, and management of various nonodontogenic pain disorders.[7–16]

Referral of Pain

Because dental pain is such a common cause of orofacial pain, the clinician can be easily drawn to an odontogenic diagnosis. This is especially true when the patient convincingly reports that the pain is felt in a particular tooth. It is a frustrating experience for both the patient and the clinician when toothache continues long after sound dental treatment has been completed. It is not uncommon to hear patients report a history of multiple endodontic procedures followed by an extraction, without reduction of pain. This type of experience is quite humbling to the clinician who is expected to be successful in managing various types of dental pain.

The clinician must be aware that sometimes pain felt in the teeth does not originate from the dental structures. In other words, the site at which the patient perceives the pain (the tooth) is different from the source of the pain. These types of pain are broadly called *referred pains* and are relatively common in the face and oral structures. When a referred pain is felt in a tooth, it is classified as a *nonodontogenic toothache*. Nonodontogenic toothaches can pose significant diagnostic challenges to the clinician.

Theory of convergence

There are several proposed mechanisms that attempt to explain the nonodontogenic toothache. The most common is pain referral. Although several mechanisms for referred pain have been proposed (see chapter 8), a widely accepted hypothesis is convergence[17] (Fig 19-1). The convergence hypothesis proposes that certain afferent sensory neurons have peripheral terminals that innervate different tissues while their central terminals converge on the same second-order projection neuron located in the trigeminal nuclear complex. This hypothesis is supported by strong experimental data that indicate that afferent neurons from multiple peripheral tissues indeed have central terminals that converge on the same trigeminal projection neuron, which receives sensory input from dental pulpal neurons[18] (see Fig 8-17). In fact, it has been estimated that about 50% of all pulpal neurons converge with other neurons on the same trigeminal projection neurons.[19]

These heterotopic pains are further enhanced by the fact that the constant barrage of nociceptive input from deep structures alters the central neurons, lowering their thresholds.[20,21] This phenomenon, known as *central sensitization*,[22–26] is described in further detail in chapter 8.

The hypothesis of convergence has important clinical implications because it explains how the site of pain perception by the patient can be different from the location of the source or origin of nociceptor activation. In the example illustrated in Fig 19-1, the tooth is perceived as the site of pain perception but the source of nociception is actually the masseter muscle.

It is important for the clinician to understand that any source of deep pain input can lead to pain referral; in the head and neck, this referral may be felt in the teeth. It is equally important to realize that pain from pulpal nociceptors can be referred to other craniofacial structures, such as the preauricular region.[27,28] It is not uncommon for a patient experiencing significant pulpal pain to report that the pain is felt in a large portion of the areas innervated by the mandibular and maxillary divisions of the trigeminal nerve.

Other studies have demonstrated that, as pain intensity increases, the incidence of pain referral to other regions also increases. For example, a study of 400 patients with odontogenic pain indicated that its intensity, but not its duration or quality, was significantly associated with pain referral to other craniofacial structures[29] (Fig 19-2). In this study, about 90% of all patients reporting moderate to severe odontogenic pain (5 or more on a scale of 0 to 10) also reported pain referred to nearby craniofacial regions. Thus, as pain intensity increases, there is an increased likelihood that the patient will report pain in associated structures. The most

common areas of extraoral pain referred from first molar odontogenic pain[29] are shown in Fig 19-3. In summary, it is important to realize that craniofacial pain originating from nondental structures can refer pain to teeth, and, conversely, pain originating from pulpal nociceptors can refer pain to other craniofacial structures.

When a patient reports to the dental office with a complaint of pain, it is the primary responsibility of the clinician to find the source of the pain and direct treatment toward eliminating it. This is the only way the patient's pain can be eliminated. It is therefore imperative that the clinician appreciate the wide variety of presentations of orofacial pain. The clinician must have the diagnostic skills to be able to differentiate the *site* of pain from the *source* of pain. Patients will quickly report the location in which they feel the pain but have no understanding that this may not be the actual source of the pain. Clearly, referred pain can pose a significant problem for the clinician.

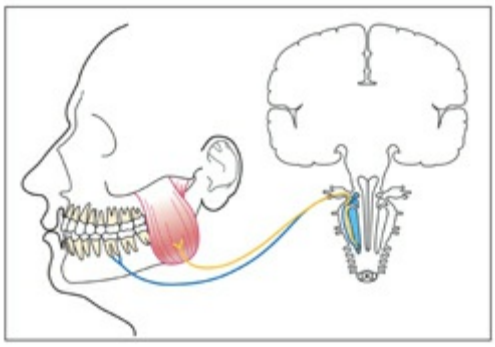

Fig 19-1 Convergence of afferent fibers from dental pulp on the same projection neuron in the trigeminal nuclear complex that receives afferent input from the masseter muscle. (Reprinted from Hargreaves and Keiser[17] with permission.)

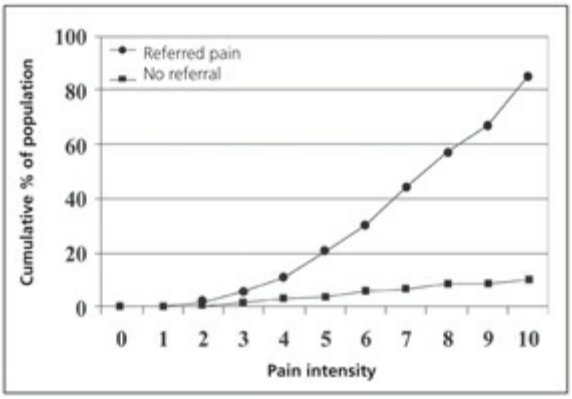

Fig 19-2 Relationship of pain referral to pain intensity in 400 patients reporting odontogenic pain (posterior teeth only). Pain intensity was measured on a 0 to 10 integer scale (0 = no pain, 10 = extreme pain). Referred pain was determined by how patients marked areas of pain perception on a mannequin. (Modified from Falace et al[29] with permission.)

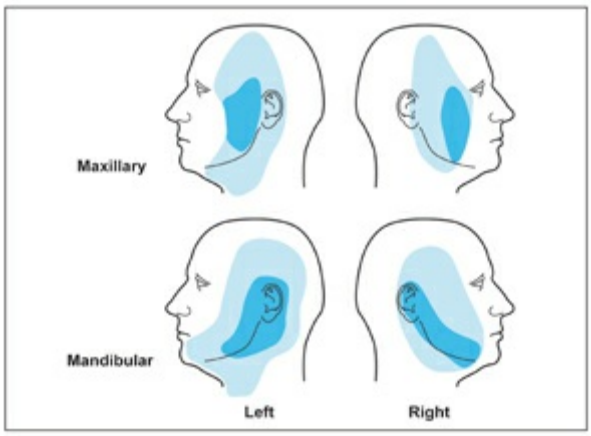

Fig 19-3 Extraoral referral patterns of pain originating from maxillary right (n = 27), mandibular right (n = 31), maxillary left (n = 38), and mandibular left (n = 47) first molars. The dark blue shading indicates the most frequent areas of pain referral; the light blue shading indicates less frequent areas of referral. Referred pain does not generally cross the midline. (Modified from Falace et al[29] with permission.)

Differentiation between site of pain and source of pain

There are four clinical rules that can help the clinician differentiate a site of pain from a source of pain (Box 19-1 and Fig 19-4). The proper use of local anesthesia can greatly assist in this task.[7,9,29–31]

Box 19-1	**Rules to exclude primary (odontogenic) and confirm referred (nonodontogenic) pain**
1. Local stimulation of the site of pain does not increase the pain. 2. Local stimulation of the source of pain increases the pain at both the source and the site of pain. 3. Local anesthetic blocking of the site of pain does not decrease the pain. 4. Local anesthetic blocking of the source of pain decreases the pain at both the source and the site of pain.	

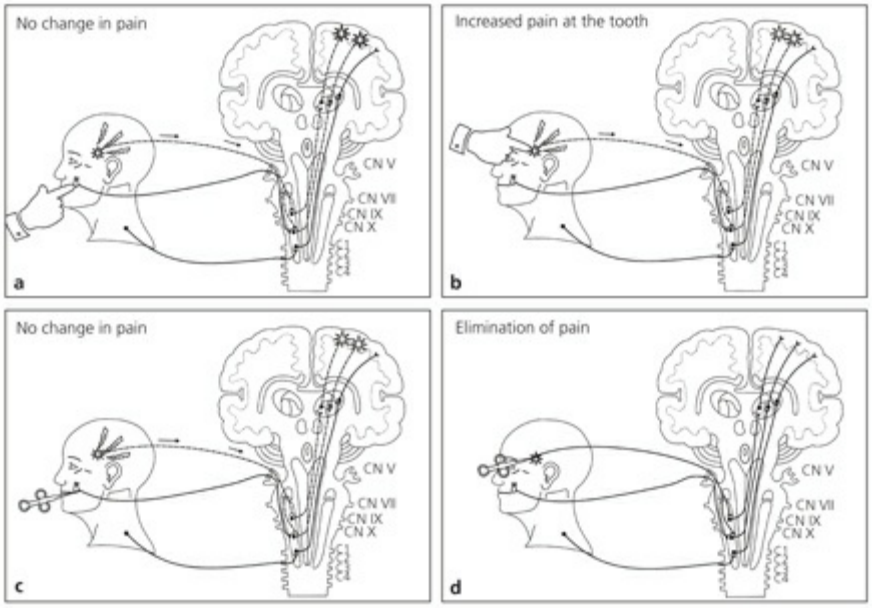

Fig 19-4 Four important clinical rules that help differentiate odontogenic toothache from nonodontogenic toothache. *(a)* Local provocation of the site of pain (a painful tooth) does not increase the pain. *(b)* Local provocation of the source of the pain (a myofascial trigger point in the temporalis muscle) increases the pain not only at the source but also at the site (increasing the tooth pain). *(c)* Local anesthesia of the site of pain (the painful tooth) does not decrease the pain. *(d)* Local anesthesia of the source of the pain (the temporalis trigger point) decreases the pain not only at the source but also at the site (tooth pain is eliminated). (Reprinted from Okeson[7] with permission.)

1. Local stimulation of the site of pain does not increase the pain

If the toothache is the primary source of pain, then provocation of the suspected toothache should increase the pain. Although thermal allodynia of the suspect tooth is a hallmark feature of pulpitis (odds ratio of 9.0 versus acute periradicular periodontitis) while mechanical allodynia is a hallmark feature of periradicular pain (odds ratio of 6.9 versus pulpitis), these provocative stimuli should still elicit pain when applied to the suspect tooth.[32] This is not the case with a nonodontogenic toothache. Because the perceived source of the toothache is not the same as the site of nociceptor activation, local provocation of the toothache (the site) will not change the pain. A lack of response to local provocation should be a key that makes the clinician suspect a nonodontogenic toothache.

2. Local stimulation of the source of pain increases the pain at both the source and the site of pain

When a toothache is suspected to be a site of referred pain, sometimes the true

source of nociceptive activation can be found relatively easily, such as with myofascial trigger point pain. Once a trigger point is identified, it should be stimulated and the patient asked if he or she feels the pain anywhere else. Often the patient will report pain not only in the area of palpation (the trigger point) but also radiating to the site of pain, the tooth. This clinical finding helps confirm that the perceived toothache is actually due to referred pain; treatment should be directed to the myofascial pain condition and not the tooth.

As discussed later in this section, some sources of pain that produce nonodontogenic toothache are not easily located or palpated, making this rule difficult to clinically demonstrate. Instead, the history should be used to gather information. Questioning the patient regarding conditions that exacerbate the toothache can be very valuable in identifying the true source of pain. In addition, understanding the characteristics of the pain (quality, duration, onset, etc) may also give the clinician insight as to the etiology of the pain source.

3. Local anesthetic blocking of the site of pain does not decrease the pain

The injection of a local anesthetic is a very valuable diagnostic tool in identifying the source of toothache. When a toothache is the primary source of pain, anesthetizing the tooth will immediately reduce or eliminate the pain. When this occurs, the dentist must identify the local cause of nociception (ie, pulpal, periradicular, periodontal, or mucosal) and provide the appropriate dental treatment.

However, when a local anesthetic fails to eliminate the toothache, referred pain should be suspected. The clinician has to be careful, however, as to how he or she asks the patient about the pain after anesthesia. Often the clinician will ask if the patient is "numb." The patient's answer will not provide the needed information because the patient will certainly feel numb in the tissues that have been anesthetized, that is, the tooth and adjacent soft tissues. The proper question is, "I know you are numb, but does it still hurt?" This is a different question and directed to the information needed to make the diagnosis. If the patient says, "I am very numb, but there is no change in my pain," then the clinician has reasonable evidence that the anesthetized tissues are not the structures that have to be treated to resolve the pain. The use of a local anesthetic is basic to differentiating dental pain and should be used whenever the source of pain is questionable.

Patients with severe pain in mandibular molars may not experience complete anesthesia following nerve block injection. Indeed, in one clinical trial, an inferior

alveolar nerve block produced successful anesthesia in only about 38% of patients with irreversible pulpitis of the mandibular molars.[33] The clinician may be misled to believe that the incomplete response to anesthetic injection indicates that the patient's pain is referred pain. The success rate of anesthetic for irreversible pulpitis of the mandibular molars improves to 88% when the inferior alveolar nerve block is combined with intraosseous supplemental injection.[33] Thus, the combined injections in these cases provide a reasonably good test of pain referral when patients continue to perceive pain even while the adjacent soft tissues are numb.

4. Local anesthetic blocking of the source of pain decreases the pain at both the source and the site of pain

When anesthetizing the tooth does not alter the toothache, the true source of the pain has to be identified. When the true source is found (as with myofascial trigger points), the source should be anesthetized (trigger point injection) for confirmation. Anesthesia of the true source will eliminate not only the source of pain but also the site of referred pain. Therefore, the toothache will be resolved immediately. When this occurs, the clinician has identified the true source of the toothache, and therapy should be directed toward resolving this source.

Although the use of local anesthetic with these rules can be very helpful, false-positive results are possible. Thus, the local anesthetic test must be considered in the context of the other findings in each case. The local anesthetic test provides only an approximate localization because multiple teeth are anesthetized even when injections are given by intraosseous or intraligamentary routes.[34,35] Similarly, if a clinician cannot reproduce the patient's chief complaint (eg, lingering pain to cold stimulus in a patient reporting thermal sensitivity), then alternative origins of the pain should be considered, including even teeth previously provided with nonsurgical root canal treatment.[36]

Odontogenic Toothache (Pulpal and Periradicular Pain)

Fortunately for the dentist, most patients reporting dental pain have symptoms that

are caused by dental structures (see chapters 7 and 8). Examples of odontogenic sources of toothache include reversible pulpitis, irreversible pulpitis, acute apical periodontitis, and acute apical abscess.[15] However, the clinician should always consider other potential sources of pain, especially when a local etiology is not obvious, to develop an accurate differential diagnosis for all patients.[13,32,37]

Common clinical features of odontogenic dental pain[11,13–15,37] are listed in Box 19-2 and are more thoroughly discussed in earlier chapters of this textbook. Although this list provides a useful summary of characteristic features of odontogenic pain, the skilled clinician will realize that not all patients present with exactly the same findings. Indeed, pain of pulpal origin is often characterized by difficulty in localizing the source of pain. In contrast, pain originating from acutely inflamed periradicular tissue is generally much easier for the patient to localize.[7] Thus, *common features* does not imply ubiquitous features, and several of those clinical findings listed in Box 19-2 are specific to certain diagnoses. For example, discriminant analysis of 74 patients with orofacial pain indicated that pain caused by pulpal pathosis is significantly associated with thermal allodynia (ie, temperature sensitivity; odds ratio of 9.0 versus apical periodontitis; $P < .001$), whereas pain caused by apical periodontitis is significantly associated with mechanical allodynia (ie, digital or percussion sensitivity; odds ratio of 6.9 versus pulpitis; $P < .01$).[32]

Box 19-2	Common features of odontogenic dental pain
• Presence of etiologic factors for an odontogenic origin of pain (eg, caries, leakage of restorations, trauma, fracture) • Ability to reproduce chief complaint during examination • Relief of pain provided by local anesthetic injection • Unilateral pain • Pain qualities: dull, aching, throbbing • Localized pain* • Sensitivity to temperature* • Sensitivity to percussion or digital pressure*	

*Diagnosis specific.

Nonodontogenic Toothache

Although nonodontogenic toothache is less common than odontogenic toothache, it is certainly common enough to be a clinical problem. The clinician must always

remember that effective management of the pain can only begin once the true source of pain has been located. Many structures of the face and neck can refer pain to the teeth. The clinician must understand these structures and the clinical characteristics of each because this knowledge will assist in making the correct diagnosis. Some of the common clinical findings for nonodontogenic toothache are listed in Box 19-3. More specific clinical symptoms are described for each type of nonodontogenic toothache in the following sections.

