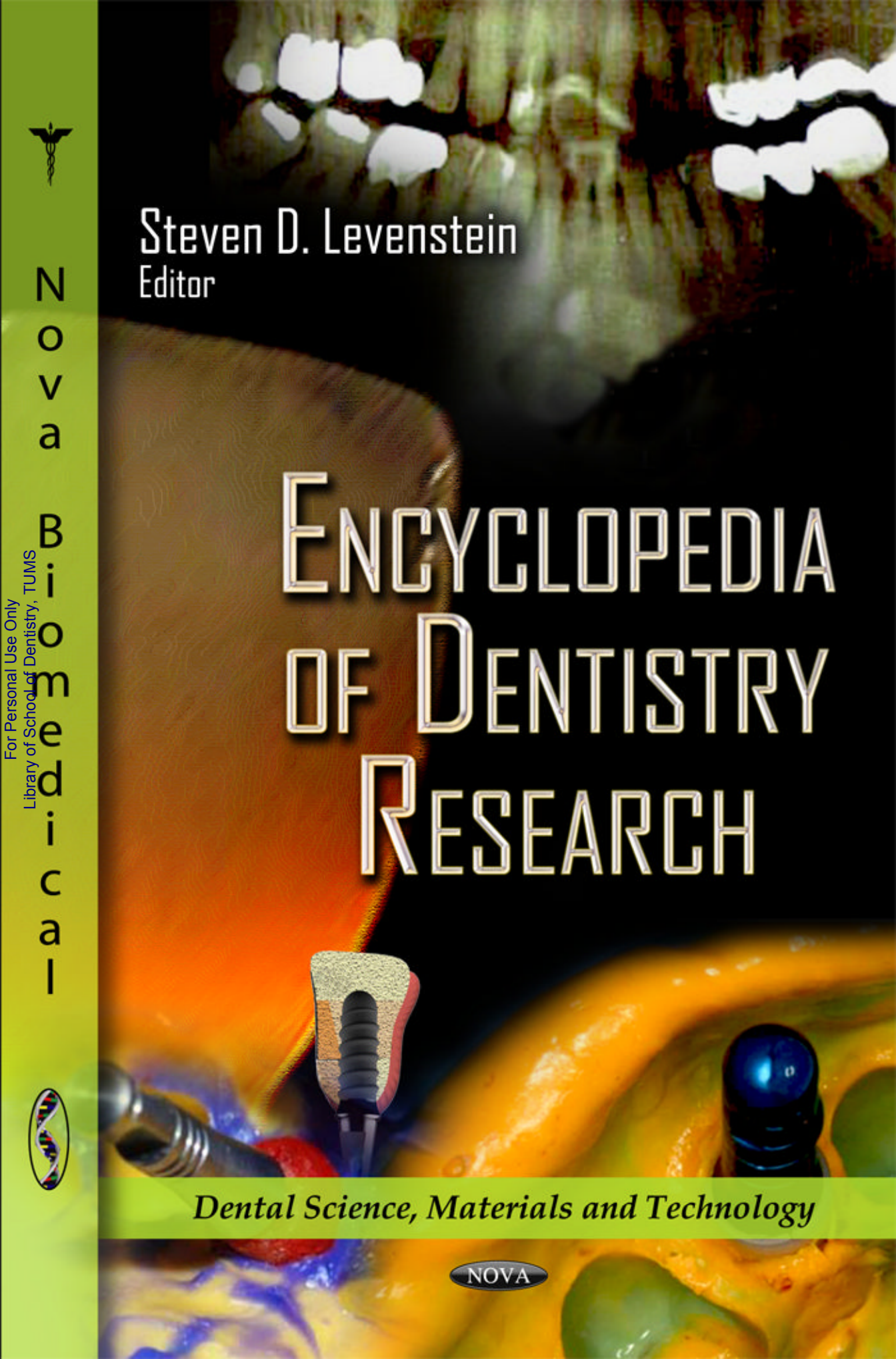

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(2 VOLUME SET)

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VOLUME 1

PREFACE

This book presents current research in the field of dentistry. Topics discussed include dental composites with nano-scaled fillers; mapping the structure, composition, properties and dental erosion in human enamel; dental polymer nanocomposites; development of low-shrinkage dental composites; placing and loading dental implants; biomechanics of oral implants; tape casting in the development of dental biomaterials; risk factors for chronic periodontal disease; the role of antimicrobial peptides in periodontal disease; treatment of periodontitis and genetic polymorphisms and periodontitis.

Chapter 1 - Resin composites have been used for various purposes in restorative dentistry. Specific resin composite materials have been manufactured for the express purpose of core restorations. Currently, resin composite core material has also been recommended for luting endodontic posts due to the simplicity and homogeneity of using the same material for the post and core placement. The utilization of resin-based luting agents, both for resin cements and resin composite core materials for post cementation is reviewed in this chapter. There are three types of existing resin cements that are used for cementation of endodontic posts: etch-and-rinse, self-etch, and self-adhesive cement systems. The research results are presented to compare the bond strength values of various types of resin cements. Emphasis is placed on the use of contemporary adhesives combined with dual-cure resin composite core materials for bonding fiber posts in root canals. The bond strength data obtained from various bonding strategies are shown in this chapter. The efficiency of using the one-step and two-step self etch adhesive system for bonding to the root canal is described. In addition, the optimal bonding technique for bonding resin composites both to the fiber post surfaces and root canal dentin are revealed with the bond strength data incorporated. For core build-up materials, the issues of the polymerization mode of core build-up resin composite and incompatibility between adhesives and resin composites are discussed. This chapter also reviews the mechanical properties of resin composite core materials including the ultimate tensile strength and microhardness values of various resin composite core build-up materials obtained from the author's research. The advantages and disadvantages of the auto-mix and hand-mix resin composites are shown with verification by SEM micrographs. Particularly, the composite core materials currently available in the market with their compositions and characteristics are summarized in this chapter.

Chapter 2 - Over the past years, polymeric dental materials attained a considerable progress due to the tremendous development of multifunctionalized methacrylates, since these offer a widespread possibility to generate composite resins through a suitable choice of

the monomers structure and the proportion implied in the three-dimensional network formation as well as by varying the hard inorganic filler (loading, nature, shape, size, distribution, orientation, adhesion, etc). Recent aspirations and approaches to perfect the composites structure have shown that there are difficulties in the systematic upgrading of these materials because all acrylic formulations presented a volumetric shrinkage through polymerization, accompanied by a polymer ageing. Moreover, the high percentage of nonpolymerized functions significantly affects the physical properties of the formed composites, reason for that the urethane di(meth)acrylates have been initially proposed for reducing the high viscosity of diglycidyl methacrylate of bisphenol A (BisGMA) and achieving an adequate conversion in the final resins. However, besides other characteristics (adhesion to tooth substrates, insufficient material properties, etc), minimization polymerization shrinkage and increasing the degree of (meth)acrylate conversion after ambient photopolymerization of the composite are one of the most important research tasks in this field.

From this perspective, the present review will report their results concerning the synthesis and characterization of new urethane dimethacrylate monomers with and without carboxyl groups, BisGMA analogous or liquid crystalline monomethacrylates following especially the structural/compositional effects derived from their incorporation as co-monomer candidates for formulating dental composites with different fillers, in order to elucidate the photopolymerization behaviour and the specific properties (polymerization shrinkage, morphology, hydrophilicity, mechanical parameters) of the experimental specimens. Complementary, data on the crack propagation in some composites subjected to Vickers indentation will be also critically discussed.

Chapter 3 - Developed almost half a century ago, dental composites, consisting of a polymeric resin matrix and silanized glass or ceramic fillers, presented opportunities never before equaled in modern dentistry. The resin matrix is usually cured (hardened) by photo-initiated free radical polymerization. Camphorquinone (CQ) is a commonly used visible light initiator and ethyl-4-(N,N'-dimethylamino) benzoate (4-EDMAB) is a commonly used accelerator. The monomer of 2,2'-bis-[4-(methacryloxypropoxy)-phenyl]-propane (Bis-GMA) has been widely used as the dental base monomer since it was invented. Bis-GMA is a very viscous, honey-like liquid. To improve the handling qualities, a low viscosity diluents monomer, such as tri-(ethylene glycol) dimethacrylate (TEGDMA), is added to thin the resin. In the Bis-GMA/TEGDMA dental resin systems, Bis-GMA functions to limit the volumetric shrinkage induced by photopolymerization and to enhance the resin reactivity, while TEGDMA provides for increased vinyl double bond conversion. Compared to dental amalgams, the composites possess better esthetic property, have fewer safety concerns, and show reasonably satisfactory clinical results. Consequently, the composites have been widely adopted by the dental profession as the restorative material of choice. However, current dental composites are far from ideal and/or perfect; for example, mechanical properties of the composites still require significant improvements particularly for large stress-bearing posterior restorations that involve the replacement of cusps. Herein the authors report that innovative dental composites reinforced with nano-scaled fillers including polyhedral oligomeric silsesquioxane (POSS), fibrillar silicate, and electrospun glass nanofibers were prepared, characterized, and evaluated. The results indicated that the incorporation of small mass fractions of nano-scaled fillers substantially improved flexural strength, elastic modulus, and work of fracture values of dental composites. The mechanical properties of the

composites could be further improved by optimizing the chemical compositions and surface treatment methods of the nano-scaled fillers. The authors envision that the uniform distribution of nano-scaled fillers into dental composites could result in the development of next generation dental composites, which would be particularly useful for large posterior restorations.

Chapter 4 - The structure-property relationship and dental erosion in human dental enamel composites is reviewed. The phase composition, microstructure and mechanical properties as characterized by grazing-incidence synchrotron radiation diffraction, atomic-force microscopy, scanning electron microscopy and Vickers indentation are described and discussed. The existence of distinct graded changes in crystal disorder, phase abundance, crystallite size and hardness within these enamel ceramics is highlighted. The phenomenon of load-dependent hardness in enamel but load-independent hardness in the dentine is highlighted and discussed. An in-situ monitoring technique of dental erosion in tooth enamel ceramics when immersed in soft-drinks is described. Atomic absorption results suggest that the increasing weight loss in tooth enamel during dental erosion in soft drinks can be attributed to the continuous leaching of Ca^{2+} ions, in addition to phosphorus, oxygen, and hydrogen. The effect of dental erosion on the hardness of enamel is also discussed

Chapter 5 - Dental resin composites have been commonly used as restorative material for dental treatment. A resin composite is a dispersion-strengthened material composed of silica glass and dimethacrylate resin. In order to enhance the chemical bonding between the silica and matrix resin, the silica glass is treated with a silane coupling agent, which has a methacryloyl group at its terminal end. As a consequence of the bonded filler phase, these materials have much better mechanical properties than did unfilled resins.

The mechanical properties of dental resin composites such as compressive strength, diametral tensile strength, flexural strength, and fracture toughness have been experimentally studied in relation to the filler content and particle size. On the other hand, several computational studies on failure behavior of dental resin composites as determined by finite element analysis have been published.

This article provides an overview of the relationship between filler loading condition and mechanical properties of dental resin composites from experimental and computational points of view.

Chapter 6 - Methacrylic compounds, like bis-phenol A glycerolate dimethacrylate (Bis-GMA), triethyleneglycol-dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), urethane-dimethacrylate (UDMA) and 1,4-butanediol dimethacrylate (BDDMA) are used as polymerizable components of composite resins and some cements utilized in dentistry and in other medical fields.

After performing dental restorations, amounts of uncured monomers are released either into the oral cavity or in pulpal tissues whence they can leach into the blood circulation causing, or contributing to, local or systemic adverse effects. Since the intracellular mechanisms of the aforesaid effects are still not completely clear, many *in vitro* studies with methacrylic monomers have been performed in the attempt to explain them. These studies have underlined that monomers display genotoxic, allergenic, cytotoxic, estrogenetic and mutagenic activity. Moreover, these monomers alter lipid metabolism, glutathione concentration, reactive oxygen species production, energy metabolism cell cycle and behave as differentiating agents on human promyelocytic HL-60 cell line. The last property was especially intriguing because HL-60 cells possess high telomerase activity, a phenotype

related to their immortalized status. Telomerase, adding telomeric repeats to the 3'-end of telomeres, protects chromosomes from the telomeric attrition associated with the 'end-replication problem'. Telomerase activity is present in human stem cells, progenitor cells, and germ cells but is undetectable in the vast majority of adult somatic tissues. During cell differentiation telomerase looses its function of synthesis and maintenance of the telomeric units.

In this work, the authors verified whether the differentiation of HL-60 cells, induced by Bis-GMA, HEMA, TEGDMA, UDMA or BDDMA, is also accompanied by a decrease of telomerase activity.

The results show that all monomers and all-*trans*-retinoic acid (ATRA) – used as positive control – induce cell differentiation.

Moreover, cells treated with TEGDMA, HEMA, UDMA, BDDMA or ATRA display a decrease of telomerase activity (about 50%) in respect to untreated cells. On the contrary, Bis-GMA does not provoke any alteration of enzymatic activity.

These observations suggest that the ability of some methacrylic monomers to induce differentiation of promyelocytic leukemia cells may be mediated by their capacity to determine a down-regulation of telomerase activity.

Chapter 7 - The authors have developed psychological scales to assess the states and behavior of dental patients with a particular focus on self-efficacy theory. When developing such scales, it is essential to verify their reliability and validity. The authors describe classic methods for developing scales to assess the psychological states of dental patients. These methods can also be applied to other psychological theories. Reliability factors, consistency, and stability are discussed, and validity factors—including content validity, criterion-related validity, and construct validity—are explored. In their past studies, the authors developed a self-efficacy scale for self-care among periodontal disease patients, and proved its effectiveness in predicting loss to follow-up for long-term periodontal treatment. Here, the authors also show the clinical applications of the psychological scales, whose reliability and validity has been confirmed.

Chapter 8 - Nanotechnology is offering us the ability to design materials with totally new characteristics. Nanotechnology is also known as molecular nanotechnology or molecular engineering is the production of functional materials and structures in the range of 0.1 to 100 nanometers-the nanoscale-by various physical or chemical methods.Today the revolutionary development of nanotechnology has become the most highly energized discipline in science and technology. The intense interest in using nanomaterials stems from the idea that they may be used to manipulate the structure of materials to provide dramatic improvements in electrical, chemical, mechanical and optical properties.

Chapter 9 - Developed over 40 years ago, dental composites have been widely adopted by the profession to replace traditional dental amalgams. These composites as restorative materials are extensively used in dentistry because they are safer and easier to use. Today, dental nanocomposites have wide spread applications, due to their physical and mechanical properties, excellent aesthetic and lack of side effects for patient and dentistry. When the hard tissue of teeth is damaged by dental caries and cavity preparation, the use of dental nanocomposites is the best treatment.

Dental composites consist of hard inorganic particles dispersed in a soft organic resin matrix. Properties of the composites are greatly influenced not only by the properties of their

fillers but also by the chemical structure of the monomers, which are used in the matrix phase. Basically, a dental composite is a mixture of particles within an acrylic monomer that is polymerized during application. In more detail dental composites consist of four major components which are an organic polymer matrix, inorganic filler particles, coupling agent, and the initiator-accelerator system. The resin forms the matrix of the composite material, binding the individual filler particles together through the coupling agent. Development of 2, 2-bis-[4-(methacryl-oxypropoxy)-phenyl]-propane (Bis-GMA) and dental composites by Bowen and their introduction to restorative dentistry was so successful that they were soon accepted as an esthetic filling material. The most widely used resin in dental composites is based on the copolymer prepared from a combination of Bis-GMA and triethylene glycol dimethacrylate (TEGDMA). TEGDMA is usually added to Bis-GMA in order to achieve workable viscosity limits since the latter monomer possesses very high viscosity ($>10^6$cP) due to the intermolecular hydrogen bonding. In Bis-GMA/TEGDMA dental resin systems, Bis-GMA functions to limit the polymerization induced volumetric shrinkage and to enhance resin reactivity, while TEGDMA provides for increased vinyl double bond conversion.

Although Bis-GMA has widely been used as the main monomer in most resin composite systems due to its superior aesthetic quality, simple operation technique, enhanced mechanical strength, less shrinkage, higher modulus, and reduced toxicity because of its lower volatility and diffusivity into the tissue, and the composites have undergone significant development since their advent, they still have shortcomings limiting their application. Lack of good adhesion to the tooth structure and polymerization shrinkage are the most important problems. Considerable interest has been devoted to synthesizing new monomers to provide alternative monomers to overcome the problems.

BTDMA as a dimethacrylate monomer containing carboxylic acid groups in its structure has been shown that can interact with the Ca^{2+} ions of the tooth structure so it has the potential to provide better adhesion properties in dental composites.

Chapter 10 - Inherent donor-site limitations with respect to tissue rejection and disease transfer are shortcomings of autografting and allografting. The development of bone-like biocomposites that induces and promotes new tissue formation at the required site would therefore be desirable.

The self organized calcium phosphate/collagen composite has been drawn attention in tissue reconstruction. The mechanical properties and biodegradability of the biocomposites need to be managed in parallel. The cross-linking between calcium phosphate particles and collagen molecules is a critical parameter in this regard. The improved cross-linking properties of the bone-like collagen composites with or without cross-liking reagents have been explored in past decade. The size distribution, composition, and crystallization of the particles may have another important role in the self organization. This review describes current strategies in development of artificial bon-like biocomposites for use in craniofacial orthopedics and periodontal repair.

Chapter 11 - Diagnostic medical and oral dental radiography comprises 82% of all man-made radiation exposure of the population in Albania. Although dental radiography does not make a major contribution to radiation dose, the authors performed a detailed assessment of radiation risk from panoramic and oral dental radiography, evaluating the annual effective dose in dentistry clinics of Tirana.

Since 1996, a computerized registration system of occupational exposure was established and the central national dosimetric register allows authorized users with basic knowledge of

programming to make various inquiries on persons or statistical analyses in connection with doses, has also been improved.

The measures of effective doses were carried out using TLD-100 cards for different parameters of X-ray tubes. After exposure of patients, the authors carried out measurements of crystals by TLD Harshaw Reader 4500 apparatus, furnished by IAEA. The assessment of TLD-100 cards (crystals) was performed through the TLD-REMS program, and processing of the results was carried out by RAIS program. A National Dose Registry is established for this purpose, which contains radiation doses for occupational exposures and patients of their study etc. The doses were evaluated for mandibular and maxillary incisors, canines, premolars and molars, given to more as 1000 patients aged 5-60 years. The average dose was 4,1 mGy (miligrey) and dose limits were from 0,7 – 144 mGy. The reference level recommended by IAEA and ICRP for dental radiography examinations is 7 mGy (surface dose), while the Albanian Commission of Radiation Protection (ACRP) has evaluated and recommended the value of 5 mGy. The dose from 5 mGy in oral dental radiography examinations is equivalent to effective dose of 5 μSv (microsivert).

In practically all techniques the films for dental procedures of the radiography are basically same. The examinations of dental radiography performed by panoramic apparatus are a unique film technique that allows the dentist to view the entire dentition and related structures, from condyle to condyle, on one film. Oral dental radiographies in their study are carried out using ISO 2 Agfa film plaque; ISO E- Safety, ISO 3665/5799 maximum 23 0C; λ= 500 nm. Dental radiography is one of the largest single group examination performed, although the effective dose per radiograph is small. The individual risks from dental radiography are low, but it has identified a significant potential for reduction in the collective dose and for improvements in the diagnostic quality of dental radiography.

The economic impact of their recommendations suggestion to cover all aspects of dental radiography: training and examination regimes for dentist and staff, patients' selection and clinical justification for radiography, diagnostic interpretation, equipment and procedural aspects, and finally the question of quality assurance in dental radiography.

The measurements obtained in their study showed that the system of radiation protection of X-ray machines in operation phase in all dental practices, where the authors performed activities was satisfactory levels, ensuring protection of staff patients and population.

Chapter 12 - The objective of this study was to investigate, the vaporization of alloying elements, variation in tensile strength and changes in the microstructure of the recycled Ni-Cr based dental alloys used in the preparation of metallic restorations like crowns and bridges. The commercial dental laboratories have the practice of using material left out in the form of sprue and buttons after every casting of a metallic restoration. The use of recycled alloys showed weaker tensile strength values and also inclusion of burnt oxides, silica and investment material after every repeated melting. The grain boundaries formed were found non uniform and scattered when observed under SEM (scanning electron microscope). The study established the quality of these restorations depends on the composition of the new alloy. During the conduct of experiments ten tensile test specimens having 30 mm gauge length and 5 mm diameter were casted for each group of repeated melting. The results were recorded and it was observed the considerable quantity of alloying elements vaporized. The loss of alloying elements resulted in decrease of tensile strength from a maximum of approximately 695 Mpa to a minimum 470 Mpa. Further, the fractured surfaces showed the

presence of foreign particles under metallurgical microscope. Theoretical validation was also carried out using Hall-petch equation. Finally, it was recommended to use new (fresh) alloy to have a good quality of metallic restorations with a considerable reduction in failure rates.

Chapter 13 - Dental composite resins have revolutionized modern clinical dentistry. They are widely used for restoring teeth and cosmetic dentistry due to their esthetic and handling properties. Despite their wide applications, present day composite resins shrink when cured. This polymerization shrinkage generate stresses which affect the marginal seal between the tooth/restoration interfaces leading to secondary caries, post-operative sensitivity, tooth fracture, bond failure and marginal leakage. Other problems associated with current dental composites include inadequate wear resistance and the leaching of uncured organic monomers. The development of low shrinkage resins is therefore an important research focus in dentistry and remains a challenge. In this review, different polymerization techniques such as soft-start, pulse cure and pulse delay used to minimize shrinkage clinically will be discussed. The effect of the different light-curing techniques on the crosslink density of composites will also be reported. Recent developments of low shrinkage composites including some of their work on silsesquioxane in the laboratory will also be highlighted.

Chapter 14 - During the last century, much has been learned about the process of dental caries, a localized destruction of tooth tissue by plaque microorganisms that ferment dietary carbohydrates into organic acids which then cause dissolution of tooth mineral. Teeth are constantly going through cycles of mineral loss (when oral pH is below the point at which tooth mineral begins to dissolve) and repair (neutral and/or basic pH conditions that favor the redeposition of mineral). The net loss or gain in mineral over time ultimately determines whether tooth decay (demineralization) will advance, stabilize and/or regress. The major goal of clinical intervention is the preservation of tooth structure and the prevention of lesion progression to the point where restoration is needed. Caries prevention strategies have focused on reducing bacterial growth, neutralizing oral acids and using various remineralizing agents. Traditionally, remineralizing caries-arresting approaches are based on calcium and phosphate ion delivery through the use of dentifrices, chewing gums and mouthwashes, and systemic and/or topical fluoridation. However, restoring the lost mineral by using remineralization solutions containing calcium and phosphate ions often fails clinically because of the low solubility of calcium phosphates, particularly in the presence of fluoride ions and their inability to incorporate into plaque and localize at the tooth surface. Incorporation of fluoride into dental materials is viewed by many as the scientific cornerstone for caries prevention. It is generally recognized that fluoride regenerates damaged tooth enamel via incorporation in tooth mineral as fluoroapatite or fluoride-enriched hydroxyapatite, therefore decreasing the solubility of tooth enamel. Fluoride has been less effective as dentin remineralizing vehicle. In addition to the various fluoride treatments, remineralization of enamel has been successfully achieved by two distinct new technologies: a) casein-phosphopeptide stabilized amorphous calcium phosphate (ACP) in the form of mouthrinses and sugar-free chewing gums and b) ACP based polymeric composites. Dental applications of ACP also include varnishes, dentifrices and desensitizing agents. The development of the ACP based remineralizing composites is discussed in this Chapter with emphasis on the structure-composition-property relationships and comprehensive physicochemical evaluation of composites formulated for various dental applications.

Chapter 15 - Implant dentistry provides unique and predictable methods for the replacement of missing teeth. The need for provisionalization of implant-supported prostheses

is increasing. Interim denture is a prosthesis designed to enhance esthetic, provide stability and function for a limited of time and should be replaced by definitive prosthesis after a period of time.

A challenge exists for prosthodontist in treatment planning and providing treatment for the patient before surgical phases. The interim restoration is an important diagnostic tool and a key factor for patient, dentist, technician and surgeon communication.

Immediate provisionalization of implants at the time of placement can provide a tooth-like restoration for the patient. In addition, interim restorations are frequently used for immediate or early occlusal loading of implants, for soft tissue management to obtain a natural emergence profile and during the fabrication period of the definitive prostheses.

Achieving esthetics in the anterior maxilla depends on natural looking teeth enveloped by normal soft tissue. Soft tissue will recede if not supported by alveolar bone, tooth structures or restorations, resulting in an esthetic failure.

Interim restorations can be either fixed or removable but certain key principles should be followed to prevent deleterious effects on the tissue in the edentulous space.

Although implant-supported provisional restorations are usually fabricated in the laboratory, there are some clinical situations that demand the direct fabrication of provisional restorations, especially when there is a high esthetic demand.

Various techniques to make implant-supported interim restorations have been described in the literature. This chapter includes indications and the types of interim restorations in implant dentistry. The interim restorative materials and different techniques for making interim restorations will also be discussed.

Chapter 16 - The treatments of oral cancer prevent many patients from bearing conventional prostheses. These patients are, thus, candidates for oral rehabilitation with osseointegrated implants. Anatomical and histological therapy-induced changes decrease their success rate.

Ablative surgery leads to anatomical modifications, with loss of keratinized tissues, loss of bone and dental anchorage.

Reconstructive surgery aims to restore the oral anatomy but the quality and the volume of soft tissues do not always allow a functional prosthesis. The use of microvascular flaps for reconstructive surgery has transformed prosthetic rehabilitation after large ablative surgery. Their poor resorption and their success allow the use of dental implants supporting a fixed or movable prosthesis.

The most important adverse effect of external radiotherapy concerns bone, as it decreases its healing potential through hypocellularity and hypovascularization, which lowers the success rate of the osseointegration.

The factors of osseointegration which the authors can control are the site of implantation, the waiting period between radiotherapy and the implant surgery, the irradiation dose and the conditions of loading. Animal studies and clinical series gain widespread backing: osseointegration is possible in radiated bone and in fibula, scapula or iliac grafts, but it takes a longer time to obtain an intimate contact between the implant and the bone. The implant surgery can be planned before or after radiotherapy. The success rate decreases with radiation doses around to 50 to 60 Grays. The preferential sites for implantation are the anterior mandible or maxilla, which present a lower dose of radiation and an easier access (less concerned by the limitation of the mouth opening).

The success rate of dental implants in microvascular flaps is around to 80-90%; nonetheless there is a difference between the rate of osteointegration and the percentage of loading, due to the new anatomy and the orientation of the implants. Moreover the management of soft tissues is often difficult.

This will be avoided by the use of local or locoregional grafts of keratinized tissues and the planning of the implants placement thanks to surgical guides, which development is the next evolution in the rehabilitation of oral cancer patients.

Chapter 17 - This commentary will detail emerging themes of personal and professional interest to the authors concerning the development of shared learning between undergraduate dental students and trainee dental technicians. Collaboration between health care professionals is not a new phenomenon. Interprofessional training and working practices in health care are well established in such fields as nursing and social care. Indeed, in practical terms, the maintenance of health demands such a wide range of expertise that it is highly problematic for any single health profession to deliver care in isolation. However, interprofessional education presents many challenges: the participants inevitably bring with them their essential differences, culturally, technically, vocationally, and of course, professionally. As a result, barriers exist at many levels. In some training contexts, this has led to cultural developments that collectively work against effective interprofessional collaboration, including the formation of rigid professional boundaries, the persistence of stereotypes and limited knowledge about each others' roles due to the isolation of professionals during training. For interprofessional learning to be successful, prejudices must be broken down, and there must be willingness among all involved to engage in the process.

Their research addresses such issues and discusses possible structural solutions, examining in particular a programme of interprofessional curriculum development.

Chapter 18 - Current concerns about facial aesthetics play major roles in our lives. In dentistry, it is increasingly common for patients to search for aesthetic restorations because the desire to have a good-looking smile is prevalent in today's society. Due to the easy access to information through various media, patients are able to constantly monitor the growing trend of aesthetic dental materials. New alternatives for aesthetic prosthetic resolutions, including all-ceramic crowns, are available on the market, and persuasive manufacturer's advertisements suggest to both professionals and patients that these materials have superior qualities. With the promise of optimized aesthetics by the elimination of the metal in the metal-ceramic fixed prostheses, a new reality has become part of the daily routine. Several manufacturers and experts emphasize that all-ceramic crowns allow light transmission, which demonstrates improved aesthetic properties when compared to metal-ceramic crowns. In daily practice, dental clinicians should be aware of the real optical benefits provided by these restorations. While the light reaches the buccal face of crowns towards the lingual, clinicians should consider the following: is the total transmission of light through an all-ceramic crown really important? Additionally, will it really result in a more aesthetic appearance? The aim of this chapter is to present to readers with the various properties of all-ceramic crowns by comparing them, in a critical way, to the clinical reality of dental professionals.

Chapter 19 - Immediate loading of dental implants is an international demand.

The field of dental implants is currently in a dilemma concerning whether to load implants immediately or after what's defined as a "healing period".

In both situations, a common factor is always a measure of success, namely osseointegration.

Osseointegration can be easily defined as: Direct implant-bone interface. Through the years many definitions were introduced, but it all falls within the above mentioned sentence.

However, in comparing implant-bone relations, authors did compare it with ankylosed teeth. Such a relation is in favor with implants because it lacks the main problem of ankylosed teeth, namely, root resorption.

Achieving osseointegration involved several factors. Most authors related this to health, implant materials, surfaces, asepcis, minimum trauma, primary stability and a healing undisturbed period.

Maintaining osseointegration became a strongly studied issue involving atraumatic surgical technique, the amount, type of prosthetic loading and to a lesser degree, oral hygiene levels.

Chapter 20 - Vertically deficient alveolar ridges represent a challenge for appropriate prosthetic rehabilitation. Nowadays osteointegrated implant use is the most popular treatment modality in such cases. A variety of techniques have been used to establish a suficant osseous tissue for supporting dental implants Onlay grafting and guided bone regeneration, are the frequently used surgical procedures. Distraction osteogenesis is an alternative augmentation technique providing the sufficient enlargement of the bone and soft tissue volume for the placement of dental implants. The purpose of this chapter was is to define the techniques and compare the complications and implant survival rates in localized alveolar deficiencies reconstructed by alveolar distraction osteogenesis.

Chapter 21 - Bone is responsible for many different functions in the body, including structural support, mineral storage, and physiological functions such as the formation of blood vessels. It is an extremely complex tissue that changes its form, mass and internal structure under load. The understanding of functional adaptation of bone tissue to mechanical stimuli has been mostly founded by Julius Wolff, in his greatest treatise, *Law of Bone Transformation*

Chapter 22 - Tape casting is one of the main methods used in the microelectronics industry to ensure precise thickness control and consistency in the manufacture of flat ceramics. This method uses specially formulated slurry comprising base material powder, binder, plasticizer, and dispersant that can be cast on a moving carrier surface. The major advantage of tape casting is that the thickness of the ceramic sheet can be adjusted precisely by varying the gap between the blade (the so-called doctor blade) and glass surface. Fabrication by tape casting therefore offers advantages that enable the preparation of dental biomaterial sheets. Thus, there are potentially a wide range of dental applications.

A number of studies have already reported new tape-casting fabrication methods for dental biomaterials such as bone substitutes and scaffolds for bone regeneration and dental ceramic materials for prosthetic restoration. Several studies have shown the tape-casting preparation of scaffolds for bone-tissue engineering. Studies by the present author have resulted in the development of a fiber-reinforced ceramic material that is suitable for a new type of dental prosthesis. These studies have demonstrated that tape casting is a useful technique in preparing new dental biomaterials.

This article is an overview of the development of various dental biomaterials fabricated by tape casting.

Chapter 23 - The complications caused by improper implant placement pose a significant challenge in implant dentistry. In order to improve the precision of placement, there is a trend towards computer-aided oral implantology, especially the application of CT-derived surgical

templates. Actually, there are three types of surgical guides, i.e., bone supported, mucosa supported, and tooth supported, reported in literature. As far as conventional clinical cases are concerned, these kinds of templates might be relatively stably placed on the underlying tissue such as jawbone or mucosa. However, with regard to some complex cases involving severely resorbed edentulous cases, clinical experience demonstrates that fixture of the surgical guides is not so stable due to unsatisfactory match between the templates and receptor sites. Aiming to solve this problem, a novel bone-tooth-combined-supported surgical guide is introduced in this study. With the use of a 3D-laser scanner, more detailed surface information at the level of the dentition can be obtained. Then, fusion of laser scanned dental occlusion data and CT data is realized through image registration technique. On the basis of this fusion data and the information obtained from preoperative planning software, a 3D computer model of this kind of bone-tooth-combined-supported surgical guides can be designed and finally fabricated via stereolithography technique. Because this approach is achieved using both laser scanning and CT imaging, thus improving the fit accuracy and reliability of this sort of surgical guides. Their applications in two severely resorbed edentulous cases show that the average distance deviations at the coronal and apical point of the implant were 0.66 mm (range: 0.3 to 1.2) and 0.86 mm (range: 0.4 to 1.2), and the average angle deviation was 1.84° (range 0.6°to 2.8°), therefore proves that the novel combine-supported templates are superior to the conventional ones. However, more clinical cases will be conducted to demonstrate its feasibility and reliability.

Chapter 24 - After loss of teeth in the posterior maxilla, the alveolar ridge decreases by bone atrophy and pneumatization of the maxillary sinus cavity. Maxillary sinus augmentation is a well-established technique for functional rehabilitation of partially or completely edentulous patients with severe maxillary atrophy and the goal of sinus augmentation procedures is to create bone quantity and quality in order to ensure the placement of dental implants of sufficient length and satisfying initial stability. Various bone grafting materials have been used in sinus augmentation including autogenous graft, freeze-dried bone allograft, xenograft, and alloplastic material. Attempts have been made to increase the bone formation using growth factors, such as bone morphogenetic proteins (BMPs). Platelet-rich plasma (PRP) has been used in sinus augmentation procedures but conflicting results are being reported. Tissue engineering procedure using autogeous mesenchymal stem cells combined with scaffold has been applied in maxillary sinus augmentation procedure.

In this review, the effect and efficacy of biomaterials including growth factors and mesenchymal stem cells in sinus augmentation procedures will be addressed.

Chapter 25 - CT scanning software is fast becoming a viable tool in the diagnosing of dental implant position and placement. Minimally invasive procedures may be requested by patients to reduce their anxiety and increase treatment acceptance rates. In areas where contours and width and height of bone are difficult to determine with conventional radiographic techniques, the CT scanning software allows diagnostic determination if bone quantity and quality exists and can be used to virtually place dental implants using the computer program prior to any surgical intervention. This is an outstanding tool in discussing the risks involved in surgical implant procedures and can help visualize the finished case before ever starting. Used in critical anatomic situations and for placing the implant in an ideal position in bone, CT scanning software eliminates possible manual placement errors and matches planning to prosthetic requirements. This innovative tool makes surgical placement

of implants less invasive and more predictable. Prosthetic reconstruction is thus made simpler since the implants are appropriately positioned to allow for fabrication of the final prosthesis.

Chapter 26 - The functional and esthetical rehabilitation of the maxilla with dental implants when there is a severe alveolar bone deficiency can be achieved with the help of distraction osteogenesis. Although this method offers certain advantages over conventional augmentation methods, it sometimes requires secondary bone grafting because the bone of the transport segment may be too thin and semilunar excavations may form in the distraction zone. In this situation, the simultaneous application of distraction osteogenesis with a polytetrafluoroethylene membrane and autogenous onlay block bone grafting is an alternative solution to secondary bone grafting. In addition, this technique prevents semilunar excavations in the distraction zone and increases callus volume.

Chapter 27 - The CEREC system celebrated its 20th anniversary in 2006. This technology was envisioned and began its development with two people. Drs. Mörmann and Branestini were the ones to treat the first patients with this unique chairside system in 1985. The technology at the time was not what it is today, but the ideas were born and seeded into what was available. They knew that the hardware and software would eventually "catch-up" to their idea. So it has. The idea was that a dental restoration of solid porcelain could be manufactured chairside in one visit with the aid of computer design and milling. This would eliminate the need for impressions and laboratory fabrication. Additionally it would allow the clinician full control over the restoration from beginning to end.

Chapter 28 - The therapeutic goal of implant dentistry is oral rehabilitation and tooth replacement. Considering dental implants as a treatment option provides patients with a positive, long term result. Implants have certainly developed into a viable alternative to conventional prosthetic reconstruction of edentulous areas. They provide outstanding support for single tooth replacements and are often less invasive than conventional crown and bridge techniques. However, implant dentistry has gone through many phases over the years. Modern technology and design allows us to predictably place their dental implants in immediate extraction sites and load the implants at the time of placement. Single too h reconstruction provides easy access for the patient to floss and clean the area compared with the relative difficulty in maintenance when crowns are splinted or bridges fabricated. This case demonstrates an immediate extraction of a fractured root and immediate placement of an OCO Biomedical ISI one piece implant and immediate function provided by a composite transitional crown.

Chapter 29 - Chronic periodontal diseases include a group of inflammatory diseases that affect periodontal supporting tissues of the teeth and encompass destructive and nondestructive conditions. Periodontal diseases are multifactorial and the role of dental biofilm in their initiation is primary. However, whether dental biofilm affects a particular subject, what form the disease takes and how it progresses, are all dependent of a wide variety of factors. Therefore, the objective of this chapter is to outline the risk factors described for the most prevalent chronic periodontal diseases (plaque induced gingivitis and chronic periodontitis) and to explain some basic concepts related to the current understanding of the role of these risk factors based on *in vitro*, animal and human studies. The review will focus on the factors that may be associated with a direct increase in the likelihood of occurrence of disease or an increase in its severity. The following factors will be discussed: 1) host characteristics, such as age, gender and race; 2) social and behavioral factors (socioeconomic status, cigarette smoking and emotional stress); 3) systemic factors, e.g. diabetes mellitus and

osteoporosis; 4) genetic factors; 5) tooth-level factors (root grooves, tooth position, caries, occlusal discrepancies, iatrogenic restorations, root abnormalities and periodontal parameters); and 6) the microbial composition of dental biofilm. Finally, this chapter will also present literature-based evidence on predictive factors associated with patients and tooth susceptibility for recurrence of periodontitis after the end of the active periodontal therapy and will examine the use of some prognostic models which may be useful for clinicians in the identification high-risk groups of patients.