Box 19-3	Common features of nonodontogenic dental pain

- Absence of apparent etiologic factors for odontogenic pain (eg, no caries, leaky restorations, trauma, or fracture)
- No consistent relief of pain provided by local anesthetic injection
- Bilateral pain or multiple painful teeth
- Chronic pain that is not responsive to dental treatment
- Pain qualities: burning, electric shooting, stabbing, dull ache*
- Pain that occurs with a headache*
- Increased pain associated with palpation of trigger points or muscles*
- Increased pain associated with emotional stress, physical exercise, head position, etc*

*Diagnosis specific.

There are several classification schemes for categorizing orofacial pain.[7,16,38,39] Although these diagnostic schemes share many similarities, they are not identical, and they have not all been validated in large-scale, multicenter studies. Because the goal of this chapter is to provide a useful overview of those pain disorders that can contribute to nonodontogenic toothache, a full classification of orofacial pain is not reviewed. Instead, information is provided that will help the clinician identify the orofacial pain disorder likely to be responsible for the pain referral. Once the clinician understands that pain originating from each orofacial structure will present with different clinical characteristics, differential diagnosis becomes possible.

The most common pain conditions to trigger nonodontogenic toothaches are reviewed in this section. They are myofascial toothache, sinus toothache, neurovascular toothache, neuropathic toothache, cardiac toothache, somatoform toothache, and toothache of systemic origin. Subcategories of neurovascular and neuropathic toothaches are also described. Knowing the pain characteristics for each of these disorders is an essential key to diagnosis. The etiology of each of these sources of nonodontogenic toothache and the clinical characteristics that will assist in making the diagnosis are discussed. Management considerations are only briefly mentioned because other texts provide a more thorough review of management.[4,7]

Nonodontogenic toothache of myofascial origin

Etiology

Myofascial pain is a regional myogenous pain condition characterized by local areas of firm, hypersensitive bands of muscle tissue known as *trigger points*. It is a muscle pain disorder diagnosed in more than 50% of the patients reporting to a university pain center.[40]

Myofascial pain was first described by Travell and Rinzler[41] in 1952. This pain condition arises from hypersensitive areas in muscles called *trigger points*. These very localized areas in muscle tissues and/ or their tendinous attachments are often felt as taut bands when palpated, which elicits pain. The exact nature of a trigger point is not known.[42] It has been suggested[43,44] that certain nociceptors in the muscle tissues may become sensitized by algogenic substances that create a localized zone of hypersensitivity.[45] There may be a local temperature rise at the site of the trigger point, suggesting an increase in metabolic demand and/or increase in blood flow to these tissues.[46,47] A trigger point is a very circumscribed region in which relatively few motor units seem to be contracting.[48] Because a trigger point has activity in only a select group of motor units, no overall shortening of the muscle results. The unique characteristic of trigger points is that they are a source of constant deep pain and therefore can produce referred pain to a variety of facial structures, including the teeth.

The etiology of myofascial pain is complex. Unfortunately, we lack a complete understanding of this myogenous pain condition. It is therefore difficult to be specific concerning all etiologic factors. Simons et al[47] have described certain local and systemic factors that seem to be associated, such as trauma, hypovitaminosis, poor physical conditioning, and fatigue. Other important factors are likely to be emotional stress and deep pain input. Recent research has pointed toward polymorphisms of a gene whose protein (catechol-O-methyltransferase) is involved in catecholamine metabolism,[49] and it is possible that at least certain forms of myofascial pain may have a central etiology, which is reflected by a peripheral trigger zone rather than caused by a peripheral trigger zone.

On the other hand, two studies have been conducted in twins to determine the relative contribution of genetic (ie, anatomical or physiologic) versus environmental (ie, learned) factors to masticatory muscle pain and other temporomandibular dysfunction symptoms.[50,51] The authors reported little to no heritability for self-

report of any pain. Another study reported no significant genetic influence on the dolorimetric measurement of pressure pain thresholds in 609 female-female twins.[52] Thus, the relative contribution of genetic and environmental factors on the development of myofascial pain remains an active area of research.

Clinical characteristics

Myofascial pain is often described as a deep, dull, aching muscle pain that can be associated with referred dental pain. Classic studies[47] have demonstrated that three masticatory muscles commonly refer pain to the teeth. These are the superior belly of the masseter (to the maxillary posterior teeth) and inferior belly of the masseter (to the mandibular posterior teeth), the temporal (to the maxillary anterior or posterior teeth), and the anterior digastric (to the mandibular anterior teeth).[47]

In a series of 230 cases of patients with a diagnosis of temporomandibular dysfunction, 85.0% demonstrated referred pain with palpation of muscles or trigger points, and 11.6% of these patients had pain referred to the teeth[53] (Fig 19-5). Molars were the teeth that most frequently received referred pain from muscle or trigger point palpation, and the masseter muscles were the most common source. The tempo-ralis muscle and even the temporomandibular joint (TMJ) itself also commonly referred pain to the teeth. It is therefore important that the clinician palpate these muscles for potential sources of pain referral (Figs 19-6 to 19-10).

An interesting clinical feature of a trigger point is that it may present in either an active or a latent state. In the active state, it produces central excitatory effects. Therefore, when a trigger point is active, a toothache is commonly felt. Because referred pain is wholly dependent on its original source, palpation of an active trigger point (local stimulation) often increases such pain. Although not always present, this characteristic has extremely important diagnostic value.

In the latent state, a trigger point is no longer sensitive to palpation and therefore does not produce referred pain. When trigger points are latent, they cannot be found by palpation, and the patient does not complain of toothache. In this case, the history is the only data that leads the clinician to make the diagnosis of myofascial pain. In some instances, the clinician should consider asking the patient to return to the office when the toothache is present so that the pattern of pain referral can be verified and the diagnosis confirmed.

It is thought that trigger points do not resolve without treatment. They may in fact become latent or dormant, creating temporary relief of the referred pain. Trigger points may be activated by various factors,[47] such as increased use of a muscle,

strain on the muscle, emotional stress, or even an upper respiratory infection. When trigger points are activated, the toothache returns. This is a common finding among patients who complain of regular late afternoon toothache following a very trying and stressful day.

The following are the key clinical characteristics of toothache of myofascial origin:

- The pain is relatively constant, dull, aching, and nonpulsatile.
- The pain is not altered by local stimulation of the tooth.
- Examination reveals the presence of localized, firm, hypersensitive bands within the muscle tissues (trigger points).
- Other heterotopic pains are often reported (eg, tension-type headache).
- The toothache is increased with function of the involved muscle (trigger points).
- Palpation and stimulation of the trigger points increase the toothache.
- Confirmed anesthesia of the tooth does not alter the toothache.
- Local anesthetic injection of the involved muscle (trigger points) reduces the toothache.

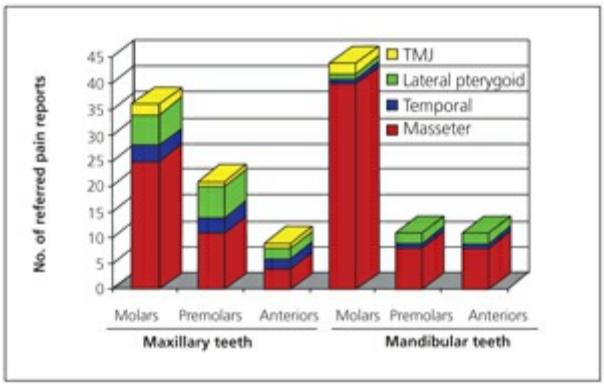

Fig 19-5 Frequency distribution of nonodontogenic dental pain referred to teeth after palpation of selected muscles in a group of 230 patients with temporomandibular dysfunction. Firm pressure was applied for 5 seconds to selected sites, and the patients were instructed to report on palpation-induced referred pain during stimulation. Muscles were palpated by applying sustained firm pressure while sliding the fingers along the length of the muscle. The entire body of the masseter muscle was palpated. The temporal muscle was palpated superior to the zygomatic arch. The lateral pterygoid muscle was palpated by applying the fifth digit along the buccal alveolar ridge just apical to the maxillary molars. The lateral pole and posterior aspect of the temporomandibular joint (TMJ) were palpated with the mouth open. (Data from Wright.[53])

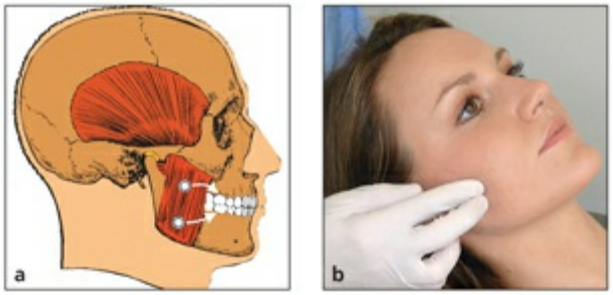

Fig 19-6 *(a)* Trigger points in the masseter muscle can refer pain to the maxillary or mandibular posterior teeth. *(b)* Palpation of the masseter muscle to assess for any pain that may be referred to the posterior teeth.

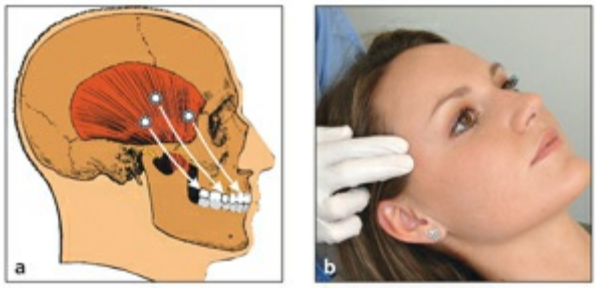

Fig 19-7 *(a)* Trigger points in the temporalis muscle can refer pain to the maxillary teeth. *(b)* Palpation of the temporalis muscle to assess for any pain that may be referred to the maxillary teeth.

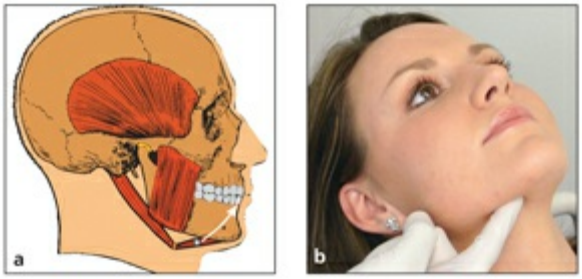

Fig 19-8 *(a)* Trigger points in the anterior digastric muscle can refer pain to the mandibular anterior teeth. *(b)* Palpation of the anterior belly of the digastric muscle to assess for any pain that may be referred to the mandibular anterior teeth.

Fig 19-9 Palpation of the sterno-cleidomastoid muscle to assess for any pain that may be referred to the face, TMJ, or teeth.

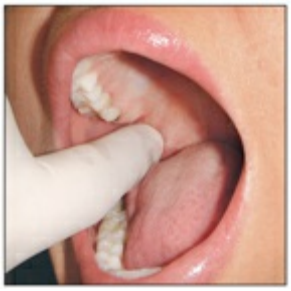

Fig 19-10 Intraoral palpation of the medial pterygoid muscle to assess for any pain that may be referred to the posterior teeth.

Management considerations

Several treatment modalities have been suggested for these patients. A partial list includes identification and elimination of contributory factors, mild analgesics, spray and stretch therapy, deep massage, biofeedback, deep heat, and injection of the trigger points. The specific combination of treatments depends on the diagnosis, the patient's responses, and the treatment philosophy of the clinician.[7,31,47,54]

Nonodontogenic toothache of sinus or nasal mucosal origin

Etiology

Pain arising from the nasal mucosa as the result of viral, bacterial, or allergic rhinitis may be expressed as referred pain throughout the maxilla and maxillary teeth in the form of a toothache. It may also display autonomic signs that are mistaken for symptoms of maxillary sinusitis. The so-called sinus headache may cause management problems because of the associated nonodontogenic tooth pain. The nonodontogenic pain is likely to arise from inflammation of the ostium, which compresses a significant concentration of nociceptors and often refers pain to the maxillary teeth.[55]

Clinical characteristics

Unlike odontogenic pain, nonodontogenic dental pain evoked by sinusitis is often characterized by pain that is not restricted to a single tooth (ie, pain may include the malar and maxillary alveolar regions), pain that may be partially relieved by a diagnostic intraoral local anesthetic block, pain that increases after percussion, and

occasional thermal sensitivity to cold.[5,9,56,57] Patients may report a sense of pressure or fullness in the infraorbital region over the involved sinus. A particularly distinguishing characteristic is the presentation of multiple maxillary posterior teeth with percussion sensitivity and a positive response to pulpal vitality testing. Reduction of pain after the intranasal application of a 4% lidocaine spray is considered diagnostic.[57] Alternatively, a swab soaked with 5% lidocaine can be placed in the middle meatus for 30 seconds for evaluation of pain reduction.[8]

Bacteria-induced sinusitis pain is characterized as a severe, throbbing, stabbing pain with a sense of pressure. More than 70% of cases are caused by *Streptococcus pneumoniae* or *Haemophilus influenzae*. [58] In cases of moderate to severe sinusitis, a positive "head-dip" test is observed (ie, pain increases when the patient lies down or places his or her head below the knees), and patients may report a purulent nasal discharge. Radiographic imaging of the sinuses, particularly a Waters view or computed tomographic scan, may reveal fluid accumulation.

Allergy-induced sinusitis tends to be seasonal in colder climates but can occur at any time in temperate climates. The pain is often characterized as a chronic, dull ache in the maxillary posterior region with a positive percussion test of molars or premolars. Interestingly, patients also may report an "itching" sensation in the maxillary teeth.

The following are the key clinical characteristics of nonodontogenic toothache of sinus or nasal mucosal origin:

- The patient reports pressure felt below the eyes.
- The pain is increased when pressure is applied over the involved sinus.
- The tooth is sensitive to percussion.
- The toothache is increased when the patient's head is lowered.
- The toothache is increased when the patient steps hard on to the heel of the foot (eg, while walking down steps).
- Local anesthesia of the tooth only partially reduc-es the pain or fails to reduce the pain at all.
- The diagnosis is confirmed by appropriate imag-ing of the sinuses (Waters view or computed tomographic scan).

Management considerations

Bacterial sinusitis is often treated with β-lactamase– resistant antibiotics such as amoxicillin with clavu-lanic acid or trimethoprim-sulfamethoxazole.[58] Allergic

sinusitis is often treated with antihistamines or decongestants.

Pain in the maxillary sinuses as well as in the maxillary and mandibular teeth also can be triggered by reduced atmospheric pressure. Several case reports have described odontogenic and nonodontogenic dental pain in patients during or after airplane flights or scuba diving.[59–62] It has been recommended that dental treatment, including root canal obturations, be completed more than 24 hours prior to exposure to reduced atmospheric pressure.[60]

There are several additional inflammatory conditions that may lead to a misdiagnosis. These include pain referred from another tooth (see chapter 8), impacted third molars,[63] eruption sequestra of bone,[64] otitis media, and foreign bodies impacted in periodontal tissues during mastication[65] or retained under surgical tissue flaps.[66] The correct diagnosis requires careful history taking, clinical examination, and interpretation of findings.

Nonodontogenic toothache of neurovascular origin

Neurovascular pain conditions are unique pain disorders only felt in the craniofacial structures. The intracranial vessels of the brain are primarily innervated by the trigeminal nerve and thus called the *trigeminovascular system*. Several pain conditions can arise from the trigeminovascular structures, two of which have been known to produce pain in the teeth. These are migraine and trigeminal autonomic cephalgia.

Migraine

Etiology

The precise etiology of migraine is still debated, but the present evidence suggests that there is a neurologic trigger in the brainstem that initiates a cascade of events that result in neurogenic inflammation of the cranial vessel, producing the headache.[67,68] For this reason, the term *neurovascular pain* is used. Although the exact mechanism and etiology are unknown, there is certainly evidence of a genetic susceptibility. Between 50% and 60% of migraine patients have parents that also experience migraines.[69,70] Migraine attacks can be precipitated by a variety of triggers, such as stress, dietary factors, altered sleep patterns, and menstruation.[71]

Clinical characteristics

Migraine is characterized by throbbing, moderate to severe, often debilitating pain. Sixty percent of the time the headache is unilateral, often reported in the temple or behind the eye. Migraine can be felt in the maxillary arch; this form is referred to as *midface migraine*. This type can be a diagnostic problem for the dentist because the pain can be felt in the teeth. The patient will often report photophobia, phonophobia, and osmophobia and will seek a dark, quiet room. The pain is aggravated by routine physical activity and sometimes even simple head movements.

Nonodontogenic tooth pain evoked by migraine can be differentiated from odontogenic pain in that the former often is characterized by pain that is not restricted to a single tooth (ie, diffuse pain); a unilateral, dull, throbbing pain quality; pain that is unrelieved by a diagnostic intraoral local anesthetic block; and pain that is not altered by intraoral thermal stimuli.[56,57] The diagnosis is often made based on reports of a temporal relationship in which the toothache subsides as the headache symptoms are reduced. Physical activity (eg, walking a flight of stairs) often increases pain intensity. Migraines are often associated with nausea, emesis, affective changes in mood, and sensitivity to light or sound.[7,56] Migraine occurs more frequently in females, particularly in those younger than 50 years.[72]

In one case report, a 35-year-old woman reported dental pain in a mandibular canine.[57] The pain persisted after extraction of the tooth. Subsequent examination revealed that the pain was associated with nausea and sensitivity to loud sounds. The patient denied experiencing an aura during the attacks. Treatment with antimigraine drugs and cessation of oral contraceptives reduced the migraine episodes.

The following are the key clinical characteristics of nonodontogenic toothache of migraine origin:

- The pain may be spontaneous, variable, and pul-satile, characteristics that can simulate pulpal pain.
- The pain is usually very intense.
- The toothache is characterized by periods of total remission between episodes (like migraine headache pain).
- The toothache may be immediately preceded by focal neurologic symptoms (an aura, that is, photopsia, scotoma, or teichopsia).
- The episodes of pain may pose a temporal behavior, appearing at similar times during the day, week, or month.

- The toothache is accompanied by photophobia, phonophobia, or osmophobia.
- The pain is frequently felt in a maxillary canine or premolar, and the patient is so convinced of the source of the toothache that dental treatment may be undertaken without hesitation, even when only minor or no dental cause is found.
- The pain may actually undergo remission following dental treatment, although recurrence is characteristic with neurovascular pains. The pain may spread to adjacent teeth, opposing teeth, or the entire face.
- The patient reveals a history of other neurovascular disorders (migraine).
- A trial of an abortive migraine medication (eg, sumatriptan) reduces the toothache.

Management considerations

Patients who experience migrainous toothache need to understand basic information regarding their pain condition. They need to know that even though the pain is very severe, it is still benign and not an aggressive tumor such as cancer. A very important management consideration is to have the patient identify any triggering factors that initiate the toothache/migraine attack. Often patients have no appreciation of the triggers. Once these factors are identified, effort is made to avoid them, so as to reduce the number of episodes of attacks. Factors that relate to diet can usually be quickly controlled. Sometimes environmental factors such as light, weather changes, and pungent odors may be more difficult to avoid. Other factors such as fatigue, sleep patterns, and stress need to be identified and addressed appropriately. Nothing is more satisfying to the patient than his or her own ability to control the number of toothache/migraine attacks. This, of course, can only occur with patient education.

Pharmacologic management is based on the frequency of the migraine attacks. Infrequent attacks are managed with abortive medications such as the triptans and other serotoninergic agents that block the 5-hydroxytryptamine 1 (5HT1) or 5-hydroxytryptamine 3 (5HT3) serotoninergic receptors,[73–76] such as sumat-riptan (Imitrex, GlaxoSmithKline) or rizatriptan (Maxalt, Merck). Ergotamine has been largely replaced by these alternatives because they have a lower incidence of adverse effects. When attacks are more frequent (one or more a week), prophylactic medications are used, such as the β-adrenergic antagonists (β-blockers). Examples of such medications are propranolol (Inderal, Wyeth), metoprolol (Lopressor, Novartis), timolol (Blocadren, Merck), nadolol (Corgard, King Pharmaceuticals), and atenolol (Tenormin, AstraZeneca).[67,77] Another group of medications used to

prevent migraine attacks are the calcium channel blockers, such as nifedipine (Procardia, Pfizer), verapamil (Calan, Searle/Pfizer; Isoptin, Abbott), and nimodipine (Nimotop, Bayer).[78] The tricyclic antidepressants have also been useful, especially amitriptyline. Clinical trials have also demonstrated efficacy for gabapentin and injection of botulinum toxin.[79–81]

Trigeminal autonomic cephalgia

Etiology

Trigeminal autonomic cephalgia is a group of primary headache disorders that are generally short in duration but severe in intensity. They are characterized by the presence of clinical signs related to autonomic activity such as lacrimation, conjunctival injection, nasal stuffiness, or rhinorrhea. These signs are usually present on the same side of the pain. Forehead sweating, facial flushing, and edema may also occur but are less common. Most trigeminal autonomic cephalgias are felt unilaterally in the maxilla, temple, or retro-orbital areas. On occasion, this pain may be felt in the teeth. The most common trigeminal autonomic cephalgia is cluster headache, which is highlighted in this section.

The etiology of cluster headache (Sluder neuralgia) is unknown but has been hypothesized to be episodic vasodilation that activates perivascular nociceptors. The term *cluster* denotes the observation that the pain episodes often last about 6 to 8 weeks and then are followed by a relatively long pain-free period. Although less common than migraines, cluster headaches are often considered to produce more intense pain.[7,57,82] Cluster headaches most often occur in male patients (male-female ratio: 6:1) in the age range of 30 to 50 years.

Clinical characteristics

Nonodontogenic dental pain evoked by cluster headache is distinct from odontogenic pain in that it is often characterized by pain that is not restricted to a tooth (ie, pain includes retro-orbital and sinus regions). The pain commonly awakens the patient from sleep, is unrelieved by a diagnostic intraoral local anesthetic block, and is not altered by intra-oral thermal stimuli of the tooth.[7,83] Cluster headaches can be triggered by drugs such as alcohol and cocaine. The pain is generally described as hot, stabbing, and paroxysmal. The pain attacks often occur at the same time of day (especially 4:00 am to 10:00 am) and often last 30 to 180 minutes.[7,57] The attacks may be associated with rhinorrhea, nasal congestion, and lacrimation from the involved eye. The pain is often distributed in the maxillary posterior teeth, sinus, and

retro-orbital regions.

A related condition is chronic paroxysmal hemicrania. This disorder has similar pain characteristics but, unlike cluster headache, is observed mostly in females and is completely responsive to indomethacin.[7,57]

Cluster headaches often evoke nonodontogenic dental pain. In one study, 43% of subjects with cluster headache were initially treated by a dentist.[84] A case report described three patients with nonodontogenic dental pain resulting from cocaine-induced cluster headaches.[85] One patient described unilateral maxillary pain in the premolar region that lasted for 30 to 120 minutes after cocaine use. Another patient reported continued nonodontogenic dental pain in the maxillary molar region. The pain persisted after endodontic treatment and subsequent extraction of the molar; only later did the patient report that the pain started about 1 to 2 hours after cocaine ingestion. The third patient also reported nonodontogenic dental pain in the maxillary premolar region after consumption of cocaine.

The following are the key clinical characteristics of toothache of cluster headache origin:

- The pain may be spontaneous, variable, and pul-satile, characteristics that can resemble pulpal pain.
- The pain is very intense and has a sudden onset.
- The toothache is characterized by periods of total remission between episodes.
- The episodes of pain pose a temporal behavior, appearing at similar times during the day.
- The toothache is reported to occur frequently for several weeks (cluster), then resolves without treatment.
- The pain is frequently felt in a maxillary canine or premolar.
- The toothache is accompanied by autonomic effects, such as nasal congestion, lacrimation, and edema of the eyelids and face, which may be mistaken for sinusitis or dental abscess.
- The patient reveals a history of other neurovascu-lar disorders (migraine).

Management considerations

As with migraine, patients who experience cluster headaches need to understand basic information regarding their pain condition. They need to know that even though the pain is very severe, it is still benign and not an aggressive disease. Probably one of the most important aspects of education is having the patient identify any triggering factors that initiate the attacks.[85] Often patients may have no understanding

of triggers. If the patient can identify a trigger, then proper avoidance can be instituted. Because the attack usually occurs shortly after the trigger, it may be easy for the patient to recognize the trigger.

Pharmacologic management of cluster headache is often difficult and not predictable. Patients with cluster headache have been treated with oxygen therapy, sumatriptan, prednisone, gabapentin, and calcium channel blockers.[7,86–88] Ergotamine has been largely replaced by these alternatives because of the lower incidence of adverse effects.

Nonodontogenic toothache of neuropathic origin

Neuropathic pain has been defined by the International Association for the Study of Pain as pain "initiated or caused by a primary lesion or dysfunction in the nervous system."[89] Neuropathic pain has its etiology in the neural tissue itself and not in the structures that it innervates.[90] Some neuropathic pains present as episodic pain conditions and some are more continuous. Toothache of neuropathic origin can likewise present as either an episodic or continuous pain.

Episodic neuropathic toothache

Episodic neuropathic pain is characterized by sudden volleys of electric-like pain referred to as *neuralgia*. When this type of paroxysmal pain is felt in a tooth, it can pose a significant diagnostic challenge to the clinician. Trigeminal neuralgia is the most common episodic neuropathic pain felt in teeth.

Nonodontogenic toothache associated with trigeminal neuralgia

Etiology. The etiology of trigeminal neuralgia (also known as *tic douloureux*) is unclear; however, demyelinization of the trigeminal nerve root by vascular compression is a common hypothesis. This theory is supported by studies demonstrating that compression of either the sciatic or trigeminal (infraorbital) nerves in rats produces models of neuropathic pain.[91–93] This demyelinization of the center nerve root causes a referred pain to be felt in the peripheral distribution of the involved branch. Therefore, nonodontogenic tooth pain can be a common part of the patient's complaint.

Clinical characteristics. The clinical presentation of a neuralgic toothache is a severe, shooting, electric-like pain that lasts only a few seconds.[5,7,10] The pain is not always restricted to a tooth but often affects a broader area. The pain is not altered by intraoral thermal stimuli. The most common branch of the trigeminal nerve involved is the mandibular branch, followed by the maxillary branch. The pain is often severe, and patients report the pain as being the most intense they have ever experienced. Often the patient is able to trace the pain radiating down the distribution of the nerve to the tooth.

With trigeminal neuralgia there is often a trigger zone that, when lightly stimulated, provokes the severe paroxysmal pain. Typically, this zone is the lip, chin, or even the tongue. The presence of this characteristic helps to establish the diagnosis. Anesthetic blocking of the trigger zone will completely eliminate the toothache and paroxysmal episodes during the period of anesthesia.

On occasion, a tooth can represent the trigger zone, posing a great diagnostic challenge for the clinician. Patients with trigeminal neuralgia frequently receive endodontic treatment for their dental pain.[94,95] Additional case reports also provide examples of the opposite diagnostic problem: Patients with odontogenic dental pain may be diagnosed as having trigeminal neuralgia.[96] In both types of misdiagnosis, the lack of response to treatment is a key factor in prompting reassessment of the differential diagnosis.

In some patients, trigeminal neuralgia initially presents with different clinical characteristics. Instead of paroxysmal pain, the pain is an aching pain in the sinus region and teeth with a duration of minutes to several hours. This condition is known a s *pre–trigeminal neuralgia*.[10,97–99] It is thought that these patients will subsequently develop trigeminal neuralgia.[98,99] The clinical presentation of pre–trigeminal neuralgia can be a significant diagnostic problem. Often the diagnosis only becomes clear once the patient starts to develop the more distinct features of trigeminal neuralgia (paroxysmal pain).

Patients with trigeminal or pre–trigeminal neuralgia commonly report nonodontogenic dental pain. In one series of 41 patients with trigeminal neuralgia and 19 patients with pre–trigeminal neuralgia, a total of 61% of patients reported an initial dental pain.[10] In another series of cases, 29% of 24 patients reported dental pain as a component of their pre–trigeminal neuralgia symptoms.[99]

The following are the key clinical characteristics of episodic neuropathic toothache associated with trigeminal neuralgia:

- The pain is a severe, unilateral, lancinating, shocklike (paroxysmal) pain felt in a tooth.
- The pain episodes are brief, lasting only 5 to 10 seconds.
- No pain between episodes is reported.
- The pain is provoked by relatively innocuous peripheral stimulation of a trigger zone. The trigger zone is commonly an extraoral site such as the lip or chin but may be the tooth.
- Very localized anesthesia of the tooth (interliga-mentous injection) will not reduce the pain unless it is also the trigger zone.
- A local anesthetic injection at the trigger zone (or a nerve block) will eliminate the episodes of paroxysmal pain and toothache during the period of anesthesia.

Management considerations. About 2% of paroxysmal neuralgias are caused by central pathologic lesions. Therefore, referral for a neurologic consultation should be considered, especially if any neurologic deficit is noted. Several treatment modalities have been recommended for patients with trigeminal neuralgia. Typically, pharmacologic management is the first course of treatment. Medications include carbamazepine (Tegretol, Novartis), gabapentin (Neurontin, Pfizer), pregabalin (Lyrica, Pfizer), baclofen (Lioresal, Novartis), oxcarbazepine (Trileptal, Novartis), topiramate (Topamax, Ortho-McNeil), lamotrigine (Lamictal, GlaxoSmithKline), and valproic acid (Depakene, Abbott).[7,95,100] Local anesthetic injection into the area of the trigger zone often relieves pain during the period of anesthesia. In a series of 24 cases, intraoral administration of capsaicin to the mucosa resulted in complete or partial relief of pain in approximately 63% of patients.[101] Surgical procedures such as percutaneous rhizotomy or nerve decompression are considered in refractory cases.[5,7,102–104]

Nonodontogenic toothache associated with glossopharyngeal neuralgia

Etiology. The etiology of glossopharyngeal neuralgia is unknown but may involve vascular compression of the ninth cranial nerve.[9,56] Accordingly, some investigators have suggested that glossopharyngeal neuralgia and trigeminal neuralgia have similar etiologies (vascular compression) involving different cranial nerves. Glossopharyngeal neuralgia is only about one-tenth as prevalent as trigeminal neuralgia.[56,82]

Clinical characteristics. Glossopharyngeal neuralgia is similar to trigeminal

neuralgia in that both disorders are characterized by episodic pain; however, patients with glossopharyngeal neuralgia report less tooth pain. The differential diagnosis between non-odontogenic dental pain evoked by glossopharyngeal neuralgia and odontogenic pain often includes the quality and duration of the pain (the former involves severe shocklike pain that lasts only a few seconds).[7,9,10,100] The pain is often elicited by swallowing; however, it may also be elicited by chewing or talking. The distribution of pain includes the posterior mandible, oropharynx, tonsillar fossa, and ear.[9,82] Other distinguishing features include pain that is unrelieved by a diagnostic intraoral local anesthetic block (unless the trigger point is in the field of anesthesia) and pain that is not altered by intraoral thermal stimuli.[7,9,101]

The following are the key clinical characteristics of episodic neuropathic toothache associated with glossopharyngeal neuralgia:

- The pain is a mild to intense, unilateral, lacerating, shocklike (paroxysmal) pain felt in the throat, ear, jaw, and tooth region.
- The pain episodes are brief, lasting only 5 to 10 seconds.
- The pain is provoked by swallowing, chewing, or talking.
- There are pain-free periods between the episodes of pain.
- Inferior alveolar nerve block does not change the pain condition.

Management considerations. Treatment for glosso-pharyngeal neuralgia includes the same medications and protocol used for trigeminal neuralgia (previously listed). Resistant cases have been treated with surgical decompression.[82]

Continuous neuropathic toothache

Continuous neuropathic pains are pain disorders that have their origin in neural structures and are expressed as constant, ongoing, and unremitting pain. The pains will often vary in intensity, but there are no periods of total remission. Continuous neuropathic pains can be felt in teeth. There are two types to be considered: neuritic and deafferentation.

Nonodontogenic toothache of neuritic origin (herpes zoster)

Etiology. Neuritic pain,[105–107] sometimes referred to as *neuritic neuralgia*, occurs as the result of alteration of the afferent fibers in a nerve trunk. It is felt as referred

heterotopic pain in the peripheral distribution of the affected nerve. It is assumed that the etiology is inflammation arising from traumatic, bacterial, viral, or toxic causes. The process alters the fibers that mediate pricking and burning pain and elevates the threshold for pricking pain but lowers it for burning pain.[105] An example of a bacteria-induced neuritis is an acute maxillary sinusitis affecting the superior dental plexus just below the sinus floor. This results in nonodontogenic pain in the maxillary anterior tooth. A similar toothache in the mandibular teeth may occur as an expression of neuritis of the inferior alveolar nerve from direct trauma or dental sepsis.

A herpes zoster infection is an example of a viral neuritis. Herpes zoster has been reported to produce nonodontogenic dental pain preceding the eruption of vesicles. These case reports are mixed, with some reporting pulpalgia-like symptoms and others reporting necrosis with the presence of periradicular lesions.[108–111]

Clinical characteristics. Neuritic toothache is characterized by a burning quality that may be accompanied by other symptoms of neuropathic pain, such as an intense, stimulating, precisely localizable pain that is accurately related in location to the site of inflammation and/or projecting to the peripheral distribution of the affected nerve. Although variable in degree, neuritic pain has a strange constancy that relates to the incidence and resolution of the inflammatory process. Its temporal behavior is less dramatic than that of other neuropathic pains.

The clinical symptoms of neuritis depend on which fibers are affected. Neuritis may present with other sensory alterations, such as hyperesthesia (increased sensitivity to stimulation), hypoesthesia (diminished sensitivity to stimulation), paresthesia (abnormal sensation), dysesthesia (an unpleasant, abnormal sensation), and anesthesia (absence of all sensation). The key usually is to recognize accompanying neurologic signs that involve other teeth or nearby structures served by the same nerve. If motor efferent fibers are present in the nerve trunk and are also affected, muscular signs become evident; these include muscular tic, weakness, or paralysis. If autonomic fibers are present, various autonomic effects become clinically evident. When the inflammatory process occurs within a bony canal, the inflammatory exudate may elicit compression effects.