Chapter 30 - The goals of periodontal therapy according to the American Academy of Periodontology are to alter or get rid of the microbial etiology and causative risk factors for periodontitis, thus arresting the progression of disease and preserving the dentition in a state of health, comfort, and function with appropriate esthetics; and to prevent the recurrence of periodontitis.

Chapter 31 - The oral cavity is a warm, moist environment, in which a number of microorganisms colonize and live in harmony as a community, a so-called biofilm. In this environment, antimicrobial peptides may play a critical role in maintaining normal oral health and controlling innate and acquired immune systems in response to continuous microbial challenges in periodontal disease. Two major families of antimicrobial peptides, found in the oral cavity, are defensin and cathelicidin. Members of the defensin family are cysteine-rich peptides, synthesized by plants, insects, and mammals. These peptides vary in length and in the number of disulfide bonds, and have a beta-sheet structure. In the oral cavity, four alpha-defensins are synthesized and stored in neutrophil granules, which are converted into active peptides by proteolytic processing, while three human beta-defensins (hBDs), hBD-1, hBD-2, and hBD-3, are predominantly produced by oral epithelial cells. The only member of the cathelicidin family found in humans is LL-37, an alpha-helical peptide that contains 37 amino acids and begins with two leucines at its NH_3-terminus. LL-37 is derived from enzymatic cleavage of a precursor peptide, namely, human cationic antimicrobial peptide-18. Clinically, differential expression of antimicrobial peptides has been reported in specific types of periodontal disease, and their presence has been shown in saliva and gingival crevicular fluid. Current evidence suggests that alpha-defensins, beta-defensins, and LL-37 have distinct, but overlapping, roles in antimicrobial and pro-inflammatory activities. Several studies have shown antimicrobial activities of hBD-2, hBD-3, and LL-37 against several periodontal pathogens, suggesting their potential role as antimicrobial agents for periodontal disease. The clinical significance of antimicrobial peptides in periodontal disease has recently been demonstrated in morbus Kostmann syndrome, a severe congenital neutropenia, in which chronic periodontal infection in young patients, resulting from a deficiency of neutrophil-derived antimicrobial peptides, causes early tooth loss. Although researchers initially focused their attention on antimicrobial activities, it is now becoming evident that defensins and LL-37 are multifunctional molecules that mediate various host immune responses, and may thus represent essential molecules of innate immunity in periodontal disease. In this chapter, basic knowledge and the clinical importance of antimicrobial peptides in periodontal disease will be discussed in detail.

Chapter 32 - This comprehensive review highlights a detailed overview related to devising a periodontal prognosis. A precise predictability of the results of a disease is profound and crucial for proper treatment planning. Since the understanding of periodontal disease has progressed to include the influence of risk factors, assigning a prognosis has become more perplexing to the clinician. Various factors that influence the overall and

individual tooth prognosis have been enumerated. The classification systems required to assign a prognosis has also been included. The potential adverse influences of both local and systemic factors have also been discussed. An experienced clinician should analyze all these factors, along with the patients attitude towards dental therapy, prior to arriving at a judgment for a single tooth or teeth. With newer trends in treatment modalities, patients can seek better options for treatment, thus improving the long term prognosis.

Chapter 33 - Background: Plaque-induced periodontitis is gingival inflammation at sites undergoing loss of connective tissue, apical migration of junctional epithelium and loss of alveolar bone. Non-surgical treatment of plaque-induced periodontitis typically involves removal of biofilm conducted through mechanical scaling and root planing (SRP) procedures. The antibiotic minocycline hydrochloride, delivered as a sustained-release product[1] used for professional subgingival administration into periodontal pockets, has been shown to be beneficial as an adjunct to conventional SRP. Use of chlorhexidine rinse is also a typical adjunct therapy to SRP procedures for chemical control of supragingival plaque. Lidocaine (2.5%) and prilocaine (2.5%)[2] provides localized anesthesia for SRP. The objective of this study is to develop and use bioluminescent recombinants of oral streptococci in determining the potential antibacterial activity of minocycline hydrochloride used either alone or in combination with the anesthetic lidocaine/prilocaine, or with the antiseptic chlorhexidine.

Methods: Recombinant plasmids containing the bioluminescence-generating *lux* gene from *Photorhabdus luminescens* were transformed into the oral bacterium *Streptococcus mutans*, strains UA159 and ATCC 25175. Transformants were verified as *S. mutans* derivatives by selection and growth on mitis salivarius agar supplemented with bacitracin, in addition to an antibody test directed specifically against *S. mutans* cell wall proteins and polymerase chain reaction experiments targeting sequences in the *S. mutans* glucosyltransferase (*gtf*) gene. *S. mutans* transformants were then subjected to growth analysis for comparison of absorbance and bioluminescence activity. Minocycline hydrochloride and lidocaine/prilocaine, or minocycline hydrochloride and chlorhexidine, were used in combination to determine the potential interactive effects of these agents on the antibacterial activity of minocycline hydrochloride.

Results: Using two distinct *S. mutans* transformants representing both strains UA159 and ATCC 25175, they observed rapid and pronounced bacteriostatic activity when using high doses of minocycline hydrochloride ($\geq$1 μg/ml), which were statistically distinct from untreated cultures (p=0.000058) when measured at the peak of metabolic activity. Reduced bacteriostatic activity was seen using lower doses. When lidocaine/prilocaine at doses >100 μg/ml is used in conjunction with minocycline hydrochloride, the authors observed an additive antibacterial effect. The *S. mutans* transformant strain UA159, when treated with chlorhexidine (0.01%) in conjunction with either high (1 μg/ml) or low (0.1 μg/ml) doses of minocycline hydrochloride, displayed reduced levels of cell mass accumulation, as measured by absorbance, that were additive when both antimicrobial agents were deployed. Bioluminescence determinations, which are a direct measure of metabolic activity and an indirect measure of cell number when cells are in logarithmic stage of growth, displayed similar reductions when cultures were treated with minocycline hydrochloride and chlorhexidine used singularly or in combination.

[1] Brand name for minocycline hydrochloride used as a sustained release product is Arestin

[2] Brand name for the lidocaine (2.5%) and prilocaine (2.5%) anesthetic is Oraqix.

Conclusions: The *S. mutans* lux transformants serve as sensitive biosensors in the determination of antimicrobial activity, and can rapidly monitor inhibition of bacterial metabolism. The authors conclude that the anesthetic lidocaine/prilocaine does not interfere with the potent bacteriostatic activity of minocycline hydrochloride, and actually has an additive antibacterial effect. The potent bacteriostatic activity of minocycline hydrochloride can also be complemented with the addition of chlorhexidine. The application of the *lux* biosensor system in the assessment of minocycline hydrochloride and lidocaine/prilocaine, or minocycline hydrochloride and chlorhexidine, represents its first use in examining antimicrobial drug interactions in periodontology.

Chapter 34 - Periodontitis is a chronic inflammatory disease which destroys the tooth-supporting tissues. This disease is initiated by bacteria; in particular, faculative anaerobic Gram-negative microorganisms. Several types of these pathogens initiate periodontal disease, and the host response determines the disease progression and ultimate tissue damage. The early periodontal lesion (gingivitis) is characterized by the presence of large numbers of T cells and macrophages within the gingiva, while the presence of beta (B) and plasma cells characterize the advanced lesion.

Chapter 35 - Oral epithelia represent the first physical and chemical barrier against bacterial invasion and colonization of the underlying tissues. This protection results from the production of epithelial innate immune responses, including the secretion of cationic antimicrobial peptides with a large spectrum of activity against pathogenic microorganisms. Among these antimicrobial cationic peptides, ß-defensin 2 (hBD-2) is expressed in the gingival epithelia upon stimulation by microorganisms or inflammatory mediators such as interleukin-1β or tumour necrosis factor-α. The aim of the present study was to investigate the effect of AV119, a patented blend of two sugars from avocado, on the induction of hBD-2 in two epithelial cell lines and a primo-culture of gingival epithelial cells. Culture supernatant from epithelial cells treated with AV119 was also evaluated for its antimicrobial activity against the periodontopathogen *Porphyromonas gingivalis*. Cell ELISA assays revealed that AV119 induces the production of hBD-2 by all the epithelial cells tested. Minimal Inhibition Concentration assay also showed that the culture supernatant of epithelial cells treated with AV119 possesses antibacterial activity. In conclusion, their data revealed that AV119 component, through hBD2 induction and antibacterial activity, could be considered for potential use in the control of oral mucosal infections and reduction of microbial tissue invasion during periodontitis.

Chapter 36 - It is evident that periodontitis is the cause at least in part of the cases of cheilitis granulomatosa. Considering that periodontitis is extremely common, it is paradoxical that cheilitis granulomatosa is relatively rare, although mild cases might well be overlooked. Since most of the bacterial species found in periodontitis are not virulent by themselves, the notion of 'endogenous infection' might be reconsidered for the pathogenesis of cheilitis granulomatosa. Since only a small fraction of bacteria (~1%) can be cultured by conventional culture system, it should be necessary to employ PCR-based molecular approaches for identifying bacteria in diseases of unknown etiology. In the future, development of DNA-array system for identifying bacteria (or organisms) might be a promising approach for identifying the bacteria.

Chapter 37 - Periodontitis is a complex disease which is associated with multiple factors, including host immune responses, and genetic, behavioral and environmental factors. It is generally accepted that genetic polymorphisms can modulate host immune responses to

bacterial challenge, hence influencing subjects' susceptibility to periodontitis. Genetic association with periodontal disease experience has been a subject of interest for more than a decade. With the completion of Human Genome Project, genetic association studies emerged in many fields of research including research into periodontitis, one of the most common human diseases. This chapter summarizes past and current research approaches with respect to periodontal disease experience and genetic polymorphisms, and suggests anticipated directions of future studies.

Chapter 38 - Coronary heart disease (CHD) shares a number of features with chronic periodontitis (CP) including risk factors such as smoking and diabetes; an aetiopathogenesis implicating a number of microbial species, as well as chronic inflammation. However, the link between these two conditions remains unclear. The prevalence of CHD increases with age and is higher in males than females. CP is a chronic inflammatory condition which destroys the supporting tissues of teeth and also increases in prevalence with age. Immune responses against heat shock proteins (HSP) can be cross-reactive among bacterial and human antigens. There is evidence that microbial HSP65 and human HSP60 is involved in periodontal disease and CHD and may therefore provide a mechanistic link between CP and CHD. The aim of this study is to investigate immune responses to the human HSP60 and microbial HSP65 in patients with CP and CHD and relate these to the level of inflammation. The authors collected serum samples from 100 male subjects divided into 4 groups, each matched for age: (a) Healthy control group with minimal gingivitis, (b) CP, (c) CHD with gingivitis (d) CHD with CP. ELISA was used to determine the levels of serum anti-HSP and C-reactive protein (CRP) in the 4 groups. Peripheral blood mononuclear cells were also isolated from these 4 groups and stimulated with HSPs. Significant lymphoproliferation was seen in CHD with or without CP when stimulated with human HSP60. CRP and serum anti-human HSP60 IgG were elevated in the patients groups compared to the healthy control group, but not serum anti-microbial HSP 65 IgG,. In view of the potential confounding effects of smoking in CP and CHD, a group of current smokers (n=24) were also recruited to investigate whether smoking affects HSP immune responses.There was no significant difference in HSP-induced lymphoproliferation between smokers and non-smokers in any of the four groups. There was a significant correlation between CRP and lymphoproliferative responses to Human HSP60.

This study shows that serum anti-human HSP60 IgG and serum CRP are raised in CHD with or without CP. In CHD with or without CP, serum CRP levels correlated significantly with human HSP60-induced lymphoproliferation, but not with anti-HSP antibody levels.

Chapter 39 - Morbidity and mortality from oral cancer are high and this has not improved in decades in spite of extensive research. A significant portion of research is concentrated on chemoprevention. However, advances in this field have not translated into a visible change in mortality and morbidity. In addition, existing chemoprevention strategies have two important obstacles: toxicity and reversal of the effects after cessation of treatment. Chronic infection and inflammation have been linked to carcinogenesis in a few organs. For oral cancer, substantial evidence has accumulated for the role of *human papillomavirus* (HPV). However, the development of an effective preventive vaccine strategy for oral cancer is still years away and the target population is largely unexplored. Therefore, safe and practical additional approaches are necessary to change the status quo of oral cancer. Periodontitis is a chronic oral infection caused by inflammatory reactions in response to gram negative anaerobic bacteria in the endogenous dental plaque. It leads to irreversible destruction of tissues around

teeth clinically detectable as periodontal pockets and alveolar bone loss. Periodontal pockets have been suggested as reservoirs of HPV. Chronic proliferation and ulceration of the pocket epithelium may help HPV's initial infection and persistence. Their preliminary results from existing data at Roswell Park Cancer Institute suggest a robust independent association between the history of periodontitis and incident oral cancer. Their next step is to test the synergy between periodontitis and HPV for the risk of oral cancer. If this is true, it will translate to practical and safe prevention and treatment strategies. This chapter will review the evidence supporting the association between chronic periodontitis and oral cancer as well as HPV-periodontitis synergy.

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Chapter 1

RESIN COMPOSITES FOR POST CEMENTATION AND CORE BUILD-UP

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ABSTRACT

Resin composites have been used for various purposes in restorative dentistry. Specific resin composite materials have been manufactured for the express purpose of core restorations. Currently, resin composite core material has also been recommended for luting endodontic posts due to the simplicity and homogeneity of using the same material for the post and core placement. The utilization of resin-based luting agents, both for resin cements and resin composite core materials for post cementation is reviewed in this chapter. There are three types of existing resin cements that are used for cementation of endodontic posts: etch-and-rinse, self-etch, and self-adhesive cement systems. The research results are presented to compare the bond strength values of various types of resin cements. Emphasis is placed on the use of contemporary adhesives combined with dual-cure resin composite core materials for bonding fiber posts in root canals. The bond strength data obtained from various bonding strategies are shown in this chapter. The efficiency of using the one-step and two-step self etch adhesive system for bonding to the root canal is described. In addition, the optimal bonding technique for bonding resin composites both to the fiber post surfaces and root canal dentin are revealed with the bond strength data incorporated. For core build-up materials, the issues of the polymerization mode of core build-up resin composite and incompatibility between adhesives and resin composites are discussed. This chapter also reviews the mechanical properties of resin composite core materials including the ultimate tensile strength and microhardness values of various resin composite core build-up materials obtained from the author's research. The advantages and disadvantages of the auto-mix and hand-mix resin composites are shown with verification by SEM micrographs. Particularly, the composite core materials currently available in the market with their compositions and characteristics are summarized in this chapter.

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INTRODUCTION

The practice in restorative dentistry has been continuously changing with the introduction of innovative techniques and materials. Since Dr. Ray L. Bowen (1962) developed composite material using Bis-GMA resin [1], resin composites have been thereafter widely used for various purposes in dentistry. For cementation, resin-based materials have been adapted from the restorative resin composite to use as a luting agent for indirect restoration such as inlays, onlays, crowns, bridges, and endodontic posts. Unfilled-methacrylate resins were first used for cementation. However, these early products were unsuccessful because of their high polymerization shrinkage, poor mechanical properties, and poor biocompatibility. Most current resin cements therefore utilize higher molecular weight resin such as Bis-GMA. Fillers were added to the resin to improve their properties and decrease polymerization shrinkage. Some resin cements are available with adhesive properties due to the specific functional monomer added for the capability of bonding chemically to dentin. Several studies reported the superior properties of resin-based luting agents over the conventional cements [2-6]. They provided good mechanical properties, higher bond strength values, and lower solubility. For this reason, resin cements are presently popular for cementation purposes. Although the zinc phosphate cement was set as a gold standard for cementation of metal cast post and core, the development of the fiber-reinforced resin composite post, so called "fiber post", have raised the utilization of resin-based material for luting endodontic posts into root canal.

Besides the cementation function of resin composite materials, specific resin composite materials have been manufactured for the express purpose of core restoration, which act as a foundation for crowns or fixed partial dentures when coronal tooth structure is significantly lost. Core materials must be strong enough to resist the occlusal loading during function. Moreover, they must be retentive enough to resist any dislodgement force. The handling characteristics of core build-up material should facilitate treatment procedures as well. Three kinds of materials are currently used for direct core build-up; amalgam, glass ionomer-based materials, and resin composite. Amalgam was formerly used for the core build-up purpose. The major advantage of amalgam is the high compressive strength and low technique sensitivity. However, it retains in the tooth by mechanical retention, whereupon the tooth structure sometimes requires extensive preparation to create a box-like cavity. Additionally, the esthetic and slow setting problems reduce the amalgam usage. In this era, patients are more concerned about aesthetics and the tooth-colored restorations are increasingly required. The dark color of the amalgam core is contra-indicated for translucent tooth-colored restoration. On the other hand, glass ionomer materials have a color closer to dentin and they have a favorable characteristic in fluoride releasing. However, glass ionomer-based materials were found to be the weakest among the three types of material in terms of compressive strength, tensile strength, flexural strength, and elastic modulus [7,8].

Resin composites have increased in their popularity for core build-up purposes especially when the prefabricated posts are used for radicular retention. It was reported that the tensile and flexural strength of light-cured resin composite core materials were superior to those of amalgam, but the compressive strength was lower [7]. A resin composite core has a favorable appearance beneath the all ceramic or indirect composite restoration. Various shades of core materials are also available in order to match the color of restorations. With the significant

improvement of contemporary adhesive systems, resin composite core materials can be strongly bonded to the tooth structure and subsequently enhance the final strength of the tooth-core element. Resin composite is usually the material of choice used for a foundation of compromised teeth with a thin dentin wall, which is prone to fracture if a stiff material is used for restoration [9]. Additionally, the concept of "monoblock" has often been discussed recently. This concept supports the utilization of materials that have similar mechanical properties to the tooth structure allowing the stress distribution analogous to the natural tooth. Resin composite meets this requirement because it has a modulus of elasticity comparable to dentin, while amalgam or other restorative metals have a higher stiffness [10;11]. Finally, the short setting time of resin composite allows clinicians and patients to receive more benefit. Clinicians can prepare the abutment teeth and take impression in a single visit after the composite core placement. However, composite materials create polymerization shrinkage or contraction stress after curing. Clinicians should select the proper materials and bonding techniques to obtain the optimal properties of resin composite and durable adhesion between the resin composite and the tooth structure.

RESIN COMPOSITE FOR POST CEMENTATION

Endodontically treated teeth that have severe coronal damage are mostly restored with posts and cores before placement of the final restorations. The primary function of a radicular post is to provide retention for a core [12]**.** The post is cemented into the root canal and the core is retained by an apical extension. Presently, aesthetic non-metal fiber posts are increasingly used because their modulus of elasticity are comparable to dentin, producing a stress field similar to that of a natural tooth and resulting in a reduction of root fractures [13-16]. However, the flexibility of fiber posts allows the post slightly to bend during function. Consequently, debonding of the fiber post could occur and has been found to be a major cause of failure in endodontically treated teeth restored with a fiber post and composite core [17]. The high quality adhesions at the resin-dentin and resin-post interfaces are important to prevent debonding of the interface. Resin-based luting agents are recommended to be used for fiber post cementation because they provide good bond strength to the post and dentin surface compared with other luting mediums [3,6]. Additionally, the stiffness of resin composites is comparable to a dentin and fiber post, the restoration behaves more closely to a monoblock.

There are two types of resin-based materials currently recommended for bonding endodontic posts into a root canal; resin cement and self- or dual-cure resin composite core materials in combination with contemporary adhesives. Light-cure flowable resin composites may be suitable for luting in the terms of viscosity because they can flow and create a thin film between the post and root canal wall. However, if sufficient photo-irradiation to the resin is not possible in the deep region of the post space, this type of resin should be avoided. Some light transmitting fiber posts were claimed to be used with a light-cure material, but the efficiency of curing light was found to dramatically decrease along the post [18;19]. Thus, a self-cure or dual-cure version of a resin-based luting agent should be selected for bonding in root canals.

Utilization of Resin Cements for Post Cementation

Resin cements were first developed by using unfilled methacrylate resin. Later, the fillers were added to improve mechanical properties and reduce polymerization shrinkage. Presently, most of the existing resin cements are the composite material containing resin monomers, mainly Bis-GMA, with the variations of filler loading. Some cements utilize a specific resin with a functional group that can chemically bond to the tooth structure. According to the bonding substrate modification, there are three major categories of the resin cements that are commonly used. The first one is the "etch-and-rinse" system, it utilizes phosphoric acid etching that completely dissolves the smear layer and creates a zone of demineralized dentin. After rinsing the acid, hydrophobic resins, with or without adhesive, are applied to the demineralized collagen network to achieve micromechanical retention (i.e. Variolink II (Ivoclar Vivadent, Schaan, Liechtenstein); Calibra (Dentsply Caulk, Milford, DE, USA); Nexus (Kerr Corporation, Orange, CA, USA)). Conversely, in the second system, "self-etch" resin cement utilizes adhesive primer containing high concentrations of acidic resin monomers to simultaneously demineralize and infiltrate the smear layer-covered dentin prior to resin luting (i.e. Panavia 21, Panavia F, Panavia F 2.0 (Kuraray Medical Inc., Tokyo, Japan); Multilink (Ivoclar Vivadent, Schaan, Liechtenstein)). Thirdly, a further reduction in working steps has been accomplished with the recent introduction of "self-adhesive" resin cements (i.e. RelyX Unicem (3M ESPE, St.Paul, MN, USA); Maxcem (Kerr Corporation, Orange, Calif); BisCem (Schaumburg, IL, USA); MonoCem (*Shofu Dental*, San Marcos, CA, USA)), which do not require any pre-treatment of tooth substrates.

As a result of various types of resin cement being commercially available, many studies attempted to compare their bonding strengths when they are used to bond a fiber post to root canal dentin. Some studies demonstrated higher bond strengths achieved by using the etch-and-rinse system compared to self-etch and self-adhesive resin cements [20;21]. On the other hand, some studies reported that self-adhesive resin cement, RelyX Unicem, demonstrated higher bond strength than the others [22;23]. It should be noted that the bond strength data collected from previous studies was divergent and could have come from different experimental conditions. From the study of our research group evaluating the regional push-out bond strength of fiber posts to root canal dentin using three types of resin cement [24], the results (Table 1) showed that the self-adhesive resin cement, RelyX Unicem, provided the highest average bond strength value in every region but statistically significant difference was not presented at the coronal region. The highest bond strength values at the coronal post space of all tested resin cements might be the result of sufficient light energy that irradiated from the coronal end. Moreover, the coronal portion had good accessibility for bonding procedures. At the middle and apical region, RelyX Unicem showed significantly higher bond strength than those of self-etch resin cement (Panavia F 2.0) and the etch-and-rinse system (Variolink II). Variolink II and Panavia F 2.0 seemed to provide similar bond strengths at all regions except the apical region where the bond strength of Variolink II was the lowest at only 2.38 MPa.

Table 1. Regional push-out bond strength values (mean±SD) in MPa of fiber post to root canal dentin using three types of resin cement

	Variolink II	Panavia F 2.0	RelyX Unicem
Coronal	$10.92\pm7.52^{A,*}$	12.56 ± 4.64^{A}	$18.72\pm8.67^{A,*}$
Middle	$6.59\pm2.62^{M,*}$	$6.24\pm2.50^{M,*}$	$17.05\pm6.30^{N,*}$
Apical	2.38 ± 1.80^{X}	$5.13\pm1.90^{Y,*}$	$15.80\pm8.67^{Z,*}$

*Mean values with the same letter in each row and asterisks in each column indicated no significant difference. ($P<0.05$)

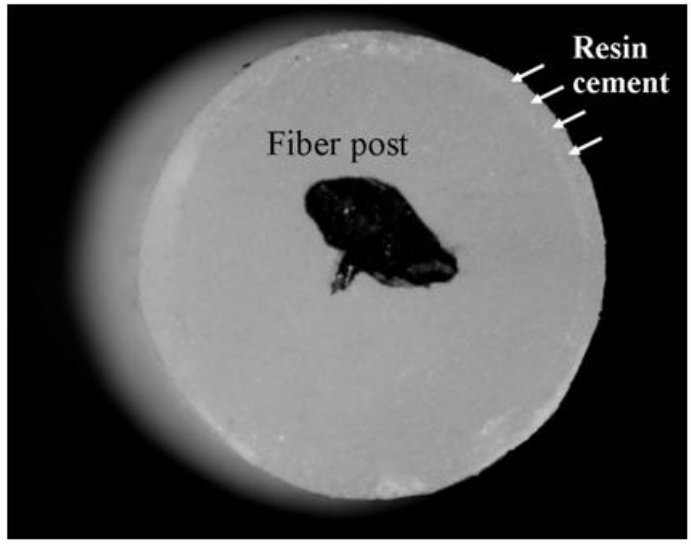

Figure 1. Representative photograph obtained from a stereomicroscope showing the failed post after a thin-slice push-out test. This failure pattern was classified **as** adhesive failure between the **r**esin cement and root canal dentin. The fiber post was surrounded with a resin cement layer.

Regarding the regional difference, the bond strength of resin cement to the root canal wall decreased in the deeper region. However, there were no statistical differences in bond strength among the three regions when RelyX Unicem was used. This study found that more than 90% of specimens adhesively failed at the interface between the luting resin and root canal dentin (Figure 1), not the fiber post interface.

The reason for high bond strength values of RelyX Unicem might be due to the superior moisture tolerance behavior and the less technique sensitivity of this material. When the root canal is irrigated before post placement, some water might remain on the dentin surface and especially within the dentinal tubules due to poor visibility and difficulty in water removal. Furthermore, the narrow tubules hold water by surface tension, making it difficult to displace water with the resin [25]. RelyX Unicem has high moisture tolerance behavior because it has a typical monomer in the constituent that can react with basic salts and tooth apatite in the tooth structure. The monomer reaction is done through the functional groups which are modified by phosphoric acid. Water is consequently formed in this neutralization. This step will increase hydrophilicity, improve adaptation of the luting agent to the tooth structure, and enhance moisture tolerance. Moreover, the specific functional group of resin monomer

composed in RelyX Unicem was able to chemically interact with the hydroxyapatite [26]. Similarly to a previous study, Bitter et al. [22;23] investigated the push-out bond strength of six luting agents used for fiber post bonding and found that the RelyX Unicem provide a significantly higher bond strength compared with other materials.

It was speculated that self-adhesive resin cement might not be able to act as a good acid to dissolve the smear layer and demineralize root canal dentin because of mild etching capacity compared to separate phosphoric acid etching prior to bonding. By using a scanning electron microscope (SEM), the hybrid layer and resin tags formation at the interface between root canal dentin and resin cements were evaluated. It was found that no resin tags were formed in the group of RelyX Unicem (Figure 2). The hybrid layer could not be indicated as well. The manufacturer claims that RelyX Unicem has typical monomers containing at least two phosphoric acid groups and a minimum of two double bonded carbon units (C=C) per molecule which provide enough acidity of pH 1 at the beginning, increasing to pH 5 within 5 minutes, and up to pH 7 within 24 hours (data from manufacturer). However, the rapid rise in pH of RelyX Unicem could prevent the demineralization process of the root canal dentin resulting in the extinguishment of the hybrid layer. Previous studies evaluating the interface of RelyX Unicem and dentin could not detect the hybrid layer as well [23;27]. Agreeing with our results, the highest bond strength was obtained even though the SEM characteristic appeared unfavorable. It seems that characteristics shown in SEM photomicrographs might not be able to represent the quantitative bond strengths. In this case, chemical interaction between the methacrylated phosphoric acid ester in RelyX Unicem and hydroxyapatite might be more important to the bond strength than the micromechanical retention from the hybrid layer.

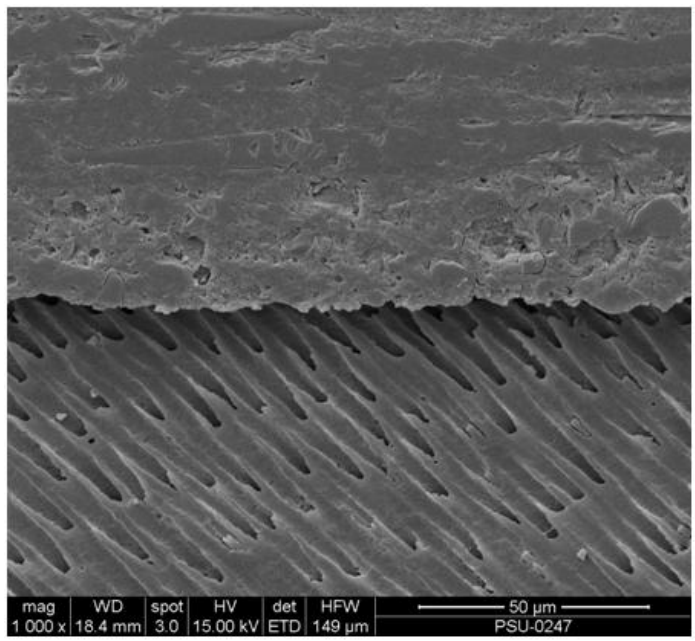

Figure 2. Representative SEM micrograph showing the interface of a fiber post bonded to root canal dentin using the self-adhesive resin cement, RelyX Unicem. Resin tags and hybrid layer did not exist. Filler-contained resin observed at the interface indicated the absence of a hybrid layer.

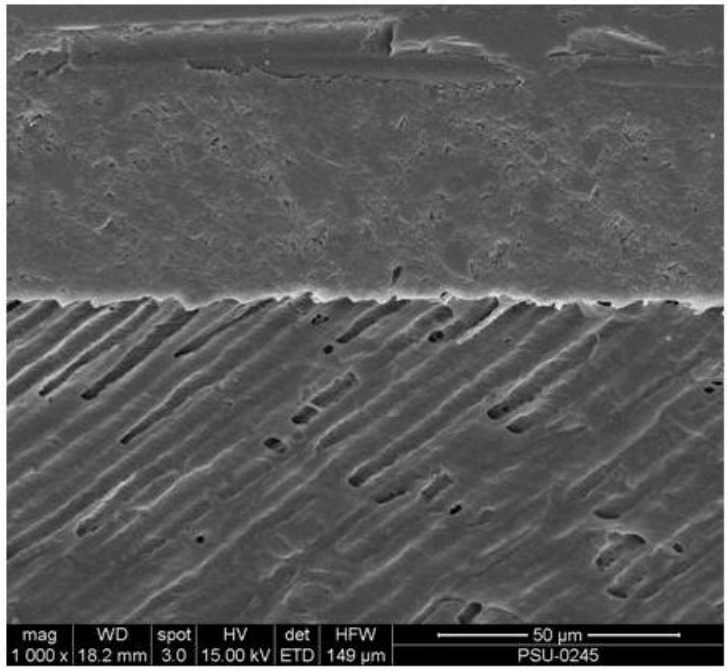

Figure 3. Representative SEM micrograph showing the interface of a fiber post bonded to root canal dentin using a self-etch resin cement, Panavia F 2.0. Hybrid layer with a few short resin tags were observed.

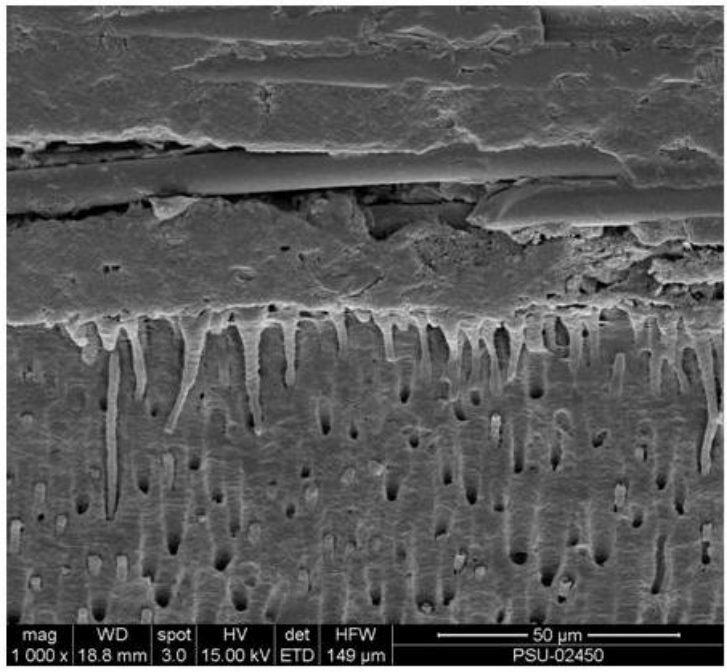

Figure 4. Representative SEM micrograph showing the interface of a fiber post bonded to root canal dentin using an etch and rinse resin cement, Variolink II. Numerous resin tags were formed.

For the resin cement utilizing self-etch primer, Panavia F 2.0, the average bond strength values were lower by approximately 2-3 times than those of RelyX Unicem in every region. The pH value of the Panavia ED Primer A+B mixture is 2.1 [28], which is higher than that of RelyX Unicem. A SEM micrograph revealed no resin tags formation, but a thin hybrid layer could be observed (Figure 3). Likewise, Salz et al. [28] examined the dentin surface after ED Primer application and found that this primer barely etched through the smear layer and the formation of resin tags was not indicated. Similar to RelyX Unicem, even though this interface appearance seemed to be disadvantageous for the bonding, bond strength values of Panavia F 2.0 were statistically comparable to RelyX Unicem at the coronal region and higher than variolink II at the apical region. 10-methacryloyloxydecyl hydrogen phosphate (MDP), a key monomer contained in ED Primer and the resin of Panavia F 2.0, was found to be a very effective functional monomer providing a good chemical interaction to the hydroxyapatite [29].

The conventional etch and rinse resin cement system, Variolink II, provided a very favorable appearance in a SEM micrograph (Figure 4) showing numerous resin tags. However, the bond strength values obtained from Variolink II were generally lower than the other two systems, especially at the apical portion. Bitter et al. reported the same characteristic of Variolink II as this result [23]. Several resin tags were found, but its bond strengths were lower than those of RelyX Unicem and Panavia F 2.0. Nevertheless, some previous studies reported the high bond strength between fiber posts and root canals cemented with Variolink II [21;30].

Controversial results obtained from different studies might be explained by technique sensitivity for the etch and rinse cement system [31]. In the application procedures of Variolink II, moist dentin is required after etching and rinsing to prevent collagen collapse, which is critical for good bond strength. However, the limitation of accessibility and visibility during the bonding procedure was an obstacle for moisture control especially in the apical region, where the water tends to remain at the bottom of the canal. Panavia F 2.0 seems to have less technique sensitivity compared with the etch-and-rinse system. The ED primer is applied followed by air blowing to remove solvents from the primer, and the luting resin is then inserted. However, it is fairly difficult to evaporate the water content in primer inside the deep and narrow canal. Some remaining solvent might deteriorate the adhesion at the deeper portion. Self adhesive resin cement has the least technique sensitivity because it can be used without any pre-treatment of the dentin surface. The clinicians are often satisfied with this simple working step. The only factor that would make differences in research results obtained from this latest product is the dentin characteristic prepared in each experiment. If any root canal filling material or sealer remained in the canal, they would weaken the bonding effectiveness since the self-adhesive resin cement would bond to the canal obturated material instead of the dentin. Phosphoric acid etching and rinsing may be beneficial for uncleaned dentin surfaces. In most experiments, the investigators could not control the dentin characteristic before bonding. Thus, results comparing the effectiveness of the three categories of resin cement are still contentious.