The symptom complex therefore depends on the types of fibers affected, the degree of change, the peripheral distribution of the affected fibers, and the state of the inflammatory process. A knowledgeable examiner can utilize these clinical symptoms to locate the site of inflammation. The relationship between the clinical symptoms and inflammatory process is strictly anatomical. The effects are directly

the result of peripheral fiber involvement and do not represent central mechanisms.

The following are the key clinical characteristics of toothache of neuritic origin:

- The pain is a persistent, nonpulsatile, often burning pain felt in a tooth.
- The toothache is accompanied by other neurologic symptoms (eg, paresthesia, dysesthesia, or anesthesia). "dead" or "strange."
- The associated gingival tissue may be affected.
- The onset of the toothache followed an infection or trauma (eg, sinusitis, viral infection, or trauma).

Management considerations. The management of neuritic toothache begins with understanding the etiology of the inflammation. When a bacterial source is suspected, antibiotics are indicated. Other treatments needed to eliminate the infection should be undertaken. When a viral infection is suspected, antiviral medications such as acyclovir, valacyclovir, or famciclovir can be helpful.[112,113] When no obvious infection exists, administration of steroids should be considered.[114,115] Steroids can reduce swelling of the nerve tissue, which may be extremely important when neuritis affects a nerve that exits a cranial foramen.

Nonodontogenic toothache of deafferentation origin (atypical odontalgia)

Etiology. *Deafferentation* is a loss of normal afferent input to the central nervous system. Deafferentation can result from physical, chemical, or thermal trauma to the nerve. Deafferentation symptoms in the orofacial region are commonplace. Only a few of these conditions, however, elicit pain. The most frequent complaints are anesthesia and paresthesia following injury of the mandibular nerve that is incidental to the removal of teeth. However, in some instances, deafferentation can result in pain. When this type of pain is felt in the region of a tooth, it is often referred to as *atypical odontalgia*[116,117] or sometimes *phantom toothache*.[118,119]

The origin of deafferentation pain appears to be associated with central plasticity in the trigeminal nuclear complex of the brainstem.[120] In some instances, there may be a sympathetic component to the pain.[121] Although no large-scale clinical trials have been conducted, atypical odontalgia has been estimated to be 10 times more prevalent than trigeminal neuralgia and may include up to 3% of patients receiving pulpal extirpation procedures.[54,122] The possibility of a psychosocial etiology has been supported in some studies[123] but not others.[116,121,124–126]

Clinical characteristics. Patients with deafferentation toothache often report a history of trauma or ineffective dental treatment in the area.[127] In a study of 42 patients with atypical odontalgia, 86% of the patient population was female, and 78% reported maxillary pain; of 119 reported areas of pain, the most common were the molar (59%), premolar (27%), and canine (4%) regions.[124,126] The pain may change in location over time; some studies have reported pain shifting in up to 82% of the subjects.[121,128] Thermography has been proposed as a diagnostic test, and, although the differences in facial thermal asymmetry between control subjects and patients with atypical odontalgia are statistically significant, they are not large in magnitude (18%).[129]

It is not unusual for patients with atypical odontalgia to have received multiple endodontic procedures or extractions for their dental pain.[124,128,130–133] In many of these cases, the lack of response to treatment was a key factor in prompting reassessment of the differential diagnosis.[54] In one case report, the lack of an effect of a local anesthetic injection in reducing the intensity of pain was a significant finding that prompted consideration of nonodontogenic dental pain.[133]

The following characteristics of deafferentation toothache can be used to differentiate it from odontogenic pain: diffuse pain; pain that is not always restricted to a tooth (eg, the area may be edentulous); pain that is almost always continuous; a pain quality that is often described as a dull, aching, throbbing, or burning sensation; pain that may or may not be relieved by a diagnostic intraoral local anesthetic block; pain that often lasts more than 4 months; and pain that is not altered by intraoral thermal stimuli.[7,9,56,121,126,131,134]

The following are the key clinical characteristics of toothache of deafferentation origin:

- The toothache is a continuous pain but often var-ies in intensity. There are no periods of remission.
- The most common locations for pain are the max-illary molar and premolar areas.
- The pain location may change over time but usually remains in the same nerve distribution.
- The patient is usually a middle-aged woman with a history of trauma to the painful region.
- The pain is not changed by local provocation.
- The effect of local anesthesia is unpredictable.

• The toothache is nonresponsive to dental therapies.

Management considerations. Like many continuous neuropathic pains, deafferentation toothache can be difficult to manage. Most of the success comes from addressing the central mechanisms that seem to dominate the condition. There is some evidence that tricyclic antidepressants may be of some benefit.[126,133–135] It is likely that the beneficial effect of these drugs is not related to the management of depression but instead to the analgesic effects of relatively low dosages of the tricyclic antidepressants. These medications inhibit the reuptake of serotonin and norepinephrine, thus increasing the effectiveness of the descending inhibitory system. Dosages ranging from 10 to 100 mg seem to be adequate for pain relief; however, total pain elimination is rare.

Another drug that that may be helpful is gabapentin (Neurontin).[136] Gabapentin may be slowly titrated until pain is reduced or a maximum dosage of 3,600 mg is reached.[137] The greatest side effect is drowsiness, and that is why the medication is slowly titrated to a therapeutic dose. Pregabalin (Lyrica) is a medication that is similar to gabapentin and has also been shown to be useful with neuropathic pain.[138–140] Pregabalin can be slowly titrated up to 300 mg/day or until adequate pain relief is achieved.

The topical application of capsaicin to the site of the painful tooth may also be of benefit.[141] Earlier data suggested that capsaicin applied to the painful tissue appeared to deplete the C fiber of substance P, thus reducing its ability to further stimulate the second-order neuron,[142,143] although this does not appear to be the mechanism for capsaicin analgesia. More recently, it has been found that capsaicin is a transient receptor potential vanilloid 1 (TRPV1) ago-nist that profoundly desensitizes this pain receptor, reducing nociceptive transmission.[144–146] Capsaicin ointment should be applied to the painful area 4 times a day for 3 to 4 weeks.[142,147,148] If this treatment is too painful, the capsaicin ointment can be mixed with a topical anesthetic.

Other medications, such as amitriptyline, carbamazepine, and even ketamine,[149] have been suggested in topical forms to reduce pain. Although documentation is lacking, this is a relatively conservative approach and may be considered. If pain relief is achieved but the topical medication is washed away quickly, a thin acrylic resin stent can be fabricated to retain the medication on the painful site (Fig 19-11).

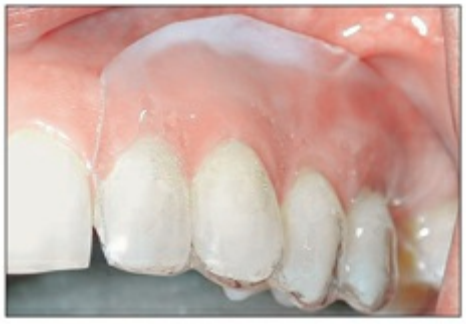

Fig 19-11 Thin acrylic resin stent placed over the teeth and gingival tissue to hold the topical medication over the site of pain.

Nonodontogenic toothache of cardiac origin

Etiology

It has been known for years that cardiac ischemia can refer pain to the arm, neck, face, and even the teeth.[97,150–153] The exact mechanism is unknown but is likely related to convergence of nociceptive input originating from the myocardial ischemia carried by the vagus and thoracic nerves as they enter the central nervous system and ascend to the cortex. This phenomenon, accompanied by central sensitization, creates a pattern of pain referral to the face, neck, and arm.

It is important that the clinician understand this pattern of pain referral because immediate diagnosis and referral to the appropriate health care professional is critical. The difficulty in correctly diagnosing an acute myocardial infarction is demonstrated in the frequency of missed diagnoses found in emergency rooms, which ranges between 2% and 27%.[154–156] One-fourth of missed diagnoses were found to result in death or potentially lethal complications for the patient.[154] Patients with atypical cardiac symptoms were more likely to be discharged from emergency departments than patients with typical symptoms.[155] An absence of chest pain and a lack of ST elevation in electrocardiograms were found to be the main predisposing factors for a missed diagnosis.[156] In line with this, patients with suspected myocardial infarction but who never experienced chest pain were found to run a three times higher risk of death than patients presenting with chest pain during emergency room evaluation. These same individuals who never developed chest pain had an eight times greater risk of death than patients whose chest pain resolved before receiving hospital care.[157,158]

Clinical characteristics

Dentists should be particularly aware of the incidence of jaw and tooth pain that occurs as a secondary manifestation of cardiac pain.[153,159] Although other clinical evidence of cardiac distress (such as substernal chest discomfort and left arm and neck pain) is usually present, sometimes the dental symptoms may be the only ones reported by the patient. Patients with only dental complaints will certainly come to the dentist with the anticipation that dental therapy will solve the problem.[150] Great care should be exercised in such situations.

In a recent study,[160] craniofacial pain was reported in approximately 40% of patients who experienced a cardiac ischemic event and was the sole symptom in 6% of patients. The data suggest that 1 in every 15 patients experiencing a myocardial ischemic event or a myocardial infarct will report pain only in the area of the jaw. The areas most frequently affected were the upper part of the throat (82%), the left mandible (45%), the right mandible (41%), and the left TMJ and ear region (18%). Nonodontogenic toothache was reported in three patients (4%), affecting mandibular teeth bilaterally in two patients and the maxillary left teeth in one patient.

The quality of the pain may help the clinician differentiate this type of nonodontogenic toothache. Although dull, diffuse pain is common, the patients will often complain of pressure. This symptom is common with cardiac pain but very infrequently reported with odontogenic toothache.[29]

As with other nonodontogenic toothaches, a lack of adequate dental cause for the pain complaint should always be an alerting sign. Failure to arrest the pain with effective local anesthetic blocking of the tooth confirms that the primary source of pain is not the tooth.[7,153]

The following are the key clinical characteristics of toothache of cardiac origin:

- The pain is a deep, diffuse toothache that may sometimes pulsate.
- The toothache has pressure and burning qualities.
- The toothache has a temporal behavior that increases with physical exertion or exercise.
- The toothache is associated with chest pain, ante-rior neck pain, throat pain, and/or shoulder pain.
- The patient has a prior history of cardiovascular disease.
- The toothache is decreased with the administration of nitroglycerin tablets (already prescribed by the patient's physician).

Management considerations

A complete health history is essential when this type of nonodontogenic toothache is evaluated. The patient may not share his or her entire medical history because he or she is in the dental office for tooth pain and not having any typical cardiac symptoms. When a cardiac toothache is suspected, an immediate referral to the appropriate medical personnel is mandatory.

Nonodontogenic toothache of psychogenic origin (somatoform toothache)

Etiology

A complete classification of orofacial pain conditions consists of two broad categories, or *axes*.[7] Axis I includes pain conditions that have their origins in the somatic (body) structures. For many clinicians, this represents most pain disorders because their training has been directed to appreciating how tissue injury, inflammation, and dysfunction can lead to painful conditions. When no obvious physical evidence of disease is present, the clinician may often describe the tooth pain as an *idiopathic toothache*. [57] Unfortunately, this term is of no benefit to the patient because a pain of unknown origin cannot be effectively treated.

Every clinician must understand that a tooth can be painful in the absence of any dental pathosis. In fact, this chapter is dedicated to these conditions. However, up to this point, each nonodontogenic toothache that has been discussed has had a physical condition (Axis I) that explained the nociceptive process that leads to the referred pain experience in the tooth. In each instance, the management of the pain has been directed toward resolving that physical condition.

There are instances, however, when pain is a significant complaint in the absence of any tissue injury or disease. These conditions fall into Axis II, which represents the psychologic conditions that may ether be the origin of or a significant contributing factor to pain disorders. Acknowledgment of the existence of both Axis I and Axis II factors is basic to the effective understanding and management of orofacial pain conditions.[7]

Psychosocial, behavioral, cultural, and environmental factors can impact pain.[7,161,162] These factors can influence a patient's interpretation and report of pain.

For example, one study reporting on cultural differences in pain language found that 82% of Mandarin-speaking Chinese reported tooth preparations as producing a *sourish* sensation, whereas only 8% of English-speaking Americans used this term.[163] In another example, environmental factors were found to influence pain reports. In this study, female subjects reported significantly greater anxiety and lower thresholds for electrical pulpal stimulation when tested in a dental operatory than they did when tested in a research laboratory setting.[164] These studies illustrate the fact that the skilled clinician must interpret the patient's pain report with due consideration given to psychosocial and cultural factors. Moreover, clinicians should appreciate the fact that their own psychosocial and cultural backgrounds may influence how they interpret a patient's pain report.

There are a few psychologic (Axis II) conditions that are strongly associated with pain complaints. The term *somatoform pain disorder* is used to describe a cognitive perception of pain that has no demonstrable physical basis.[165–168] Somatoform pain disorders can certainly pose a significant diagnostic problem for the clinician evaluating orofacial pain complaints. The clinician might suspect this condition if the patient's pain is not associated with any evidence of local somatic tissue changes. This assumption may be unjustified, however, considering the many pain disorders that have been described in this chapter that are not associated with any obvious source of local tooth pathosis. The clinician must therefore always be mindful of somatoform pain disorders so that an improper diagnosis does not lead to mistreatment.

Clinical characteristics

Although it is not always easy to diagnose a somatoform nonodontogenic toothache, there are certain clinical characteristics that will help the clinician. In general, patients will report their toothache as having abnormal, unconventional clinical presentations. Patients may report pain from multiple teeth that often changes in location and character. The pain may even cross anatomical distributions of peripheral nerves. The pain is often reported as being present for a long duration (chronic pain) and having not been responsive to previous appropriate treatment. The toothache has no identifiable etiology.[7,57,169]

The following are the key clinical characteristics of toothache of somatoform (psychologic) origin:

- The pain is reported in many teeth and/or other sites.

- The pain jumps from tooth to tooth or to other locations.
- There is a general departure from normal or physi-ologic patterns of pain.
- There is a lack of response to reasonable dental treatment.
- There is an unusual and unexpected response to treatment.
- The toothache is chronic and often unchanging.
- The patient presents with chronic pain behavior (eg, frequent use of the health care system, unusual dependence on others, reclusive nonfunctional lifestyle, or significant use of medications).
- There is no identifiable source of pain, and the clinical characteristics do not fit any other pain condition.

Management considerations

Somatoform pain disorders are mental disorders and are best treated by a qualified psychologist or psychiatrist. If the clinician suspects that the patient has this condition, a referral to the appropriate health care professional is indicated. Irreversible dental procedures should be avoided because they are very likely to lead to an unsatisfactory outcome.

Nonodontogenic toothache of systemic origin

Certain systemic conditions can result in tooth pain, including malignant neoplasia, diabetes, sickle cell anemia, and developmental disorders. For example, patients with sickle cell anemia may report dental pain that signals an impending sickling crisis rather than an indication of a local, dental pathosis.[170,171] In a 12-month study, 68% of 51 patients with sickle cell anemia reported dental pain with no evident dental pathosis, yet in other studies, no differences were observed in patients with the sickle cell trait compared to controls.[172,173] Yet there is also some evidence that sickle cell anemia may lead to pulpal necrosis[174] (see chapter 20).

Several studies have reported on various neoplastic diseases associated with nonodontogenic dental pain. Although the incidences are rare, these reports describe dental pain as an initial or severe symptom in patients with glioblastoma multiforme[175]; metastases from breast, lungs, or prostate[176–178]; osteoblastoma[179]; carcinoma[180–182]; sarcoma[181]; non-Hodgkin lymphoma[183,184]; and Burkitt lymphoma.[185] Key findings that prompted consideration of a nonodontogenic origin

of the dental pain included subsequent altered sensation (eg, paresthesia or anesthesia), positive responses to pulpal testing when a periradicular radiolucency was present, failure of dental treatment, the diffuse or spreading nature of pain, unusual-appearing radiographic lesions (eg, diffuse borders involving multiple teeth or a moth-eaten trabecular pattern), and lack of etiologic factors.[175,176,179,180,183,184] In one series of 763 patients with nonspecific jaw pain, 1.2% of the population had pain that was caused by metastases located in the mandible.[177]

Patients with multiple sclerosis may present with nonodontogenic dental pain.[186] Initially, this may be the only symptom related to multiple sclerosis, which places a difficult diagnostic burden on the clinician. Later, the emergence of electric shock–like pain, as well as the lack of evidence of dental pathoses, contributes to expansion of the differential diagnosis and prompts evaluation for multiple sclerosis. Multiple sclerosis is typically diagnosed in young women.

Pulpal pain correlated with the menstrual cycle has been reported by some authors.[187] Evidence suggests that pulp tissue may contain both estrogen and progesterone receptors.[188] Additional studies are required to determine the prevalence, mechanisms, and differential diagnostic evaluation of this form of odontalgia.

In some instances, the systemic condition may actually interact with pulp or periradicular tissue, leading to dental pathoses. When this occurs, there may be a true odontogenic toothache caused by a systemic condition. Endodontic treatment should be performed on the nonvital tooth, but the systemic disorder also has to be appropriately addressed. Management may be referral to the proper health care provider.

The effects of systemic disorders on tooth pain, as well as clinical characteristics and management, are reviewed in more detail in chapter 20.

Conclusions

Toothaches of nondental origin require accurate diagnostic identification of the true source of the patient's pain. The most important step toward proper management is for the clinician to be suspicious that the pain is not of dental origin. The cardinal warning symptoms of a nonodontogenic toothache are as follows:

- The patient reports spontaneous toothaches involving multiple teeth.
- There is inadequate local dental cause for the pain.
- Effective injection of a local anesthetic for the offending tooth does not reduce or eliminate the pain.
- The toothache has a stimulating, burning, or non-pulsatile quality.
- The toothache is constant, unremitting, and nonvariable.
- The toothache is persistent or recurrent.
- The toothache fails to respond to reasonable dental therapy.

Hargreaves[189] has recommended that clinicians who treat pain patients remember the classic tale of the blind men describing the elephant. In this tale, each blind man described the elephant as a completely different animal, depending on whether he was touching the trunk, ears, or legs. Similarly, clinicians tend to interpret the symptoms and results of the clinical examination based on their own focus or training. Clinicians should consider the "whole animal" when assessing patients' reports of dental pain. Many painful disorders can result in nonodontogenic dental pain. Accordingly, before focusing on the planned dental treatment, clinicians should carefully consider the clinical findings and the differential diagnosis.

To ensure appropriate diagnosis of patients reporting dental pain, clinicians should follow these guidelines:

- Be diligent in establishing a differential diagnosis.
- Reproduce the patient's chief complaint.
- Determine the etiology of the dental pain. Is the pain of dental origin?
- Consider all local anesthetic injections as diagnostic. Evaluate the patient's response.
- Monitor the treatment outcome.
- Know other area health care professionals who diagnose and treat pain (eg, orofacial pain dentists, neurologists, psychologists, radiologists, and otolaryngologists) and consult with them when necessary.

Effective pain control is a hallmark of clinical excellence, and this starts with active consideration of the differential diagnosis of odontogenic and nonodontogenic dental pain.

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Interrelationship of Pulp and Systemic Disease

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It is conceivable that for any given systemic disease, the dental pulp may become involved. This involvement is often a two-way street, as evidenced by the example of diabetes mellitus. It is well established that poorly controlled diabetes mellitus increases the patient's risk of suffering an oral infection, including pulpitis. Conversely, poorly controlled oral infection can adversely impact diabetes control. Unfortunately, few studies have purposely assessed the relationship between systemic disease and the dental pulp, leaving much of the profession's current understanding to be derived from empiricism and sporadic case reports.

The clinician must always consider two questions when managing a patient who presents for care. First, does a systemic condition underlie or contribute to the patient's chief complaint? Second, does the health status of the patient affect the delivery of dental care? To illustrate the first question, consider the decision points the clinician must address to appropriately manage the patient suffering from acute sinusitis who presents with odontalgia. To illustrate the second question, consider the deliberative processing required in determining whether or not to perform apical surgery on a tooth previously exposed to therapeutic irradiation to treat head and neck cancer. In both cases, the clinician must interpret the local dental findings in the

context of the patient's overall health to develop an effective treatment plan.

The purpose of this chapter is to briefly review selected conditions that may, directly or indirectly, affect the dental pulp and the delivery of treatment for various pulpal diseases (see also chapters 2, 17, and 19). Because most of the topics presented in this chapter represent extensive subjects of study in and of themselves, only a succinct overview is possible. The reader is encouraged to seek additional detailed information as necessary.[1–5]

Infectious Diseases

Human immunodeficiency virus

AIDS represents the predominant clinical manifestation of advanced infection with the human immunodeficiency virus (HIV).[6] As of December 2009, the World Health Organization estimated that 31.4 to 35.3 million individuals (14.8 to 17.2 million women, 14.4 to 15.4 million men, and 1.6 to 3.4 million children younger than 15 years of age) worldwide were infected with HIV; of these, 2.3 to 2.8 million became newly infected in 2009.7

The available scientific evidence clearly reveals a dynamic process in which the initial and ongoing immunologic response to HIV infection is not only unsuccessful in clearing HIV but paradoxically paralleled by a progressive reduction in immunocompetence.[8] While a cure remains elusive, the introduction of antiretroviral therapy (ART) has essentially turned this once almost universally fatal infection into a manageable chronic illness. For most HIV-infected patients, ART effectively stabilizes and normalizes the immune system.

Several oral lesions have been shown to occur in association with HIV-induced immunosuppression.[9–11] The most strongly associated lesions in adult patients are mucosal and include candidiasis (erythematous and pseudomembranous), hairy leukoplakia, Kaposi sarcoma, non-Hodgkin lymphoma, and periodontal disease (linear gingival erythema, necrotizing ulcerative gingivitis, and necrotizing ulcerative periodontitis).[12] For pediatric patients, the most strongly associated lesions are candidiasis (erythematous, pseudomembranous, and angular cheilitis),

herpes simplex infection, linear gingival erythema, parotid enlargement, and recurrent aphthous stomatitis (minor, major, and herpetiform). The risk of developing these lesions is inversely related to the $CD4^+$ counts. Thus, the presence of oral lesions may serve as good clinical markers to signal a loss in therapeutic efficacy of ART.[13,14]

Although HIV has been identified in dental pulp[15] and in periradicular lesions of pulpal origin,[16] its direct contribution to pulpal disease remains unknown (Fig 20-1). Studies to assess the caries risk in HIV-infected patients have yielded conflicting results, and it is likely that numerous factors, such as the patient's immune status, dietary habits, medication profile, and salivary status, all contribute to caries risk.[17–19] The impaired immune response observed in patients with low $CD4^+$ counts does not appear to be associated with an increase in endodontic complications after root canal treatment,[20,21] and three recent studies have confirmed that HIV-infected patients respond well to and benefit from indicated root canal therapy.[22–24]

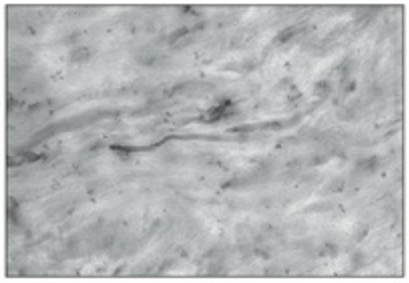

Fig 20-1 Evaluation of the presence of HIV in human dental pulp using in situ hybridization histochemistry. Dental pulp tissue from an HIV-positive patient was fixed, sectioned, and incubated with one of two biotinylated oligonucleotide probes complementary to a region of messenger RNA specific to HIV. Sections were incubated, washed, and developed. A positive signal is visible as a dark spot in the figure. (Reprinted from Glick et al[15] with permission.)

Herpesvirus infections

Herpesviruses are ubiquitous pathogens that have the ability to establish latency in the infected host.[25] There are eight identified human herpesviruses: herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6, human herpesvirus 7, and human herpesvirus 8.26 Primary infections are often asymptomatic and typically occur during childhood.

The pathophysiology of HSV-1 viral infection is well established. During the

primary infection, the virus is transported via retrograde axonal transport to regional sensory ganglia (typically the trigeminal ganglia), where it establishes latency.[27] During the latent phase, the virus exists in a quiescent immunologically shielded state. However, shedding of HSV has been demonstrated in the saliva of asymptomatic healthy adults. Although the clinical relevance of this finding remains to be determined, it reinforces the need for strict adherence to infection-control procedures in the dental office to minimize the risk of occupational exposure.[26]

Clinically apparent recurrent infections are estimated to occur in 15% to 40% of individuals harboring latent HSV-1.28 Well-documented triggers associated with HSV recurrence include sunlight, trauma, menstruation, fever, immunosuppression, decompression of the trigeminal nerve, and irritation by dental instruments. Three clinical forms of recurrence are recognized: *(1)* recurrent herpes labialis, *(2)* intraoral recurrence, and *(3)* recurrence mimicking a primary infection. Intraoral recurrent lesions typically present as a focal clustering of vesicles affecting the keratinized mucosa (gingiva or hard palate). The vesicles quickly rupture and coalesce to form the characteristic shallow erosion. The pain may be so severe as to interfere with eating and speaking. HSV-1 infection has been proposed as a potential etiologic factor of pulpal necrosis and should be considered in the differential diagnosis of odontalgia (see chapter 19). However, the overall impact of HSV infection on pulpal disease remains unknown. In one study assessing 31 patients with an apical abscess or cellulitis of pulpal origin, HSV was detected (using polymerase chain reaction) in only 1 of 31 specimens.[29] In contrast, another study revealed the presence of HSV in 15 of 50 necrotic pulps assessed by polymerase chain reaction.[30]

The classic clinical presentation of a primary VZV infection is termed *chickenpox*. Similar to HSV, VZV establishes latency in neuronal ganglia (cranial nerve, dorsal root, and autonomic ganglia).[31] Clinical VZV recurrence is called *zoster* (or *shingles*). Factors that predispose an individual to zoster include conditions of immunocompromise (eg, lymphoma, Hodgkin disease, leukemia, or AIDS) and advanced age.[32] Zoster usually manifests a prodrome of mild to moderate tingling, itching, burning, boring pain, or numbness affecting the affected dermatome.[33,34] Within 3 days, the characteristic erythematous papular rash develops, which quickly progresses to vesicles and pustules. Constitutional signs and symptoms of fever, chills, malaise, and headache may also be present.

Resolution typically occurs within 3 weeks and may lead to altered pigmentation or scarring of the affected area. The most common complication of zoster is

postherpetic neuralgia, a condition characterized by pain or hyperesthesia that persists after clinical resolution has occurred. An estimated 50% of patients over the age of 60 years who experience zoster are at risk for postherpetic neuralgia.

An estimated 13% of zoster cases involve the head and neck.[34] Zoster of the maxillary or mandibular branch may affect dentoalveolar structures such as the pulp and periodontal ligament, leading to pulpal death, tooth exfoliation, internal root resorption, tooth neuralgia, and osteonecrosis[34–37] (Fig 20-2). The degree to which zoster directly contributes to pulpal disease is unknown, with only a few case reports noted in the literature.[38,39] Further confounding the issue, prodromal zoster pain not directly involving the dental pulp may mimic pain of pulpal origin.[40]

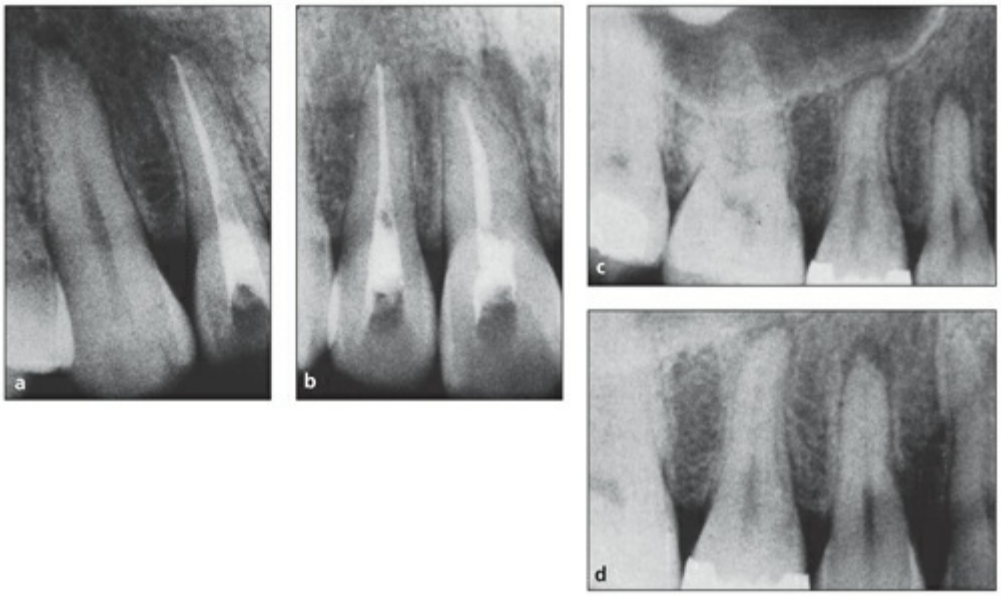

Fig 20-2 Periradicular radiographs of a patient 5 months after a herpes zoster outbreak. Note the periradicular radiolucencies of the maxillary left canine *(a and b)* and first and second premolars *(c and d)*. (Reprinted from Goon and Jacobsen[38] with permission.)

Recent reports have indicated that both CMV and EBV may play an important role in the etiopathogenesis of periapical pathoses.[41–44] CMV establishes latency in monocytes and T lymphocytes, and EBV establishes latency in B lymphocytes. Both viruses may upregulate the production of numerous proinflammatory cytokines such as interleukin 1β, interleukin 6, and tumor necrosis factor α. These findings have led some to postulate that both CMV and EBV may contribute to the pathogenesis of periradicular pathosis, either as a result of direct infection or through virus-induced immune system alteration.

Infection during fetal and childhood development

Maternal rubella (German measles)

Maternal infection during pregnancy may directly affect the developing fetus, leading to changes that vary from inconsequential to severe enough to result in fetal death.[45] Fortunately, it appears that the most commonly encountered maternal infections cause no significant harm to the developing fetus. The outcome of maternal infection depends largely on the infectious agent involved, the maternal immune response, and the stage of fetal development when the infection occurred. Commonly reported orofacial defects attributed to maternal infection usually encompass structural defects such as cleft lip and palate and various tooth malformations.

One of the more commonly cited examples of a maternal infection that adversely affects tooth development is rubella (German measles).[46–49] Potential effects on the developing teeth vary from none at all to significant changes in terms of size, shape, and color (Fig 20-3). In light of current vaccination protocols, maternal rubella is now a rarely observed phenomenon.

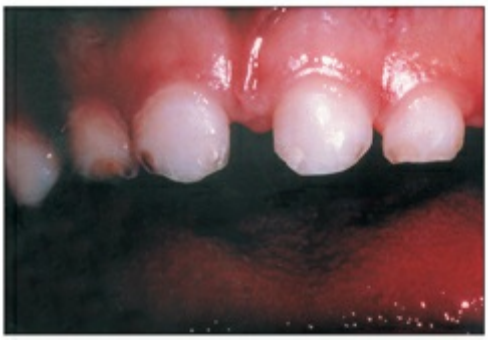

Fig 20-3 Patient with rubella. Note the discoloration of the maxillary anterior teeth.

Childhood infections

Paralleling the effects of maternal infection on the fetal tooth germ, infectious processes that occur during childhood may adversely affect tooth development. Once again, the high prevalence of childhood illnesses weighed against the actual low occurrence of tooth defects indicates that the overall risk is low. The actual etiopathologic mechanisms leading to tooth germ damage are poorly understood and may involve direct injury to the developing tooth by the infectious agent and/or metabolic changes (eg, fever or altered calcium metabolism) associated with the infection[50,51] (Fig 20-4).

Clinical findings include quantitative defects (eg, pits, grooves, or partial or complete loss of enamel) and qualitative defects (eg, the presence of white or discolored enamel with a smooth surface and normal thickness).[52] Because enamel is continuously elaborated during tooth development, it may be possible to crudely estimate the age at which the infectious process occurred by the location of the linear defect on the enamel surface of the tooth.

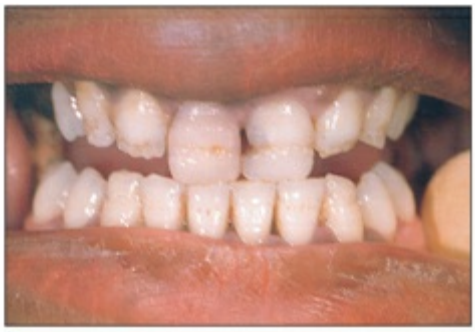

Fig 20-4 Enamel hypoplasia. Note the linear defect in enamel formation that may occur from any of the exanthematous fevers (eg, rubella, measles, or chicken- pox). The position of the linear defect depends on the age of morphodif-ferentiation of the different permanent teeth at the time of the infection.

Genetic and Developmental Disorders

Taurodontism

Taurodontism is a developmental disturbance of teeth that results in abnormally large pulp chambers at the expense of root length.[53–55] The cause is unknown but likely involves aberration or failure of epithelial root sheath differentiation.[54] Both the primary and permanent dentitions may be affected.

The overall prevalence of taurodontism is estimated to be about 3%. Most cases occur as an isolated trait, but the presence of taurodontism in association with several syndromes and diseases has been reported[56–76] (Box 20-1).

Taurodontism may affect one or more teeth; most cases involve molars and, to a lesser extent, premolars. The radiographic appearance is characteristic, revealing elongated pulp chambers and shortened roots.[65,77–79] The altered pulp chamber and canal morphology of the taurodont may complicate the provision of endodontic

therapy because identification of the canal may be compromised and affected teeth are more prone to manipulative fracture[77,78] (Fig 20-5).