Table 2. Currently available resin composite core materials

Product	Manufacturer/Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
Absolute Dentin	Parkell Inc, Edgewood, NY, USA	Fluoride-containing highly filled resin composite	Auto-mix	Dual-cure	Yes	Methacrylate ester monomer, silane treated glass filler, fluorided-containg glass, benzoyl peroxide, initiators, stabilizers
Bis-Core	Bisco Inc, Schaumburg, IL, USA	Highly filled resin composite	Hand-mix	Light-cure (Base paste) /Dual-cure	No	Base: Bis-GMA, UDMA, glass filler, fused silica Catalyst: Bis-GMA, TEGDMA, glass filler, fused silica
Build-It F.R.	Pentron Clinical Technologies, LLC, Wallingford, CT, USA	Fluoride-containing fiber-reinforced resin composite	Auto-mix	Dual-cure	Yes	Mixture of Bis-GMA, UDMA, HDDMA, silane treated bariumborosilicate glass fillers, silane treated chopped glass fibers, pigments, initiators, stabilizers, UV absorber
Clearfil Core New Bond	Kuraray Medical Inc, Tokyo, Japan	Filled resin composite	Hand-mix	Self-Cure	Yes	Universal: Bis-GMA, TEGDMA,glass filler, colloidal silica, accelerators, pigments, and others Catalyst: Bis-GMA, TEGDMA,glass filler, colloidal silica, initiators and others
Clearfil DC Core	Kuraray Medical Inc, Tokyo, Japan	Filled resin composite	Hand-mix	Dual-Cure	Yes	Universal: Bis-GMA, TEGDMA, silanated colloidal silica, barium glass, N,N-Diethanol p-toluidine Catalyst: Bis-GMA, TEGDMA, silanated colloidal silica, barium glass, d,l-camphorquinone, benzoyl peroxide
Clearfil DC Core Automix	Kuraray Medical Inc, Tokyo, Japan	Filled resin composite	Auto-mix	Dual-Cure	Yes	Universal: TEGDMA, methacrylate monomers, silanized glass fillers, silica microfillers, chemical/photoinitiator Catalyst: Bis-GMA, TEGDMA, silanized glass fillers, silica microfillers, chemical/photoinitiator
Clearfil Photo Core	Kuraray Medical Inc, Tokyo, Japan	Filled resin composite	One paste system	Light-cure	No	Bis-GMA, TEGDMA, silanateded glass powder, silanateded barium glass powder, dl-camphorquinone
CompCore	Premier Dental Products Co., Plymouth Meeting, PA, USA	Fluoride-containing resin composite	Hand-mix	Self-cure	N/A	Blend of multifunctional methacrylates, initiator, amine

Table (Continued)

Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
CompCore AF Flow	Premier Dental Products Co., Plymouth Meeting, PA, USA	Flowable nanofilled resin composite	Auto-mix	Dual-cure	N/A	TEGDMA, co-initiator, photoinitiator, fumed silica
CompCore AF Stack	Premier Dental Products Co., Plymouth Meeting, PA, USA	Nanofilled resin composite	Auto-mix	Dual-cure	N/A	TEGDMA, co-initiator, photoinitiator, fumed silica
CompCore AF Twist	Premier Dental Products Co., Plymouth Meeting, PA, USA	Nanofilled resin composite	Hand-mix	Self-cure	N/A	TEGDMA, co-initiator, fumed silica
Core-Flo	Bisco Inc, Schaumburg, IL, USA	Flowable resin composite	Hand-mix	Self-cure	Yes	Base: Ethoxylated Bis-GMA, TEGDMA, glass filler, silica Catalyst: Bis-GMA, TEGDMA, glass filler
Core Paste XP	Den-Mat Holdings, LLC, Santa Maria, CA, USA	Filled resin composite	Auto-mix	Dual-cure	Yes	Glass fillers in methacrylate resin
Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
Core Restore 2	Kerr Corporation, Orange, CA, USA	Filled hybrid resin composite	Hand-mix (Dual-cure)/ One-paste (Light-cure)	Dual-cure/Light-cure	No	BisGMA, TEGDMA, EBPADMA, redox initiator, photoinitiator, barium glass, silica
CosmeCore	Cosmedent Inc., Chicago, IL, USA	Microhybrid resin composite	Auto-mix	Dual-cure	Yes	Diurethane dimethacrylate, Bis-GMA, diacrylate, barium aluminum boron silicate glass, dibenzoyl peroxide, silica fume
Encore original	Centrix Inc, Shelton, CT, USA	Hybrid composite with/without fluoride	Hand-mix	Self-cure	Yes	Bis-GMA resin composite
Encore AF	Centrix Inc, Shelton, CT, USA	Fluoride- containing hybrid composite	Hand-mix	Self-cure	Yes	Bis-GMA resin composite
Encore D/C Automix	Centrix Inc, Shelton, CT, USA	Fluoride- containing hybrid composite	Auto-mix	Dual-Cure	Yes	Bis-GMA resin composite
FluoroCore	Dentsply Caulk, Milford, DE, USA	Fluoride- releasing resin composite	Hand-mix	Dual-cure	N/A	Base: UDMA, barium boron fluoro-alumino silicate glass Catalyst: UDMA, barium boron fluoro-alumino silicate glass, aluminum oxide, benzoyl peroxide

Table (Continued)

Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
FluoroCore 2	Dentsply Caulk, Milford, DE, USA	Fluoride- releasing highly filled resin composite	Auto-mix	Dual-cure	N/A	UDMA, di- & tri-functional methacrylates, barium boron fluoroaluminosilicate glass, camphorquinone (CQ) photoinitiator, photoaccelerators, silicon Dioxide, benzoyl Peroxide
Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
FormCore DC	J. Morita USA Inc., Irvine, CA, USA	Flowable resin composite	Auto-mix	Dual-cure	N/A	Mixture of resin based on Bis-GMA, catalysts, stabilizer and pigments
Gradia Core (Unifil Core EM)	GC Corporation, Tokyo, Japan	Hybrid composite	Auto-mix	Dual-cure	Yes	Base paste: fluoro-alumino-silicate glass, methacrylic acid ester, silicon dioxide, photo initiator, inhibitor, pigment Catalyst paste: fluoro-alumino-silicate glass, methacrylic acid ester, silicon dioxide, initiator, inhibitor, pigment
Light-Core	Bisco Inc, Schaumburg, IL, USA	Fiber-reinforced resin composite	One paste system	Light-cure	No	Bis-GMA, ethoxylated Bis-GMA, glass filler, fiber
LuxaCore	DMG, Hamburg, Germany	Filled resin composite	Auto-mix	Self-cure	Yes	Barium glass and pyrogenic silicic acid in a Bis-GMA-based dental resin matrix
LuxaCore Dual	DMG, Hamburg, Germany	Filled resin composite	Auto-mix	Dual-cure	Yes	Barium glass and pyrogenic silicic acid in a Bis-GMA-based dental resin matrix
LuxaCore Z Dual	DMG, Hamburg, Germany	Zirconium dioxide and nano filler-reinforced resin composite	Auto-mix	Dual-cure	Yes	Barium glass, pyrogenic silicic acid, nanofillers and zirconium oxide in a Bis-GMA-based dental resin matrix
Multicore Flow	Ivoclar Vivadent AG, Schaan, Liechtenstein	Flowable resin composite	Auto-mix	Dual-cure	Yes	Bis-GMA, UDMA, TEGDMA, barium glass fillers, Ba-Al-fluorosilicate glass, ytterbium trifluoride, highly dispersed silicon dioxide, catalysts, stabilizers, pigments
Multicore HB	Ivoclar Vivadent AG, Schaan, Liechtenstein	Highly viscous resin composites	Hand-mix	Dual-cure	Yes	Bis-GMA, UDMA, TEGDMA, barium glass fillers, Ba-Al-fluorosilicate glass, ytterbium trifluoride, highly dispersed silicon dioxide, catalysts, stabilizers, pigments

Table (Continued)

Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
Paracore	Coltène/Whaledent AG, Altstätten, Switzerland	Fluoride-containing resin composite	Auto-mix	Dual-cure	Yes	Methacrylates, fluoride, barium glass, amorphous silica
RapidCore	Centrix Inc, Shelton, CT, USA	Fluoride- containing hybrid composite	Auto-mix	Self-Cure	Yes	Bis-GMA resin composite
Rebilda DC	VOCO, Cuxhaven, Germany	Flowable resin composite	Auto-mix	Dual-Cure	Yes	Mixture of UDMA, DDDMA, Bis-GMA
SuperCure	Centrix Inc, Shelton, CT, USA	Barium borosilicate glass- filled hybrid composite	One paste system	Light-cure	Only with clear fiber post	Bis-GMA resin composite
SuperCure Q	Centrix Inc, Shelton, CT, USA	Quartz glass reinforced hybrid composite	One paste system	Light-cure	Only with clear fiber post	Bis-GMA resin composite
Product	Manufacturer/ Supplier	Material type	Hand-mix/ Auto-mix	Self-/Light-/Dual-cure	Cementation of fiber post (Yes/No)	Composition
Ti-Core/Ti-Core Natural	Essential Dental Systems Inc, S. Hackensack, NJ, USA	Titanium/Lanthanide-reinforced fluoride-releasing resin composite	Hand-mix	Self-cure	Yes	Aromatic/aliphatic dimethacrylate reinforced with titanium or lanthanide (Ti-Core Natural)
Ti-Core Auto E	Essential Dental Systems Inc, S. Hackensack, NJ, USA	Lanthanide- reinforced fluoride- releasing resin composite	Auto-mix	Dual-cure	Yes	Bis-GMA resin composite with lanthanide
UnifilCore	GC Corporation, Tokyo, Japan	Hybrid composite	Hand-mix	Dual-cure	Yes	Paste A: UDMA, dimethacrylates, fluoro-alumino-silicate glass, silicon dioxide, photo/chemical initiator Paste B: UDMA, dimethacrylates, fluoro-alumino-silicate glass, silicon dioxide, photo/chemical initiator

Abbreviation: Bis-GMA: bisphenol-A-glycidyldimethacrylate
DDDMA: dodecandioldimethacrylate
EBPADMA: ethoxylated bisphenol A dimethacrylate
HDDMA: hexane diol dimethacrylate
TEGDMA: triethylene glycol dimethacrylate
UDMA: urethane dimethacrylate;

Table 3. Mean±SD in MPa of microtensile bond strength of three types of fiber post to dual-cure resin composite core materials treated with different post surface treatments

Snowpost	No treatment		Adhesive (LB)		Adhesive &LC		Silane (PB+PBA)		Silane &LC
Upper	8.6±3.4	NS	9.8±2.8	P<0.05	15.0±4.3	NS	16.5±5.1	P<0.05	25.7±5.5
Middle	7.9±2.7	NS	9.1±3.9	P<0.05	14.6±5.0	NS	13.7±4.0	P<0.05	23.8±6.0
Bottom	7.8±2.9	NS	8.3±2.5	P<0.05	13.0±3.4	NS	13.0±3.8	P<0.05	21.9±5.0
Aestheti-Plus									
Upper	10.5±3.0	P<0.05	18.7±6.0	NS	20.5±5.3	P<0.05	37.0±8.7	NS	33.3±9.5
Middle	8.0±1.8	P<0.05	14.0±5.0	NS	17.9±3.0	P<0.05	35.1±8.8	NS	28.2±5.7
Bottom	8.1±1.5	P<0.05	14.2±4.2	NS	16.3±4.1	P<0.05	34.4±8.7	NS	29.6±7.0
Light-Post									
Upper	12.5±3.3	P<0.05	31.6±9.2	NS	40.5±7.5	P<0.05	53.2±5.3	NS	49.3±10.8
Middle	8.6±4.6	P<0.05	28.7±6.9	P<0.05	38.8±4.6	P<0.05	46.2±5.7	NS	45.5±8.5
Bottom	8.7±2.7	P<0.05	20.5±5.8	P<0.05	30.6±5.8	P<0.05	43.9±7.4	NS	45.9±10.0

Note: NS demonstrates no significant difference and P<0.05 indicats significant difference between two columns.
LB: Clearfil Liner Bond 2V, PB+PBA: the mixture of Photobond and Clearfil Porcelain Bond Activator, LC: Light curing

Utilization of Dual-Cure Resin Composite Core Materials for Post Cementation

Resin composite used with a light-transmitting post was introduced as an effective method for restoring a weakened root or flared canal for more than a decade [9;32]. Later, it was reported that resin composite core materials combined with adhesive systems is a comparable, and in some ways superior, method for luting endodontic posts compared to the resin cement [33]. A recent study was convinced of the effectiveness of using core build-up resin composite for fiber post cementation over resin cements in terms of push-out strength [34]. Traditionally, resin composite core materials had a somewhat high viscosity in order to facilitate sculping of the core by hand instruments. When they were introduced for using as a luting agent, only cases needing high cement thickness were selected. However, most of the currently available resin composite core materials provide a favorable consistency for cementation purpose. The injectable syringe/cartridge or the auto-mix systems are presently occupying the market for core build-up resin composite. Most of them are recommended for use in cementation of endodontic posts further than core build-up purpose. This method has become popular and it increased the convenience for clinicians in using the same material for post luting and core build-up. Technically, this method reduces the interfaces occurring from different resin used (resin cement and core resin). Table 2 lists the compositions and characteristics of currently available resin composite core materials gathered from commercial manufacturers.

There are three modes of the polymerization method of resin composite core materials; self-cure, light-cure, and dual-cure. Self-cure resin composite was originally used for core build-up. However, problems such as slow setting time and inferior properties and bond strength compared to the light-activated resin [35;36] reduced the use of self-cure resin composite. Light-cure resin composite provided the highest degree of conversion, good mechanical properties, and prompt setting after curing. However, resin at the deep region may not be completely polymerized due to the limitation of light energy in a deep and narrow canal. The dual-cure mode is the most appropriate activation method for resin polymerization in the canal. Dual-cure resin composite composes of both chemical- and photo- initiators. The resin that receives light-activation can be rapidly cured after light exposure with a high degree of conversion. Polymerization in the deep region is ensured by the chemical activation. For this reason, the dual-cure type of resin composite core material is recommended for cementation of endodontic posts.

Adhesion of dual-cure resin composite core material to fiber posts

Laboratory and clinical research have found that failures of fiber post and core restorations often occurred through decementation between the fiber post-resin and/or resin-root canal dentin interfaces [17;37;38] as a result of inadequate bond strength between their interfaces. Many studies have been performed to investigate the bond strength between fiber posts and luting resin. For bonding to the post surface, different surface treatments have been used to create a reliable bond. The method of increasing the chemical interaction and wettability to the post surface is simple, and it can keep the post surface undamaged. A silane coupling agent and adhesive resin are often used to apply on the post surface to improve wettability and sometimes creates chemical bonding between posts and luting resin.

Evidently, application of a silane coupling agent and adhesive to the post surface before luting was found to enhance the bond strength to fiber post surfaces [39;40].

Regional microtensile bond strength values of the silica-zirconium glass fiber posts (Snowpost, Carbotech, Ganges, France) and quartz fiber posts (Aestheti-Plus and Light-Post, RTD, Grenoble, France) with different surface treatments are shown in Table 3 [39;40]. This experiment was designed using two kinds of post surface treatments, a dual cured bonding agent, Clearfil Liner Bond 2V (LB) and a silane coupling bonding agent, Photobond (PB) with Clearfil Porcelain Bond Activator (PBA). The results revealed that application of the adhesive to the post surface significantly improved the bond strength to fiber posts except for Snowpost, which is the non-translucent glass fiber post. Light-curing to the adhesive is important for a stronger bond if Snowpost were used. On the other hand, the quartz fiber post might have better light transmission properties compared to the Snowpost. The bonding agent surrounding Aestheti-Plus posts would indirectly cured by photons passing through the quartz fibers in the post during photo-irradiation of the DC Core from the top of the cavity, resulting in better mechanical properties of the bonding agent to Aestheti-Plus post than to Snowpost. Aestheti-Plus therefore provided better bond strength even though the adhesive was not exposed to light before luting. In can be understood that the application of the adhesive with sufficient light curing can improve the bond strength to the fiber post.

For the silanization method, there are two types of silane systems: the single phase prehydrolyzed and the two-component system for immediate hydrolysis. The mixture of PBA and PB is a two-component system. PBA contains γ - methacryloxy propyltrimethoxy silane (γ-MPS), which is rapidly hydrolyzed in the presence of an acidic monomer in PB. After mixing, the alkoxy groups of γ-MPS were hydrolyzed into silanol groups that can bond with silica through the formation of siloxane bonds [41;42] Both the glass fiber of Snowpost and quartz fiber of Aestheti-Plus and Light-Post contain silica. Therefore, higher bond strengths could be obtained in the groups treated with a silane coupling agent compared with the group that received no surface treatment and the groups treated with adhesive alone.

Studies investigating the effect of silane revealed controversial results. Concurring with the results showed in this chapter, three studies supported the use of silane to enhance bond strength between a luting resin composite to fiber post surfaces [43-45]. In the contrary, some studies did not observe the improvement of bond strength with silanization [46-48]. Theoretically, silane coupling agents improve the adhesion between two interfaces by increasing wettability to the post surface and chemically interacting with the silica contained substance. Some luting resins have good flow ability and they perform a good wetting on the post surface. In this case, silanization may exhibit a negligible result in improving the bond strength to fiber posts. Secondly, the formation of a siloxane bond occurs only with silica-contained fibers or filler, not resin part. If the fiber post surface is covered with the resin, silane may not act as a coupling agent to promote the adhesion between the two surfaces. On the other hand, if the fibers are exposed at the surface, silanization is beneficial for the bond strength. Additionally, the use of silane is a prudent procedure. Their efficiency is dependent on silane selection, application technique, and preservation of the bottle. A warm air stream after silane application was found to be effective for evaporating the solvent and drying silane [49;50]. The prehydrolyzed silane coupling agent is prepared in one bottle. It is ready for use because the silane has already activated. This type of silane molecule tends to form bonds together when the solvent is evaporated from the bottle and the liquid become thicker. This

process may turn the coupling agent to the separating agent. Before application, the clinician should inspect the silane coupling agent whether it is clear in consistency. For the two-component system, fresh activated silane is prepared by mixing unhydrolyzed silane with the acidic monomer. Furthermore, a resin monomer mixed with silane may assist in chemical bonding if methacrylate-based fiber posts are used.

Various chemical surface treatments were proposed in order to enhance the silanization efficiency. Monticelli et al. [51] found that treating the post surface with potassium permanganate followed by silane application significantly improved the bond strength to fiber post surface compared with a sole silane treatment. Surface treatment with Hydrogen peroxide or hydrofluoric acid plus silane were also reported to be a method that improved adhesion to fiber posts [52;53]. However, the SEM micrographs showed radical alteration of fiber posts after immersion in 4% hydrofluoric acid gel for 60 seconds. The fibers were detached from the resin matrix and the integrity of the fiber post was lost [52]. Tribochemical coating such as the Cojet system (3M ESPE, Seefeld, Germany), which consists of airborne-particle abrasion with Al_2O_3, coated with SiO_2, and then silanization, was another method that enhanced the bond strength [54;55], but the concern about fiber post damage should be taken when particle abrasion is used.

According to the light-transmitting ability of fiber posts, there are two types of posts. The non-translucent post is opaque in color restraining the light-transmitting property of the post, whereas the translucent post allows the light to be transmitted through fibers and clear resin matrix. Through microhardness evaluation, previous researches have found that this type of post offers the possibility of transmitting light into the canal to achieve a greater depth of cure for the resin composite [9;56;57]. Comparing bonding efficiency between translucent and non-translucent fiber posts, it was found that the translucent fiber post significantly improved the adhesion between a post surface and dual-cure resin composite except for the control group where the post surfaces were not applied with any adhesive or silane (Figure 5). Aestheti-Plus and Light-Post were fabricated from the same manufacturer with the same composition of 60% quartz fiber and 40% epoxy resin. For Light-Post, different epoxy resin is used to create the translucent resin matrix increasing light transmitting ability. The bonding agent and luting resin composite surrounding the Light-Post may have been more polymerized by the light transmitted through the post resulting in the higher bond strength.

It is commonly assumed that light-cure adhesive or light-cure luting resin can be used when a light-transmitting post is inserted. In fact, a portion of the light energy is absorbed by the surrounding resin composite during transmission through the post, resulting in a reduction in light energy in deeper regions. Reduction of light energy along the translucent posts has been well documented. Robert et al. [19] found that the hardness of the resin composite surrounding the translucent fiber post, which correlated with the degree of conversion, decreased when composite depth increased. A reduction of light energy during transmission would produce attenuation of polymerization of the resin at the bottom region. It has been reported that the bond strength of dual-cure resin composite to the fiber post surface may have been dependent upon the mechanical properties of the adhesive [39;58]. When the adhesive at the deeper region could not be completely cured as the upper part, the bond strength was inferior.

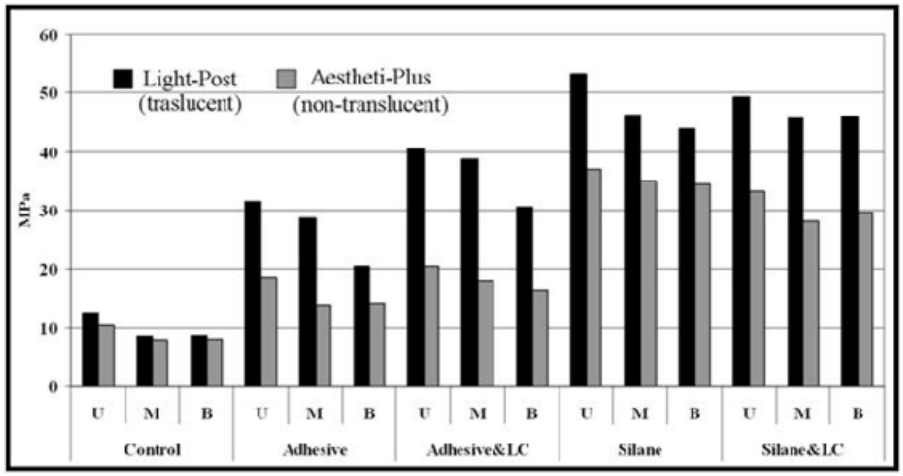

Figure 5. Bar graph showing the comparison of regional microtensile bond strengths between translucent post (Light-Post) and non-translucent post (Aestheti-Plus) for each surface treatment.

For the aspect of adhesion to fiber post surface, it can be summarized that the translucent or light-transmitting post should be selected to enhance the bond strength to the post surface. Application of the adhesive or silane coupling agent is advantageous for the bonding. However, the enhancement relies on the type of fiber post, the type of adhesive or silane including their application technique.

Adhesion of dual-cure resin composite core material to root canal dentin

Difficulties in bonding to the root canal system

In reviewing the literature, there were plenty of studies investigating the bonding of the resin composite to the coronal dentin substrate. However, those results cannot be implied when bonding to root canal dentin because there are many factors that can weaken adhesion to the root canal wall compared to coronal dentin. The reduced visibility for bonding inside the root canal is one major problem. It is somewhat difficult for an operator to control bonding procedures and apply a consistent layer of any dentin conditioners or adhesives in a deep and narrow canal. Another interfering factor is the unfavorable geometry of the root canal system for bonding [59]. The configuration factor or C-factor, being the ratio of the bonded to unbonded surface, is often used as a quantitative measure for the geometry of the cavity preparation for bonding [60]. The greater the percentage of unbonded surfaces, the less stress is generated from polymerization contraction. Unbonded surfaces allow plastic deformation, or flow, within the resin mass during polymerization. It has been reported that the C-factor in endodontic post luted cavities may exceed 200, whereas the C-factor of an intracoronal restoration is in the range of only 1 to 5 [37]. The very restricted system of the root canal would be a detrimental factor to deteriorate the bonding quality of fiber posts. Bouillaguet et al [37] reported that the bond strength obtained from the root canal system was lower than that of flat prepared radicular dentin when the same bonding procedures were conducted. This indicates that the geometry of the root canal has an adverse effect to the bonding.

Variations in the root structure such as accessory root canals, areas of resorption, pulp stones, and varying amounts of irregular secondary dentin may influence bonding to root canal dentin [61]. Moreover, in endodontically treated teeth, SEM analysis revealed large areas covered by smear layers, debris, and sealer/gutta-percha remnants on canal walls along the post space [62]. The sealer remaining in the drilled post space was found to have an effect on the bond strength [63;64]. The canal irrigating agents such as sodium hypochlorite (NaOCl) could weaken the bond strength to root canal dentin as well, but its effect may depend on the type of bonding agent [65-69].

Mechanical properties and the structure of root canal dentin are dependent on the region [70-72]. It was reported that tubule density in the coronal region of root dentin is higher than that of the apical region, and the diameter of the tubules decreases in an apical direction [73]. However, Liu et al [71] found that dentin location did not affect the microtensile strength of the bovine root dentin. Additionally, it was revealed that bond strength to root canal dentin was not influenced by dentin depth and tubule density when a self-etch adhesive system was used [70].

It can be realized from the above problems that many factors can weaken the bonding of fiber posts to root canal walls. Accordingly, the clinician should therefore circumspectly select the proper bonding technique in order to obtain the optimal bonding performance to the radicular dentin.

Utilization of Two-Step Self-Etch Adhesive with Dual-Cure Resin Composite Core Materials for Bonding to Root Canal Dentin

Currently, clinicians can use various “etch-and-rinse” or “self-etch” adhesive systems for bonding to root canal dentin. For etch-and-rinse adhesive systems, moisture control on the etched dentin subsurface is necessary prior to bonding because exposed collagen fibrils collapse after drying and prevent penetration of resin monomers into demineralized dentin, resulting in lower bond strength to dentin [74-77]. However, it is difficult to control the surface wetness in a complex cavity such as a deep and narrow post space within root canal dentin. In contrast, self-etch adhesive systems are generally less technique sensitive because this system simultaneously demineralizes and penetrates resin monomer into dentin [78;79] and avoids the rinsing and drying steps [31;80].

Most clinicians generally use the dual-cure mode of adhesives for bonding to root canal dentin because of their ability to self-polymerize in the absence of light in deeper regions of the post cavity. However, an adverse chemical reaction was reported to occur between chemically-activated resin composite and acidic resin monomers [81-83]. Self-etching primer contains acidic monomers, and consequently, a high concentration of uncured acidic monomers would be present within the primed dentin surface. Therefore, the compatibility of dual-cure adhesive with the self-curing mode and self-etch primed dentin is questionable. The light-cure adhesive resin is not commonly used for bonding to root canal dentin because of the major concern in inadequate light energy to cure the bonding resin at the apical regions. However, the results [84] as shown in Table 4 revealed that light-cure adhesive resin seemed to be effective for bonding root canal dentin. The light-cure adhesive system, Clearfil Liner Bond 2V (Kuraray Medical Inc, Tokyo, Japan), provided higher bond strength than that of the

dual-cure system, Clearfil Liner Bond 2V at both the coronal and apical region. The mechanical properties of the light-cure adhesive resin may have been greater than those of dual-cure adhesive resin [85]. On the other hand, for light-cure SE Bond (Kuraray Medical Inc, Tokyo, Japan), although the μTBS obtained from the coronal region was comparable to that of light-cure Clearfil Liner Bond 2V, the bond strength significantly decreased at the apical region. This may have been due to the different irradiation times for Clearfil Liner Bond 2V and SE Bond. The manufacturer of Clearfil Liner Bond 2V adhesive recommended 20 seconds light curing, whereas 10 seconds was recommended to cure SE Bond adhesive. In the root canal, the light-irradiation to adhesive was limited from the orifice of the post cavity; therefore, the adhesives applied to the root canal wall were light-irradiated from the lateral side. A longer irradiation time might improve the accession area of light energy in the post cavity, and improve the bond strength at the apical region. Moreover, light accessibility through the root canal is limited and, in the clinical situation, remaining coronal tooth structure or adjacent teeth might hinder placement of the light tip close to the root orifice and inadequate polymerization of the adhesive could occur [86].

Table 4. Mean±SD in MPa of microtensile bond strength of four self-etch adhesive systems with different curing mode used for bonding dual-cure resin core materials to root canal dentin

	Dual-cure Photobond	Light-cure (20s) Liner Bond 2V	Dual-cure Liner Bond 2V	Light-cure (10s) SE Bond
Coronal	33.3±4.2^{A}	51.0±11.8^{B}	43.7±11.7^{A,B}	52.2±15.7^{B}
	p < 0.05	NS	NS	p < 0.05
Apical	18.4±5.7^{a}	46.4±7.1^{b}	31.2±7.4^{c}	26.7±12.1^{a,c}

Note: # NS demonstrates no significant difference.
P<0.05 indicates significant difference between coronal and apical regions.

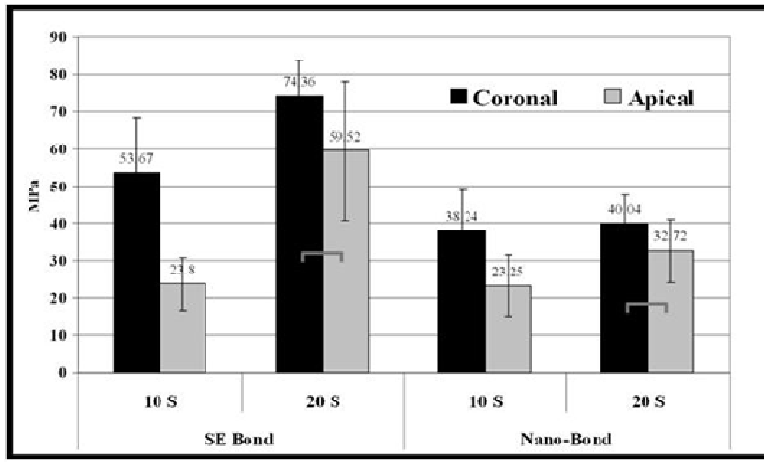

Figure 6. The chart shows regional microtensile bond strengths of light-cure adhesive used in bonding dual-cure resin composite core materials to root canal dentin. Horizontal brackets indicate no significant difference.

To confirm the effect of light energy, an experiment was performed to evaluate the effect of light exposure time to the adhesive on the regional bond strength of a dual-cure resin composite core material to root canal dentin using self-etching priming adhesive systems [87]. The results (Figure 6) revealed that the microtensile bond strength obtained from light-cure adhesive resin (SE Bond and Nano-Bond (Pentron Clinical Technologies, LLC, USA)) significantly decreased at the apical region when light-curing was performed for only 10 seconds. Light-irradiation time for 10 seconds according to the manufacturer's instructions might be enough to reach the optimal light energy for bonding to the flat dentin, where the light can be directly irradiated to the adhesive surface. However, due to the limitation of light accessibility through the root canal and the parallel light-exposure direction to the adhesive surface, 10 seconds irradiation would be insufficient for bonding to the apical region of the post cavity. Indeed, when the adhesive resin was cured for 20 seconds, the bond strength of SE Bond significantly improved at both coronal and apical regions, and the bond strength of Nano-Bond increased at the apical region. Extension of the photo-irradiation time might result in an increase of total light energy inside the root canal. The conversion of double bonds during polymerization might therefore be enhanced. From the results of this study, it appears that light-cure adhesive resin is effective for application to root canal dentin if the light-exposure time is sufficient. Another study evaluating the push-out bond strength confirmed that light-activated adhesive could be used in the canal [88]. Additionally, clinicians may find it financially beneficial to use only one type of adhesive resin to bond to both coronal and radicular dentin without worrying about inefficient polymerization of the adhesive resin in deeper regions.

Utilization of One-Step Self-Etch Adhesive with Dual-Cure Resin Composite Core Materials for Bonding to Root Canal Dentin

At the moment, one-step self-etch adhesive, so called "all-in-one" adhesive, is widely spread in the adhesive market, although it demonstrated ineffective results compared to multi-step adhesives [89]. Some manufacturers have recently developed a dual-cure version of the one-step self-etch adhesive used with resin composite core materials because it simplifies the application procedures for fiber post luting and core build-up. Since light penetration is limited in the deeper region compared with the upper region of the post cavity, dual-cure adhesive in the deeper region is expected to polymerize by self-cure mode with resin core materials filled into the post cavity.

One-step self-etch adhesive contains acidic resin monomer, organic solvent, and water. A major concern is the incompatibility between the acidic monomer of one-step self-etch adhesive and the self- or dual-cure resin composite. Many researchers have reported on adverse acid-base reaction between the acidic resin monomers and the tertiary amines used in the self-cure initiator systems, thereby causing the amines to lose their effectiveness as reducing agents and resulting in poor polymerization [83;90]. In the other words, this incompatibility would pose a barrier to good bonding [82;91-93]. In the deeper region of the post spaces, uncured acidic resin monomers within one-step self-etch adhesive could reduce the self-polymerization of dual-cure resin core materials. Therefore, conversely, if the acidic adhesive were to be completely cured, the adverse impact arising from the incompatibility

issue would be reduced. For this reason, it is important to irradiate dual-cure adhesives with sufficient light energy through the post space.

Besides, the mechanical properties of dual-cure resin were also found to be affected by curing strategies [94;95]. Bond strength to the tooth substrate depends on the mechanical properties of the adhesive resin, which means that dual-cure adhesive should be exposed to light – *versus* – chemical activation alone – to ensure good bond strength [58;96]. However, within the post space, high attenuation of light passing through the canal may jeopardize bonding quality especially in the deeper region. It can be presumed that using a high intensity light curing unit and/or prolonging the light-exposure time to the adhesive resins may improve the adhesion of dual-cure one-step self-etch adhesive to root canal dentin.

An experiment evaluating the effect of the light-curing unit, which provides different power densities, and the exposure time on regional bond strengths of dual-cure resin composite core material to root canal dentin bonding using dual-cure one-step self-etch adhesive was performed [97]. The results (Table 5) demonstrated that light-power density and curing time affected the microtensile bond strength especially at the apical region of the post space. The optimal curing time for dual-cure one-step self-etch adhesive applied to the 8 mm deep root canal dentin surface was 30 seconds with Optilux 501 (830 mW/cm^2) and 20 seconds with the high intensity curing unit, Hyperlightel (1350 mW/cm^2).

Interestingly, numerous specimens failed as cohesive failure in the resin including failure within the adhesive or at the interface between the adhesive and the resin composite, and mixed failure of this type with failure at the resin-dentin interface. Failure within the adhesive resin was probably a result of the undesirable properties of one-step self-etch adhesives. In the studies using two-step self-etch adhesive, Clearfil SE Bond, only a small number of specimens failed cohesively within the adhesive resin. For the one-step self-etch adhesive used in this study, the liquid B component of Clearfil DC Bond contained water, which was mixed with the liquid A component and then applied within one single step. As for two self-etch adhesives, the hydrophobic resin is generally separated from the first-step solvent-included primer. It must be highlighted that any water remaining within the adhesive layer after evaporation may weaken the properties of the resin [98]. Additionally, water trapped at the surface of the adhesive resin might interfere with the bonding between the adhesive and resin composite. Tay et al. [99] found that the reticular mode of nanoleakage could occur due to incomplete water removal from the adhesive layer. The studies regarding bond strengths of single-step adhesive have demonstrated a significant inferior microtensile bond strength of resin-dentin bonds after long term water storage, with a typical deterioration at the adhesive-composite interface [100;101]. As shown in Figure 7, honeycomb structures formed by numerous blisters were seen all over the fractured surfaces of both the adhesive and resin composite. In light of these findings, air blowing of the adhesive during bonding procedures should be performed carefully to ensure absolute evaporation of the solvent within the adhesive. However, in practical dentistry, this step may be difficult to achieve at the deep region of the post spaces.

Table 5. Mean±SD in MPa of microtensile bond strength of DC Core Automix to root canal dentin treated with the dual-cure self-etch adhesive, Clearfil DC Bond0

	Optilux 501 (830 mW/cm^2)*			**Hyperlightel** (1350 mW/cm^2)*		
	10 s	20 s	30 s	10 s	20 s	30 s
Coronal	38.9±13.7^{AB}	39.8±12.5^{AB}	38.5±10.0^{AB}	33.9±11.3 ^{A}	49.8±8.6^{B}	42.7±11.4 ^{AB}
	p<0.05	p<0.05	NS	p<0.05	NS	NS
Apical	11.1± 2.1^{a}	20.4±8.3^{ab}	28.6±11.1^{bc}	16.0±7.2 ^{ab}	36.4±13.3^{c}	30.2±10.6^{bc}

Note: #Same superscripts demonstrate no significant differences in each row.
NS demonstrates no significant differenc and P<0.05 indicates significant difference between coronal and apical regions.
* Measured with a digital radiometer (Jetlite light tester, J. Morita, Mason Irvine, CA, USA),

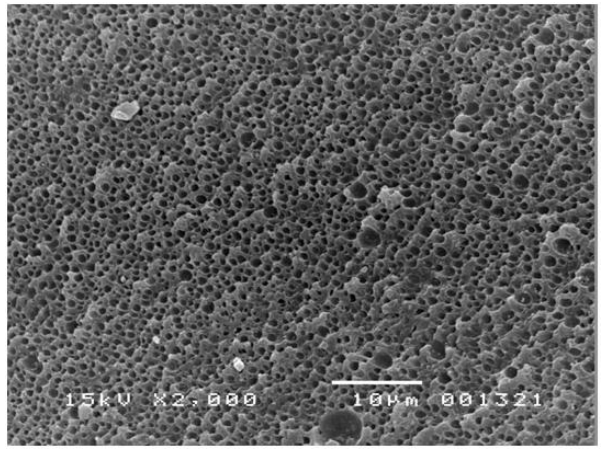

Figure 7. SEM micrographs showing the surface of one-step self-etch adhesive resin of the fracture specimen at the apical region. Honeycomb structures formed by numerous blisters can be seen all over the fractured surfaces.

Bond strengths and failure of fiber post-bonded teeth using dual-cure resin composite core materials

The results of bond strengths obtained from resin composites to the post surface and resin composites to root canal dentin have been revealed in the above discussion. However, those experiments separately evaluated the adhesions of resin composite to the post surface and resin composite to root canal dentin to determine the most favorable method for bonding each interface. In reality, when a fiber post is luted into the root canal, two different types of interfaces, post-resin composite and resin composite-root canal dentin, are created under the polymerization-stressed condition of the luting resin. Moreover, the C-factor significantly increases for fiber post bonded teeth. The restriction of any free surfaces which might be able to reduce the shrinkage stress would have an additional affect on the adhesion of fiber posts to root canal dentin [59].

Some studies have suggested that problems in adhesion might occur at the resin composite/root canal dentin interface rather than at the fiber post-resin composite interface [102;103]. On the other hand, previous research has also indicated a problem at the post-resin composite interface [55;104]. Therefore, it is still uncertain which interface is the weakest part of the fiber post restoration. Vichi et al. [105] used scanning electron microscopy (SEM)

to evaluate the interfaces created in a fiber post bonded root canal, and suggested that an absence of voids at the fiber post-resin cement interface indicates a good bond between the post surface and resin cement. However, SEM evaluation alone cannot be correlated to quantitative bond strength data. Hence, our experimental study was performed to investigate the qualities of two resin interfaces to dentin and fiber post and to identify the weaker part by evaluating the regional microtensile bond strengths of four kinds of fiber posts to root canal dentin luted with dual-cure resin composites and evaluating the failure characteristics of fiber post restored teeth [106]. The experiment was performed by using the microtensile testing method. (Figure 8, reprint with permission from Operative Dentistry, IN, USA).

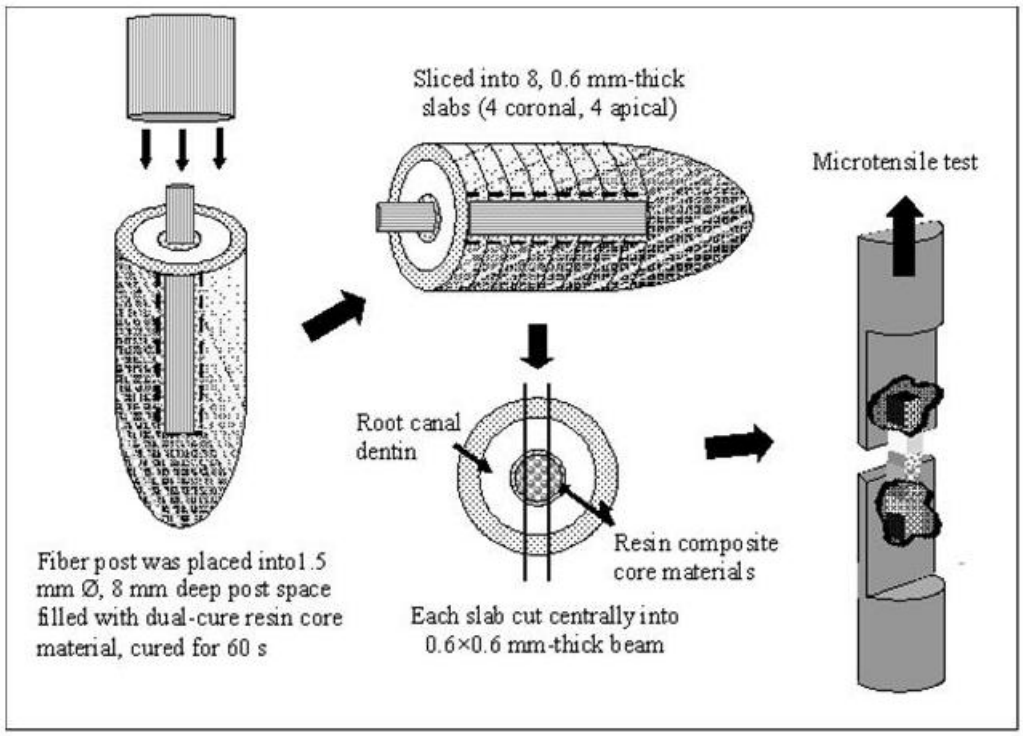

Figure 8. Schematic illustration of the fiber post bonding and microtensile bond strength testing procedures.