Box 20-1	Syndromes and conditions associated with taurodontism

Aarskog syndrome[57]
Apert syndrome[58]
CHARGE syndrome[59]
Down syndrome[60]
Ellis-van Creveld syndrome (chondroectodermal dysplasia)[61]
Gorlin-Goltz syndrome (focal dermal hypoplasia)[62]
Glycogen storage disease type III[63]
Kabuki syndrome[58]
Klinefelter (XXY) syndrome[58,64,65]
LADD syndrome[66]
Lowe syndrome[67]
McCune-Albright syndrome[58]
Menz microphthalmia syndrome[58]
Mohr syndrome[58]
Prader-Labhart-Willi syndrome[68]
Seckel syndrome[69]
Smith-Magenis syndrome[70]
Thalassemia major[71]
Tricho-dento-osseous syndrome[72]
Williams syndrome[58]
Wolf-Hirschhorn syndrome[73,74]
Triple-X syndrome (XXX); XXXX syndrome[58,75]
X-linked hypophosphatemic rickets (XLH)[76]

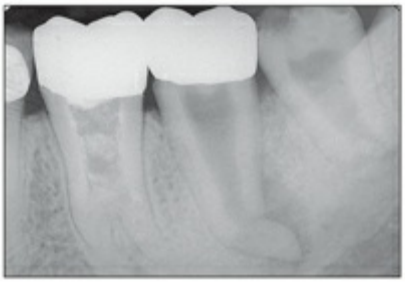

Fig 20-5 Preoperative radiograph of a taurodontic mandibu-lar right second molar. Note the relatively large pulp chamber and shortened roots with distal caries. (Reprinted from Hayas-hi[77] with permission.)

Dens in dente (dens invaginatus) and dens evaginatus

There are several examples of developmental disorders of morphodifferentiation of teeth. Dens in dente (dens invaginatus) and dens evaginatus are two of the more common disorders of morphodifferentiation that have pulpal implications. The reader is encouraged to review oral pathology textbooks for a complete discussion of other disorders of morphodifferentiation (eg, gemination, fusion, concrescence, and dilacerations).[3–5]

Dens in dente is a developmental disorder in which a portion of the crown undergoes an invagination prior to calcification. This is thought to involve an infolding of the dental papilla during development and may occur because of altered tissue pressures, trauma, infection, or localized discrepancies in cellular hyperplasia (eg, apically directed proliferation of ameloblasts). Dens in dente is characterized by a deep infolding of enamel and dentin and often involves maxillary lateral incisors[80–83] (Fig 20-6). Teeth affected by dens in dente are classified into three types: Type 1 is an enamel-lined, relatively minor defect; type 2 is an enamel-lined blind sac that invades the root; and type 3 invades the root and has a secondary foramen. The prevalence has been reported to be between 0.04% and 10.0%.[80]

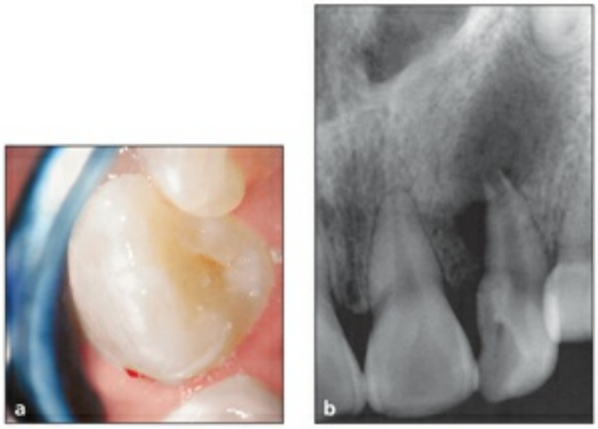

Fig 20-6 Clinical presentation *(a)* and radiograph *(b)* of a dens in dente. (Courtesy of Dr Yoav Shiloah, San Antonio, TX.)

There are several points of clinical significance in the dens in dente tooth. First, there is an increased risk of bacteria-induced pulpal necrosis, and prophylactic placement of sealants may be indicated.[80–84] Second, nonsurgical root canal treatment is difficult because the anatomical complexity makes both tissue debridement and complete obturation extremely challenging. Suggested treatment approaches include the use of ultrasonic files, calcium hydroxide dressings, and

obturation with a thermoplasticized gutta-percha technique.

Dens evaginatus is a localized outgrowth of ameloblasts that appears clinically as a globule of enamel and may be reminiscent of an accessory cusp. The most commonly affected teeth are premolars or molars. The prevalence is higher among Asians (about 15%) than among Caucasians.[5] The outgrowth is postulated to arise from proliferation and elongation of a portion of the inner enamel epithelium and associated dental papilla into the stellate reticulum of the developing enamel organ.[85,86] The resultant extension contains enamel, dentin, and pulp. The clinical significance of this disorder is that the relatively narrow shelf of enamel, once penetrated, often results in pulpal exposure (Fig 20-7).

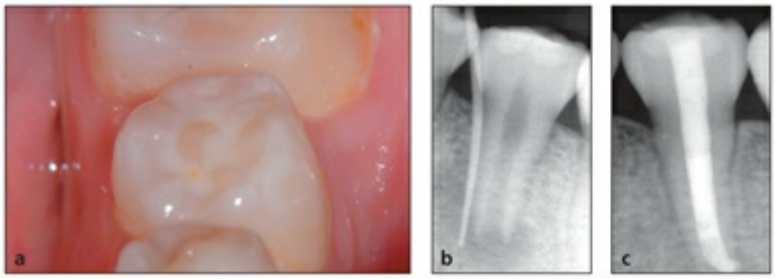

Fig 20-7 Clinical and radiographic views of dens evagi-natus in an 11-year-old boy. *(a)* Clinical view of external anatomy. *(b)* Preoperative radiograph. Note the extensive periradicular radiolucency, complex root canal systems, and immature apex. The sinus tract is traced with a gutta-percha cone. *(c)* Postoperative view illustrating the use of Collatape (Sulzer Dental) and mineral trioxide aggregate (MTA) as an internal barrier and final obturation with thermoplasticized gutta-percha. (Courtesy of Dr Neil Begley, Sequim, WA, and Dr William Schindler, San Antonio, TX.)

Dentinogenesis imperfecta

Dentinogenesis imperfecta (DI) can occur either as an isolated finding or in association with osteogenesis imperfecta.[87–93] Three types of DI are recognized. DI type I is associated with osteogenesis imperfecta and likely represents the dental manifestation of the underlying type I collagen defect. DI types II and III are attributed to mutations affecting the dentin sialophosphoprotein (*DSPP*) gene.[87,92,94] *DSPP* encodes two tooth matrix proteins (dentin sialoprotein and dentin phosphoprotein) that are involved with dentinogenesis.[92,95–97] Recent studies have demonstrated that DI types II and III occur as a consequence of altered dentin sialoprotein and phosphoprotein production.[89,91,96] DI type II is considered to be the most common dental genetic disease in humans, affecting an estimated 1 in 6,000 to

8,000 people.[87,92]

The clinical findings of all types of DI are similar and characteristic. The teeth manifest an amber-brown to blue-gray hue, an opalescent sheen, cracking or loss of enamel, and attrition[88–91,98] (Fig 20-8). Radiographic findings for DI types I and II include a bulbous crown structure, cervical constriction, short roots, and obliteration of the pulp chamber (Fig 20-9). For DI type III, the radiographic features are similar, except that the pulp chambers appear normal or enlarged (shell teeth), and periradicular radiolucencies are frequently present. The notable clinical similarities between DI types II and III, along with a similar condition called *dentin dysplasia type II*, has prompted some to speculate that these disorders represent phenotypic variations of a single disorder.[91,94] Both the primary and permanent dentitions may be affected.

The altered tooth structure associated with DI may compromise the success of endodontic therapy.[99] Pulpal obliteration may compromise access, canal identification, and chemomechanical debridement. The presence of extensive shelling may increase the risk of spontaneous or manipulative enamel fracture. The altered, and at times difficult to identify, root canals may compromise the success of surgical endodontic treatment.

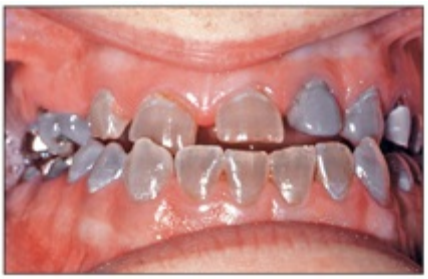

Fig 20-8 Type II DI in an 18-year-old man. Note the yellow-brown shade of the anterior teeth and the blue-gray shade of the posterior teeth. (Reprinted from Huber[90] with permission.)

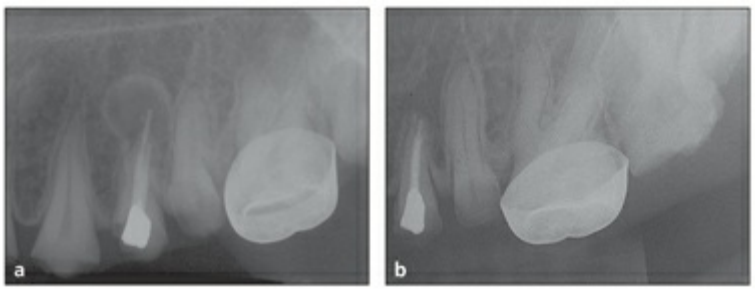

Fig 20-9 Periradicular radiographs of a patient with type I DI. *(a)* Preoperative radiograph. Note the reduced size of the pulp chamber, canal orifices, and canal systems in the first premolar as well as the presence of overextended gutta-percha and apical periodontitis. *(b)* One-year follow-up after root-end resection and MTA retrofill of the first premolar.

Amelogenesis imperfecta

Amelogenesis imperfecta (AI) comprises a hetero-genous group of heritable developmental disorders that affect both the primary and permanent dentitions in the absence of a generalized syndrome.[100–105] AI is estimated to occur in 1:14,000 patients in the United States, and autosomal-dominant, autosomal-recessive, and X-linked patterns of inheritance have been described.[102] To date, four confirmed genes for AI have been described, but others remain to be identified and mapped[101] (Table 20-1). A universally accepted nosology to describe the phenotypic diversity of AI does not exist, but most cases are clinically described as either hypoplastic, hypocalcified, or hypomatured.[101,102,106]

In addition to the universal enamel impairment observed in AI, other anomalies, such as delayed or failed eruption, crown resorption, and pulp calcification, have been reported.[107] However, the extreme phenotypic diversity observed in AI compromises comparative studies, of which there are few. In one study of 22 patients from nine unrelated families with various forms of AI, the presence of crown resorption (41%), delayed eruption (27%), and pulp calcifications (14%) was significantly greater than that observed in controls.[107] The presence of pulp calcification was highly associated with the autosomal-recessive hypoplastic form of AI.

Although the patient with AI may present significant restorative and esthetic challenges for the dental practitioner, there is no AI-specific reason to withhold indicated endodontic therapy.

Table 20-1 Proven genes for amelogenesis imperfecta*

Gene	Inheritance pattern	Phenotypic findings
Amelogenin *(AMELX)*	X-linked	Distinctive vertical banding pattern on enamel of affected females
Enamelin *(ENAM)*	Autosomal dominant	Hypoplastic (thin) enamel Mild pitting Horizontal lines in the affected enamel, especially in the cervical third
Distal-less homeobox 3 *(DLX3)*†	Autosomal dominant	Enamel hypoplasia Taurodontism

Enamelysin *(MMP20)*	Autosomal recessive	Pigmented agar-brown discoloration Rough, mottled enamel Enamel fracturing Decreased radiopacity of enamel
Kallikrein 4 *(KLK4)*	Autosomal recessive	Pigmented yellow-brown discoloration Hot/cold sensitivity Chewing sensitivity Enamel fracturing Decreased radiopacity of enamel

*Data from Ng and Messer.[101]

†Phenotype considered a variation of the trichodento-osseous syndrome.

Osteoclastic diseases

Osteoclasts, which are essential for normal tooth eruption, are highly specialized cells derived from hematopoietic cell precursors in the bone marrow and peripheral blood.[108–112] Osteoclast formation requires the presence of two essential factors found in bone marrow stromal cells and osteoblasts: macrophage colony-stimulating factor and receptor activator of nuclear factor κB ligand (RANKL).[109,113,114] Binding of RANKL with the osteoclastic receptor activator of nuclear factor κB (RANK) leads to increased nuclear factor κB (NFκB) signaling and upregulation of genes required for osteoclast differentiation and activity. Another defined modulator in the RANK/RANKL system is osteoprotegerin (OPG), which is a soluble decoy receptor that competes with RANK for the RANKL binding site.

It appears that most processes affecting bone resorption and tooth eruption act through modulation of the balance of RANKL and OPG[108–112] for RANK. Disease states that result in reduced osteoclastic activity (eg, osteopetrosis, cleidocranial dysplasia, and pyknodysostosis) commonly exhibit phenotypic delay or failure of tooth eruption, while disease states that result in increased osteoclastic activity (eg, Paget disease of bone [PDB] and juvenile Paget disease) manifest accelerated tooth loss.

Paget disease of bone (osteitis deformans)

PDB is a heterogenous, focal, progressive bone disease characterized by active bone turnover.[114,115] The risk of developing PDB increases with age, affecting an estimated 1% of individuals over the age of 40 years in the United States.[116] Men are at slightly greater risk than women. In addition to a genetic predisposition, other yet to be defined environmental factors likely contribute to the disease process.[117,118]

Mutations affecting the sequestosome 1 gene are found in up to 50% and 30% of patients with familial and sporadic cases of PDB, respectively.[119] Sequestosome 1 (also known as *p62*) is an important scaffold protein in the NFκB pathway. In terms of environmental factors, some have proposed that a latent paramyxoviral infection (eg, measles, canine distemper virus, or respiratory syncytial virus) contributes to PDB, but this issue remains unresolved.[113,118,120]

PDB may affect one (monostotic) or a few (polyostotic) bones, and most cases are asymptomatic and discovered incidentally.[116,118,121] The most commonly affected sites include the pelvis, skull, vertebra, femur, and tibia. Affected bones usually manifest an initial osteolytic phase, followed by a mixed osteolytic/osteosclerotic phase, which ultimately progresses to a disorganized osteosclerotic phase. In reality, all three arbitrary phases of PDB may exist concurrently. Common clinical signs and symptoms include osseous distortion or expansion and mild to moderate, deep, aching bone pain. Elevated serum total alkaline phosphatase levels are reflective of the increased bone activity and characteristic for the disease. While osteosarcoma is a rare complication of PDB, most cases of adult osteosarcoma occur in patients with PDB.[113]

Skull involvement is estimated to occur in about 27% of patients and may result in hearing loss and vestibular problems in up to 89% of those affected.[122] It is postulated that, in addition to direct auditory nerve impingement, the disease may adversely alter the bone density, mass, and structure of the auditory complex to contribute to hearing compromise.

Numerous dental abnormalities, such as malocclusion, hypercementosis, tooth mobility, root resorption, pulp calcification, osteomyelitis, poorly fitting prostheses, and excessive postsurgical bleeding, have been associated with PDB.[122,123] However, these findings are largely based on anecdotal case reports, and their true prevalence is unknown. In a cross-sectional survey of 292 patients with PDB, 93% of those with maxillomandibular involvement related having dental problems, compared to only 10% of those with skull or other distant bone involvement.[122] However, compared to controls, the patients with dental problems reported no significant difference in edentulism, tooth movement, change in bite, change in

denture fit, periodontal disease, or need for endodontic treatments. Many of the dental changes associated with PDB are likely explained by the dynamic nature of the disease. During the osteolytic phase, tooth mobility, shifting, and increased postsurgical bleeding may occur. More mature pagetoid changes affecting the jaws may manifest as malocclusion and/ or deformity and result in an increased risk of osteomyelitis.

Potential radiographic changes associated with PDB reflect the brisk osteoclastic and osteoblastic nature of the disease.[121,124] The characteristic lytic sign of PDB affecting the skull is osteoporosis circumscripta, whereas the characteristic sclerotic sign is the cotton wool appearance. An overall mosaic of lytic and sclerotic findings is frequently noted. Potential radiographic findings of PDB involving the jaws include hypercementosis, thickening of the periodontal ligament space, root resorption, and pulpal obliteration.[123,125–129]

Hypercementosis has been reported to occur frequently in PDB, and its presence is considered highly suggestive of PDB.[123,126,127] Large amounts of cementum may be deposited in the apical two-thirds of the roots, giving the tooth the appearance of a baseball bat (Fig 20-10). The cementum may appear to be fused with the adjacent sclerotic bone[130] (Fig 20-11), resulting in ankylosis.[128] In such a scenario, the involved teeth will be abnormally firm. Lytic activity around an involved tooth may affect the lamina dura to produce a widened periodontal ligament space. In contrast to primary or secondary hyperparathyroidism, which manifests generalized thickening of the periodontal ligament space, in PDB the widening is restricted to involved teeth. An additional lytic change that may occur is root resorption, which may clinically manifest as mobility. Paralleling the concurrent mixed lytic and sclerotic processes affecting the bone, lytic changes may affect some teeth while other teeth may demonstrate hypercementosis.