Table 6. Mean±SD in MPa of microtensile bond strength of various fiber posts to root canal dentin luted with Clearfil SE Bond and DC Core Automix

	Snowlight	FibreKor	D.T. Light-Post	GC Post
Coronal	22.8±7.1^{A}	50.1±7.8^{B}	13.2±2.6^{C}	9.6±2.6^{C}
	NS	NS	NS	NS
Apical	19.9±5.5^{a}	45.3±9.5^{b}	13.4±3.6^{c}	8.4±2.3^{c}

Note: # Same superscripts demonstrate no significant differences in each row.
##NS demonstrates no significant differenc between coronal and apical regions.

Interestingly, the results (Table 6) showed that no failure occurred on the dentin side for any of the tested posts after specimen fracture. In this experiment, the light-cure adhesive, Clearfil SE Bond, with a prolonged irradiation time of 20 seconds was used as the bonding procedure for the root canal dentin surface. This indicated that a light-cure adhesive system is possibly a good choice for bonding to root canal dentin. The dentin/resin interface, therefore, would not be a problem in a fiber post restoration when it is bonded effectively. However, using light-cure adhesive may affect the seating ability of the fiber post if the adhesive layer is thick. Application of strong air blow to spread adhesive and using paper points to absorb excess resin probably eliminates the seating problem.

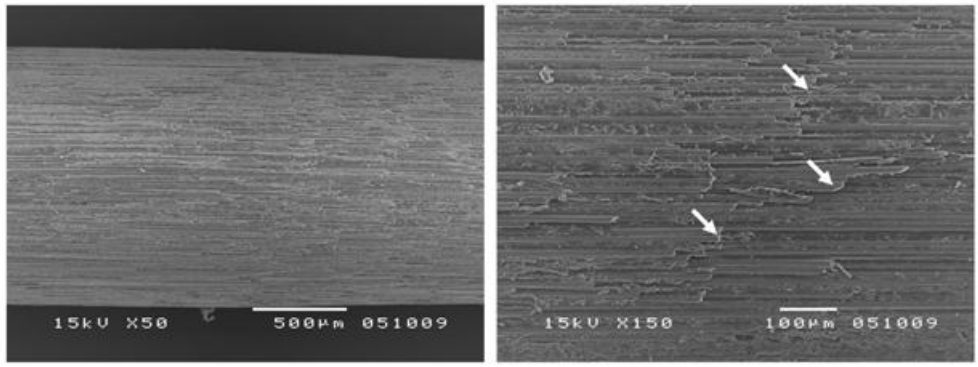

Figure 9. SEM micrographs of the surfaces of the D.T. Light post. The fibers at the surface were found to be cut in steps along the post (white arrows).

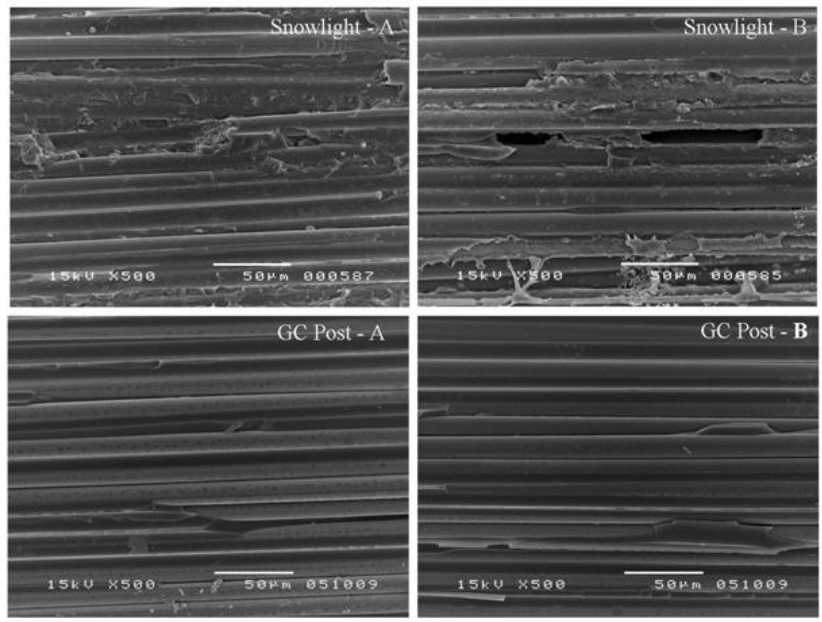

Figure 10. SEM micrographs of the fractured specimens of Snowlight and GC Post after microtensile bond strength test. Cohesive failures within the fiber post were classified for these failure patterns. The fibers and resin matrix surrounding fibers were observed at both sides of the fractured surfaces.

In this study, two kinds of failure were predominantly found: cohesive failure within the post and failure at the post-resin composite interface, depending upon the type of fiber post. The highest bond strength among the four tested posts was obtained with FibreKor post, in which most failures occurred at the post-resin composite interfaces. In the pilot study on the bonding of dual-cure resin composite to FibreKor posts under free conditions of polymerization shrinkage, the silane-treated FibreKor post produced bond strength of approximately 50 MPa, which was similar to the present results performed under constrained conditions. This indicated that adhesion at the interfaces of dentin as well as FibreKor post was sufficient to resist contraction stress of the luting resin composite even though there was a high C-factor value.

For D.T. Light-Post, all the specimens failed at the post-resin composite interface. The bond strength of D.T. Light-Post was found to be approximately four times lower than that of FibreKor at both regions. In our study utilizing Light-Post to evaluate the bond strength to a dual-cure resin core material, it was found that the average microtensile bond strength in the group that received similar post surface treatment was 53.2 and 46.2 MPa at the coronal and apical regions, respectively. Light-Post and D.T. Light-Post are composed of the same components, but they are different in shape. As a result of the anatomical taper shape of the post, fibers on the surface of D.T. Light-Post were found to be cut in steps as shown in Figure 9. In contrast, the cylindrical part of Light-Post was employed in our previous study in which the fibers were parallel to the surface. It is suggested that optimal properties of the fibers are obtained when virgin fibers are used and their properties might dramatically change when the fibers are scratched [107] The characteristic of fiber surface might be a reason for a reduction in microtensile bons strength of D.T. Light-Post compared with Light-Post.

In contrast, all the Snowlight specimens failed cohesively within the post through detachment at the fiber and resin matrix interfaces. Also, approximately 75% of GC post failed in a similar manner to Snowlight. SEM micrographs revealed that failure occurred as a result of detachment between the fiber and resin matrix of the fiber posts (Figure 10). A fiber post is a composite material in which surface treated fibers are embedded in a resin matrix. The type of silane coupling agent and silanization process might have an influence on the quality of the bond at the fiber-matrix interface of each post [108]. These results indicate that attention should be paid to adhesion between the fibers and resin matrix of the fiber post during post fabrication and also the surface characteristics of the post might be critical for teeth restored with fiber posts.

It can be concluded that the bond strengths of fiber posts to root canal dentin were various and that could be affected by the properties of the fiber post and the bonding quality of resin composite to the post surface or root canal dentin. When bonding to root canal dentin using light-cure adhesive, Clearfil SE Bond, with prolonged light-irradiation time, failures occurred either at the post-resin composite interface or within the post and not at the resin-dentin interface. Failure patterns were dependent upon the post system.

Mechanical properties of dual-cure resin composites used for post cementation

Besides the concerning with bond strengths at the resin-post-dentin interface, one more important factor is the mechanical properties of the luting resin composite. Many dual-cure resin composite core materials are available and they are different in their handling characteristics, compositions (such as matrix type, filler type, filler load) and properties (such

as polymerization ability, flexural strength, hardness). Cohesive failure within the luting resins may occur during function if their mechanical properties are inferior. Additionally, the differences in mechanical properties of luting resin may have an effect on their adhesion to tooth substrate. A previous study has reported that shear bond strengths to flat dentin surfaces were significantly influenced by the mechanical properties of resin composites [109]. However, within the root canal which has a deep post space and using a dual-cure type of resin composite, the effect of using different core materials is still unknown. Additionally, when a dual-cure resin composite is used to place a fiber post into the root canal, light-curing will be performed from a coronal direction. Resin composite at the coronal region will therefore mainly polymerize through visible light-initiated reactions, while in the apical region it will be via chemically-initiated polymerization. It has been reported that the mechanical properties of dual-cure type resin composites are better after light-activation compared with chemical-activation alone [95;110]. Therefore, the properties of dual-cure resin composites may be dissimilar at different regions of the post cavity because of the reduction of light energy in the deeper regions of the post cavity.

The microhardness test is a simple and reliable method to reflect the degree of conversion at different curing depths of resin composite [111;112]. However, microhardness values cannot be used to compare the degree of polymerization between different materials [113] because, in addition to the degree of conversion, other factors such as filler type, size or loading may affect the hardness of resin composite [114]. The flexural strength test is also useful for evaluating and comparing the strengths of various composite materials [115-118]. Due to the method of specimen preparation, comparison of the strength of the resin composite at different curing depths is difficult. Providentially, the microtensile bond strength test method has been introduced. This technique has been very useful not only for bond strength testing, but also for ultimate tensile strength testing of materials or tooth substrates [119-121]. The microtensile method is beneficial in improving stress distributions during testing and enables very small specimens to be tested [122]. The regional ultimate tensile strengths of resin composite at different depths are able to be evaluated by this technique.

By using the ultimate microtensile strength test and the Knoop hardness test as shown in Figure 9 (reprinted with permission from Elsevier Ltd.), the regional ultimate tensile strength (UTS) and Knoop hardness number (KHN) of the follwing dual-cure resin core materials have been assessed; 1) Unifil Core (UC) (GC Corporation, Tokyo, Japan), 2) Clearfil DC Core (DC) (Kuraray Medical Inc, Tokyo, Japan), 3) Build-It F.R. (BI) (Pentron Clinical Technologies, LLC, USA), and 4) Clearfil DC Core Automix (DCA) (Kuraray Medical Inc, Tokyo, Japan) [123]. The results (Table 7) revealed that the KHN values of the tested resin composites varied among the materials. Previous research has shown that various factors can affect the microhardness of resin composite, for example filler load, filler type, filler size, or resin matrix type [124-126]. The amount of fillers in the resin core materials used in this study were 68.2, 72.4, 76.8, and 77.8 percent mass for BI, DCA, UC, and DC, respectively [127]. The data showed that a ranking of KHN from low to high values was found to correspond with the amount of filler content, and this finding is in agreement with previous reports [113;128;129].

On the other hand, UTS values were found not to correspond with the amount of filler. DCA exhibited the highest average UTS at both coronal and apical regions, although the filler contents were less than those of UC and DC, and the average UTS of BI was higher than those of UC and DC although it contains fewer fillers. In this experiment, two types of resin

composite were used: a hand-mix type and an auto-mix type. SEM photographs of the cut surface of each material revealed that numerous voids could be observed in the hand-mixed resin composites (DC and UC), whereas no voids were observed in the auto-mixed resin composites (BI and DCA). The defects within the resin composite can initiate fracture at a lower load level than specimens with no defect [130;131]. Therefore, the manipulation characteristics of the resin composite might be a factor that could affect the strength of the material. Moreover, the results indicated that there were no positive correlations between UTS and KHN within each resin composite at both regions. Braga et al. [132] also found no correlation between hardness and flexural strength of the dual-cure resin cement. It seemed that UTS of the dual-cure resin composites were affected by a variety of factors. Previous studies have found that the monomer structure of the resin matrix and the filler/matrix adhesion were factors that could also influence the strength of resin composites as well [133-136].

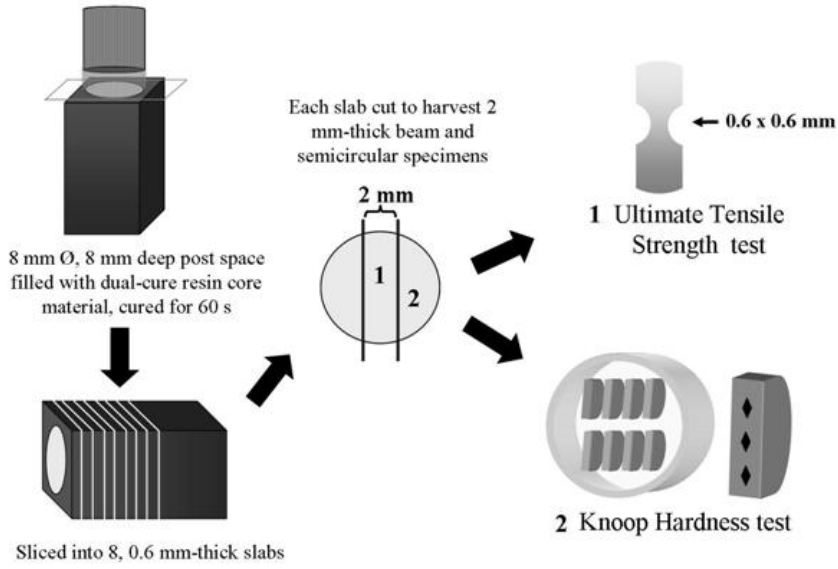

Figure 11. Schematic illustration of the specimen preparation for ultimate tensile strength and micro-hardness test.

Table 7. Mean±SD in MPa of ultimate tensile strength (UTS) and Knoop hardness number (KHN) of four dual-cure resin composite core materials

	UTS (MPa)		KHN	
	Coronal	Apical	Coronal	Apical
Unifil Core	92.3±13.7^{A}	80.6±11.2^{a}	71.4±1.8^{A}	66.6±2.8^{a}
Clearfil DC Core	106.4±22.1^{A,*}	91.6±15.6^{a,b,*}	92.7±4.2^{B}	85.7±4.2^{b}
Build-It F.R.	105.6±14.7^{A,*}	101.7±16.0^{b,*}	57.4±1.9^{C}	53.3±1.2^{c}
Clearfil DC Core Automix	126.2±11.0^{B}	105.1±11.4^{b}	67.0±3.1^{D}	58.5±1.6^{d}

Note: #Same superscript letters demonstrate no significant differences in each column.
##Asterisks demonstrate no significant differences between coronal and apical regions.

The statistical analysis revealed a significant effect of regional factor on UTS and KHN. This indicated that within the same resin composite, the properties of the material at the coronal portion were better than those at the apical portion. Previous studies have confirmed that KHN values could be used to reflect the degree of polymerization at different depths of resin composites [111;112]. Therefore, the results of this study indicated that the degree of conversion of resin composite at the coronal region was higher than that of the apical region. This could simply be explained by the direction of photo-initiation. Even though a dual-cure resin composite was used in this experiment, light-irradiation was performed from the coronal direction of the 8 mm deep post cavity. The upper portion of the resin composite may have been immediately polymerized through the photo-activated free radicals, whose degree of conversion was found to be higher than when chemically-activated [137;138]. Many studies have shown that KHN of light- and dual-cure resin composites are affected by depth of the cavity [113;139;140]. Although the present results indicated that there was no linear relationship between UTS and KHN in each material, the average UTS of the tested resin composites at the coronal region were higher than at the apical region. The regional UTS may have been influenced by regional differences in polymerization. Resin composite at the coronal region may have polymerized through photo-activation resulting in higher UTS, whereas resin composite at the apical region could have polymerized mainly through chemical-activation. Previous studies using the flexural strength test have reported photo-activated composites possess superior flexural strength to chemically-activated composite [110;132]. Anyhow, even though the photo-activated free radicals at the coronal region could induce chain propagation of the underneath resin polymer, the exact polymerization mechanism of dual-cure resin composite in very deep cavities is still unknown. It is not possible to clearly distinguish which part of the material is polymerized through photo-initiation or chemical initiation alone.

Whereas the UTS and KHN of the composite core materials in each region varied depending on the material, the bond strengths of all the tested materials were similar at both coronal and apical regions (Table 8). This indicated that the mechanical properties of the resin composite did not affect the differences in regional bond strength. On the other hand, the adhesion to root canal dentin might be influenced by the type of adhesive systems [84]. For Clearfil SE Bond, the bond strengths at the apical region were found to be significantly lower than at the coronal region when the bonding agent was light-cured for 10 seconds according to the manufacturer's instructions. We had speculated that differences in the type and concentration of the photo/chemical initiators in each dual-cure resin core material would affect the polymerization of the adhesive resin. However, the results of the present study showed that our assumption was not correct. Alternatively, the bond strength of dual-cure resin composite to dentin at the apical region could be improved by extending the photo-irradiation time to SE Bond adhesive resin to 20 seconds [87].

From this study, it can be concluded that the ultimate tensile strength and microhardness of the resin composite core materials varied depending on the type of the materials. The mechanical properties of the resin composite at the coronal region were found to be superior to those at the apical region of the post space. However, the differences in these mechanical properties did not affect the adhesion to root canal dentin when the same bonding system was used.

Table 8. Mean±SD in MPa of microtensile bond strength of various fiber posts to root canal dentin luted with Clearfil SE Bond and DC Core Automix

	Unifil Core	Clearfil DC Core	Build-It F.R.	Clearfil DC Core Automix
Coronal	51.2±14.9[A]	52.2±15.7[A]	53.7±14.7[A]	51.2±10.3[A]
	P<0.05	P<0.05	P<0.05	P<0.05
Apical	28.9±9.8[a]	26.7±12.1[a]	23.8±7.1[a]	29.1±8.9[a]

Note: #Same superscripts demonstrate no significant differences in each row.
##P<0.05 demonstrates significant difference between coronal and apical regions.

RESIN COMPOSITE FOR CORE BUILD-UP

There is a variety of resin composites that can be used for core build-up. Clinicians sometimes use the hybrid resin composite or flowable resin composite, which are normally used in direct filling, for a core-build up purpose. These types of resin composite need light-activation for the polymerization process. Thus, the incremental build-up has to be performed to ensure the complete polymerization of the resin. The major advantage of the high-filled hybrid resin composites is their packable consistency allowing the operators to simply shape a core by using hand instruments. Additionally, the high filler loading of the hybrid composites help them to obtain good mechanical properties. However, the high viscosity of the hybrid resin composite is a barrier for a good adaptation to a cavity wall or post surface due to poor wettability. Our latest research results proved that the viscosity of core materials influenced the bond strength to the post surface and their viscosity affected the selection of the post surface treatment method (in publication process). When the resin composite core materials had a high viscosity, an application of adhesive resin could significantly improve the bond strengths of fiber posts to luting resin.

On the other hand, the evaluation using SEM reported that cores built-up with flowable composites showed the highest integrity and the best adaptation onto the post [141]. However, bond strengths to fiber posts using flowable composites were relatively weak compared to the hybrid composite and bespoke core build-up resin composite [142]. Flowable resin composites contain a small amount of fillers and are consequently weak in terms of mechanical properties and wear resistance [143-145]. They may not be able to resist occlusal loading during function especially when a large bulk of resin composite is needed to be restored in a high load bearing area. Furthermore, the small filler content in low-viscosity resin composites causes high polymerization shrinkage, which can create high interfacial stresses [146;147]. For these reasons, flowable resin composites may not be a good choice for core build-up.

Specific resin composites were developed in order to serve a core build-up purpose. The products of the bespoke resin composite core materials currently available in the market are shown in Table 2. The majority of core build-up composites are prepared in a dual-cure mode, which separate the chemical initiators in the base and catalyst pastes. After mixing, the polymerization is initiated. Recently launched products are mostly provided in automix syringes or cartridges, whereas some older versions require hand-mixing. Resin composite core materials are widely different in their rheological characteristics. Some of them have an inappropriate consistency for packing with hand instruments because they tend to flow during

placement or injection. For convenience, clinicians may require a clear plastic core former for the build-up.

Polymerization Modes of Resin Composite Core Materials

Similar to other restorative resin, core build-up resin polymerized through free radial-initiated reactions. Free radicals are created by the division of an initiator molecule induced by energy from a light source or reaction with the electron donor such as a tertiary amine. For a direct core build-up resin composite, the polymerization or curing mode can be classified into three modes; self-cure, light-cure, and dual-cure. Firstly, self-cure or chemical-cure resin composites, mostly utilize the benzoyl peroxide/amine initiator system to generate free radicals. They are supplied in two pastes or two jars containing benzoyl peroxide and tertiary amine separately in each. After mixing, a redox reaction occurs between the benzoyl peroxide and tertiary amine. The benzoyls peroxide molecule split and form free radicals, and the propagation of monomers thereafter progress. The self-cure core build-up resin composite is profitable because the resin cure homogenously for the whole mass with an infinite depth of cure. Hence, it can be placed in deep cavities where light penetration is limited. Moreover, several reports demonstrated less contraction stress of the self-cure composites compared with the light initiated composites because of their slower polymerization, which resulted in the less rigidity of the cured resin [148-151]. However, the degree of conversion of resin composites solely cured with chemical activation was significantly lower than that of dual-activated (chemical- and light-cure) resin composites [151]. Besides inferior mechanical properties, the lower level of degree of conversion significantly affected the biocompatibility of resin composites. It was found that the cellular toxicity decreased as the percentage of monomer conversion increased [152]. The cytotoxicity of self-adhesive resin cements was lower when specimens were dual-cured compared to self-cured [153]. Polydorou et al. [154] also recently reported a significant higher amount of Bis-GMA and TEGDMA releasing from chemical-cure core build-up resin composite compared to a dual-cure and light-cure. A conspicuous problem that decreases the use of self-cure core build-up materials could possibly be the limited working time. Clinicians require build-up material that has a long enough working time for contouring resin composites into a core shape, and it should immediately set after shaping. The viscosity of self-cure resin composite rapidly increases over time after mixing, making it difficult to shape a core.

On the other hand, light-cure resin composites facilitate the operative procedures by providing a working time as long as the operators need, and they are abruptly hardened after light exposure. Light-activated resin composites contained a photo-initiator, frequently camphorquinone, to absorb light energy and turn to be free radicals by releasing an electron. Light-cure resin composites provide the most excellent physical properties due to the highest degree of conversion [155;156]. However, core build-up is always employed in a deep cavity of the pulp chamber where the light energy attenuates. Resin composite at the bottom of the cavity may not be sufficiently cured. High intensity light curing or a small light guide is supportive if the light-cure resin composite is used. Due to the concern in curing efficiency, the bespoke light-cure core build-up materials are therefore limited in the market. Clearfil Photocore (Kuraray Medical Inc, Tokyo, Japan), Light Core (Bisco Inc, Schaumburg, IL,

USA), and SuperCure (Centrix Inc, Shelton, CT, USA) are examples of light-cure versions of core build-up resin composite that is currently used (Table 2). Clinicians sometimes modify the light-cure filling resin composite for using in core build-up purpose because they are the same in composition and characteristics.

To overcome the inappropriate characteristics of self-cure and light-cure core build-up resin composites, a dual-cure version has been developed, and they have become the most popular type of core build-up resin composite. Dual-cure resin composite core materials are supplied in two syringes or cartridges to separate the benzoyl peroxide and amine in the different paste. Photo-initiators are added in one tube to initiate polymerization after light exposure. In the absence of light, the chemical-activated polymerization will proceed to ensure the setting of the resin. Although the dual-cure resin composites are able to polymerize in the absence of light energy, there is a consensus in research results demonstrating that light initiation is essential for the enhancement of polymerization degree and properties of resin composite [132;138;151;157].

Incompatibility between Adhesive and Resin Composite Core Materials

When the self-or dual-cure resin composite core materials are chosen, the incompatibility between the resin composite and acidic adhesive used for bonding should be considered. One-step self-etch adhesives were mostly found to be the cause of the incompatibility problem because they contained a high content of acidic monomer and they are applied in a single step without rinsing or coating with a more hydrophobic resin. The major mechanism of the incompatibility was the adverse chemical reaction between the residual acidic monomer and tertiary amine [82;92;93]. The tertiary amine acts as a reducing agent for redox initiation in the polymerization process. Therefore, polymerization of chemically-activated resin would be retarded if some molecules of amine interacted with acidic monomers. Coupling the alternative reducing agent such as sulphinic acid salt to the adhesive or adding the anion exchange resin to the amine component was effective to reduce the incompatibility [91;158]. However, the second mechanism creating this phenomenon is the permeability of simplified one-step self-etch adhesives, as they still detriment the adhesive-resin composite interface [92;159]. After polymerization, the one-step self-etch adhesive behaves as a permeable membrane allowing water to diffuse through the channels inside the adhesive layer [160-162]. By using the resin replica method, the surface of one-step self-etch adhesive, Adper Prompt L-Pop (3M ESPE, St Paul, MN, USA), after application and polymerization under stimulated pupal pressure were evaluated. Numerous water blistets were observed on the surface (Figure 12). This characteristic is critical for the curing of self- or dual-cured resin rather than the light-cured resin because their polymerization rate is slower. For light-activated resin composite, the incompatibility resulted from the permeable adhesive layer occurring when the light-irradiation was delayed or when using soft start curing methods [163]. For these reasons, clinicians should avoid using the simplified one-step adhesive in bonding self- or dual-cure resin composite core materials.

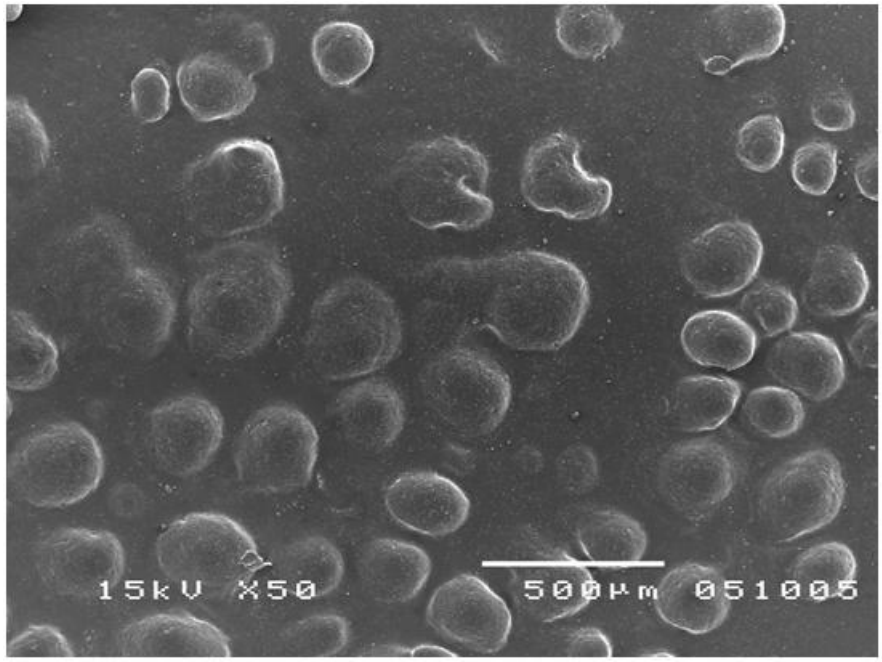

Figure 12. SEM micrographs of resin replica showing the surface of one-step self-etch adhesive, Adper Prompt L-Pop, after application and polymerization under stimulated pulpal pressure. Numerous blisters were observed.

Mechanical Properties of Resin Composite Core Materials

Ideally, the core build-up materials should bond strongly to the tooth structure and behave in the same way as dentin. Unfortunately, there is no material that has the exact characteristic as the tooth structure and can permanently bond to the tooth substrate. No adhesive system was capable of absolutely preventing microleakage [164]. Some resin composites may be inferior to metals in terms of strength; however, they provide the stiffness and stress distribution more closely to dentin [10;11;165]. Additionally, they can bond directly to the tooth structure using the adhesive without excessive extension of the cavity for macro-retention**.** In an attempt to increase the strength of the resin composite, some manufacturers reinforce the composite with fiber or metal. For examples, Build-It F.R. (Pentron Clinical Technologies, LLC, USA) was reinforced with a chopped glass fiber and Ti-Core (Essential Dental Systems Inc, NJ, USA) was reinforced with titanium and lanthanide. However, their mechanical properties were not outstanding compared to the non-reinforced resin composite (Table 7) [7;166]. The mechanical properties of core materials might depend on various factors such as the type of resin matrix, the fillers (type, size, and quantity), the coupling between fillers and matrix, and the presence of defects in the resin bulk. It should be realized that when the resin composites are placed in the highly constrained condition like in a pulp chamber, their mechanical properties might be weakened due to the contraction strain generated inside the resin bulk [167]. Therefore, the results for the mechanical properties of core materials obtained from in vitro experiments that did not simulate clinical situations might present less clinical relevance.

In 1999, Combe et al. [7] evaluated the mechanical properties of direct core build-up materials including three types of resin composites: Coradent (Vivadent Schaan, Liechtenstein) – a self-cure resin composite with cermic filler, Prisma APH (De Trey Dentsply, Weybridge, UK) – a light-cure resin composite, and Ti-Core (Essential Dental Systems Inc, NJ, USA) – a self-cure resin composite containing Ti. The visible light-cure resin composite, Prisma APH exhibited the highest values of compressive strength, diametral tensile strength, flexural strength, and flexural modulus [7]. Thereafter, two studies evaluated the fracture toughness of core build-up materials [166;168] and reported that the titanium-reinforced resin composite (Ti-Core) and the resin composite with fluoride (Fluorocore, L.D. Caulk Division, Dentsply Int., Milford, DE) had the fracture toughness comparable to amalgam and could withstand the stresses generated during mastication [166]. However, the fracture toughness and hardness of core materials was affected by thermocycling [168]. With the rapid improvement in resin composite technology, data relating to mechanical properties of the resin composite materials used in the above studies are rather obsolete. Recently, a variety of resin composite core materials have been manufactured with the development of the curing method, composition, and mixing characteristics. The study comparing mechanical and physical properties of contemporary resin composite core materials is limited. One recent study evaluated the diametral tensile and compressive strength of core materials and stated that packable resin composite, Filtek P60 (3M Dental Products, St Paul, MN,USA), light-cure nanohybrid resin composite, Grandio (Voco, Cuxhaven, Germany), and organically-modified ceramic, Admira (Voco, Cuxhaven, Germany), had compressive and diametral tensile strength values higher than those of the bespoke resin composite core material, Rebida DC (Voco, Cuxhaven, Germany) [169]. It should be noticed that the comparison was not performed within the group of bespoke resin composite for core build-up purposes.

The dimensional stability of resin composite core material is another critical property that should be addressed. Resin composites change in dimension due to the polymerization shringkage, thermal contraction and expansion, and water sorption. It was revealed that the tested bespoke resin composite core materials volumetrically expand in water up to 56 days after mixing [170]. However, their dimensional changes were significantly lower than those occurring in glass-ionomer based material. During this period, if the provisional restorations are not well prepared to prevent leakage, the dimensional change of core may undesirably affect the crown seating. The expansion of the resin composite core after final cementation may generate stresses, but the amount of stresses generated from the resin composite core was found to be insufficient to create cracks for all-ceramic restorations. On the other hand, hygroscopic expansion of resin modified glass ionomer and compomer materials led to failure of overlying ceramic [171]. Although the restoration was not cracked by a small amount of stress generated from resin composite expansion, the stress accumulated underneath the crown may cause microleakage and subsequent crown failure. Therefore, final restorations should entirely cover the core and have strong adhesion to the foundation in order to prevent any water exposure of the resin composites.

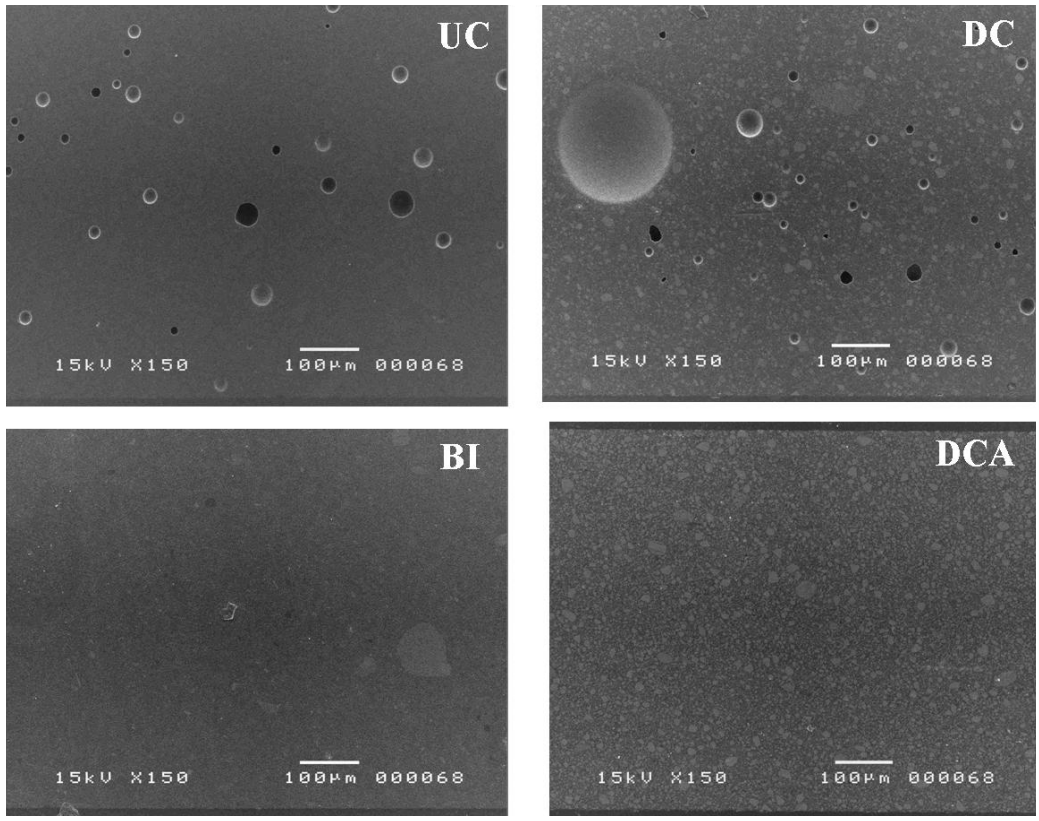

Figure 13. SEM photographs of the cut-surfaces of each core material. Numerous voids could be observed in the Unifil Core (UC) and Clearfil DC Core (DC), which are the hand-mix type resin composites, while, no voids presented on the surface of the auto-mix type resin composites, Build-It F.R. (BI) and Clearfil DC Core Automix (DCA).

Hand-Mix vs. Auto-Mix Resin Composite Core Materials

There are two types of dual-cure resin composite core materials classified by the mixing characteristic: the hand-mix type and auto-mix type. To simplify the application procedure, the recent core build-up resin composites are often supplied in auto-mix syringes or cartridges connected to a small tip, which can be placed directly into a post space or cavity. The consistency of the auto-mix injectable type of resin composite is suitable for the matrix technique. Some manufacturers provide two consistencies of resin composite for different build-up technique. For example, MultiCore (Ivoclar Vivadent Inc, NY, USA) is available in two categories: Mulicore HB for the molding technique and MultiCore Flow for the matrix technique. The hand-mix type of core build-up resin composite is beneficial in material saving. Clinicians can dispense the resin composite with the volume that they need, whereas high amounts of the material is wasted in the mixing tube if the auto-mix type is used. Concerning the material properties of resin composite obtained from the different mixing characteristics, SEM photographs of the cut surface of resin composite core build-up materials, as shown in Figure 13 (reprinted with permission from Elsevier Ltd.), revealed that numerous voids could be observed in the hand-mixed resin composites, whereas no voids were observed in the auto-mixed resin composites [123]. The ultimate tensile strength of the

auto-mix Clearfil DC Core was found to be higher than that of the hand-mix Clearfil DC Core (Table 7). Voids in resin composite might have occurred as a result of air entrapment during spatulation and probably were the weak points of the specimens, which further initiated fracture at a lower load level than specimens with no voids [131;172]. Mentink et al. observed the porosity in resin composite core restoration and reported significant fewer voids when the resin composite was inserted into the cavity by the syringe technique compared to bulk insertion. These findings signify the importance of mixing and placement technique of resin composite core materials. Some previous studies revealed that porosities in resin composite might assist in the relaxation of shrinkage stress [173]. The presence of a few empty spaces in resin composite bulk may be advantageous for the bonding. However, many of them possibly cause the cohesive fracture of the core.

CONCLUSION

Resin composites have several appropriate characteristics for usein cementation of endodontic posts and core build-ups. Clinicians may attain the benefit of using a dual-cure resin composite core material both for post cementation and core build-up. The author has conducted several studies to determine the most favorable strategy for bonding fiber posts in root canals. For fiber post cementation, application of adhesive or silane coupling agents on the post surface was found to enhance the bond strength between the resin composite and fiber post surface. Light-cure adhesive was effective for bonding to root canal dentin if sufficient photo-irradiation was performed. In the fiber post bonded teeth, when fiber posts were bonded using light-cure adhesive with prolonged photo-irradiation time and post surfaces were treated with a silane coupling agent, failures were found to occur either cohesively within the posts or adhesively at the post-resin composite surfaces, depending on the post type.

A variety of resin composite core build-up materials are currently available in the market. They are different in compositions, mixing characteristics, polymerization methods, rheological properties, and physical and mechanical properties. The selection of material should be made carefully to obtain optimal results of final restorations. Dual-cure polymerization may be the most appropriate method for composite curing in the deep cavity. However, light activation is important to achieve the superior properties of the core even though the dual-cure resin composite is used. The simplified one step self-etch adhesives compromised the bonding to the root canal due to their incompatibility with the self- or dual-cure resin composites. If it is used, application procedures should be carefully performed by entirely evaporating the solvents and irradiating with sufficient light allowing the least amount of residual uncured acidic monomer. To avoid numerous air bubbles trapping in the resin composite core build-up materials, auto-mix syringes or cartridges with a small tip, which can directly place the resin composite into the cavity, should be used

To date, bespoke resin composite core build-up materials are rapidly expanding in the dental market, but research data evaluating the properties of these materials are scarce. Additionally, information regarding the clinical evaluation of various resin composite core materials is very limited. Further research in this field is required.

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Chapter 2

DESIGN AND DEVELOPMENT OF NOVEL URETHANE DIMETHACRYLATE MONOMERS FOR USE IN DENTAL COMPOSITIONS

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ABSTRACT

Over the past years, polymeric dental materials attained a considerable progress due to the tremendous development of multifunctionalized methacrylates, since these offer a widespread possibility to generate composite resins through a suitable choice of the monomers structure and the proportion implied in the three-dimensional network formation as well as by varying the hard inorganic filler (loading, nature, shape, size, distribution, orientation, adhesion, etc). Recent aspirations and approaches to perfect the composites structure have shown that there are difficulties in the systematic upgrading of these materials because all acrylic formulations presented a volumetric shrinkage through polymerization, accompanied by a polymer ageing. Moreover, the high percentage of nonpolymerized functions significantly affects the physical properties of the formed composites, reason for that the urethane di(meth)acrylates have been initially proposed for reducing the high viscosity of diglycidyl methacrylate of bisphenol A (BisGMA) and achieving an adequate conversion in the final resins. However, besides other characteristics (adhesion to tooth substrates, insufficient material properties, etc), minimization polymerization shrinkage and increasing the degree of (meth)acrylate conversion after ambient photopolymerization of the composite are one of the most important research tasks in this field.