The potential for pagetoid changes to affect the pulp has been proposed, but few reports addressing the issue exist.[125,128,129] In one case report evaluating a mandibular third molar, internal resorption of the dentin, direct apposition of cellular hard tissue partially occluding the coronal pulp, and dystrophic calcification in the radicular region of the pulp were noted. The overall mosaic pattern observed was similar to that typically observed in the bone and cementum.[125] These potential radiographic findings may interfere with endodontists' ability to establish a proper working length, prompting some to recommend the use of an apex locator to establish the canal length.[123]

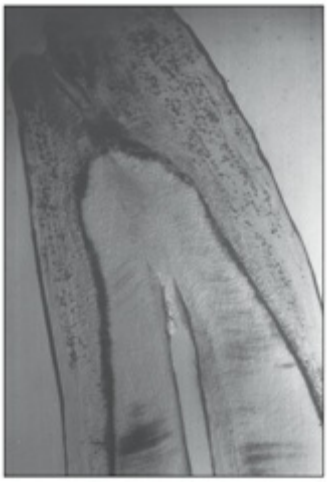

Fig 20-10 Ground section of a maxillary second premolar showing the extensive hypercementosis associated with Paget disease.

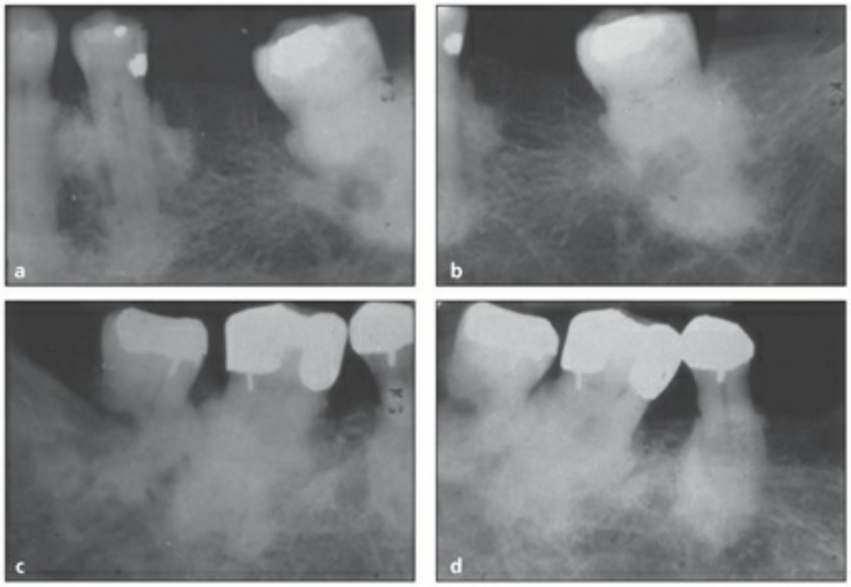

Fig 20-11 Characteristic intraoral radiographic features of Paget disease. Note the variable appearance of the canal space, periodontal ligament space, and lamina dura. *(a)* Areas of radiolucency. *(b)* Isolated sclerosis. *(c)* Isolated sclerosis and distal root resorption of the maxillary second molar. *(d)* Isolated sclerosis and hypercementosis of the second premolar. (Reprinted from Barnett and Elfenbein[130] with permission.)

Disturbances of vitamin D metabolism

The primary regulator of calcium homeostasis and subsequent bone and dentin mineralization in the body is bioactive vitamin D (1α,25-dihydroxyvitamin

D_3).[131–133] Numerous rachitic conditions relating to specific alterations in vitamin D metabolism have been identified. Both vitamin D–dependent rickets type I (VDDRI) and vitamin D–dependent rickets type II (VDDRII) are rare recessive disorders, while X-linked hypophosphatemic rickets (XLH) is passed as an X-linked dominant trait. The phenotypic presentations of all three forms of rickets have much in common, but subtle variations do exist.

Vitamin D–dependent rickets type 1

VDDRI manifests as deficient 1-α-hydroxylase production, and the responsible mutation has been mapped to chromosome 12.131 1-α-hydroxylase is a member of the cytochrome P450 superfamily of enzymes and is required for normal vitamin D activation. It acts on the circulating vitamin D precursor 25-hydroxyvitamin D_3 to form bioactive vitamin D.[132,134] Originally believed to be restricted to the kidney, 1-α-hydroxylase expression and activity occur at several extrarenal sites, including skin, lymph nodes, colon, pancreas, brain, endothelial cells, and bone.

Prominent clinical features of VDDRI include stunted growth, skeletal abnormalities, genu valgum, rachitic rosary, open fontanels, pathologic fractures, muscle weakness, and convulsions. Common laboratory findings include hypocalcemia, hypophosphatemia, elevated parathyroid hormone, high alkaline phosphatase, normal levels of vitamin D precursors, and low levels of bioactive vitamin D. A single case report of the dental findings in a patient with VDDRI noted the presence of enamel hypoplasia, large quadrangular pulp chambers, and short roots.[133] The administration of physiologic levels of bioactive vitamin D (1α,25-dihydroxyvitamin D_3) is the treatment of choice. Such therapy bypasses the deficient enzyme, thereby fostering normal development.

Vitamin D–dependent rickets type II

Mutational abnormalities mapped to chromosome 12 underlie VDDRII.[131] In this scenario, the vitamin D receptor is adversely affected, resulting in end-organ resistance to vitamin D modulation. The resultant defective intestinal calcium absorption leads to hypocalcemia and rickets. Laboratory findings include hypocalcemia, elevated parathyroid hormone levels, and hypophosphatemia. Vitamin D precursor levels are normal, and bioactive vitamin D levels are elevated. The elevated levels of bioactive vitamin D help to distinguish VDDRI from VDDRII.[131]

The clinical findings of VDDRII are often severe and usually apparent within a

few months of birth.[131] The clinical phenotype is similar to that for VDDRI. However, a fairly characteristic feature of VDDRII is sparse body hair, or *alopecia universalis*, the presence of which appears to correlate well with disease severity. Medical therapy is limited and consists of calcium infusion and the administration of pharmacologic levels of precursor and bioactive vitamin D. Because neither of these approaches addresses the receptor deficiency, results have been mixed.

Only a single report is available in the English literature addressing the dental findings associated with VDDRII.[135] The authors reported on the dental findings in three children with VDDRII. Two of the three children, both girls, exhibited no evidence of enamel hypoplasia but did manifest enlarged pulp chambers and thin dentin. Both responded well to medical therapy and experienced normal root canal and pulp chamber development. The other child, a boy, presented with a more severe phenotypic presentation of VDDRII than the two girls. On initial examination, an abscessed primary maxillary first molar was noted, and his teeth also exhibited enlarged pulp chambers and thin dentin. The abscessed tooth was extracted and submitted for histologic assessment. The histologic findings showed abundant interglobular dentin and a lack of a predental layer. This child did not respond to medical therapy, and the dental defects persisted.

X-linked hypophosphatemic rickets

The most prevalent form of rickets is caused by mutations affecting the *PHEX* gene (phosphate-regulating gene with homology of endopeptidases that maps to the X chromosome) and is termed *X-linked hypophosphatemia*.[136] The mutation responsible for XLH has been mapped to Xp22.2-p22.1, and the incidence is estimated at 1 in 20,000 persons.[70,136–138] Patients with XLH experience impaired 1-α-hydroxylase activity, reduced phosphate resorption in the kidney and intestine, hypophosphatemia, and hyperphosphaturia. The current medical treatment of choice is the prompt institution of supplemental vitamin D and phosphate therapy.

The main clinical features include severe bony deformities, impaired growth, short stature, frontal bossing, craniotabes, and a square-shaped head. The dental findings affect both the primary and permanent dentitions and include enlarged pulp chambers, enlarged pulp horns, hypocalcified and interglobular dentin, short roots, taurodontism, poorly defined lamina dura, and hypoplastic alveolar ridges[70,133,138] (Figs 20-12 and 20-13). The severity of the clinical and radiographic dental lesions is influenced by the patient's age, the type of teeth (prenatal or postnatal odontogenesis), and whether the patient was undergoing therapy prior to the time of

examination.

The finding of prominent pulp horns extending up to the dentinoenamel junction in both dentitions is regarded by some as pathognomonic for XLH.[137,139] These abnormalities place the XLH patient at risk for spontaneous dental abscesses, which occur frequently and often without any evidence of preexisting trauma or caries.[70,137,138,140,141] It is likely that attrition of the enamel allows bacterial entry into the dentinal tubules and pulp.[70,142,143] Although endodontic treatment is clearly indicated in many instances, the underlying structural defects associated with XLH may compromise the clinician's ability to obtain an adequate obturation seal.

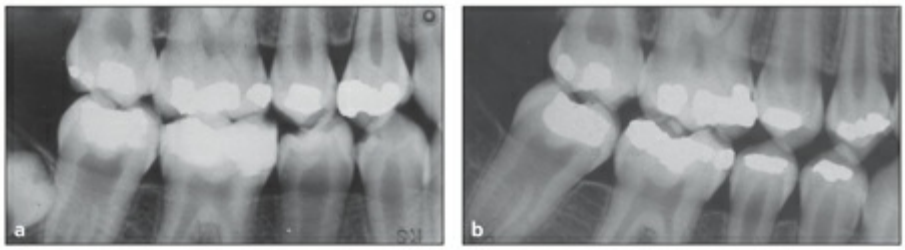

Fig 20-12 *(a and b)* Bitewing radiographs of a patient with XLH rickets. Note the enlarged pulp chambers, large root canal systems, and pulp horns that extend to the dentinoenamel junction. (Reprinted from Bender and Naidorf[139] with permission.)

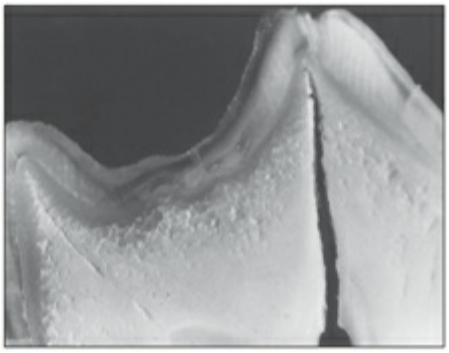

Fig 20-13 Ground section of a premolar from a patient with XLH rickets. The high risk of pulpal necrosis and subsequent periradicular lesions is readily apparent. The pulp horn defect appears as a tubular cleft covered only by a thin layer of hypoplastic enamel, showing the extension of the pulp horn to the dentinoenamel junction. Note the appearance of hypoplastic enamel and interglobular dentin. (Courtesy of Dr C. J. Witkop).

Hemoglobinopathies

Hemoglobinopathies are a group of hereditary blood disorders that affect hemoglobin. The two most commonly cited diseases of this group are thalassemia

and sickle cell anemia (SCA).

Thalassemia

Thalassemia refers to a group of clinically heterogenous and common hemolytic anemias involving reduced or absent hemoglobin production.[63,144,145] Most cases are inherited in an autosomal-recessive pattern, and an estimated 1.67% of the worldwide population carries a thalassemia trait.[145] Thalassemias vary in severity and are classified as homozygous (major), heterozygous (minor), or compound heterozygous (intermedia). The most serious type of thalassemia is the major form of β-thalassemia, also known as *Cooley anemia*.[145,146]

Hemoglobin consists of four protein subunits, typically two subunits called α-*globin* and two subunits called β-*globin*. The *HBB* gene provides instructions for making β-globin. Over 200 point mutations affecting the gene have been discovered, which helps account for the variability in β-globin–chain synthesis reduction.[145,147] In β-thalassemia, two β-globin genes that carry a severe mutation are present. The imbalance between normal α-globin production and reduced β-globin production results in severe anemia, an ineffective compensatory erythropoiesis, and iron overload. The compensatory erythropoiesis often leads to a 15- to 30-fold expansion of the bone marrow.[63,147] Iron overload underlies most of the other characteristic manifestations, including hepatosplenomegaly, skeletal changes, growth retardation, increased susceptibility to infection, endocrine dysfunction, and cardiac failure.

Medical management is multidisciplinary and centered on lifelong regular transfusions and chelation therapy. The primary cause of death in β-thalassemia is cardiac failure, usually during early adulthood, because of iron overload.[145]

Orofacial manifestations of β-thalassemia are frequently observed and generally a consequence of the extensive marrow space expansion. A characteristic "rodent" or "chipmunk" facies may be observed because of the presence of maxillary protrusion and expansion, bossing of the skull, hypertelorism, and prominent cheek bones.[63,146,148] Malocclusion is commonly observed, typically marked by overbite, flaring, increased overjet, and spacing of the teeth. Delayed dental development has been reported, as has a higher prevalence of taurodontism. Radiographically, the jaws have been described as osteoporotic with coarse trabeculation[146] (Fig 20-14).

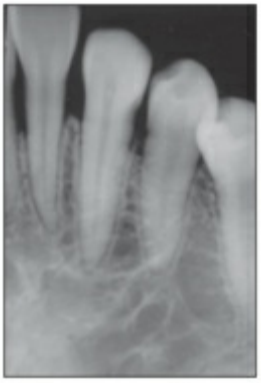

Fig 20-14 Periradicular radiograph from a patient with thalas-semia.

Sickle cell anemia

SCA represents the most common form of a group of sickling disorders that affect hemoglobin.[149] It is a recessive disorder estimated to affect 1 in 500 African Americans. Approximately 13% of African Americans are heterozygous carriers of the SCA trait. SCA is caused by a single point mutation at the sixth codon, which leads to the substitution of valine for glutamic acid in the β chain of hemoglobin.[150] When deoxygenated, affected hemoglobin undergoes polymerization, which causes the red blood cell to change into the characteristic sickle shape.[151] For those individuals with SCA, more than 70% of circulating hemoglobin is affected, whereas for those with SCA trait, less than 45% of circulating hemoglobin is affected.[151]

The sickle red cell is more fragile than a healthy red cell and cannot deform as it attempts to move through the capillaries. As a consequence, the affected red cell life span is reduced from the normal 120 days to a range of 10 to 30 days, and compensatory marrow hyperplasia is characteristic (Fig 20-15). The disruption of blood flows leads to vascular occlusions, hemorrhages, infarctions, and ischemic necrosis affecting a variety of body tissues and organs.[149,151]

Clinical manifestations of SCA include chronic hemolytic anemia, pallor, increased susceptibility to infection, and specific endorgan damage (eg, retinopathy, nephropathy, leg ulcers, and osteomyelitis of long bones and the mandible).[149] Characteristic painful episodic vaso-occlusive crises (sickle cell crises) occur in the abdomen, joints, muscles, and bones. These crises may be precipitated by dehydration, acidosis, strenuous exercise, trauma, pulmonary disease, or a prior vaso-occlusive event.

There is no cure for SCA. Medical management is multidisciplinary and targeted to relieve pain, prevent infection, prevent end-organ damage, and control

complications if they occur.[150] Heterozygous carriers generally do not manifest significant clinical disease.

Head and neck manifestations of SCA, such as marrow hyperplasia, osteoporosis, bone infarction, osteonecrosis, and osteomyelitis, are observed in 79% to 100% of SCA patients.[151] The risk that osteomyelitis will affect the jaws of patients with SCA is 200 times that found in healthy controls.[152] Radiopaque lesions, commonly observed in the posterior body of the mandible, are believed to represent a sclerotic response to a prior osseous infarction.[151] A spectrum of neuropathic manifestations may occur as a consequence of vaso-occlusive events. Examples include asymptomatic pulpal necrosis, symptomatic pulpitis without evident radiographic involvement, vague jaw pain or paresthesia, and referred odontalgia.[151,153–155] When feasible, endodontic treatment is preferred over extraction to reduce the risk of osteomyelitis. All dental care should be performed in close consultation with the managing physician. If surgery is necessary, surgical manipulation and trauma should be minimized.[156]

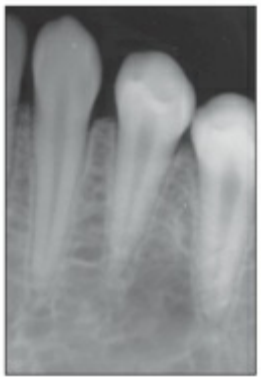

Fig 20-15 Periradicular radiograph from a patient with sickle cell anemia.