From this perspective, the present review will report our results concerning the synthesis and characterization of new urethane dimethacrylate monomers with and without carboxyl groups, BisGMA analogous or liquid crystalline monomethacrylates following especially the structural/compositional effects derived from their incorporation as co-monomer candidates for formulating dental composites with different fillers, in order to elucidate the photopolymerization behaviour and the specific properties (polymerization shrinkage, morphology, hydrophilicity, mechanical parameters) of the

experimental specimens. Complementary, data on the crack propagation in some composites subjected to Vickers indentation will be also critically discussed.

1. INTRODUCTION

Interdisciplinary approaching of the dental composite materials has received a widespread attention in the last decades, given that light-cured dental resin composites have almost replaced amalgam alloys for filling materials in small-sized operations, since amalgam has disadvantages such as toxicity, pollution, difficulty in fixing and lack of bioactivity [1, 2]. However, despite all research efforts, the monomer systems used in dental restorative applications have not changed fundamentally, reason for that various copolymers based on photopolymerizable dimethacrylates incorporating bisphenol A diglycidyl methacrylate (BisGMA) have been extensively used as the organic phase in dental applications for nearly 50 years [3-5]. These dental composite materials typically contain a mixture of rigid and viscous photopolymerizable monomers in combination with reactive diluents such as triethyleneglycol dimethacrylate (TEGDMA) that are filled up to 80 % with inorganic fillers to provide greater rigidity and toughness [6-10]. Depending on the chemical configuration of the dimethacrylates, significant differences in photopolymerization kinetics and mechanical properties of the formed dental resins, such as final double bond conversion, modulus, flexural strength and hardness, polymerization shrinkage or biocompatibility have been reported in the literature [11-15]. Therefore, besides structural factors and variations in compositions including the filler (loading, shape, size, distribution, orientation, adhesion, etc), multiple characteristics such as curing intensity and time, environment temperature, sample size and stress history may have a considerable effect on the properties of the resulting materials.

In spite of several advantages reported in the literature, the main deficiencies encountered in the current resin composites are the high values of the polymerization shrinkage, the relatively high percentage of unpolymerized (meth)acrylate functions (25-50 %) and the susceptibility to water sorption [16-21], all with negative effect on the physical/mechanical properties of the materials, for instance, marginal leakage, insufficient abrasion and inadequate resistance to wear, degradation and fatigue, toxicity, a.s.o, restricting thus the service life of them [22-26]. In order to improve the clinical performance of such materials, the chemical tasks have been primarily focused on the maximization of the filler amount and/or formulating of organic phases based on BisGMA analogous [11, 27-29], liquid crystalline or hyperbranched monomers [30-35], sol-gel polycondensates [36-38], fluorinated and spiro-orthoester derivatives [39-42], that have been tested especially in restorative dentistry. Thus, in the case of polymerizable liquid-crystalline (LC) monomers that contain new aromatic mesogens displaying nematic state at room temperature, the results demonstrated that these showed ultra-low polymerization shrinkage [30-32, 43-45]. Although the chemical routes used for their synthesis are complicated, and often produce very low yields, a number of LC diacrylates and diepoxides are known for their properties generated in the corresponding polymer networks, more important being a much less shrinkage (1.6 %) compared to the conventional reference system, BisGMA/TEGDMA/BisEMA (8.2 %) at similar degrees of conversion.

In tandem, a series of multifunctional methacrylates of various lengths were also used as co-monomers (up to 30 %) in the organic phase owing to the notable effect observed on the ultimate conversion, volumetric shrinkage and mechanical properties [46-49]. Moreover, an increased double-bond conversion in the final polymer matrix is desirable to enhance biocompatibility and reduce swelling in the later systems, compared to that of low-molecular weight monomers (diethylene glycol dimethacrylate (DEGDMA) or TEGDMA) used currently in dental filling composites.

Among the mentioned issues, performing of a better adhesiveness to dentin and enamel has required the development of new carboxylic acid functionalized monomers or phosphor-containing derivatives to be used as dental polymeric adhesives [50-54]. More recently, Moszner et al. [55-58] considered polymerizable methacrylamides as most important components of the adhesives and restorative materials, because an amide group is more resistant to water than the ester function from (meth)acrylates which deteriorates its performance. In addition to the conventional composite filling materials mentioned before, a new type of inorganic-organic hybrid dental composite resins known as ormocers (organically modified ceramics) were developed to reduce the polymerization shrinkage and to improve the stability and biocompatibility [37, 59-62].

A viable alternative to acrylic systems are the urethane di(meth)acrylates with an aliphatic (UDMA) or partially aromatic (TMX-UDMA) core structure, proposed initially for reducing the high viscosity of BisGMA that allow a higher filler loading and finally, attaining of a high conversion in the resin composites [63-67]. Though a series of urethane (meth)acrylate composites have been widely explored and exploited as dental restorative materials with improved properties, up to date, reactive urethane dimethacrylate oligomers acid functionalized and nonacid ones have not been reported. Recently, our studies were focused on the pursuing of the chemical structure effects of the synthesized macromers, and formulations composition on the photo curing process in some preliminary dental formulations. This work describes our approach to prepare new resin composites employing reactive polymerizable oligomers with and without carboxyl sequences, and BisGMA modified with photopolymerizable groups, together with other traditional dental monomers (BisGMA, HEMA, TEGDMA) in the establishing of specific properties during the formation of polymeric networks in the resin composites.

2. URETHANE DIMETHACRYLATE OLIGOMERS AND LOW MOLECULAR WEIGHT URETHANE METHACRYLATE DERIVATIVES

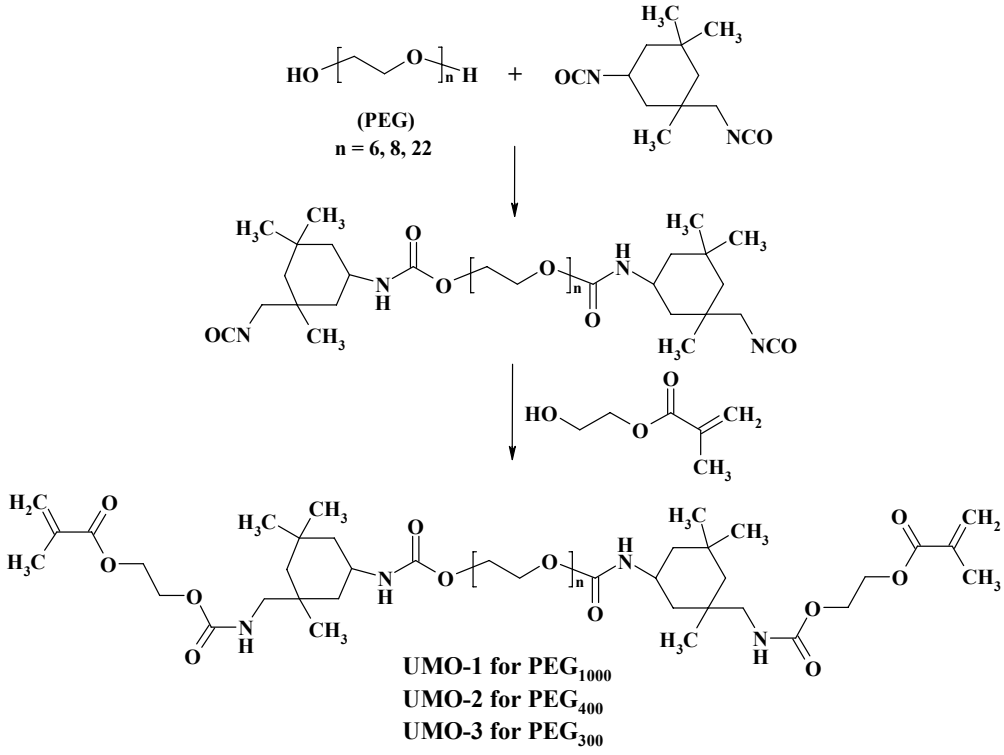
According to the recent literature data, polyethylene glycol dimethacrylates have been mainly investigated as cross-linkable oligomers, due to the generous applicability field, especially as industrial coatings, adhesive materials, dental materials, and other materials used in biomedicine [68, 69]. These oligomers can form highly cross-linked networks *in situ* by thermal or photopolymerization, so they can be used as injectable materials that can fill irregularly shaped cavities or defects [70, 71]. Polyethylene glycol dimethacrylates are generally, known as hydrophilic materials owing to the ethylene oxide sequences, which presents a very good biocompatibility but weak mechanical properties. For that reason, in

biomedical appliance, such oligomers are used in tandem with other systems, in function of the desired characteristics.

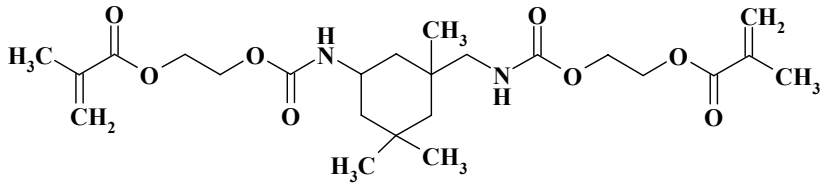
On the other hand, urethane dimethacrylates (UDMA) are increasingly employed in dental formulations, especially to reduce the polymerization shrinkage, water sensitivity and monomer leaching of the dental composites, some extremely important features of this kind of materials [63, 67, 72, 73]. Within this context, for combining the advantages offered by the polyethylene glycol structure with those of urethane moieties, the synthesis and photopolymerization behaviour of urethane dimethacrylates of oligomer type was realized with the aim to propose them as co-monomers in dental formulations.

The urethane oligomers were prepared according to the general synthetic route shown in Scheme 1, *via* condensation of the polyethylene glycol (PEG) with isophorone diisocyanate (IPDI) to yield a prepolymer with end isocyanate groups, which finally, was converted to the methacrylate oligomer by reaction with 2-hydroxyethyl methacrylate [74]. Depending on the PEG average molecular weight (M_n: 300, 400 and 1000 g/mol), three urethane macromers with methacrylic function on the both side were obtained.

For comparison, a methacrylic monomer (UAc-M) without hydrophilic polyethylene oxide in its structure has also been prepared (Scheme 2).

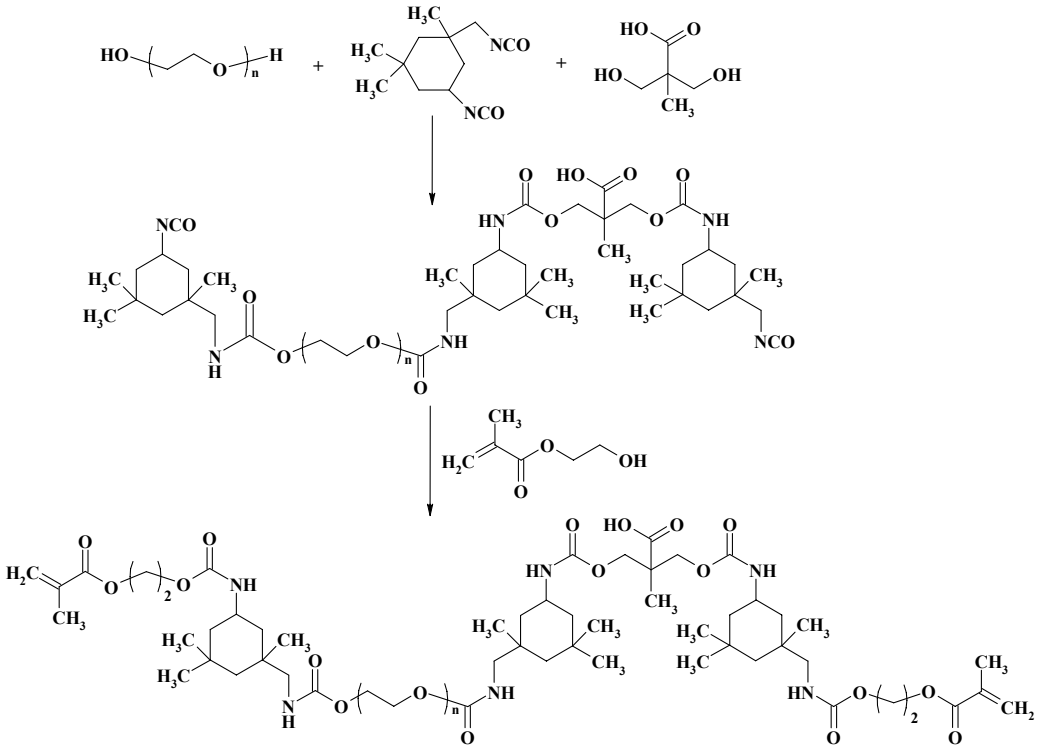


Scheme 1. Synthesis of urethane dimethacrylate oligomers (UMO).



UAc-M

Scheme 2. Structure of the acrylic monomers used in the obtaining of composite resins.



Scheme 3. Synthesis of monocarboxylic urethane dimethacrylate oligomers.

On the assumption that the incorporation of carboxylic groups into a dimethacrylate monomer was expected to be capable not only to form a cross-linked network by radical photopolymerization but also undergo an acid-base neutralization reaction with cations liberated from the glass micro(nano)particles [75], providing thus adhesion to tooth substrates, new urethane dimethacrylate oligomers containing carboxyl groups as potential candidates for dental composites were synthesized and characterized in our group [76]. The basic reaction pathway for the synthesis of such macromers containing urethane structure besides methacrylic and carboxylic groups in a single molecule is given in Scheme 3. The chemistry of the aliphatic urethane difunctional methacrylates involved classical condensation of the polyethylene glycol and 2,2-bis(hydroxymethyl) propionic acid with isophorone diisocyanate, followed of a reaction between the resulting prepoymer and 2-hydroxyethyl methacrylate. Although the reaction of isocyanate with hydroxyl groups from both monomers is performed by minimizing the occurrence of side reactions, it does not provide strict control

over the composition of the resulting product, but the simplicity of the procedure, widely exploited for preparing polyurethane anionomers [77], may be advantageous in applications for which a narrow polydispersity is not necessary.

Under these conditions, the use of PEG_{400}, PEG_{600} or PEG_{1000}, for a given molar ratio of the partners, led to three urethane macromers with different content of carboxylic groups and methacrylic function on the both side, CUDA-O1, CUDA-O2 and CUDA-O3 (Table 1). Similarly to CUDA-O1, another carboxyl macromer (CUDA-O4) was prepared by changing the molar ratio between components used in synthesis.

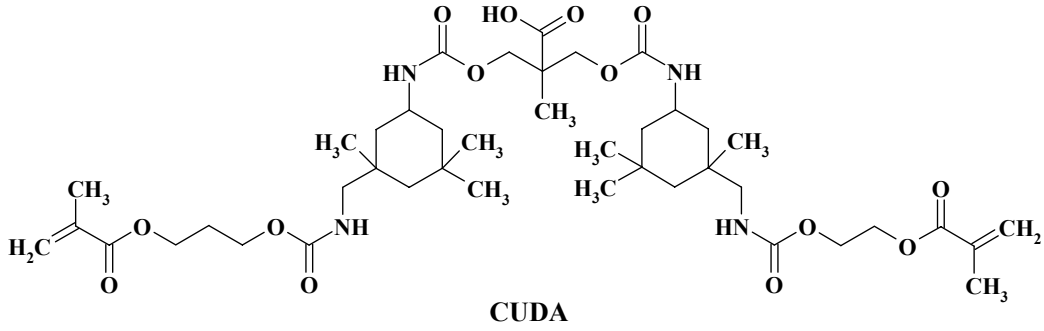
For comparison, the corresponding carboxyl acrylic monomer (CUDA) with no flexible chain in its structure has been synthesized (Scheme 4).

Following the same motif, subsequently we achieved the synthesis and characterization of acid urethane dimethacrylates bearing in a single molecule both sequences of polyethylene glycol and carboxyl groups incorporated by means of L-tartaric acid that contains a diacid function attached to the respective asymmetric carbon [78]. In this case, polyethylene glycol (PEG) and tartaric acid (TA) were reacted with isophorone diisocyanate to form a prepolymer, which finally, was functionalized to dimethacrylate by its reaction with 2-hydroxyethyl methacrylate. The idealized structure of these macromers is given in Scheme 5. However, depending on the manner in which the PEG average molecular weight varies (M_n: 400, 600, 1000 g/mol) and the ratio between partners used in synthesis, four carboxylic urethane macromers (CAd-1÷4) that differ by the length of the spacer connecting the methacrylate units on the both side were prepared to be tested as co-monomers in resin-based dental materials. Further, the cross-linked network parameters in the resulting materials were evaluated. For assessment, the corresponding low molecular carboxyl monomer (CAd-M) was obtained, too (Scheme 5).

The abbreviations and compositions of the synthesized urethane dimethacrylates are shown in Table 2.

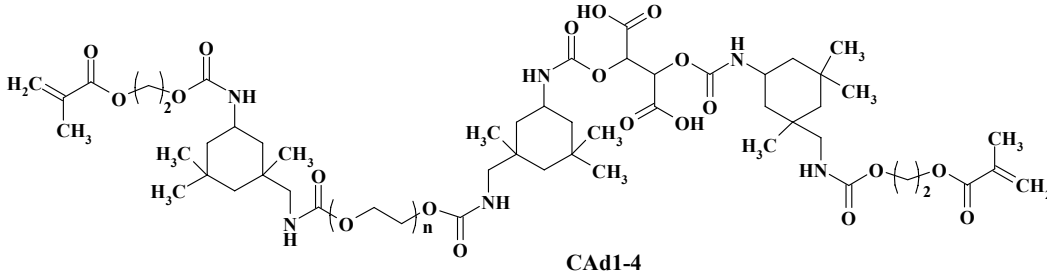
Table 1. Characteristics of the monocarboxylic urethane dimethacrylate oligomers and monomers

Sample	PEG, M_n (g/mol)	Molar ratio OH/NCO	Carboxyl content (%)
CUDA-O1	400	PEG:IPDI:DMPA:HEMA 0.5:2:0.5:2	4.6
CUDA-O2	600	PEG:IPDI:DMPA:HEMA 0.5:2:0.5:2	4.2
CUDA-O3	1000	PEG:IPDI:DMPA:HEMA 0.5:2:0.5:2	3.5
CUDA-O4	400	PEG:IPDI:DMPA:HEMA 0.8:2:0.2:2	4.3
CUDA	-	IPDI:DMPA:HEMA 2:1:1	5.3

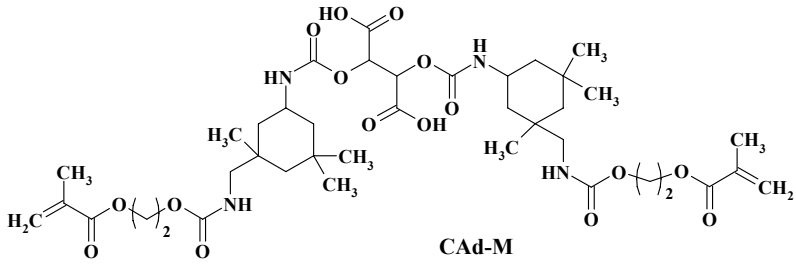


CUDA

Scheme 4. Structure of the acid acrylic monomer (CUDA).



CAd1-4

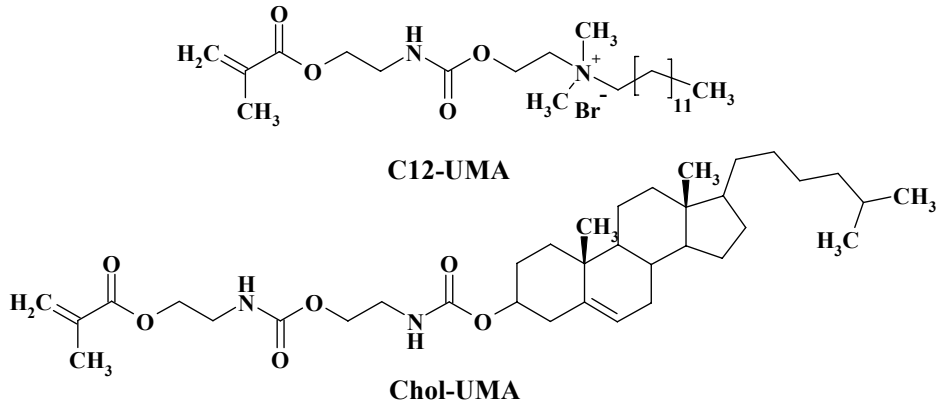


CAd-M

Scheme 5. Structure of the dicarboxylic urethane dimethacrylate oligomers CAd-1÷4 and of the corresponding monomer CAd-M.

Table 2. Characteristics of the dicarboxylic urethane dimethacrylates

Sample	PEG, M_n (g/mol)	Molar ratio OH/NCO	Carboxyl content (%)
CAd-1	400	PEG:IPDI:TA:HEMA 0.8:2:0.2:2	8.5
CAd-2	600	PEG:IPDI:TA:HEMA 0.8:2:0.2:2	7.4
CAd-3	1000	PEG:IPDI:TA:HEMA 0.8:2:0.2:2	5.8
CAd-4	400	PEG:IPDI:TA:HEMA 0.5:2:0.5:2	9.2
CAd-M	-	IPDI:TA:HEMA 2:1:2	10.5



Scheme 6. Structures of liquid crystalline urethane methacrylates.

All dimethacrylate oligomers are clear, colourless and viscous liquids, soluble in organic solvents as acetone, chloroform, methylene chloride, tetrahydrofuran (THF) and dimethylsulfoxide (DMSO), as well as in the photocurable acrylic monomers encountered in commercial resin composites (TEGDMA, BisGMA). The viscosities values obtained for urethane macromers were between 2.3-2.5 Pa s.

Complementary, other strategy has been taken in study for the preparation of reactive polymerizable urethane methacrylates with liquid crystalline properties to be used in dental formulations besides common partners, employed in such materials. Also, attempts were made for obtaining BisGMA modified with photopolymerizable groups. Hereby, we synthesized two urethane monomethacrylates bearing specific groups that present liquid crystal (LC) behaviour, namely C12-UMA with long alkyl chain and quaternary ammonium group and Chol-UMA containing cholesteryl moiety [79], whose structures are depicted in Scheme 6.

Because the monomer liquid crystals are one of the most promising candidates for reducing cure shrinkage and consequent stresses in modern dental composites, it was necessary to investigate the properties of the synthesized photopolymerizable mesogens. In Figure 1 is shown the LC behaviour of C12-UMA observable by optical microscopy in polarized light at room temperature and after its heating up to 60 ^{o}C, and cooling up to room temperature (a, b). Moreover, would be expected that the presence of bromide as counterion of ammonium quaternary structure in C12-UMA to induce radiopacity.

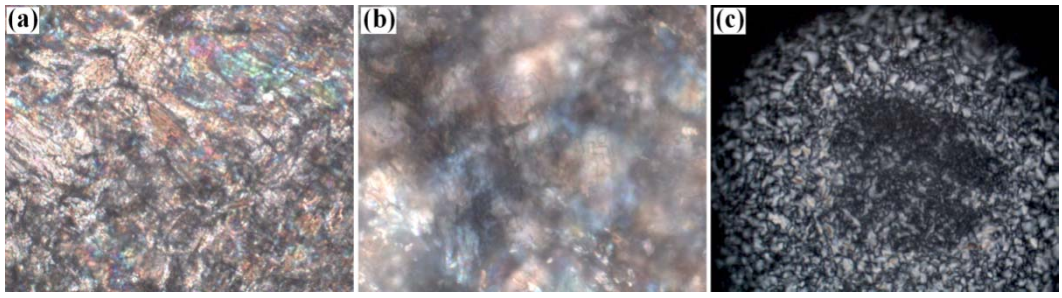
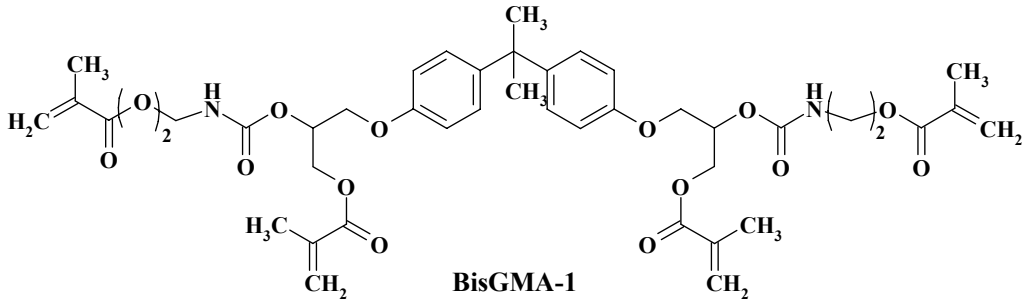


Figure 1. Optical microscopy images in polarized light for C12-UMA (a, b) and Chol-UMA (c).



Scheme 7. Structure of the urethane methacrylic modified BisGMA.

Compared with C12-UMA, in the case of Chol-UMA (Figure 1, c), other texture was identified in the corresponding image taken at room temperature.

As above mentioned, one of the major drawbacks of the dental photopolymerizable matrixes is the occurrence of volumetric contraction during the dimethacrylate photopolymerization. This situation often critically, has imposed the finding of new preparative methods for the obtaining of dental materials with improved properties, especially aiming at the development of BisGMA derivatives [11, 12, 27-29]. Thus, the literature data support that a reduction of the BisGMA viscosity will allows the incorporation of a large amount of inorganic phase, together with a decrease of the amount of the diluent monomers which tend to adversely affect the properties of the resulting dental composites, such as increasing water sorption, water solubility and polymerization shrinkage, to name the most important ones.

Therefore, to reduce the viscosity of BisGMA, mainly attributed to the hydrogen linkages formed between the hydroxyl groups from its structure, and also for an increase of the photopolymerizable network density, we have proposed a new method for the synthesis of BisGMA analogous namely, through the chemical modification of an important percent of hydroxyl groups (almost 90 %) with groups of urethane methacrylic type. In Scheme 7 is given the structure of the resulting derivative BisGMA-1.

3. PHOTOPOLYMERIZATION

A fundamental parameter in the development of dental composites is represented by the double bond conversion which is responsible for the formation of cross-linked polymeric networks applicable as dental materials. To provide valuable insight into kinetics of the viscous urethane dimethacrylates involved in light-induced polymerization systems with emphasis on the correlation of some parameters (initiators, irradiation source, intensity), we studied the curing kinetics at ambient temperature in the presence of specific partners, commonly encountered in dental composites. Theoretically, the FTIR spectroscopy can be used to determine the degree of the curing reaction, since both the stretching vibration of the double bond located at 1637 cm^{-1} and the bending vibration at about 816 cm^{-1} can disappear after UV exposure of the sample, indicating photopolymerization of the above macromers.

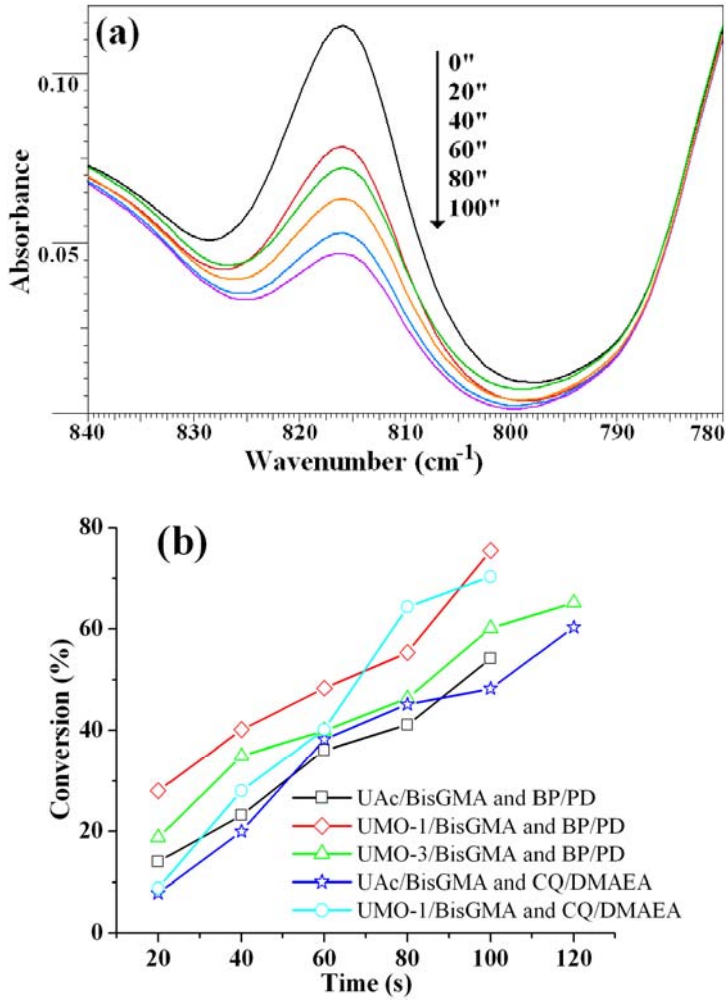


Figure 2. Modification of the double bond absorption band in the FTIR spectrum for the mixture UMO-1/BisGMA (1.5:4 weight ratio) and BP/PD upon irradiation (a) and the evolution of the conversion with UV irradiation time for the acrylic function from the photopolymerizable mixtures (b). *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

Since the photosensitizer is a very important additive that governs the photo polymerization process of these composites, a first study on the kinetics of the free-radical polymerization of urethane dimethacrylates initiated with benzoyl peroxide (BP)/1-phenyl-1,2-propanedione (PD) was investigated comparatively to a system based on a combination of camphorquinone (CQ) and tertiary amine (dimethylaminoethylacrylate, DMAEA). The photopolymerization pursued through FTIR spectroscopy showed that the absorption band positioned at 816 cm^{-1} corresponding to the aliphatic double bond of the UMO-1 macromer taken in mixture with BisGMA (1.5:4 weight ratio) and BP/PD (1/0.5 wt. %) decreased with irradiation time, whereas the absorption bands in the vicinity remain unchanged (Figure 2, a).

Hence, the obtained result suggested that the photopolymerization of the methacrylic oligomer UMO-1 in the company of BisGMA, photoinitiated by the couple BP/PD, occurred within 100 sec of irradiation (around 75 % conversion). Figure 2, b illustrates the relation between irradiation time and the transformation degree (degree of conversion) for the acrylic

function in both monomers (UMO-1/BisGMA). As it can be seen, a double bond conversion of about 30 % is attained at the beginning of the copolymerization (20 sec UV exposure) owing to the higher mobility of the reacting chains. In analogy with UMO-1, a conversion of 65 % after 120 sec of irradiation was reached for UMO-3/BisGMA that contains shorter segments of poly(ethylene oxide). Although such monomers incorporate flexible spacer between the acryl groups, as the polymerization progresses, the mobility of the reaction environment becomes even more restricted and thus begins to affect unfavourably the final conversions of them. Under these circumstances, both propagation and termination are diffusion-limited processes.

Returning to the urethane methacrylic monomer which does not contains flexible aliphatic spacer group (UAc-M) and taken in combination with BisGMA (1.5:4 weight ratio), the cure profile is characterized by a reducing of the transformation degree in the initial irradiation step (20 sec) at approximately 14 % conversion (Figure 2, b), followed by a moderate response to further polymerization. Finally, it was found no appreciable increase in conversion upon prolonged exposure (54 % after 100 sec of irradiation). Even though the monomer viscosity was initially lower and photopolymerization of the UAc-M/BisGMA led to a lower conversion than that corresponding to the oligomeric form, this result could be related to the chemical structure and viscosity of the monomer mixture used in this experiment, without to exclude other factors, such as morphology.

The effect of the typical photoinitiator couple (CQ/tertiary amine) which produces free radicals on exposure to UV irradiation of the above mixture has been also explored by FTIR spectroscopy. From Figure 2, b it is also evident that the maximum conversion attainable during copolymerization of the UMO-1/BisGMA (1.5:4) containing 0.5 wt. % CQ and 1 wt. % DMAEA is of about 70 % after 100 seconds of UV irradiation, whereas the UAc-M/BisGMA reached a conversion of 10 % (20 sec), and 60 % (120 sec), respectively. In spite of the presence of differing initiating species, the final conversions, better for the system that contains oligomers, are a result of the high cross-linking density in the resin formulation which limits the mobility of the reacting species.

In another study [80] it was examined the vanishing of the double bond absorption band from the FTIR spectrum of a formulation containing UMO-1/TEGDMA (1:2 weight ratio) and 4-(dimethylamino)-phenylacetic acid (DMPheAA)/camphorquinone (CQ) upon irradiation with UV/visible light (Figure 3). It may be observed that through irradiation, the absorption band at 1636 cm^{-1} is completely vanished after 180 s with the formation of the resin network. This information indicates that the studied systems exhibited a similar trend to other multifunctional dimethacrylates and upon photopolymerization they are capable to form complex polymeric networks, in which the spacer connects rigid polymethacrylate main chains. Subsequently, the DC for the investigated dimethacrylates is about 55 % after 40 s of irradiation, a satisfactory result for the dimethacrylate urethane dental composites.

In order to evidence the photopolymerization behaviour of the monocarboxyl urethane dimethacrylate oligomers in the presence of noncarboxylic urethane dimethacrylate macromers, we have prepared monomer mixtures, which together with DMPheAA/CQ as photoinitiating system were irradiated with UV/vis light (λ = 400-500 nm). Therefore, in the case of the system CUDA-O1/UMO-1 (1/9 weight ratio) the absorption band characteristic to the aliphatic double bond at 1637 cm^{-1} gradually decreased with increasing exposure time, whereas the carbonyl double bond at 1716 cm^{-1} remained unchanged (Figure 4, a).

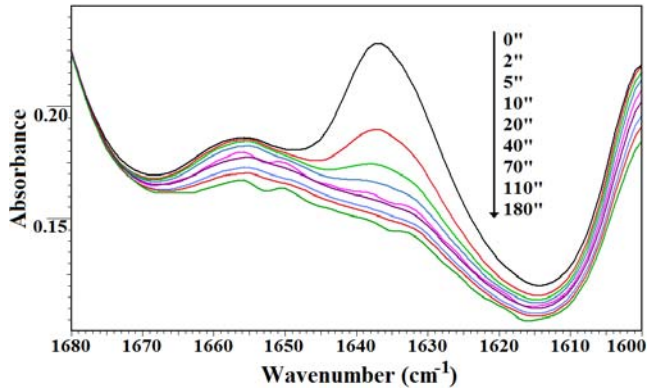


Figure 3. Modification of the FTIR double bond absorption band of a mixture containing UMO-1/TEGDMA (1:2 weight ratios) and DMPheAA/CQ upon irradiation. *Reprinted with permission of [Ref. 80], Copyright (2008) – INOE.*

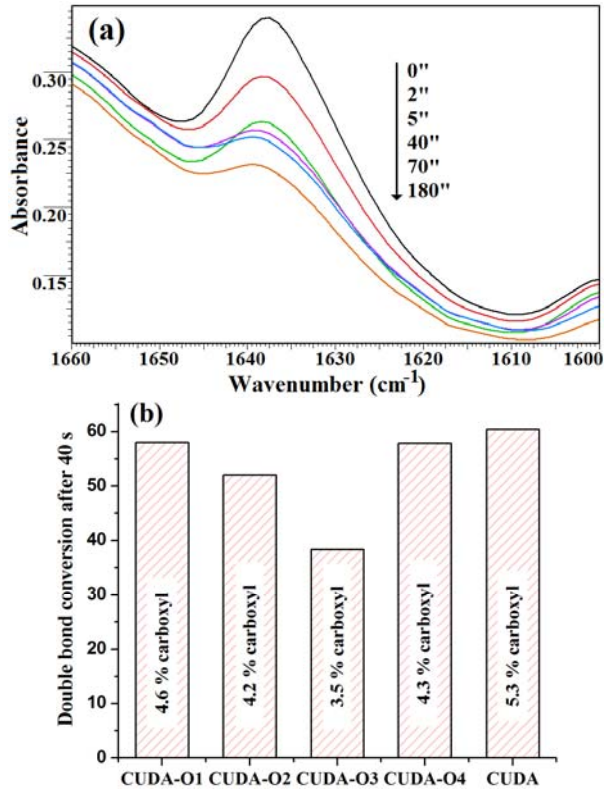


Figure 4. Modification of the double bond absorption band from the FTIR spectrum of a formulation based on CUDA-O1/UMO-1 (10/90 wt. %) and DMPheAA/CQ upon irradiation (a), and (b), double bond conversion degree of all monocarboxyl dimethacrylates at 40 s of irradiation (also in mixture with UMO-1). *Reprinted with permission of [Ref. 76], Copyright (2007) – Wiley.*

Figure 4 (b) displays the transformation degree of the methacrylic function for all urethane monocarboxyl dimethacrylates of oligomer or monomer type (CUDA-O1÷4/UMO-1) determined after 40 s of UV/visible irradiation as calculated from the differences of

absorbance between peak top and baseline at 1637 cm^{-1} in the FTIR spectra of the sample, before and after irradiation.

Compared with the first formulation, the photopolymerization of CUDA-O2 or CUDA-O3 in mixture with UMO-1 showed diminished conversions of 52 % and respectively, 38 %, when irradiated for 40 sec. However, in the above composites a maximum conversion value (58 %) was reached just for CUDA-O1 that contains shorter flexible chains of PEG and implicitly, more carboxyl groups (4.6 % COOH). When CUDA-O1 was replaced with CUDA-O4, which differs only through the carboxyl content, a minor change in conversion (56 %) of both macromers was noticed, indicating that the carboxyl group seems to be one of the factors responsible of the conversion realized in composites formulated with a carboxyl acid macromer. To obtain additional information, the photopolymerization of the corresponding carboxyl urethane monomer (CUDA) and UMO-1 (1:9 weight ratio) was investigated, the cure profile of this mixture with more carboxyl units reflecting an easy increase of the transformation degree (Figure 4, b). Therefore, the relatively good conversion (61 %) attained for the latter, suggested that the content of carboxyl group had indeed a significant influence during the photopolymerization process. Excepting CUDA-O3, that presented a small conversion, in each resin system exposed to prolong irradiation (over 100 sec) the degrees of conversion over 65% were determined. Analysed from this point of view, the composite resins incorporating carboxylic urethane acrylic monomer or carboxyl oligomer based on PEG_{400} in tandem with another urethane macromer is more advantageous.

Since the chemical structure and the relative concentration of each multifunctional macromer can have a marked effect on the mobility and kinetics of polymerization, the photobehaviour of the urethane dicarboxyl dimethacrylates was monitored upon their exposure to UV/vis light, using the photoinitiator system CQ/DMPheAA, (0.5/1 wt %). Under specified conditions of photopolymerization, the oligomers are subsequently homopolymerized up to the formation of a hard polymer. As shown in Figure 5, a, for the macromer CAd-4 the absorption band specific to the aliphatic double bond at 1637 cm^{-1} monotonically decreased with increasing irradiation time, without to observe changes of the carbonyl double bond (1716 cm^{-1}).

Therefore, in the absence of any kind of diluent co-monomer and filler, the degree of vinyl conversion (DC) attained after 35 seconds of irradiation of the CAd-4 is about 60 % in the formed polymeric resin. In line with this finding, DC values of 55-80% were observed for other light-activated dental carboxyl dimethacrylate resins, as reported in the literature [81]. Following the same homopolymerization process for each of the dicarboxylic monomers taken in study, it was found that there was no substantial change in the double-bond conversion of the acid urethane dimethacrylates (CAd-1, CAd-2, CAd-3 and CAd-M) after 35 s of UV/vis irradiation (Figure 5, b). This remark is not in agreement with expectations, because the double-bond conversion should increase monotonically with increasing distance between the polymerizable groups and the flexibility of the spacer from monomer (for instance, the DC ranged from 0.68 in the case of non-acid polyethylene glycol dimethacrylate (PEG200DMA) to 1.00 in PEG600DMA) [46]. At this point we could only suggest a tentative explanation for the above result, namely, the existence of stronger hydrogen bonding interactions in our urethane dimethacrylates that induce the assembly of photocured composites with greater strength than those where there are no hydrogen bonds.

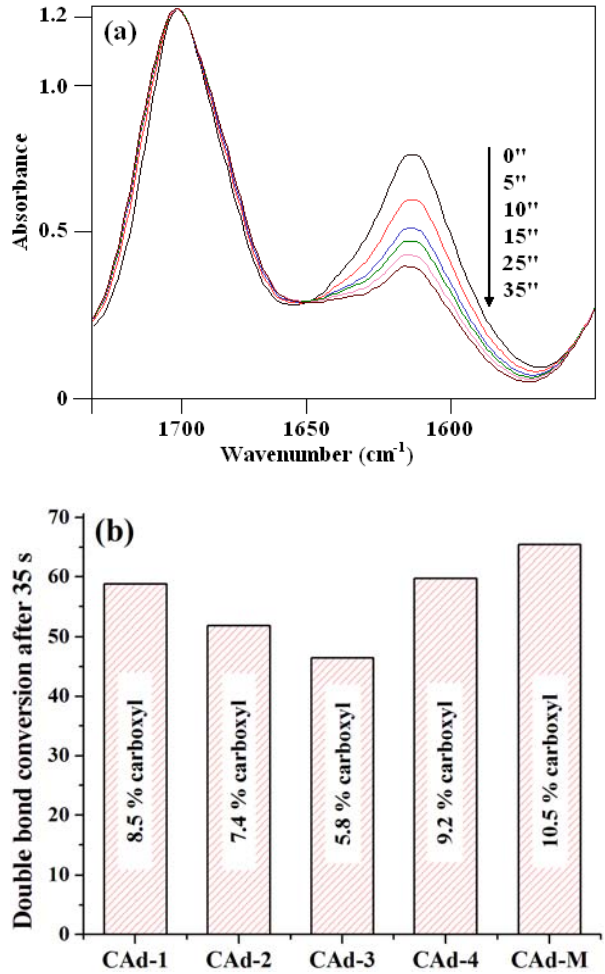


Figure 5. Modification of the double bond absorption band from the FTIR spectrum of CAd-4 oligomer and DMPheAA/CQ upon irradiation (a), and double bond conversion degree of all dicarboxyl dimethacrylates after 35 s of irradiation (b). *Reprinted with permission of [Ref. 78], Copyright (2009) – SPSJ.*

4. FLUORESCENCE STUDY FOR MONITORING PHOTOPOLYMERIZATION

Another option for examining the photopolymerization process is the use of fluorescence probe that give information about the rotational mobility of the molecules, in the surrounding matrix. This choice is motivated by the fact that an important application of fluorescent probes in polymer chemistry consists in the *in situ* monitoring of the polymerization process [82]. In a highly viscous medium in which the mobility of the molecules or molecular fragments is strongly reduced, it is of great interest to observe the changes in fluorescence intensity that accompanies the slow-down in the fluorophore mobility as the matrix viscosity increased upon completion of the curing reaction in the dental resin formulations. Photopolymerization, going from a monomer of relatively low viscosity to a polymeric

network, causes large changes in the mobility of the molecules that comprise the medium. Consequently, a modification in monomer fluorescence intensity as the irradiation time increased was remarked, reflecting the extent of photocuring process in such composites, where only viscosity changes owing to the polymerization reaction may be invoked to explain such a trend.

Over time, has been proved that fluorescence probe methods are very useful in the investigation of many aspects concerning the polymer structural changes, because its high specificity, selectivity and short response time. For this reason, 4-chloromethylphenyl-carbamoyl-1-oxymethylpyrene was chosen as spectroscopic probe and potential photosenzitizer [83] to provide data about the curing process produced in a dental formulation, in which this was inserted. Fluorescence spectra of the monomer mixture UMO-1/BisGMA (1.5:4), BP/PD (1/0.5 wt. %) in the presence of $5x10^{-4}$ M pyrene derivative before and after subsequent photopolymerization are displayed in Figure 6. Firstly, the sample was irradiated with an Hg lamp (500 W) and excited at 353 nm.

In this case, the vibronic fluorescence spectrum is typical for the pyrene fluorescence presenting four peaks at 377 nm (I_1), 385 nm (I_2), 397 nm (I_3) and 411 nm (I_4), all attributed to the fluorophore molecular emission. For determining variations in the relative fluorescence contribution of individual fluorophore in the system, the fluorescence spectra were deconvoluted using a set of simultaneous equations [84, 85]. At a first glance, an excimer emission expected to become visible from the encounter between an excited pyrene and a pyrene molecule in its ground state or through direct excitation of aggregates (dimers) cannot be detected in our pyrene-doped system, since excimer formation, beside the probe concentration, is a viscosity dependent process.

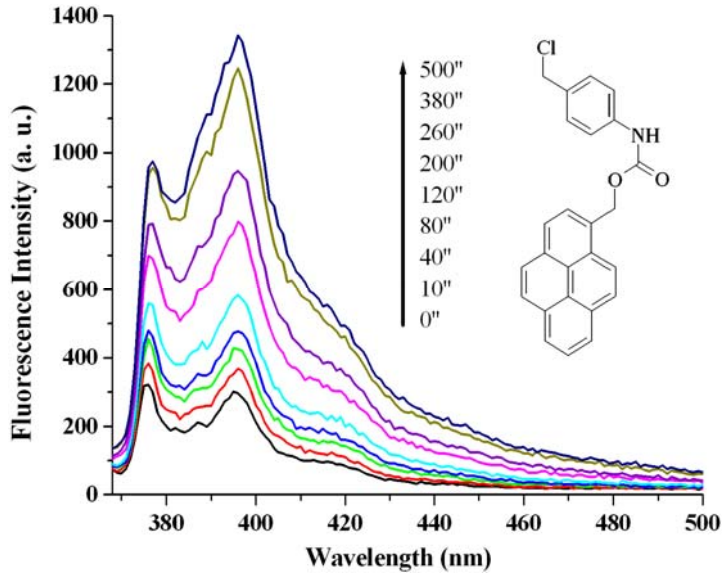


Figure 6. Fluorescence profile for the system based on UMO-1/bisGMA (1.5:4 weight ratio), BP/PD and pyrene derivative with UV irradiation time, excited at 353 nm. *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

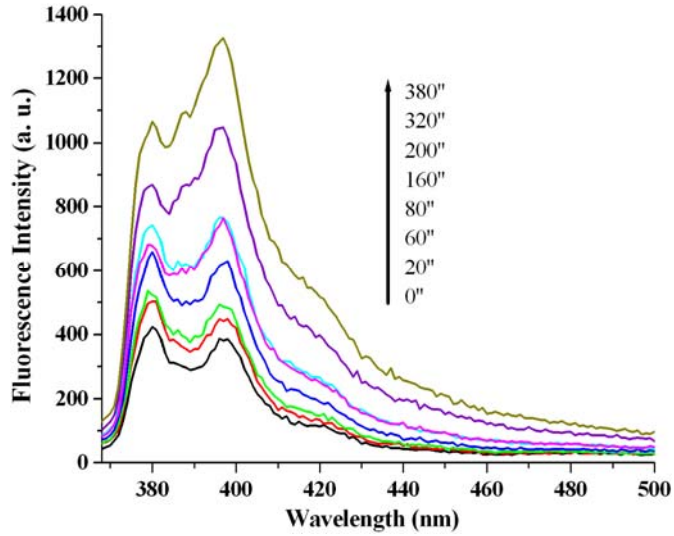


Figure 7. Effect of the UV irradiation times on the fluorescence spectrum of urethane dimethacrylate monomer UAc-M/BisGMA (1.5:4), BP/PD and pyrene derivative (λ_{ex} = 353 nm). *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

Therefore, the free-radical photopolymerization of the above acrylics was monitored against reaction time and correlated with fluorescence data. Naturally, from the in situ polymerization experiments one may expect a substantial increase in fluorescence intensity of pyrene at certain time intervals. Indeed, there is a change in fluorescence intensity that accompanies the slow-down in the fluorophore mobility as the matrix viscosity increases upon completion of the curing reaction of the monomers up to the formation of more rigid product. This increase in monomer fluorescence intensity with the irradiation time (up to 500 sec) could reflects the extent of photocuring in such materials, where only viscosity changes owing to the polymerization may be invoked to explain this trend. Moreover, the higher viscosity once the top surface was cured, the pyrene molecules excited in the singlet state cannot lose thermal energy so that their return to the fundamental state is possible just through photon emission (fluorescence). Similarly, an increase in the monomer fluorescence of the pyrene probe was used to measure the rate of polymerization and the onset of the gel effect of the system (point at which the system vitrifies) during the free-radical polymerization of methylmethacrylate as reported by Pekcan and Yilmaz [86], or cross linking copolymerization of styrene and divinylbenzene presented by Okay et al. [87].

On the other hand, the ratio of pyrene monomer fluorescence intensity at 377 nm (I_1) to that at 397 nm (I_3), defined as I_1/I_3 ratio, gives information about its local environment of the pyrene into our polymeric network. For example, the pyrene emission I_1/I_3 ratio for UMO-1/BisGMA varies slightly from 0.33 to 0.46 with increasing UV irradiation time (500 sec). Hence, the magnitude of I_1/I_3 suggests small changes in the intensity ratio of the first and third vibration bands, placing pyrene in a more hydrophobic environment of the polymeric matrix. A careful analysis of the total area (I_1-I_4) of the monomer fluorescence indicates a significant increase of about 4.3 times.

As one can see in Figure 7, in the case of UAc-M/BisGMA (1:1) used in formulation, the influence of urethane dimethacrylate structure on the I_1/I_3 ratio as a function of irradiation time suggested an analogous response, the value of I_1/I_3 ranging from 0.4 to 0.5 upon UV

exposure (380 sec). For the given resin, the total area (I_1-I_4) of the monomer fluorescence increases of 3.3 times.

Secondly, changing the irradiation source (400-500 nm UV/vis light) and the system taken in study to produce a more consistent formulation with workable viscosity, namely the monomer mixture UMO-1/BisGMA (1.5:4, 67.43 %), TEGDMA (32.57 %), initiated by DMAEA/CQ, in which about 50 % filler (Aerosil/Sr-Zr glasss 1:1) was added, the partitioning of pyrene in hydrophobic environments can be followed by the same gradual increase in pyrene monomer emission. Thus, by exposure to UV/vis irradiation conducted with a curing light dental device (BLUE SWAN led) the aforementioned monomers start to polymerize, so that after 40 sec, the formation of a clear, hard glassy solid is visible. Figure 8 (plot a) reveals the change in the monomer fluorescence intensity of pyrene, excited at 353 nm, with the curing time that confirms again, that there is no excimer in the obtained glassy materials. The variation of I_1/I_3 with the irradiation time is quite similar to that observed upon photopolymerization of UMO-1/BisGMA (1.5:4) initiated of BP/PD (40 sec) (Figure 6). In such a case, the total area (I_1-I_4) of the fluorescence intensity increased more than 45 % (Figure 8, plot b) compared to the non-irradiated sample, and this effect probably arises from a diminishing in collision-induced fluorescence quenching.

Since the hardening time could be adjusted by varying the composition of the formulation, two modifications were operated. If Aerosil/quartz (1:1) is included as filler, the I_1/I_3 value changed from 0.42 to 0.48 and the total area (I_1-I_4) of the fluorescence intensity increased with 44% (not shown). Such finding suggested that the filler nature has not as effect a qualitative difference in pyrene fluorescence in this case, where the viscous UMO-1/BisGMA monomers exhibited almost the same effective activation energy. When the system based on UMO-1 (67%) and TEGDMA (32.57%) initiated with CQ/DMAEM does not contain BisGMA, the I_1/I_3 ratio presented comparable values with those discussed for the formulation in which the BisGMA was included. It should be mentioned that for the same irradiation time (40 sec) the total area (I_1-I_4) of the monomer fluorescence intensity increases with 120 % by using of Aerosil/quartz in the latter. Figure 9 (plot a, b) shows the influence of composition on the profile of fluorescence vs. irradiation time for UMO-1/TEGDMA and Aerosil/quartz initiated of CQ/DMAEM by UV/vis light. This result indicates a significant enhancing of the curing process compared to Aerosil/Sr-Zr glass, where the total area (I_1-I_4) increased just with 50 %. It is difficult to explain the contribution of the Aerosil/quartz filler and its interactions that seems to be responsible for the highest fluorescence intensity as the monomers continue to react for the formation of highly cross-linked, rigid, and glassy polymeric network in the absence of BisGMA.

Compared to UMO-1, for the polymerization of low molecular weight urethane dimethacrylate (74.65 % UAc-M), TEGDMA (25.35 %), DMAEM/CQ, pyrene derivative and filler (Aerosil/Sr-Zr glass, 1/1), the total area (I_1-I_4) of the fluorescence intensity decreased at 22 % during the polymerization reaction (Figure 10).

This means that the monomer structure and the interactions performed in this system can cause a lower mobility of monomer molecules during polymerization accompanied of a decrease of flexibility of the corresponding polymeric network containing fluorescent pyrene probe. Therefore, the fluorescence data for all studied formulations are in good agreement with those determined by FTIR spectroscopy, this fact being in the favour of a higher reactivity of the urethane dimethacrylate oligomers during the curing reaction with the formation of dental polymeric networks comparatively with the UAc-M monomer.

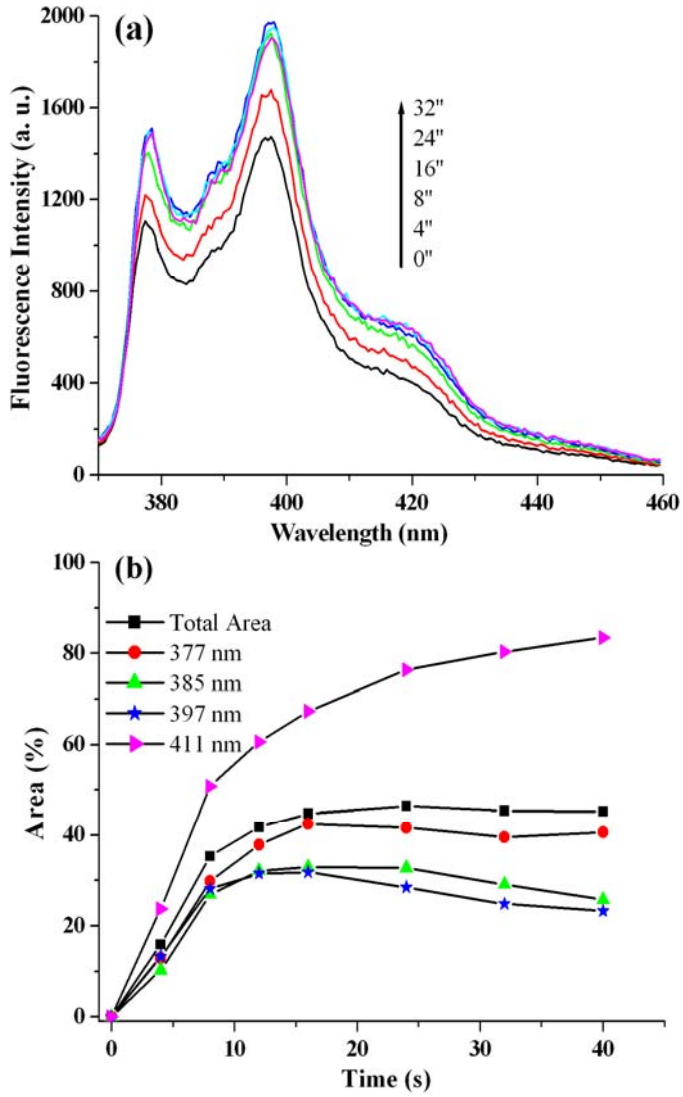


Figure 8. Fluorescence spectrum for noncarboxylic urethane dimethacrylate oligomer UMO-1/BisGMA (1.5:4 wt. %), TEGDMA, DMAEA/CQ, Aerosil/Sr-Zr glass (1:1), and pyrene derivative exposed to irradiation with visible light, and excited at 353 nm (a) and (b), plot of the deconvolution results of monomer fluorescence (I_1-I_4). *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

In order to develop an understanding of the intrinsic properties of the carboxyl urethane macromers proposed for testing in dental composites, where there are multiple intermolecular interactions between all components, we have also studied the fluorescence dynamic of pyrene derivative introduced into some dental formulations. Hereby, the fluorescence spectra of a sample that contains 3 wt. % CUDA-O1 and 27 wt.% UMO-1, initiated with DMPheAA/CQ (1/0.5 wt. %), in which about 70 % filler (Aerosil/Sr-Zr glass, 1/1) and 1% pyrene methanol was incorporated, are given in Figure 11, a.

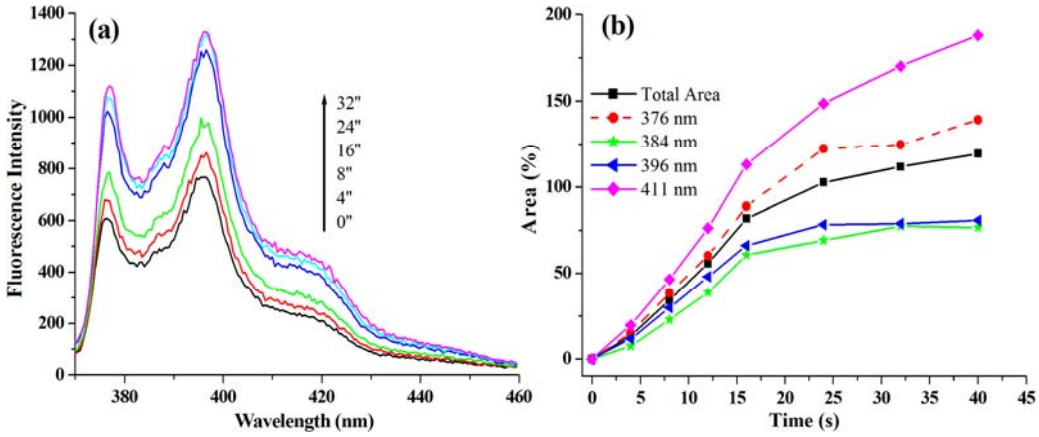


Figure 9. Effect of the filler (Aerosil/quartz, 1/1) on the fluorescence spectrum for urethane dimethacrylate oligomer (UMO-1)/TEGDMA, DMAEA/CQ, and pyrene derivative during irradiation with visible light and excited at 353 nm (a) and (b), plot of the deconvolution results. *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

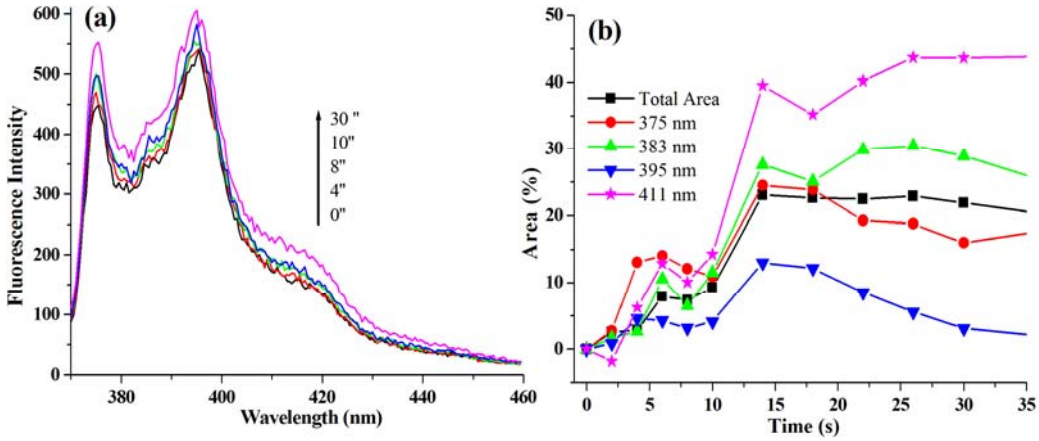


Figure 10. Fluorescence spectrum for noncarboxylic urethane dimethacrylate monomer UAc-M/TEGDMA, DMAEA/CQ, Aerosil/Sr-Zr glass, and pyrene derivative during irradiation with UV/visible light and excited at 353 nm (a) and plot of the deconvolution results (b). *Reprinted with permission of [Ref. 74], Copyright (2009) – Wiley.*

It may be observed that by exposure of this formulation to UV/vis irradiation, both urethane dimethacrylates of oligomer type start to polymerize leading to a hard glassy solid (after 40 s). Similarly, the vibronic fluorescence spectrum is typical for the pyrene fluorescence reflected of four peaks centred at 378 nm (I_1), 386 nm (I_2), 397 nm (I_3) and 418 nm (I_4), attributed to a monomer fluorescence originated from the solitary fluorophore molecular emission. The deconvolution results (Figure 11, b) showed that the excimer emission cannot be detected in the resulting composite, because this process depends on the mobility of the excited pyrene molecule, distance between them and implicitly, of surrounding microviscosity, the lifetimes of an excited form, as well as number of pyrene molecules. Moreover, the dynamical response of pyrene molecule suggests that the spontaneous emission intensity decreases as the irradiation time increased, and we interpret this change in interaction between the pyrene probe and its surroundings in the context of

strong intermolecular interactions. Monitoring the changes in fluorescence intensity as the matrix viscosity increased upon completion of the curing reaction in resin composite, it is clearly that the pyrene molecules excited in the singlet state cannot lose thermal energy, so that their return to the fundamental state is possible just through photon emission (fluorescence). Our observation of decay for pyrene methanol in a formulation containing a small quantity of carboxyl urethane dimethacrylate could be accounted for by the fact that, in the polymerizable matrix was also incorporated the filler of Aerosil/glass type. We believe that the origin of this decay in monomer fluorescence intensity lies in the ability of the carboxylic groups to interact with cations liberated from Aerosil/glass that allow the formation of carboxylate units acting as quencher of fluorescence.

A point of ambiguity appeared in the interpretation of this result comes from the fluorescence measurements performed on the composites that contains the noncarboxylic urethane oligomers for which, the total area of the fluorescence intensity increased comparatively to the non-irradiated samples (Figures 7-10), effect probably arisen from a diminishing in collision-induced fluorescence quenching. To distinguish between two discrete possibilities concerning the fluorescence intensity dependence of the composite structure exposed to UV/vis irradiation, the monocarboxylic urethane dimethacrylate CUDA-O1 was replaced in the formulation with acrylic acid. Indeed, the emission spectrum obtained is essentially the same as that for CUDA-O1 from the first composite, where is noticeable an ample reducing in the monomer fluorescence intensity as a function of irradiation time (Figure 12, a). Therefore, the spectral alterations of the fluorescence intensity when the carboxylic acid function is contacted with filler for even just 40 sec reflect probably the ability of the polymer for electrostatic interactions of the carboxylic groups with releasing of positively charged ions on the glass surface. Once the carboxylate units appear in matrix, these can quench the fluorescence of pyrene *via* electron or energy transfer mechanism [88]. Figure 12, b summarizes the results of spectral deconvolution in the case of using acrylic acid as co-monomer of the dental formulation.

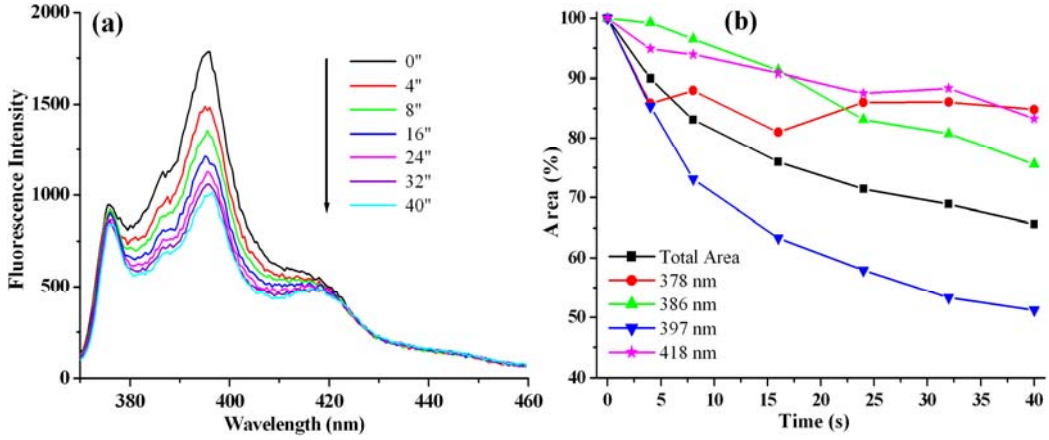


Figure 11. Monitoring the photopolymerization of a mixture of CUDA-O1, UMO-1, DMPheAA/CQ and Aerosil/Sr-Zr glass (1/1) *via* fluorescence probe (pyrene methanol) at λ_{exc} = 353 nm (a) and the evolution of the monomer fluorescence intensity area during irradiation (b). *Reprinted with permission of [Ref. 76], Copyright (2007) – Wiley.*

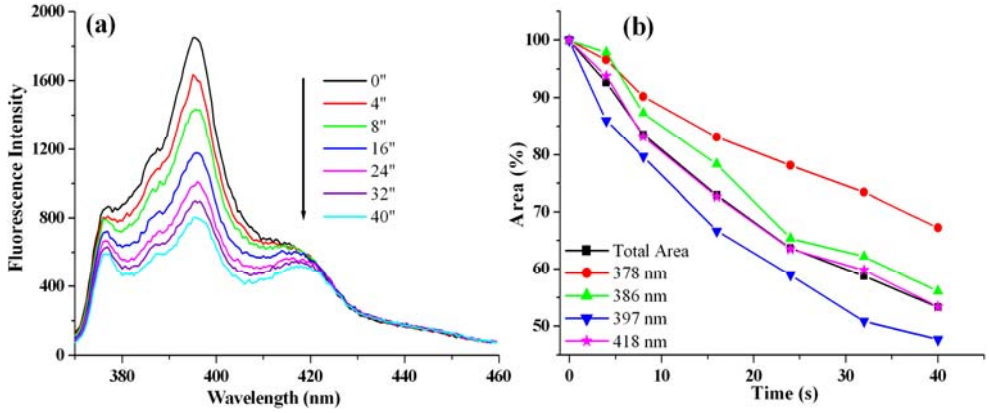


Figure 12. Fluorescence spectra for the formulation containing acrylic acid, UMO-1, DMPheAA/CQ, pyrene methanol and Aerosil/glass (1:1) exposed to irradiation (a) and relationship from the total area of monomer fluorescence and irradiation time (λ_{exc} = 353 nm) (b). *Reprinted with permission of [Ref. 76], Copyright (2007) – Wiley.*

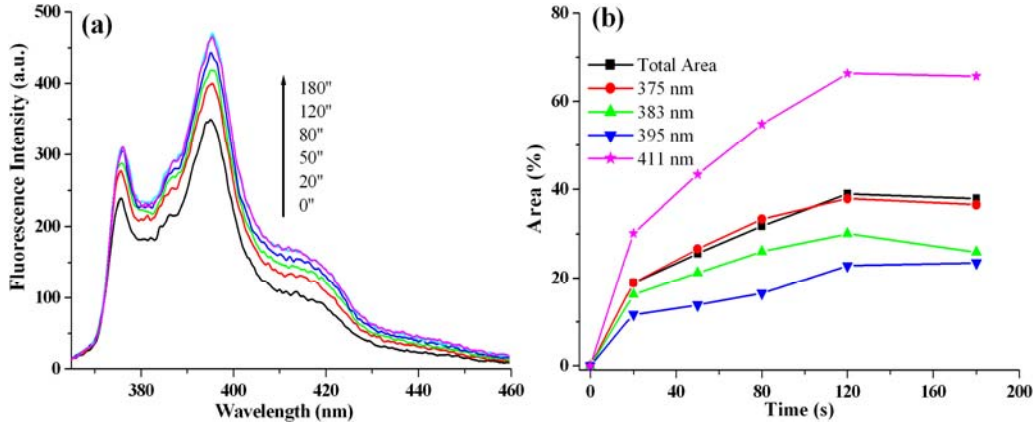


Figure 13. Monitoring the photopolymerization of CAd-3 initiated by DMPheAA/CQ, mixed with Zr/Sr glass (70 wt.%) *via* fluorescence method using pyrene methanol probe (λ_{exc} = 353 nm) (a) and the evolution of the monomer fluorescence intensity area (b). *Reprinted with permission of [Ref. 78], Copyright (2009) – SPSJ.*

For the above system, the magnitude of I_1/I_3 suggests very small changes in the intensity ratio of the first and third vibrational bands, placing the pyrene in a hydrophobic environment of the hybrid composite. In this context, for such resin the total area (I_1-I_4) of the monomer fluorescence indicates an important decrease of 33.25%, when was used a urethane dimethacrylate monomer with carboxylic group.

Subsequently, the specific properties of the molecular microenvironment during the photopolymerization of dicarboxylic urethane dimethacrylates in the presence of a small amount of pyrene methanol were evaluated, too. In Figure 13 (a) are given the fluorescence spectra recorded for a formulation based on CAd-3, pyrene methanol (0.5 wt. %), DMPheAA/CQ (1/0.5 wt. %) and about 70 wt. % Zr/Sr glass as filler. Upon irradiation of this combination with UV/vis light and exciting at 353 nm, the above carboxylic urethane oligomer starts to polymerize s and after 180 sec, a clear and glassy solid was formed.

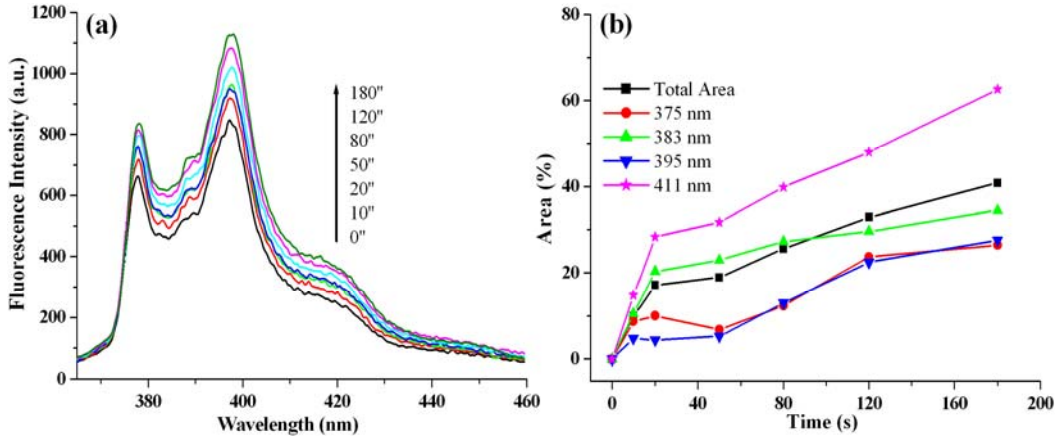


Figure 14. Monitoring the photopolymerization of the CAd-M dimethacrylate in the presence of DMPheAA/CQ/pyrene system and Zr/Sr glass filler, *via* fluorescence method (a) and plot of the deconvolution results (b). *Reprinted with permission of [Ref. 78], Copyright (2009) – SPSJ.*

The fluorescence spectrum of this combination is similar to that recorded in the case of the noncarboxylic or monocarboxylic oligomers, being typical to the pyrene fluorescence. It is evident that the photoresponse of pyrene molecule indicated that the spontaneous emission increases in intensity with irradiation time (Figure 13, b). This seems to be consistent with a picture where the fluorescence quenching was rather restricted in the polymerization process, owing to the distribution of the involved species that limited the pyrene-pyrene hydrophobic interaction. Thus, the non-radiative energy process is diminished and consequently, the photon emission is preferred as the microviscosity continues to increase in the above composite incorporating a high amount of inorganic filler. On the other hand, from the intensity ratio of the first and third vibrational bands (I_1/I_3 ratio), it can be noted that the pyrene emission I_1/I_3 ratio varied slightly from 0.35 to 0.32 with increasing irradiation times (180 sec), suggesting a hydrophobic environment around the molecular probe in the polymeric matrix.

The similarity in results continues with formulation prepared with acid dimethacrylate monomer (CAd-M) used together with DMPheAA/CQ, Zr/Sr glass and pyrene probe (Figure 14).

As it can be seen from figure 14, the examination of data rising from the fluorescence is fitting to those previously discussed. Obviously, the microviscosity changes that occur during polymerization confirms that the rapid modifications in fluorescence intensity can be viewed as measure of hardening sample during photopolymerization of the urethane monomers to produce highly cross-linked polymer networks with desirable properties.

5. SURFACE PROPERTIES - CONTACT ANGLE

To evaluate a possible applicability of these new monomers in dental materials, the water contact angles (CA), which are an indicative of the wetting properties of the composite resins, were determined. Thus, for some formulations based on 18.25 % urethane dimethacrylate oligomers (UMO-1, UMO-2 or UAc-M), 48.25 % BisGMA, 32 % TEGDMA and

DMPheAA/CQ (1/0.5 wt. %) and 50 % wt. Aerosil/Zr-Sr glass (1/1), the contact angle are represented in Figure 15.

It can be remarked that the presence of longer hydrophilic PEG segments in the structure of urethane dimethacrylate tested in the formulation of composite (S1, CA: 40.51^{o}) produced photocured resins with relatively low contact angle compared to that which contains shorter segments (S2, CA: 46.39^{o}), when their surfaces were in contact with a drop of bidistilled water. In comparison with S2, a small increase of the contact angle to 52.54^{o} was found by using of urethane acrylic monomer (UAc-M) in the S3 formulation. However, it is clearly that the length of the flexible chain is responsible for the modification of the contact angle in the S3 formulation, without to neglect the effect of hydrophobic BisGMA. Moreover, for a formulation in which the BisGMA was totally replaced by UMO-1 oligomer, the contact angle is substantially decreased (S4, CA: 13.11^{o}), due to the hydrophilic nature of the polymeric sequence.

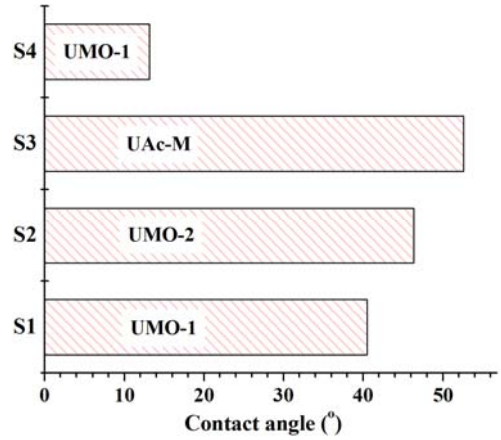


Figure 15. Illustration of contact angle values for some composites based on noncarboxylic urethane dimethacrylates.

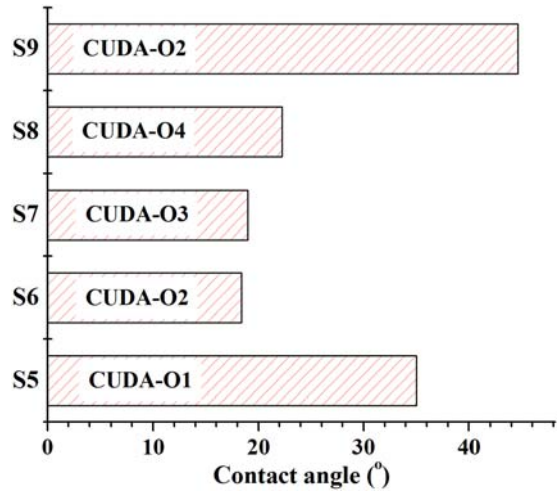


Figure 16. Diagram of contact angle values for some composites based on monocarboxylic/ noncarboxylic urethane dimethacrylates.

Subsequently, the water contact angles for a series of composite specimens formulated with monocarboxylic urethane macromers were measured to assess their hydrophilicity. Hereby, for composites prepared employing the monocarboxyl macromers and UMO-1 derivative (1/9 wt. ratio), DMPheAA/CQ, and a filler (Aerosil/Zr-Sr glass, 1/1), the values determined for contact angles are included in figure 16.

Taking into account the fact that, in composites S5-S8, the gravimetric ratio of the components is similar (10 % monocarboxylic derivative and 90 % noncarboxylic UMO-1), we can affirm that for the samples containing carboxylic urethane macromer with long flexible sequence (PEG_{600}/CUDA-O2, PEG_{1000}/CUDA-O3), the measured contact angle exhibited a smaller value (CA: 18.39°; 19°) comparatively to sample S5 based on PEG_{400} (CA: 35°), confirming the hydrophilic nature of PEG sequences. Such result suggests a favourable distribution of the hydrophilic soft segments which in contact with water are portioned at the surface as a consequence of the mobile hydrophobic urethane structure retreating from the surface. Compared to CUDA-O1 (S5), a small decreasing of the concentration of carboxyl group in CUDA-O4 (S8) produces a decreasing of the contact angle value to 22.5°. However, it is evident that the length of flexible chain caused rather a modification of the contact angle between the droplet and the surface. On the other hand, the presence of BisGMA into the S9 formulation (5.46 % CUDA-O2, 21.8 % UMO-1, 72.67 % BisGMA), had as effect an increasing of the contact angle (44.62°), the hydrophobicity difference being mainly generated by the presence of BisGMA.