Other inherited disorders affecting the oral cavity

Gaucher disease

Gaucher disease, the most commonly occurring lysosomal storage disorder, is a recessively inherited deficiency of the enzyme acid-β-glucosidase.[157,158] Acid-β-glucosidase breaks down the glycolipid glucocerebroside to yield glucose and ceramide. Insufficient enzyme availability leads to accumulation of glycolipid throughout the reticuloendothelial system, particularly in macrophages. Gaucher

disease is classically divided into three types, based on the presence or absence and rate of progression of neurologic manifestations.[158,159] Type I Gaucher disease is the most frequently occurring genetic disorder affecting Ashkenazi Jews, among whom the carrier rate is about 1 in 15.158 Most patients with Gaucher disease respond well to the administration of recombinant acid-β-glucosidase (enzyme replacement therapy).[158,159]

The clinical manifestations of Gaucher disease vary greatly, ranging from death in utero to inconsequential. Common signs and symptoms include hepatosplenomegaly, thrombocytopenia, and anemia. Skeletal manifestations affect an estimated 75% of patients and include osteopenia, lytic lesions, pathologic fracture, deformity, chronic and acute bone pain, bone infarcts, and osteonecrosis.[157,160,161] An Erlenmeyer flask–like appearance of the distal head of the femur is considered a pathognomonic sign for Gaucher disease.[161,162]

In a small survey, radiographic involvement of the jaws, consisting of enlarged marrow spaces, cortical thinning, endosteal scalloping, external root resorption, or radiolucencies of the jaws, were noted in 25 of 28 patients studied.[162] Involvement of the mandibular body and the premolar and first molar region of the mandible has also been noted[163] (Fig 20-16). Bender and Bender[163] reported on the dynamic nature of Gaucher disease, following 11 affected patients over a period of 13 to 60 years (Table 20-2). They noted lesion enlargement and temporary bone regeneration that occurred after local curettage, tooth extraction in proximity to the lesion, or long-bone fracture. These changes were eventually replaced by further lytic destruction. While extraosseous extension of Gaucher lesions is considered rare, reports of extraosseous extension into masseter muscle and sphenoid sinuses have been published.[160,161,164]

Aside from the previously mentioned external root resorption, specific dental and gingival changes associated with Gaucher disease appear to be minimal.[162] However, these patients should be closely followed and should undergo regular radiographic assessment to monitor osseous involvement because they may be at increased risk for developing osteomyelitis or pathologic fracture.[165] Many of these patients undergo splenectomy as part of their disease management, which may increase their risk of infection. Finally, the commonly observed thrombocytopenia places the patient with Gaucher disease at risk for increased postsurgical bleeding.[162]

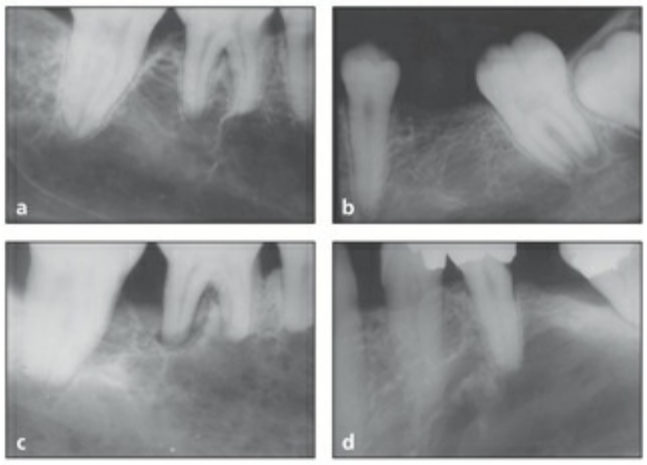

Fig 20-16 Radiographic presentation of Gaucher disease. *(a and b)* Uniform bilateral periapical radiolucency of the mandibular second premolars and first molars. *(c)* Twenty-year follow-up revealing evidence of periapical root resorption on the second premolar and first molar. The resorption only occurs in the apical portion of the roots. *(d)* Twenty-year follow-up of the contralateral side reveals less periapical root resorption. (Reprinted from Bender and Bender[163] with permission.)

Table 20-2 **Dental observations in 11 patients with Gaucher disease (type 1)**

Mandibular radiolucency	Maxillary radiolucency	Osteopenia	Sclerotic changes	Biopsy	Hemorrhagic diathesis	Radiologic root resorption
+	+	+	-	-	-	+
+	+	+	-	-	-	-
+	-	+	-	+	+	+
+	0	+	-	+	+	-
+	+	+	-	-	+	-
+	0	+	+	0	-	-
+	0	+	+	0	-	+
+	0	+	-	0	+	+
+	0	+	-	+a	+a	-
+	+	+	+	+	0	+b
+	0	+	0	+	0	0

+, present; -, absent; 0, not mentioned; +a, autopsy; +b, histologic resorption. (Reprinted from Bender and Bender[163] with permission.)

Other disorders

Numerous other uncommon or rare inherited disorders may manifest involvement of the oral cavity. A selected few are summarized in Table 20-3.[74,166–184]

Table 20-3 Other inherited disorders affecting the oral cavity

Disease	Mode of inheritance	Pathogenesis	Clinical manifestations
Fabry disease[166-167]	X-linked recessive	Deficiency of lysosomal enzyme α-galactosidase	Acroparesthesias, angiokeratomas, hypohidrosis, corneal opacity, hearing loss, malocclusion, diastemas, mucous retention cysts
Oculocerebrorenal syndrome (Lowe syndrome)[67,168,169]	X-linked recessive	Deficient phosphatidylinositol 4,5-bisphosphate-5-phosphatase	Hydrophthalmos, cataracts, mental retardation, renal tubular dysfunction, large pulp chambers, dysplastic dentin, periodontal disease
Cyclic neutropenia[170-173]	AD	Cyclic production of white blood cells (21-day periodicity)	Increased susceptibility to infection, progressive periodontal disease, oral ulcerations
Papillon-Lefèvre syndrome[174-177]	AR	Defective cathepsin C activity	Palmoplantar hyperkeratosis, severe early-onset periodontitis leading to early tooth loss
Chédiak-Higashisyndrome[178-180]	AR	Defective intracellular protein trafficking to and from the lysosome	Severe immunologic defects, reduced pigmentation, mild bleeding tendency, progressive neurologic dysfunction
22q11.2 deletion syndrome (DiGeorge syndrome)[181]	AD	Deletion of approximately 30 genes on chromosome 22	Cardiovascular defects, craniofacial anomalies, ear defects, immunologic problems, parathyroid abnormalities, kidney

			abnormalities
Primary oxalosis type I[182-184]	AR	Deficiency of alanine-glyoxylate aminotransferase	Progressive nephrolithiasis/ nephrocalcinosis and eventual renal failure, extrarenal calcium deposits, slate-gray teeth, odontalgia, pulpal calcifications, root resorption

AD, autosomal dominant; AR, autosomal recessive.

Endocrine Disorders

Diabetes mellitus

Diabetes mellitus is a serious endocrine disorder characterized by an absolute or relative insulin insufficiency or target resistance to insulin activity. In the United States, an estimated 25.8 million individuals have diabetes, and an estimated 7.0 million are undiagnosed.[185] Patients with poorly controlled diabetes manifest chronic hyperglycemia and are at increased risk for myriad complications, including infection.[186–189] The five classic complications of diabetes are *(1)* retinopathy, *(2)* nephropathy, *(3)* neuropathy, *(4)* macrovascular disease, and *(5)* impaired wound healing.[187,189–191]

Hyperglycemia leads to the creation and accumulation of advanced glycation end-products (AGEs), which are believed to contribute directly to the etiopathogenesis of diabetes.[187,189,191–196] AGEs impair normal homeostatic collagen turnover, leading to the accumulation of a more mature, less soluble collagen in the vessel wall; subsequent vessel wall thickening and reduction in lumen size; and a decrease in tissue perfusion.[187,189,195] AGE-altered collagen is also more prone to bind with low-density lipoprotein to form atheromas, which contribute to large vessel disease.[187] At the immunologic level, hyperglycemia results in impaired chemotaxis, adherence, and phagocytosis of polymorphonuclear leukocytes.[187] AGE interaction with monocytes and macrophages results in the creation of a proinflammatory

phenotype.[187,191,193,197]

While it is axiomatic that adequate medical control of diabetes is essential to the maintenance of oral health, the impact of poor oral health (eg, periodontitis or periradicular abscess) on the control and progression of diabetes must be understood. A two- to three-times greater rate of periodontal disease has been observed in individuals with diabetes. This is understandable, given the fact that the typical area of the susceptible periodontium is roughly the size of the palm.[198] Such a large inflammatory burden may negatively impact glucose control in the diabetic patient and confer an increased risk of developing diabetes-related complications.[188–191,194,197–199]

Although there are few studies addressing the relationship between diabetes and pulpal disease, logical points of intersection to consider would include diabetes-induced microvascular, neuropathic, and inflammatory changes. It appears that diabetic patients in need of endodontic treatment are more likely to present with larger periradicular lesions, harbor more virulent pathogens, experience perioperative symptoms, and experience a higher incidence of therapeutic failure than control patients.[186,200–205]

The increased risk of infection and potential poor wound healing observed in the diabetic patient has led some to advocate the administration of antimicrobial prophylaxis prior to dental treatment, particularly in the individual with poorly controlled diabetes.[206] However, there have been no studies directly addressing the issue, and the overall complexity of diabetes would seem to confound any attempted study. It is clear that any infection, including pulpal disease, in the diabetic patient must be managed promptly and aggressively. As a rule, diabetic patients under good disease control can be generally regarded as normal patients, whereas patients who are either suspected of having diabetes or whose diabetes seems under poor control should be referred for medical evaluation prior to the delivery of dental care. Medical consultation is further recommended for any anticipated dental treatment, such as extensive surgery, that can adversely impact the patient's glucose control.

Adrenal dysfunction

Cortisol, a naturally occurring glucocorticoid of the adrenocortex, and the catecholamine epinephrine are the primary modulators of the stress response.[207–210]

Cortisol stimulates peripheral fat and protein catabolism to serve as substrates for the hepatic production of glucose.[211] Well-established anti-inflammatory and immunomodulatory effects of cortisol include preventing leukocyte migration from the circulation into the extravascular space, reducing the accumulation of monocytes and granulocytes at inflammatory sites, and suppressing the production of numerous cytokines and other proinflammatory mediators. Cortisol also acts in a permissive role to allow other hormones such as catecholamines and angiotensin II to modulate cardiac contractility, vascular tone, and blood pressure.[212–214] Finally, cortisol provides negative feedback to the hypothalamus and the anterior pituitary gland (hypothalamic-pituitary-adrenal [HPA] axis) in regulating corticotropin-releasing hormone and adrenocorticotropin-releasing hormone.[207,210,215]

Conditions of insufficient cortisol production, regardless of the etiology, are termed *Addison disease*. [216] Most cases of glucocorticoid deficiency develop insidiously, presenting with nonspecific signs and symptoms such as lethargy, anorexia, nausea, weight loss, and hypoglycemia. These patients are typically prescribed empirical replacement glucocorticoid therapy.[217] Conditions of endogenous cortisol excess are termed *Cushing syndrome*.[211,218,219] However, the most frequent cause of glucocorticoid excess is iatrogenic. The clinical findings of cortisol excess correspond directly to the severity and duration of glucocorticoid excess and include truncal obesity, violaceous striae of the skin, buffalo hump, facial fullness (moon facies), facial plethora, acne, hirsutism, easy bruising, muscle wasting, and myopathy.

There are no disease-specific oral manifestations of Addison disease. Some individuals manifest a patchy brown (bronzing) pigmentation of the face (with superimposed areas of vitiligo), the buccal mucosa, the tongue, and, less frequently, the lips and gingivae. Head and neck manifestations of Cushing syndrome include facial plethora, moon face, hirsutism, and acne. Affected children may exhibit delayed or arrested dental development paralleling the overall growth retardation that may occur with glucocorticoid excess.[220,221] Chronic exposure to excess glucocorticoid appears to stimulate odontoblast-like cells within the dental pulp, resulting in narrowing of the dental pulp chamber or complete pulpal obliteration[222–224] (Fig 20-17).

Patients with Addison disease are inherently unable to produce sufficient levels of adrenocorticotropic hormone or cortisol to meet physiologic demand. Similarly, chronic exogenous glucocorticoid exposure can suppress the HPA axis, and therefore patients on chronic glucocorticoid therapy may not be able to produce

sufficient levels of adrenocorticotropic hormone and cortisol to meet physiologic demand.[211,225–227] The concern in clinical practice is that, for either situation, an overwhelming stressor (surgery, sepsis, or fever) may precipitate an Addi-sonian crisis (adrenal insufficiency with hypotension and shock).[228–230] Accordingly, supplemental glucocorticoids ("stress dose" steroids) are recommended perioperatively for patients with documented or presumed HPA axis suppression when they are to undergo a stressful medical procedure. Fortunately, it appears that the risk of an Addisonian crisis during an outpatient dental procedure is low or very low.[217,230] Consequently, patients need only to take their usual daily glucocorticoid dose on the day of the procedure. If moderate or major surgical stress is anticipated under general anesthesia and the patient has documented or presumed HPA axis suppression, then appropriate stress doses of perioperative steroids are indicated.

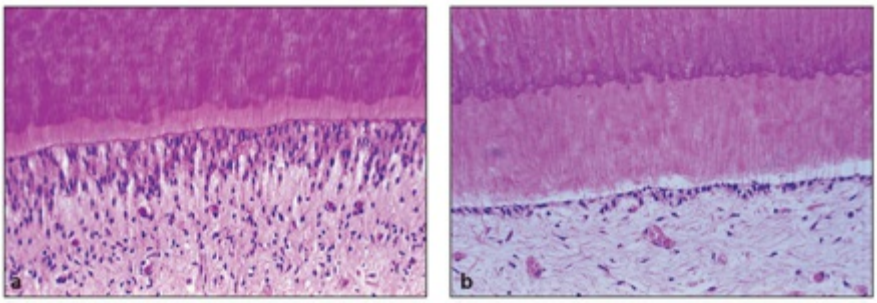

Fig 20-17 Comparison of predentin thickness in teeth collected from a healthy patient *(a)* and a patient who was chronically treated with glucocorticoids, suffering from renal failure, and undergoing dialysis *(b)*. The predentin is about four times wider in the healthy patient than it is in the chronically ill patient (hematoxylin-eosin stain). (Reprinted from Wysocki et al[224] with permission.)

Cancer

There are few reports of malignancies affecting the dental pulp. The risk that a primary malignancy will arise from pulpal tissue appears to be extremely remote.[231] In reviewing the dental literature published over a 100-year span (1870 to 1970), Stanley[129] reported fewer than 20 case reports of pulpal cancer. Furthermore, if a primary pulpal malignancy were to develop, the enclosed nature of the pulp chamber increases the likelihood that the tumor growth will adversely interrupt the pulpal vascular network, resulting in self-strangulation. A more likely, albeit still rare, scenario occurs when the pulp or periradicular area is infiltrated by a hematologic malignancy or is the target of a solid tumor metastasis.[232–251]

Regardless of the specific malignancy involved, the presenting signs and symptoms are often nonspecific and mimic routine inflammatory pulpal or periodontal disease.[235,237,238,243,244] Thus, the presence of findings such as odontalgia, percussion sensitivity, periradicular radiolucencies, and gingival inflammation and swelling often raise little suspicion. However, the presence of more worrisome findings, such as altered sensation (eg, anesthesia or paresthesia), asymmetric widening of the periodontal ligament space, loss or thinning of the lamina dura, and moth-eaten or ill-defined radiolucencies, warrant increased suspicion (Fig 20-18). Finally, a prior history of malignant disease, especially of the breast, lung, adrenal glands, kidney, prostate, thyroid, and colon, should raise the clinician's suspicion of a malignancy even more.[252,253]

Therapeutic interventions to treat cancer may adversely affect structures of the head and neck, including the dental pulp.[254–261] Probably the most frequently incurred therapy-induced damage affecting adult patients occurs as a consequence of head and neck irradiation therapy. Potential signs and symptoms associated with head and neck irradiation include mucositis, irreversible salivary gland impairment, taste aberration, radiation caries, and osteoradionecrosis.[256] While developed teeth appear to be fairly resistant to the effects of irradiation, it has been suggested that radiation-induced collagen damage within the dental pulp results in impaired odontoblastic metabolism, which in turn contributes to poor dentinal hydration.[261] It has been further suggested that impaired dentin homeostasis contributes to the increased caries risk observed in the postirradiation patient.

Dental infection and dental trauma (eg, extraction or apical surgery) increase the risk for development of osteoradionecrosis. As a consequence, endodontic treatment is the preferred alternative to extraction in managing problematic teeth in the patient who has undergone head and neck irradiation.[256,258,262] Fortunately, the success of conservative endodontic treatment in managing previously irradiated teeth appears to be on par with that of treatment in control teeth.[258]

Interventional chemotherapy and radiation therapies may adversely affect craniofacial development in the growing child.[255–257,261,262] In principle, any therapy that may directly kill odontogenic cells, inhibit their metabolism, or block or alter signaling and cell communication may result in aberrant tooth development.[255] Resultant abnormalities include hypodontia, root stunting, microdontia, enlarged pulp chambers, and retention of primary teeth. The observed abnormalities reflect the stage of dental development during which the interventional therapy was prescribed. From an endodontic perspective, therapeutic success may be

compromised by the aberrant tooth development.

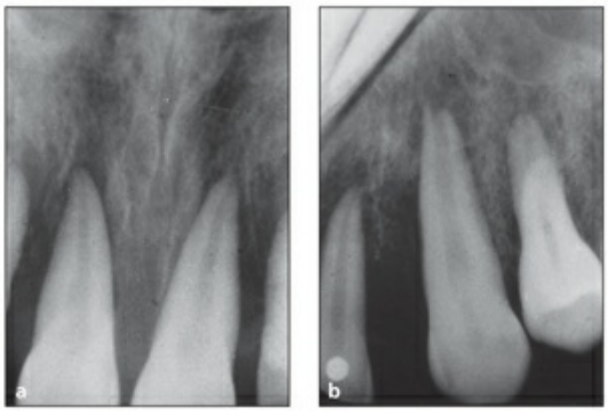

Fig 20-18 *(a and b)* Periradicular radiographs of a 13-year-old patient with acute monocytic leukemia. Note the uniform widening of the periodontal ligament space.

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