In the case of dicarboxyl urethane dimethacrylates, were prepared a higher number of formulations with variable compositions, for which the water contact angle were determined [78, 89]. Consequently, in order to realize facile displays of all composites, in Table 3 are detailed the samples structures.

From the graphical representation of contact angles as a function of the dicarboxyl urethane dimethacrylate type (Figure 17), it can be observed that the resin composites are hydrophilic with the contact angles varying between 24.8° and 62.2°, owing to the PEG sequences.

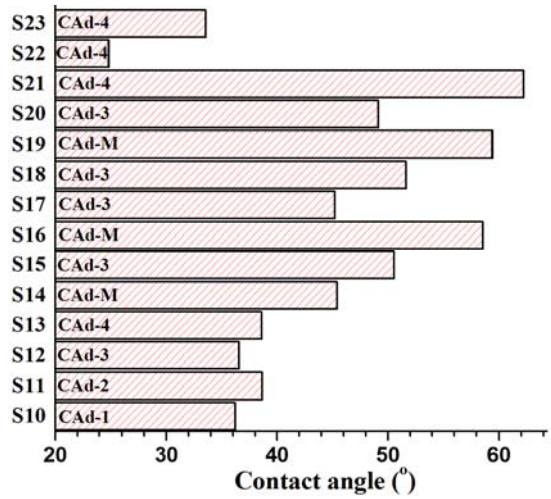


Figure 17. Diagram of contact angle values for some composites including dicarboxylic urethane dimethacrylates.

Table 3. Composition of the samples prepared for contact angle determination, initiated with CQ/DMPheAA

Sample*	CAd-1	CAd-2	CAd-3	CAd-4	CAd-M	UMO-1	BisGMA-1	BisGMA	TEGDMA	HEMA
	(wt. %)									
S10	15	-	-	-	-	-	-	49.25	34.25	-
S11	-	15	-	-	-	-	-	49.25	34.25	-
S12	-	-	15	-	-	-	-	49.25	34.25	-
S13	-	-	-	15	-	-	-	49.25	34.25	-
S14	-	-	-	-	15	-	-	49.25	34.25	-
S15	-	-	15	-	-	-	-	40	43.5	-
S16	-	-	-	-	15	-	-	40	43.5	-
S17	-	-	15	-	-	-	-	40	-	43.5
S18	-	-	15	-	-	-	40	-	43.5	-
S19	-	-	-	-	15	-	40	-	43.5	-
S20	-	-	15	-	-	-	40	-	-	43.5
S21	-	-	-	5.5	-	46	-	48.5	-	-
S22	-	-	-	5.5	-	46	48.5	-	-	-
S23	-	-	-	51.5	-	-	48.5	-	-	-

* samples S10-S14 contain 70 % wt. zirconium silicate nanopowder, S15-S23 contain 60 % wt. Aerosil/Zr-Sr glass (1/1)

Given these results is obviously that the modification of carboxyl concentration in composite formulations incorporating urethane dimethacrylates is not a determining element for a significant variation of the contact angle values. Moreover, the filler variation causes hydrophobicity changes, generally, the composite including zirconium silicate nanopowder presenting a higher hydrophilicity comparative to Aerosil/Zr-Sr glass formulations.

Another option to assess the compatibility between the organic and inorganic phases and the formation of compact homogeneous materials was the use of scanning electron microscopy (SEM) investigations in fractured surfaces.

The SEM analyses were made on fractured specimen surfaces by selecting resin composites comparable with those tested for contact angle. Therefore, for the composites S10-S14 and for a composite with similar composition in which the dicarboxylic derivative was replaced with UMO-1 (S24), the SEM examination revealed a good compatibility between the inorganic matrix formed of zirconium silicate nanopowder and the organic constituents, since in the SEM images are identifiable large homogeneous domains, where no voids are observed (Figure 18, a-f). The fracture edges are clear and sharper, feature specific for the hard materials, confirming an intimate interaction between the components taken into formulation. However, in the recent research, nano-sized particles and ceramic whiskers are added to dental resins due to the fact that the high surface reactivity of nano-sized particles provides a much tighter bonding to the resin matrix than conventional-sized particles [6].

In our systems, the observed aggregates/agglomerates are formed by filler crystals stacking into bundles and have dimensions under 5 μm. Also, it may be noticed that between the composites comprising the CAd-1 ÷ CAd-4 oligomers (S10-S13) and the composite S14 which include the monomer CAd-M are not visible important differences, suggesting an equivalent linking of the oligomers and monomer in the resin formulations. In addition, the comparison of SEM images for the composites S10-S14 based on dicarboxylic dimethacrylate derivatives (Figure 18, a-e) with that containing dimethacrylate without carboxyl fragments (Figure 18, f), sustains that the microstructure of the materials is not fundamental changed, as evidenced by this technique.

The morphology of the resin composites S15 and S17 containing a different filler (Aerosil/Zr-Sr glass) revealed a regular and homogeneous appearance (Figure 18, g, h), where the formation of porous cavities due to filler debonding seems to be minimal. Moreover, the filler distribution into the organic matrix is relatively uniform, in fracture being observed only few filler particles with dimensions under 5 μm. This result indicates a favourable adhesion between filler and matrix on one hand, and a good interaction between the filler and the urethane dimethacrylate by means of the carboxyl groups situated along an oligomeric backbone, on the other hand. However, it may be noticed that for the S17 composite (with HEMA in formulation), the fractured surface has an elevated roughness as compared with S15 sample, observation which confirm also the high water sorption/solubility of the composites containing HEMA. Additionally, the filler variation determines that the S15 and S17 samples to have a softer look in contrast with the S10-S15 composites.

For a given application is also important to examine the crack opening profile in the case of resin composites after they have been subjected to Vickers indentation, since fracture properties and implicitly, fracture mechanism are key features of such materials. This fact may be related to initiation of the first microcracks formed mainly by decohesion on the particle-matrix interface or by cleavage of the particles [90]. Interestingly, there were published results indicating a good correlation between the hardness of dental restorative

composites and the inorganic filler content [91], including the resin composition, nature of the organic/inorganic interface and the inorganic particle size distribution [92].

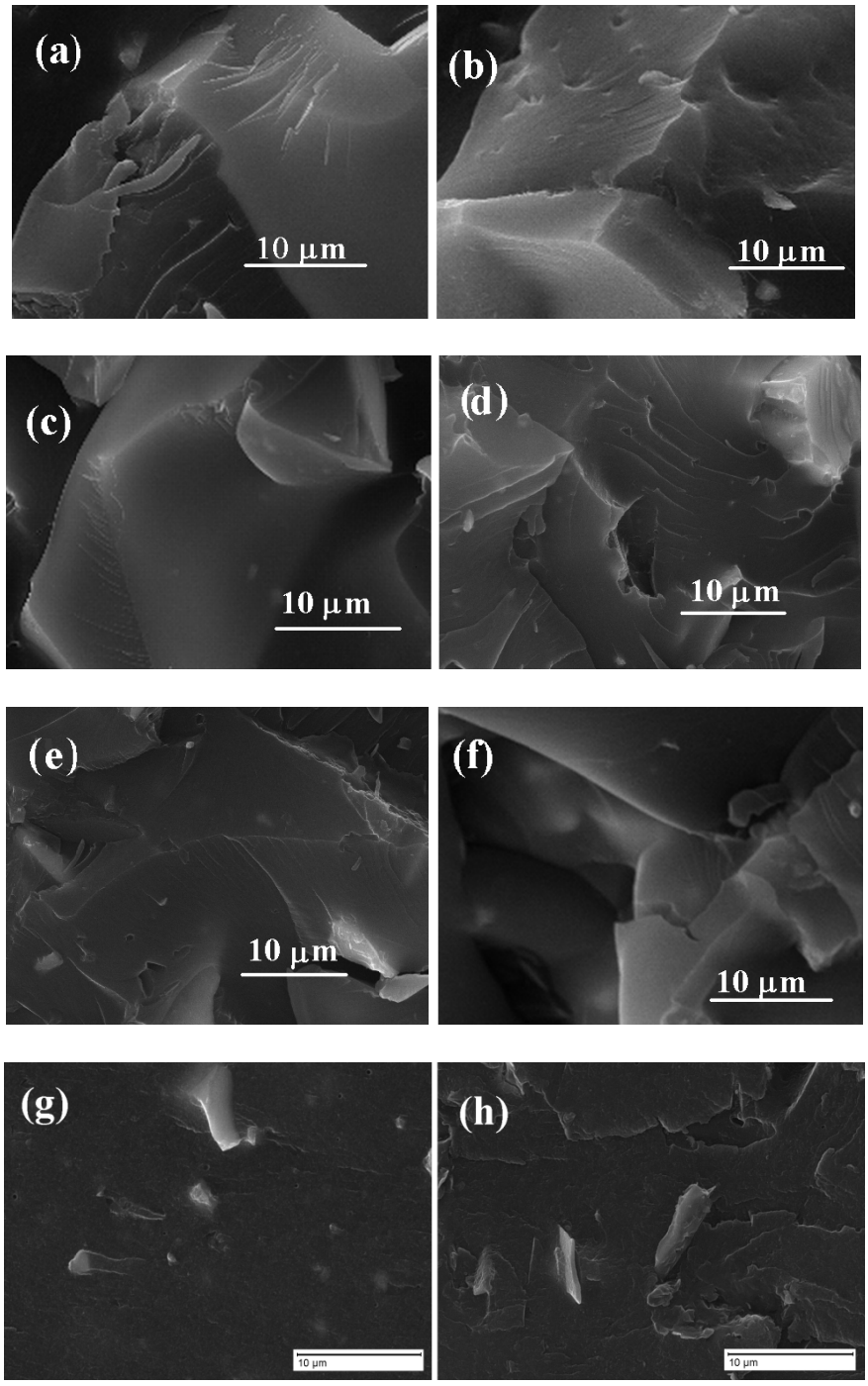


Figure 18. SEM micrographs of S10 (a), S11 (b), S12 (c), S13 (d), S14 (e), S24 (f), S15 (g) and S17 (f) resin composites in fracture. *Reprinted with permission of [Ref. 78], Copyright (2009) – SPSJ (a-f) and reprinted with permission of [Ref. 89], Copyright (2009 –Romanian Academy (g, h).*

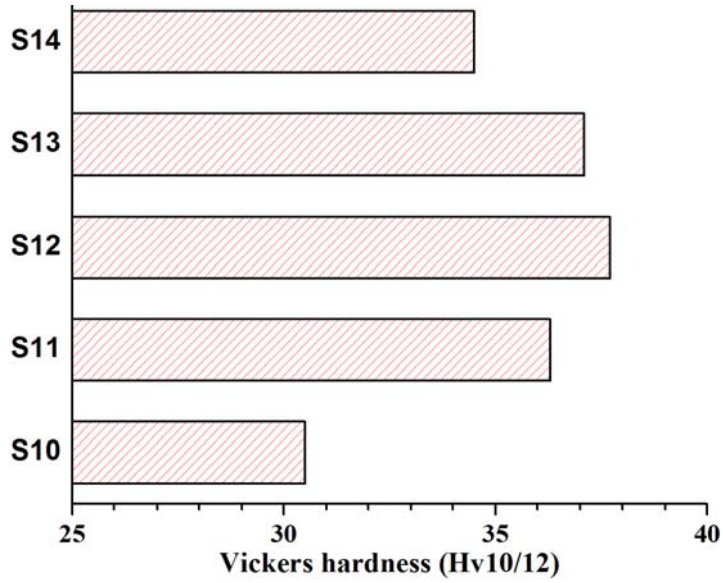


Figure 19. Vickers hardness for S10-S14 resin composites based on dicarboxylic urethane dimethacrylates and containing 70 % wt. zirconium silicate nanopowder.

Consequently, the values of the Vickers hardness of the polymer composites S10-S14 were in the range of 30.5 to 37.7 (Figure 19), the results suggesting that the hardness of the polymer composites varied slightly because all composites contain the same inorganic filler but different organic resin matrices.

Moreover, there was a small difference (of only 3.2) in the values of the Vickers hardness of the composites incorporating the urethane dimethacrylate monomer (CAd-M, S14) or the urethane dimethacrylate oligomer with the highest PEG molecular weight (CAd-3, S12) that sustain the similarity of the resin composites under investigation.

To analyze the surface damage caused by the Vickers indentation, two methods of optical examination were employed, namely, polarizing optical microscopy (POM) and atomic force microscopy (AFM). The optical microscopy image of the resin composite S12 (Figure 20, a) showed the characteristic Vickers indents on the surface, where four specific line are visible, but without to generate at a microscopic level the radial and lateral cracks alongside the four main lines generated of indentation. Compared to that of POM, the AFM image of the same composite (Figure 20, b) evidenced the presence of smooth surface and a relatively uniform topographical pattern, where a linear crack seems to appear from an edge within the pyramid, resulting thus in an alteration of the surface, observable at a microscopic level. Moreover, the characteristic crack had a deep of 250 nm, as indicated the profilometric curve attached to Figure 20, b. A similar crack was detected in the AFM image of S14 composite (unshown here) suggesting rather that the crack propagation in such composites follows most probably a relatively linear pattern. This observation is crucial for explaining the first cracks developed in dental resin composite, reason for that additional investigations will be further taken into consideration in our laboratory.

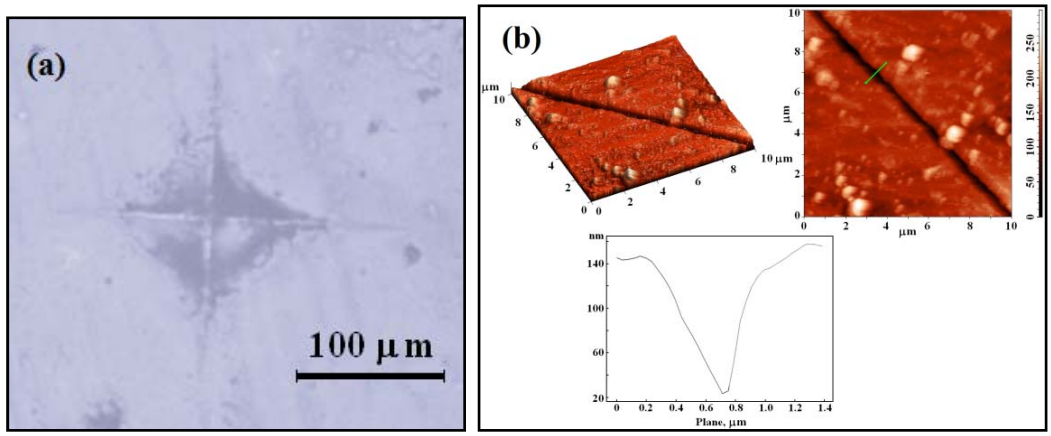


Figure 20. Optical microscopic image of the Vickers indentations in resin composite S12 (a) and the 2D and 3D AFM micrographs of the crack in the S12 composite as well as the clear changes in the depth profile. *Reprinted with permission of [Ref. 78], Copyright (2009) – SPSJ.*

6. Water Uptake and Solubility Measurements

Most important characteristics of these materials are related to the water sorption and leaching of small molecules, two processes which take place simultaneously, when composite resins are in contact with water and oral fluids. For this reason, water sorption is a key parameter which guarantees viability and quality of such materials. Normally, water sorption is a diffusion-controlled process that produces in the organic resin matrix and mainly depends on hydrophilicity [93] and cross-linking density of the cured resins [94]. Therefore, hydrophilic constituents increased water sorption values and thus, it is expected that the composites enclosing dicarboxylic urethane dimethacrylates of oligomer type to behave adequately. The analysis of the data included in Figure 21 (a), obtained on some of the previously described composites (Table 3), allow us to affirm that the water sorption values determined for the dental composite resins comprising the urethane dimethacrylate oligomers varied between 1.68 and 2.57 wt. %, these depending on many factors (especially composition of the samples and the filler nature). However, it can appreciate that the measured values are similar or even smaller than the literature ones [65, 95-97].

A complementary process that occurs together with water sorption is the release of small, unreacted or insufficiently bonded molecules, namely water solubility. The composites incorporating HEMA as comonomer (S17 and S20) presented a higher percent of water solubility caused probably of itself hydrophilic nature. Additionally, HEMA having a small molecule that contains only a single double bond, could favour the significant leaching out of the monomer from the formed material. Significant water solubility was recorded also for the composite S14 containing the acid urethane monomer CAd-M, behaviour probably due to an incomplete connecting of the low molecular weight dimethacrylate in the resulting photocrosslinked matrix. However, we can summarize that the results obtained are good, in agreement to those reported for other dental materials [98, 99].

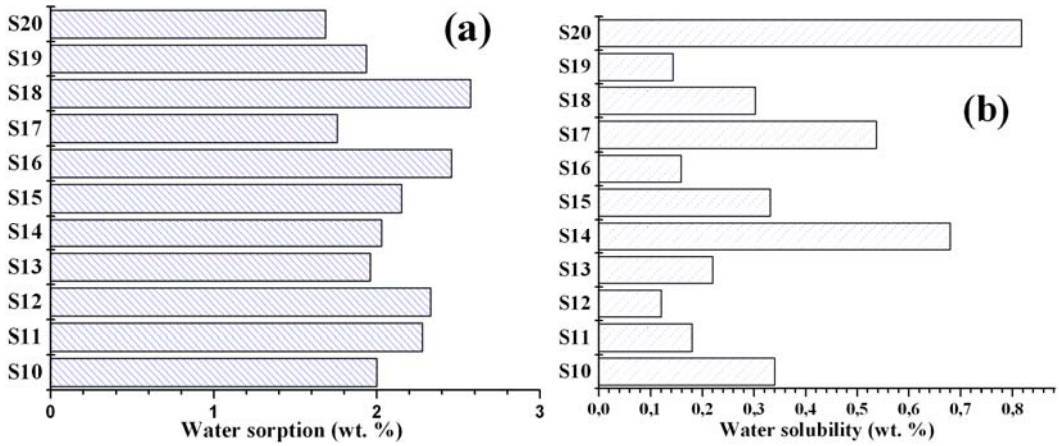


Figure 21. Water sorption (a) and water solubility (b) characteristics of some experimental resin composites.

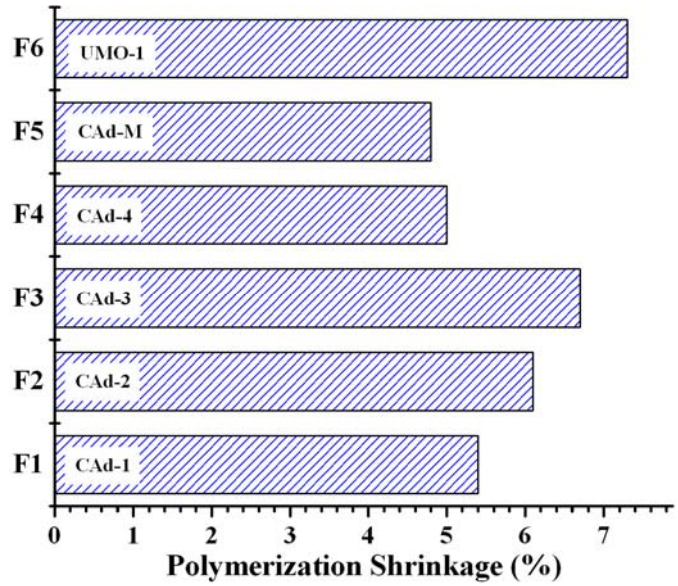


Figure 22. Polymerization shrinkage changes for some dental urethane dimethacrylates.

7. POLYMERIZATION SHRINKAGE

In the field of modern dental composites the polymerization shrinkage (PS) continues to be one of the unresolved issues. Preponderantly, the commercially available dental resin composite materials utilize BisGMA as major monomer in the resins, although his main drawback is related to its high viscosity, which complementary demand the employing of diluents to enhance the handling of composite pastes. The monomers frequently used for this purpose are TEGDMA and HEMA, which unfortunately have less desirable effects on the properties of the resin, since they increases water sorption and polymerization shrinkage [72, 100, 101]. In this context, we have considered appropriate to evaluate the polymerization

shrinkage values for a series of dental formulations based on dicarboxyl urethane dimethacrylates. Consequently, we have prepared a number of formulations based on a mixture of dicarboxyl urethane dimethacrylates (CAd):BisGMA:TEGDMA:CQ:DMPheAA in the gravimetric ratio 15:49.25:34.25:0.5:1, and to evidence the influence of carboxyl groups on the shrinkage profile of the samples, a supplementary model based on urethane dimethacrylate without carboxyl groups (UMO-1) was prepared, too. Investigations of the polymerization shrinkage (determined by picnometric method) for the formulations F1–F6 without using any filler revealed that the obtained results are comparable with the literature data, taking into account that the amount of urethane dimethacrylate oligomer included in each specimen, is of only 15 wt.% (Figure 22).

It can be remarked that the incorporation of polyether sequences with variable length (PEG_{400}–PEG_{1000}) in the organic matrix led to an increasing of the polymerization shrinkage (PS: 5.0÷6.7 vol%) as compared to the formulation comprising the monomer CAd-M (PS: 4.8 vol%). It means that the degree of shrinkage of a composite is directly related to the molecular weight of the monomer, the amount of monomer from the composite and its conversion [11, 61, 102]**.** Thus, in our case, the lower conversion degree obtained for the CAd-3 oligomer gives also the highest polymerization shrinkage (PS: 6.7 vol%), when this was incorporated in the F3 formulation. For other photopolymerizable systems, the polymerization shrinkage values are between 5.26 and 6.06 vol%, comparable with those determined for dental formulations containing similar components [103]. The polymerization shrinkage measured for F6 formulation that contains UMO-1 oligomer had the highest value (PS: 7.3 vol%), denoting that the existence of carboxylic groups with adhesive properties into photopolymerizable mixtures, confering them a slow improvement of the volumetric shrinkage, probably due to a tightly packaging of the polymer network after polymerization. This result tends to confirm that the using of more adhesive bonding agents could counteract polymerization shrinkage and the resulting stresses [104].

8. MECHANICAL PROPERTIES

Diametral tensile strength (DTS) and compressive strength (CS) are significant properties for dental composites and are regularly used in the evaluation of mechanical parameters of the dental material formulations [12, 105-107]. In our case, the study of mechanical characteristics was performed on composites containing dicarboxylic urethane dimethacrylate oligomers and monomer mixed with BisGMA and TEGDMA together with 70 wt.% zirconium silicate nanopowder (samples S10-S14 from Table 3) or 60 % Aerosil/Zr and Sr glass (samples S15-S20 from Table 3). The diametral tensile and compressive strength results, included in Figure 23, showed that generally, the mechanical parameters for the reported composites are rather comparable to those reported for some glass ionomer cements (DTS: 26.8-40 MPa; CS: 134-200 MPa) [108].

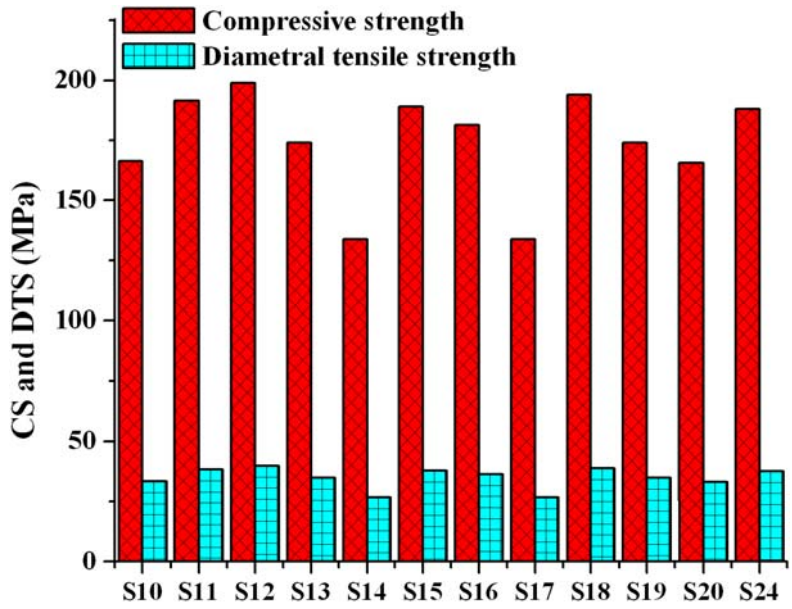


Figure 23. Compressive (CS) and diametral tensile strength (DTS) for the experimental resins.

It may be remarked that the lower values for the diametral tensile and compressive strengths are recorded in the case of composites that contain HEMA, followed then of those comprising the CAd-M low molecular monomer, when is supposed that the short molecular chains induced an inferior cohesion between the organic and inorganic matrixes. On the other hand, the incorporation of modified BisGMA, namely BisGMA-1 determined a slight increasing of the mechanical parameters, comparative to the resin composites prepared by using commercial BisGMA, results attributed to an augmented concentration of double bonds. Moreover, the mechanical parameters of the noncarboxylic composite (S24) are comparable to those measured for the carboxylic homologues, suggesting that the presence of carboxyl groups into the formulations is not a determining factor in the improvement of mechanical features.

CONCLUSION

In conclusion, in the present work, we have demonstrated that the chemical structure of the photopolymerizable urethane dimethacrylates could play an important role in the formation of polymeric network into dental resin composites. A new class of carboxylic monoacid and diacid functionalized dimethacrylates containing urethane groups and polyethylene oxide sequences of variable length (Mn: 300-1000 g/mol) were prepared via classical “ionomer” methodology, to be investigated in dental formulations comparatively with non-acid analogues of oligomer or monomer type. In tandem, the obtaining of BisGMA modified with urethane and methacrylate units involved an addition reaction of a monoisocyanate, while the synthesis of LC monomers was performed through the use of addition reaction or nucleophilic substitution and addition reaction.

The approach commented here has many features and advantages: (i) the formation of polymer networks after relatively short time of irradiation (35 s- 180 s) was observed by FTIR and fluorescence spectroscopy, (ii) the chemical structure of the monomer has effect on the mobility and reactivity of the formed network, more reactive being the oligomeric forms (degree of conversion between 40 and 80 %), (iii) by controlling the nature and amount of urethane dimethacrylate in the monomer mixture (BisGMA, modified-BisGMA, TEGDMA, HEMA), the resin composite properties could be specifically directed towards dental restorations or adhesives applications (iv) the filler (Aerosil/Sr-Zr glasss, Aerosil/quartz, Zr/Sr glass; zirconium silicate nanopowder)–dependent resin composite fluorescence studies revealed that the formation of hard materials (irradiation up to 500 s) is deeply related to the microviscosity changes occurred during photopolymerization, fact reflected of rapid modifications in fluorescence intensity, and (v) the contact angle determinations sustain the hydrophilic nature of the resins, while the values of polymerization shrinkage, diametral tensile and compressive strengths of some specimens indicated that the incorporation of dimethacrylate urethane oligomers in the organic matrix led to materials with good properties, which could recommend them as potential dental materials.

The research is currently focussed on these directions including the behaviour of LC monomers in different formulations and the results will be published elsewhere.

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Chapter 3

DENTAL COMPOSITES WITH NANO-SCALED FILLERS

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ABSTRACT

Developed almost half a century ago, dental composites, consisting of a polymeric resin matrix and silanized glass or ceramic fillers, presented opportunities never before equaled in modern dentistry. The resin matrix is usually cured (hardened) by photo-initiated free radical polymerization. Camphorquinone (CQ) is a commonly used visible light initiator and ethyl-4-(N,N'-dimethylamino) benzoate (4-EDMAB) is a commonly used accelerator. The monomer of 2,2'-bis-[4-(methacryloxypropoxy)-phenyl]-propane (Bis-GMA) has been widely used as the dental base monomer since it was invented. Bis-GMA is a very viscous, honey-like liquid. To improve the handling qualities, a low viscosity diluents monomer, such as tri-(ethylene glycol) dimethacrylate (TEGDMA), is added to thin the resin. In the Bis-GMA/TEGDMA dental resin systems, Bis-GMA functions to limit the volumetric shrinkage induced by photopolymerization and to enhance the resin reactivity, while TEGDMA provides for increased vinyl double bond conversion. Compared to dental amalgams, the composites possess better esthetic property, have fewer safety concerns, and show reasonably satisfactory clinical results. Consequently, the composites have been widely adopted by the dental profession as the restorative material of choice. However, current dental composites are far from ideal and/or perfect; for example, mechanical properties of the composites still require significant improvements particularly for large stress-bearing posterior restorations that involve the replacement of cusps. Herein we report that innovative dental composites reinforced with nano-scaled fillers including polyhedral oligomeric silsesquioxane (POSS), fibrillar silicate, and electrospun glass nanofibers were prepared, characterized, and evaluated. The results indicated that the incorporation of small mass fractions of nano-scaled fillers substantially improved flexural strength, elastic modulus, and work of fracture values of dental composites. The mechanical properties of the composites could be further improved by optimizing the chemical compositions and surface treatment methods of the nano-scaled fillers. We envision that the uniform distribution of nano-

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scaled fillers into dental composites could result in the development of next generation dental composites, which would be particularly useful for large posterior restorations.

Keywords: Dental Composites, Polyhedral Oligomeric Silsesquioxane (POSS), Fibrillar Silicate, Electrospinning, Nanofibers, Mechanical Properties

INTRODUCTION

For over 40 years, dental composites consisting of resin matrices and inorganic fillers have been accessible in the field of dentistry [1]. This new age restorative material has replaced the old silver amalgams that have been the material of choice for over 150 years. While dental amalgams are considered durable and convenient, they have a number of problems associated with them. For example, the shiny metallic look they present can be considered unattractive. There is also speculation that this material can have medical concerns with toxic effects from the mercury content. Dental amalgams also play a negative role in the environment with issues relating to waste disposal [2]. New efforts have been put forth to synthesize a polymer resin-based restorative material (such as dental composites) that can possess an "invisible" repair of teeth rather than the traditional silver color of the amalgams [2]. To achieve the right consistency of dental composites, it is important to have the right mass fraction of the components. There are two main components that make up dental composites; the first is a methacrylate-based monomer resin matrix with a mass fraction in the range of 25-35%, while the second component is a glass or ceramic filler with a mass fraction around 70-75% [1]. The search for a resin system for restorative materials has been underway. Such a system should fulfill the following requirements including (1) a large monomer molecule that can decrease shrinkage from polymerization, (2) reduced polarity to mitigate water absorption and plasticization, (3) sterically-hindered to increase the elastic modulus of the resin, (4) having multiple functional groups per unit allowing for extensive cross-linking, (5) to be non-toxic, and (6) to be inexpensive [2].

The most common base monomer used in dental composites is 2,2'-bis-[4-(methacryloxypropoxy)-phenyl]-propane, better known as (Bis-GMA) [3,4]. The advantages for using Bis-GMA compared to its smaller-sized monomer counterparts are that it has lower volatility and diffusivity into tissues, as well as the formation of higher moduli polymers while keeping volumetric shrinkage to a minimum [7]. However, there is almost always a tradeoff between advantages and disadvantages. Bis-GMA is a highly viscous fluid, making the handling quality somewhat challenging [1]. Additionally, Bis-GMA has an average lifetime of 5 years, whereas the average lifetime expectancy of dental amalgams is 15 years [5]. Furthermore, under common dental polymerization conditions, the Bis-GMA methacrylate double bond conversion can be low. To counter these problems, tri(ethylene glycol) dimethacrylate (TEGDMA) is added to Bis-GMA. TEGDMA is a monomer with low viscous properties that can be used to thin the resin. Together, the Bis-GMA/TEGDMA system works hand-in-hand limiting the volumetric shrinkage induced by photopolymerization, enhancing the resin activity, and increasing the conversion rate of the methacrylate double bonds [8]. While Bis-GMA/TEGDMA resin has shown good clinical results, there are still problems with the strength and wear properties of the dental composites [9-13]. Having two hydroxyl groups on the Bis-GMA molecule causes high viscosity (due to

the formation of hydrogen bonds) and facilitates water absorption. Too much water absorption can plasticize the matrices of dental composites and promote hydrolytic degradation. This leads to less desirable properties of dental composites such as relatively short longevity [7, 14-16].

The resin matrix is cured (hardened) by a process known as photo-initiated free radical polymerization. In order for the reaction to occur, a photo-initiator such as camphorquinone (CQ), as well as a co-initiator such as ethyl-4-(N,N'-dimethylamino) benzoate (4EDMAB), are needed,. During photo-initiation, CQ is promoted to an excited state by absorbing radiation energy from the visible blue light. Once in the excited state, CQ reacts with 4EDMAB to form an intermediate complex that degrades to create free radicals, initiating the polymerization. A side effect of the polymerization process is volumetric shrinkage. It is not uncommon to see dental resins experience 6 to 10% volumetric shrinkage during this process; several disadvantages related to volumetric shrinkage include resin-filler de-bonding, decrease in marginal adaptation in dental restorations, and internal stress development [1]. The ideal dental composites have low volumetric shrinkage and high double bond conversion.

The second components of dental composites are ceramic and/or glass fillers. Most dental composites are reinforced by inorganic fillers. Commercially, amorphous SiO_2 (glass) is referred to most in dental composites; due to the refractive index of glass being similar to dental resins, creating the translucent look mimicking that of human teeth. In addition, the properties of glass reinforced composites generally meet the basic necessities of dental restorations. Conventionally, particle sizes of glass in dental composites are in the range from tens of nanometers to a few microns, reinforcing the dental composite. Nevertheless, glass particle properties such as strength, toughness, and durability are found to be sub par for the expanded use of large stress-bearing posterior restorations that involve the substitution of cusps. Interestingly, one of the main reasons for adding glass particles to resins is to strengthen the materials, but in the same sense, it also destroys the materials. The stress created mainly from chewing food, carries through the boluses of food to the surfaces of glass particles projecting from the occlusal surface. Because resin matrices are substantially softer than the glass particles they encompass, a lot of the stress is transmitted through the particles into the resins. Any irregularity in shape from submerged portions of the glass particles causes the stress to increase significantly. This problem causes small cracks around the particles, therefore weakening the matrices nearby [6].

Mechanical properties from the dental composites can be obtained through a standard three point flexural testing method where the flexural strength (S_F), Young's Modulus (E_Y), and diametral tensile strength (DTS) are measured. S_F can best be thought of as the maximum amount of stress inflicted upon a material at its time of rupture (bending strength). E_Y is a property that measures the level of stiffness that an elastic material can create. By using the stress range from which Hooke's law holds true, E_Y can be found from the slope of the stress of the material over its strain [2]. Finally, DTS is used to measure how a material responds to a tensile load. Basically, if the maximum strain of a sample is surpassed, the sample fails [2]. The following sections involve three research studies: (1) evaluation of dental restorative composites containing polyhedral oligomeric silsesquioxane methacrylate (POSS-MA), (2) fabrication and evaluation of Bis-GMA/TEGDMA dental resins/composites containing nano fibrillar silicate, and (3) electrospun nano-scaled glass fiber reinforcement of Bis-GMA/TEGDMA dental composites.

Table 1. Codes and formulations of the resin systems [1]

	POSS-MA (mass fraction)	BIS-GMA (mass fraction)	TEGDMA (mass fraction)
POSS1	0 %	50 %	50 %
POSS2	2 %	48 %	50 %
POSS3	10 %	40 %	50 %
POSS4	25 %	25 %	50 %
POSS5	50 %	0 %	50 %

Evaluation of Dental Restorative Composites Containing Polyhedral Oligomeric Silsesquioxane Methacrylate

Research efforts have suggested that the organic/inorganic hybrid monomer of POSS-MA could be used to completely or partially replace Bis-GMA in order to improve the mechanical properties of dental composites. The POSS-MA compound can have 8, 10, or 12 silicon atoms and comes in the form of a liquid with a honey-like appearance that mixes well with Bis-GMA and TEGDMA. Five different mixture ratios of POSS-MA and Bis-GMA were studied to find the most favorable results while the percentages of diluent monomer TEGDMA were held constant in all systems [1].

Materials

For this study, the POSS-MA monomer was purchased from Hybrid Plastics (Fountain Valley, CA). The base monomer Bis-GMA was supplied by Freeman Chemicals (Port Washington, WI), and the TEGDMA monomer was purchased from Aldrich Chemical Company (Milwaukee, WI). For the photo-initiation process, both CQ and 4EDMAB were also purchased from Aldrich Chemical Company. The filler used in this study was a finely milled silanated barium oxide glass powder, provided by L.D. Caulk/Dentsply Company (Milford, DE) [1].

Formulations

The initiator and co-initiator used were CQ and 4EDMAB. These compounds were added to each resin system in order to allow for visible light curing. The mass fraction of CQ and 4EDMAB used was 0.2% and 0.8%, respectively. A mass fraction of 70% of silanated fine milled barium glass powder was used; whereas the filler level for mercury dilatometer measurements (discussed later) was 77.5%, due to the fact that the filled paste at 70% is inadequate in viscosity to be evaluated by mercury dilatometer [1].

Volumetric shrinkage

A mercury dilatometer produced by the Paffenbarger Research Center of the American Dental Association Foundation (Gaithersburg, MD) was used to measure the volumetric shrinkage from the photopolymerization process. The dilatometer can be described as a mercury-filled tube that has one end clamped against a glass slide, allowing dental composites

to polymerize once initiation has begun. This procedure is measured using a visible light-curing unit (MAX100, L.D. Caulk Company, Milford DE). A linear variable differential transducer (LVDT), joined to a Teflon plunger, sits on top of the mercury column and is used to measure the volume change of mercury. In order to account for the change in volume from temperature fluctuations, a thermistor is used to record the temperature of mercury. A computer is used to measure the output of the thermistor and LVDT, where the volume changes of mercury are corrected for any temperature fluctuations by means of a least-squares algorithm method [1].

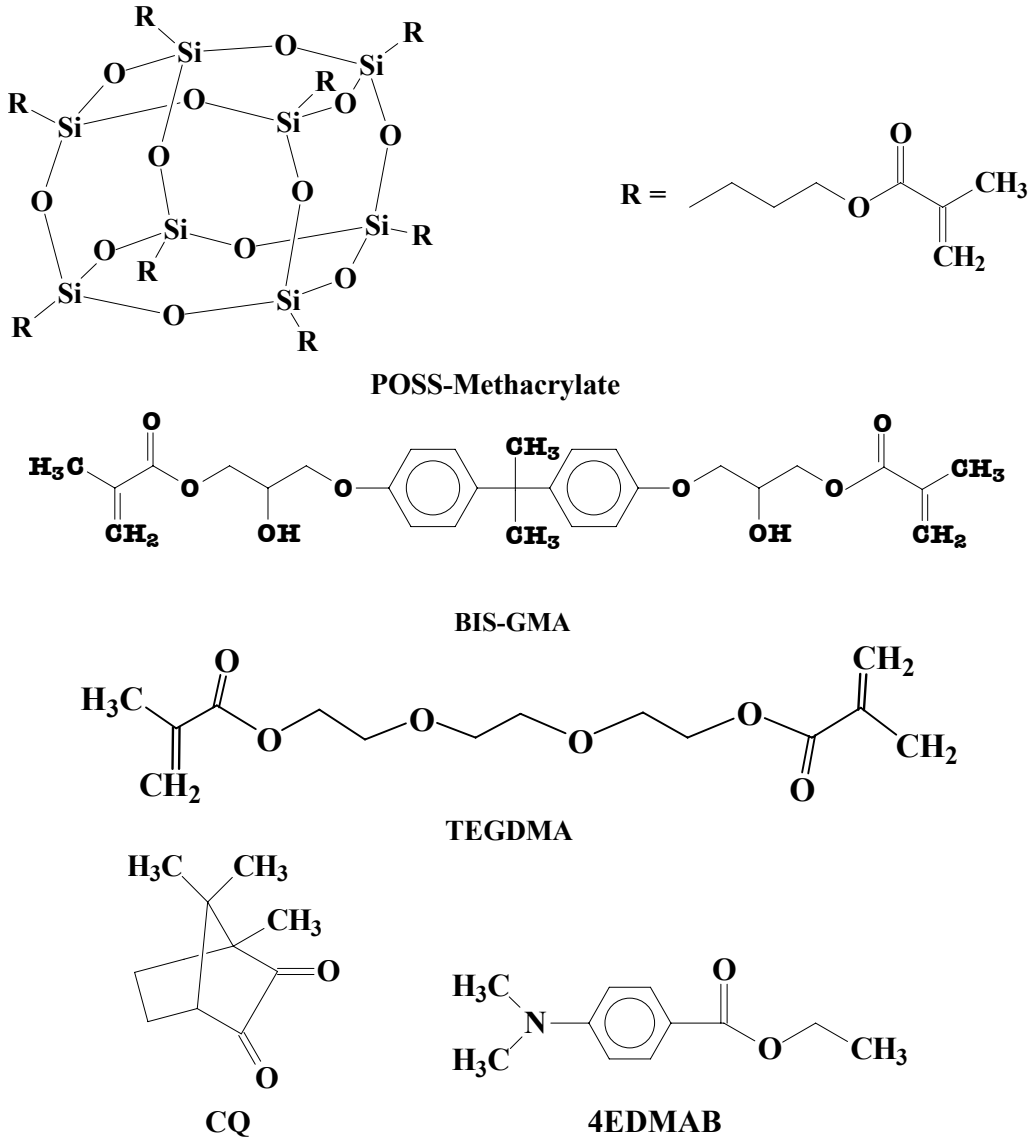


Figure 1. Molecular structures of the monomers and photo-initiation system [1].

X-ray characterization

Small Angle X-ray Scattering (SAXS) was performed at the National Synchrotron Light Source, Brookhaven National Laboratory. In this portion of the study, the samples used were cured resins without fillers. These samples were cured and stored at room temperature and ambient conditions for one week before characterization were conducted. Defined by a double multi-layer monochromator, the wavelength of the incident X-rays was 0.1366 nm. The synchrotron X-rays (600 μm beam size) were collimated using a three pinhole collimator. The scattering angle was standardized, by using silver behenate and the data acquisition times were set at 60 s. A rotating anode X-ray generator (Rigaku Dmax-2200 diffractometer, 40 kV, 40 mA, Rigaku/MA, USA), with Cu K_α radiation (λ = 0.154 nm) was used to carry out Wide Angle X-ray Scattering (WAXS) measurements [1].

Double bond conversion

Near infrared (NIR) spectroscopy was used to analyze the degree of methacrylate double bond conversion, as well as the photopolymerization rate of POSS resins. This technique determines the conversion of double bonds in methacrylate resins by following the overtone of that =C-H band near 6167 cm^{-1}. Fourier transfer infrared (FTIR) was used to conduct this experiment. The spectrometer used was a Nicolet Magna-IR™ 550 FTIR (Nicolet Inc., Madison, WI) and was purged with dry air [1].

Mechanical properties

The main purpose was to improve three mechanical properties associated with dental composites: flexural strength (S_F), elastic modulus (E_Y), and diametral tensile strength (DTS). Both FS and E_Y were conducted using the standard three point bending test method (ASTM F417-78), whereas DTS of the composites was tested using a standard DTS test method (ADA Specification No. 27). Six specimens with length of ~25 mm, width and thickness of 2 mm (for the three point bending test), and diameter of 6.3 mm and thickness of 3 mm (DTS test) were prepared for each of the composite systems through photo-curing the specimens for 1 min on each side. Each specimen was tested on a computer-controlled universal testing machine (model 5500R, Instron Corp., Canton, MA), with a crosshead speed of 0.5 mm/min for S_F and E_Y, and 10 mm/min for DTS. Each mechanical property can be calculated using the following equations:

(1) $S_F = 3PL/2WT^2$
(2) $E_Y = (P/d)(L^3/4WT^3)$
(3) $DTS = 2P/\pi DT$

Where P corresponds to the load fracture, L is the distance between two supports (set at 20 mm), W represents the width of the sample, T is the thickness of the sample, D is the diameter, d signifies the deflection (in millimeters) at load P [1].

Table 2. Volumetric shrinkage (%) of the composite systems. Each sample was filled with a mass fraction of 77.5% fine milled silanated barium glass powder during the mercury dilatometer measurements [1]

Samples	1	2	3	4	Average	SD
POSS1	3.68	3.89	3.67	3.45	3.67	0.18
POSS2	3.57	3.92	3.27	3.43	3.55	0.27
POSS3	4.09	3.40	3.92	4.05	3.86	0.32
POSS4	4.20	4.22	4.38	3.78	4.15	0.26
POSS5	3.97	3.95	4.21	3.71	3.96	0.20

Results and Discussion

Volumetric shrinkage

Volumetric shrinkage coincides with the methacrylate double bond conversion, and with the degree of conversion, cured composite properties can experience dramatic changes [17]. Higher double bond conversion increases the mechanical strength. However, increased conversion and increased shrinkage produce unwanted results like resin-filler debonding, internal stress development, and reduced marginal adaptation in dental restorations. In order to have an ideal composite, polymeric dental composite material should include both low volumetric shrinkage, and high double bond conversion.

The POSS-MA samples were measured for volumetric shrinkage. The results were promising, showing very little volumetric shrinkage for both partial and full substitution of Bis-GMA with POSS-MA, considering typical resins experience a 6 to 10% volumetric shrinkage [18]. All the samples had statistically the percent volumetric shrinkage. These results are encouraging, suggesting that the POSS-MA with 8, 10 or 12 polymerizable methacrylate double bonds did not promote large volumetric shrinkage.

X-ray characterization

In order to explore whether or not the POSS components were isolated in the composites, a technique known as small angle X-ray scattering (SAXS) was used (Figure 2a). The abscissa unit "q" represents the scattering vector, which can be calculated by using the equation $q = 2\pi\sin(2\theta/\lambda)$, where 2θ is the scattering angle. Figure 2a shows that as q increases, the intensity levels off suggesting that the three monomers were well mixed in all five samples and a homogenous network was developed. Also, the intensity increases in relation to the amount of POSS added, which can be explained by the POSS particles scattering creating a higher intensity compares to that of the polymer part. A second technique, known as wide angle x-ray scattering (WAXS) was used to show that all POSS composites had no definite form (Figure 2b). The POSS crystal peaks could not be identified from Figure 2b, even when there was complete substitution of Bis-GMA (POSS1). This proves that POSS-MA was not isolated in the composite resins [1].

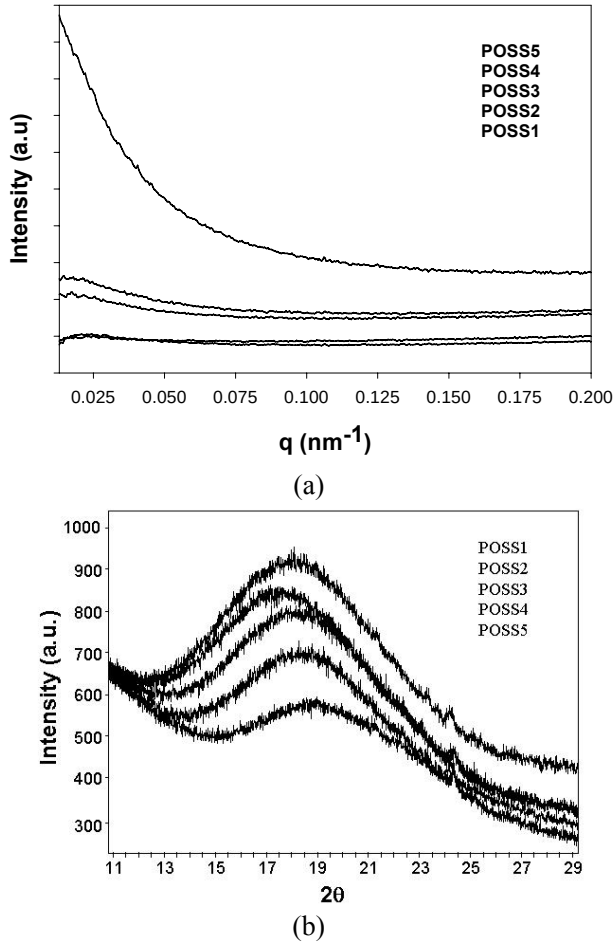


Figure 2. SAXS (a) and WAXS (b) profiles of the POSS resins [1].

Real time near infrared (RT-NIR) study

The NIR spectrum consists of fundamental bands such as combination and overtone bands that absorb in the mid-IR spectrum region. Most bands are too weak to record in the NIR region; with the exception of C-H, O-H and N-H. The =C-H band can be found in the wavenumber region of 4743 cm^{-1}, with its first overtone at 6167 cm^{-1}. Since 4743 cm^{-1} is found in the combination region, the baseline dramatically drops in the vicinity, making it difficult to measure peak areas. As a result, the overtone band at 6167 cm^{-1} was used for determining the degree of methacrylate double bond conversion; even though its absorptivity was lower than the 4743 cm^{-1} combination band. The left arrow "A" found in Figure 3, shows the decrease in intensity of =C-H absorbance as a result of polymerization. The middle arrow "B" shows the water band. During photo curing, it was noted that water peaks shifted approximately 20 cm^{-1} to higher wavenumbers and once photo curing was complete, water shifted back to its normal position. This is due to the increase in temperature from polymerization; the released heat, weakened the hydrogen bonds, causing water to exist as free water (5250 cm^{-1}) instead of the normal hydrogen bonded water (5200 cm^{-1}) [19].

Finally, arrow "C" located at 4620 cm^{-1}, shows the absorption of the phenyl group found in Bis-GMA.

The methacrylate double bond section of Figure 3 (arrow "A") was enlarged (Figure 4) to indicate the differences in absorbance of =C-H as photopolymerization progresses. Once the curing light was turned on, it was noted that the absorbance dropped dramatically during the first 30 seconds, and then continued at the same level for the extent of time. This observation concludes that the majority of methacrylate double bonds were in fact polymerized directly after photo initiation [1].

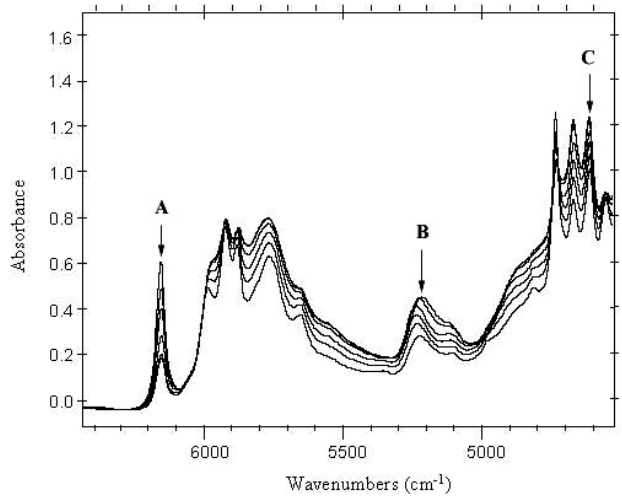


Figure 3. Real time NIR spectra of POSS3 resin irradiated for 1 min. Shown are the spectra at different collection times. From top to bottom, the spectra were linked at 0.512 (immediately before the curing light was turned on), 0.605, 0.636, 0.682, 0.993, and 4.981 min, respectively [1].

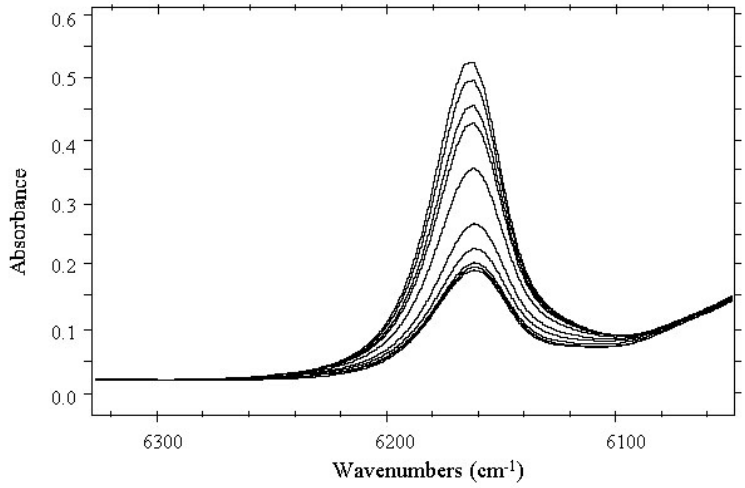


Figure 4. The magnified methacrylate double bond region in the real time NIR spectra of POSS3 resin subjected to irradiation for 1 min. Spectra were collected at different times, from top to bottom, 0.512 (immediately before the curing light was turned on), 0.558, 0.589, 0.605, 0.636, 0.682, 0.745, 0.993, 1.505, and 4.981 min, respectively [1].

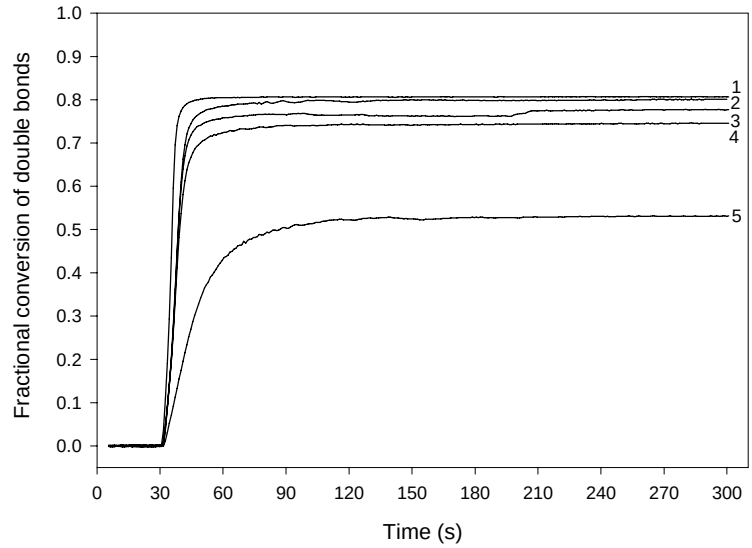


Figure 5. NIR cure profiles showing the extent of methacrylate double bond conversion versus time for POSS resins. (1) POSS1, (2) POSS2, (3) POSS3, (4) POSS4 and (5) POSS5 [1].

Integration was performed on the peak in Figure 4 in order to acquire NIR curing profiles. By using the absorbance of the methacrylate double bonds before curing as references, the degrees of methacrylate double bond conversion were acquired from conversion of NIR cure profiles (Figure 5). The results showed that the replacement of Bis-GMA with POSS-MA changed both the methacrylate double bond conversion and the photo-polymerization rate. It is evident that as the POSS content increases, the photopolymerization rate decreases and the double bond conversion is slower. POSS1 containing only Bis-GMA and TEGDMA and no POSS-MA had methacrylate double bond conversion as high as 81%. Substitution ratios of 2, 10 and 25% Bis-GMA with POSS-MA (POSS2, 3 and 4) were all found to have a reduced double bond conversion rate at around 75%. Finally, the complete replacement of Bis-GMA with POSS-MA (POSS5) shows the reduced double bond conversion to be significantly lower at 53% [1].

Mechanical tests

The main purpose was to test the flexural strength (S_F), elastic modulus (E_Y) and diametral tensile strength (DTS) of the POSS composites (Figure 6). The numbers found inside each column represent the mass fraction of POSS-MA in that sample. The letters in the columns represent any significant differences compared to the other columns. The asterisk signifies that the means are different compared to the control (POSS1). Having small amounts of POSS in the composite (10% or less) gave reasonable increases in S_F when the POSS content was increased. However, once the POSS content reached a certain level (25% or higher) the mechanical were found to drastically decrease as shown in Figure 6a. Figure 6b shows the E_Y of the five samples. There is a similar trend when dealing strength, except POSS2 displayed the highest E_Y with steady decrease as the POSS content increases. Finally, Figure 6c shows the DTS of the five samples. The trend was found to be somewhat different compared to the previous two the S_F and E_Y. The first four samples (POSS1, 2, 3 and 4) showed similar results with POSS3 having a slight advantage. Once the Bis-GMA was

completely replaced with POSS-MA, the DTS dropped substantially compared to the other four samples. The reason for the dramatic decrease can be explained by the high mass fraction of POSS-MA, which causes a higher inorganic content in the resin matrix. This results in a matrix that is fairly brittle, causing the DTS to decrease. Also, having large amounts of POSS-MA in a composite causes over-crosslinking, this is due to the 8, 10 or 12 polymerizable methacrylate groups. Over-crosslinking is a similar reaction to that of the vulcanization of rubber, which causes composites to be brittle and fairly weak [1].

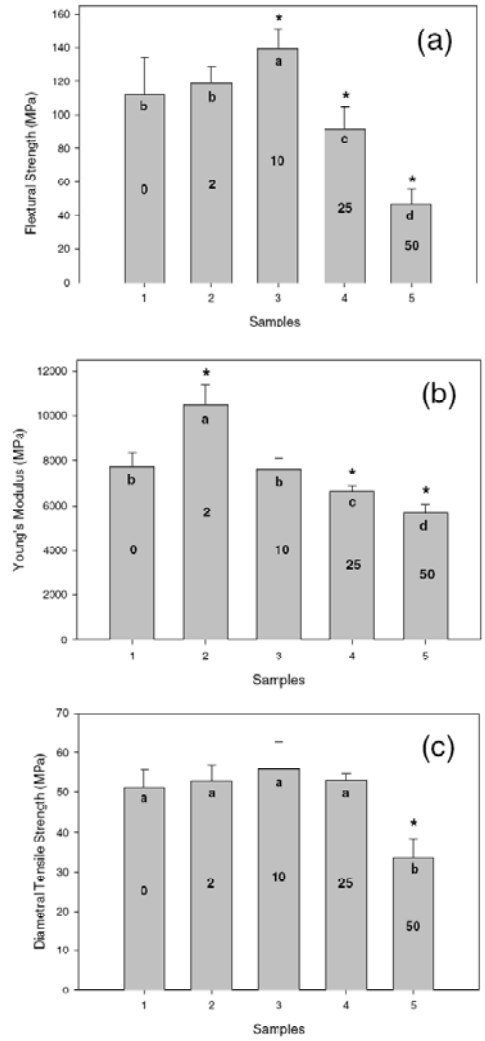


Figure 6. Mechanical properties: (a) flexural strength, (b) Young's modulus and (c) diametral tensile strength, of POSS composites. In each bar diagram the numbers on the abscissa indicate the respective POSS mixture (1=POSS1, 2=POSS2, 3=POSS3, 4=POSS4 and 5=POSS5). The numbers inside each column show the mass fraction of POSS-MA in that sample. One-way ANOVA showed that the groups are significantly different $P<0.001$ (Different letters indicate significant differences in an all-pairwise multiple comparison procedure, Holm-Sidak method, $\alpha=0.05$). The asterisk (*) indicates that the means are different from the control, POSS1 (Multiple comparison vs. a control group, Holm-Sidak method, $\alpha=0.05$) [1].

Fabrication and Evaluation of Bis-GMA/TEGDMA Dental Resins/ Composites Containing Nano Fibrillar Silicate

As mentioned earlier, the mass fraction of glass or ceramic fillers constitute 70-75% of dental composites. Exploration for new age fillers that can account for such problems as high volumetric shrinkage from polymerization, high water absorption and plasticization, and low elastic modulus of the resin is underway. Fibrillar silicate (FS) is made from nano-scaled single crystals (fibers) and is in a class of hydrated magnesium/aluminum silicate. The most common type of FS is attapulgite/palygorskite, which is most abundant in the United States and China. The FS used during this experiment was attapulgite and was purchased from China. Silicate single crystals are the main structural units of FS and are 100-3000 nm in length and 10-25 nm in diameter; these single crystals stack/agglomerate into particles with their sizes ranging in the microns [20,21]. The benefits of these FS fillers are that their nano-scaled single crystals contain a high degree of structural perfection and superior mechanical properties. Another important factor in the study of FS fillers is that it is fairly easy to separate into nano-scaled single crystals and to uniformly disperse throughout dental matrices. This is due to the larger spacing of the aggregated single crystals in FS when compared to the silicate layers in montmorillonite. Hence, even if metal ions in FS are not chemically substituted with surfactants like tertiary amine ions, they can be separated into nano-scaled single crystals by diffusing FS agglomerates/particles in polar solvents such as ethanol, followed by mechanical stirring at a rapid pace [21]. The purpose of this study was to explore the reinforcements of Bis-GMA/TEGDMA resins (without conventional glass fillers) and composites (with conventional glass filler) with different mass fractions of nano-FS. It is believed that by evenly distributing the silanized FS nano-scaled single crystals (fibers) into Bis-GMA/TEGDMA dental resins/composites will result in substantial improvement of the mechanical properties (flexural strength, elastic modulus and work-of-fracture).

Fabrication

Materials

The materials used for this study are as followed: Bis-GMA, TEGDMA, CQ, 4EDMAB, 3-methacryloxypropyltrimethoxy (MPTMS, a coupling agent), *n*-propylamine and (anhydrous) ethanol, all of which were obtained Sigma-Aldrich Co. (Milwaukee, WI). Also, purified FS powder (1250 mesh, white/gray in color) was purchased from Dalian Global Mineral Co. (Dalian, China). A finely milled 7% (mass fraction) silanized barium borosilicate glass powder (V-117-2707) was the conventional glass filler used in this experiment and was purchased from Esstech Co. (Essington, PA) [4].

Separation and silanization of FS

The FS (powder) was first added in ethanol that had a mass fraction of 5%, the suspension was mixed thoroughly for 4 hrs at a rate of 400rpm using a Heidolph RZR 50

Heavy Duty Stirrer. The suspension was then placed into a rotary evaporator containing MPTMS with a mass fraction of 10% to FS and *n*-propylamine with a mass fraction of 5% to FS. Finally, the mixture was then heated to 90 °C until it was dry [4].

Dispersion of silanized fs into dental matrix

Different quantities of silanized FS with mass fractions varying from 0 to 7.5% were placed in the dental resin systems. The systems consisted of 49.5% Bis-GMA, 49.5% TEGDMA, 0.2% CQ and 0.8% 4EDMAB. A total of three dispersion methods were examined in order to find the optimal method to distributing the silanized FS as highly separated nano-scaled single crystals into the dental matrices. In addition, the systems of Bis-GMA/TEGDMA/FS were combined with conventional glass fillers (mass fraction of 50% to Bis-GMA/TEGDMA) in order to prepare the dental pastes [4].

Method A: The first method involves placing silanized FS into neat TEDGMA, followed by vigorous stirring for 2 hrs at 400 rpm using a Heidolph RZR 50 Heavy Duty Stirrer. Next, Bis-GMA, CQ, and 4EDMAB was added followed by mechanically stirring the suspension for an additional 30 min at 400 rpm.

Method B: This method deals with placing silanized FS into a mass ratio of 10/90 of TEGDMA/ethanol respectively. The mixture was then mechanically stirred at a vigorous rate of 400 rpm for 2 hrs. Subsequently, vacuum evaporation was used to remove the ethanol from the mixture. Finally, Bis-GMA, CQ, and 4EDMAB were placed in the mixture, followed by mechanical stirring for an additional 30 min at 400 rpm.

Method C: The last method has silanized FS being placed in a 90% (mass fraction) ethanol diluted dental resin system. The mixture was then mechanically stirred for 2 hrs at 400 rpm. Finally, vacuum evaporation was used to remove the ethanol from the system, followed by 30 min of mechanically stirring the mixture at 400 rpm.

Characterization and Evaluation

Photopolymerization Kinetics

The study of photopolymerization kinetics of Bis-GMA/TEGDMA dental resins containing different mass fractions of silanized FS was conducted using real time near infrared spectroscopy (RT-NIR). The spectrometer used was a Bruker Tensor-27 FT-IR spectrometer with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. The absorption band of the vinyl double bond (6100-6250 cm^{-1}) was studied *in situ* by RT-NIR for 5 min at ambient conditions [4].

Mechanical properties

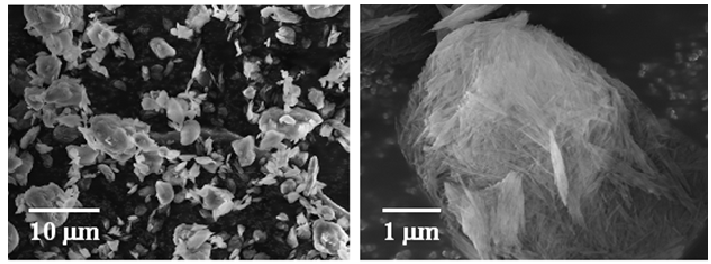
The samples were tested using a standard three-point flexural test (ASTM D 793) that had a span of 20 mm at a crosshead speed of 0.5 mm/min using a computer-controlled universal mechanical testing machine (QTEST™/10, MTS Systems Co., USA). A commercial software of Analysis of variance (ANOVA) (Winks, TexaSoft, Cedar Hill, TX), was used to perform the statistical analysis of the acquired data of flexural strength, elastic

modulus, and work-of-fracture. Work-of-fracture is defined as the energy required in order to fracture a sample [4].

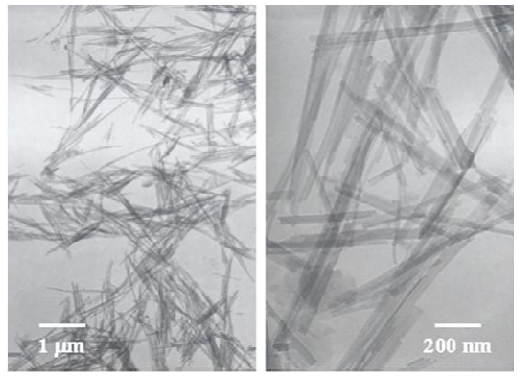
Results and Discussion

Separation and silanization of fs

Several impurities were found in the naturally occurring FS minerals, including silica and carbonates. All impurities were removed from the as-received FS (powder). The FS agglomerates/particles consisted of nano-scaled single crystals (fibers) which had diameters in the tens of nanometers and lengths in microns; as evident in Figure 7a, containing SEM images of the as-received FS. A few FS nano-scaled single crystals were found to be stacked together in bundles, and then the fibers/bundles further aggregated into agglomerates ranging from submicrons to several microns in size. The silanization step was performed (as described above in the Fabrication section) on the as-received FS (powder). TEM images of the silanized and separated FS nano-scaled single crystals (fibers) can be found in Figure 7b. These TEM samples were prepared by taking the silanized FS and dispersing it in ethanol (mass fraction of ~1%), which was then mechanically stirred using a Heidolph RZR 50 Heavy Duty Stirrer at a rate of 400 rpm for 30 min. Afterward, the carbon-coated TEM grids were set in the uniform suspension and were removed swiftly. The TEM grids evaporated once the ethanol in the suspension left and were then studied [4].



(a)



(b)

Figure 7. (a) Representative SEM images of the as-received FS powder. (b) TEM images of separated and silanized FS nano-scaled single crystals [5].

Dispersion of silanized fs into dental matrix

The three dispersion methods discussed in the Fabrication section can be seen in Figure 8 with the letters A, B and C representing methods A, B and C respectively. These images display the representative fracture surfaces of the photo-cured Bis-GMA/TEGDMA (50/50 mass ratio) dental resins that were filled with 2.5% (mass fraction) silanized FS. Figure 9 shows the three method samples after they were microtome for TEM examination. The silanized FS in the Bis-GMA/TEGDMA dental resin from method A were found to be agglomerates, as evident in Figures 8A and 9A. Small portions of the FS were observed as separated single crystals (seen in Figure 8A as small bright dots, with virtually no separated single crystals identified in the TEM images). This implies that the direct dispersion of the silanized FS in dental monomers does very little in separating FS into nano-scaled single crystals. A higher success rate of separation and dispersion were found in "Methods B" and "C", because the FS nano-scaled single crystals were easier to identify in the SEM/TEM images. These results imply that by using ethanol during the dispersion process, it is possible to improve the degree of separation and dispersion of the silanized FS. However, a few agglomerates were still found in Figure 9B and 9C. This could be due to the fact that the ethanol solutions with TEGDMA and/or Bis-GMA/TEGDMA could have difficulties separating the silanized FS compared to pure ethanol containing no TEGDMA and/or Bis-GMA/TEGDMA. In addition, once the ethanol is removed/evaporated, there is a possibility that some of the separated FS nano-scaled single crystals could re-aggregate into agglomerates. Additional studies are being performed in order to provide better dispersing methods/procedures. "Method C" showed the best potential when compared to "Method B" because a higher percentage of separated FS nano-scaled single crystals were present when looking at the SEM/TEM images (Figures 8B and 8C and Figure 3B and C). It was then determined that "Method C" was the best dispersion method for preparing the samples for photopolymerization kinetics and mechanical property measurements [4].

Photopolymerization kinetics

To determine whether or not Bis-GMA/TEGDMA dental resins containing nano-FS could be photo-cured, the kinetic process photopolymerization was performed. RT-NIR spectroscopic analysis was used to measure the rate of conversion of the vinyl double bonds in dental resins. This was done by following the overtone bands at 6100-6250 cm^{-1}. The use of this technique to study the photo-curing behaviors of dental methacrylate-based resins was demonstrated by Stansbury and Dickens [24]. After photo-curing for 30 s, a RT-NIR spectra was taken of the 2.5% (mass fraction) nano-FS filled Bis-GMA/TEGDMA dental resin (Figure 10a). The four lines from top to bottom represent the time the spectra were taken at 8 s (immediately before turning on the curing light), 12 s, 16 s, and 60 s respectively. The absorbance of the vinyl double bond varied in situ with photopolymerization, as indicated by the spectra. A dramatic drop was observed during the first 10 s (after the curing light was turned on). In order to collect NIR curing profiles, the absorbance peak from Figure 10a was integrated. The absorbance of the vinyl double bond before photopolymerization was used as a reference; these NIR curing files were then converted to the degrees of vinyl double bond conversion (Figure 10b). The tangent of the curves at each time spot in Figure 10b represents the photopolymerization rate at that specific time. All the systems of Bis-GMA/TEGDMA dental resins filled with different mass fractions (up to 7.5%) of nano-FS were found to be

photo-cured in under 15 s; therefore giving approximately a 90% vinyl double bond conversion rate (Figure 10a and 10b). There was virtually no change in vinyl double bond conversion or the polymerization rate when adding nano-FS into Bis-GMA/TEGDMA [4].

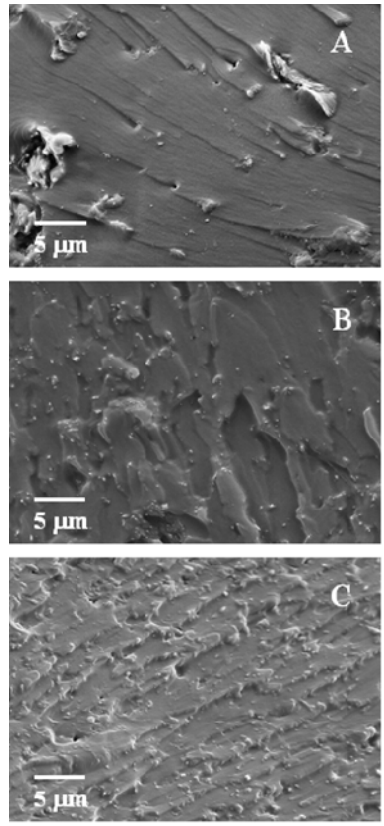


Figure 8. SEM images of representative fracture surfaces of 2.5% (mass fraction) nano-FS filled Bis-GMA/TEGDMA dental resins. The letters A, B and C represent different dispersion methods [5].

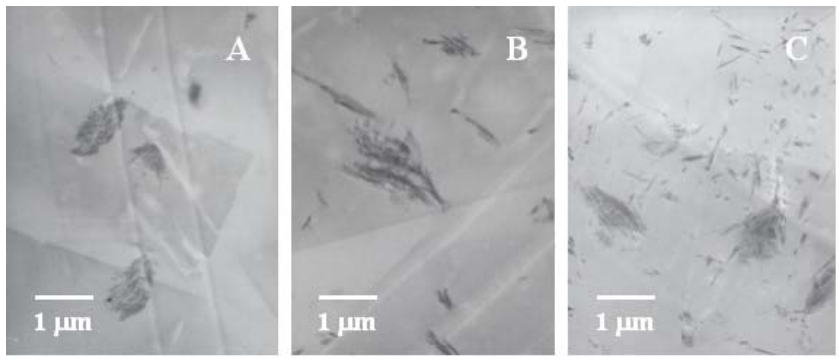


Figure 9. TEM images of 2.5% (mass fraction) nano-FS filled Bis-GMA/TEGDMA dental resins. The letters A, B and C represent different dispersion methods [5].

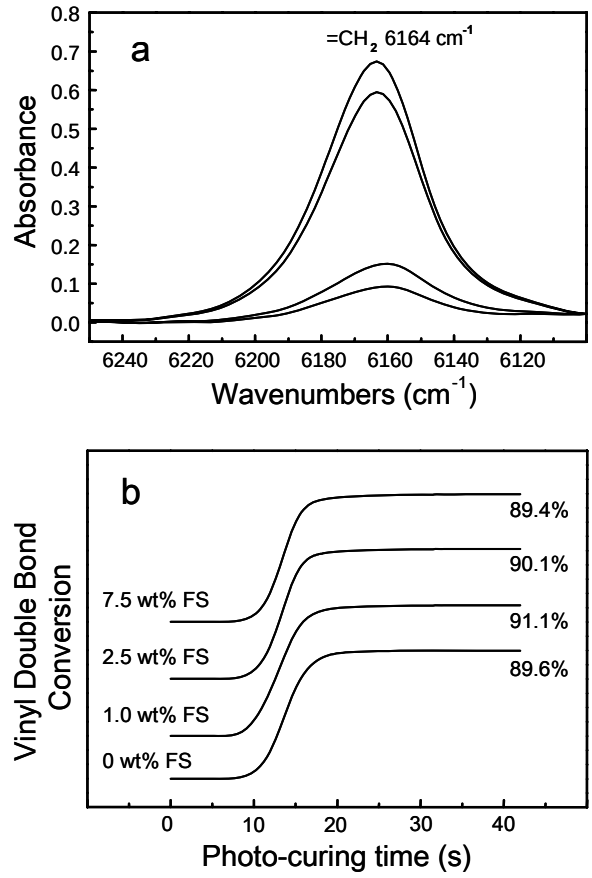


Figure 10. (a) RT-NIR spectra of 2.5% (mass fraction) nano-FS filled Bis-GMA/TEGDMA resin after photo-curing for 30 s. Spectra were collected at different times, from top to bottom, 8 s (immediately before the curing light was turned on), 12 s, 16 s, and 60 s, respectively. (b) RT-NIR photo-curing profiles of Bis-GMA/TEGDMA dental resins filled with various mass fractions of nano-FS. The profiles are offset for clarity [5].

Mechanical properties

Figure 11 displays the results of flexural strength (S_F), elastics modulus (E_Y) and work-of-fracture (WOF) of the Bis-GMA/TEGDMA (50/50 mass ratio) dental resins/composites with different mass fractions of the silanized nano-FS. The resins/composites that didn't contain any nano-FS were used as the control samples. A total of six measurements were taken with each datum in the plot representing the mean value and the error bars signify one standard deviation. The values of S_F, E_Y and WOF all showed significant improvements (Figure 11) from the substitution of nano-FS into the Bis-GMA/TEGDMA dental resins (without conventional glass fillers). The S_F, E_Y and WOF for the unfilled/neat resin (mean ± standard deviation, n = 6) were (90 ± 4) MPa, (1.8 ± 0.2) GPa and (5.1 ± 0.8) kJ/m^2, respectively. The resin filled with 1.0% (mass fraction) nano-FS resulted in S_F, E_Y, and WOF increasing to (126 ± 4) MPa, (2.1 ± 0.2) GPa, and (9.1 ± 1.0) kJ/m^2, respectively. These represent an increase in S_F by 40%, E_Y by 16.7%, and WOF by 78.4%. There was no further improvement of mechanical properties when increasing the mass fractions of the nano-FS. The resin containing 2.5% (mass fraction) nano-FS gave S_F, E_Y and WOF values of (128 ± 6)

MPa, (2.4 ± 0.4) GPa and (8.6 ± 1.1) kJ/m^2 respectively. The resin containing 7.5% (mass fraction) nano-FS resulted in S_F, E_Y and WOF values of (133 ± 7) MPa, (2.8 ± 0.3) GPa and (7.9 ± 1.1) kJ/m^2. There data show no significant improvement in S_F, E_Y, and WOF when impregnating silanized nano-FS into Bis-GMA/TEGDMA dental resins. Nonetheless, it is important to note that nano-FS needs to be highly separated into nano-scaled single crystals and evenly distributed into the dental resin. It is not plausible to obtain an effective reinforcement if the nano-FS existed primarily as agglomerates/particles, which operate as the mechanical weak points (structural defects) [4].

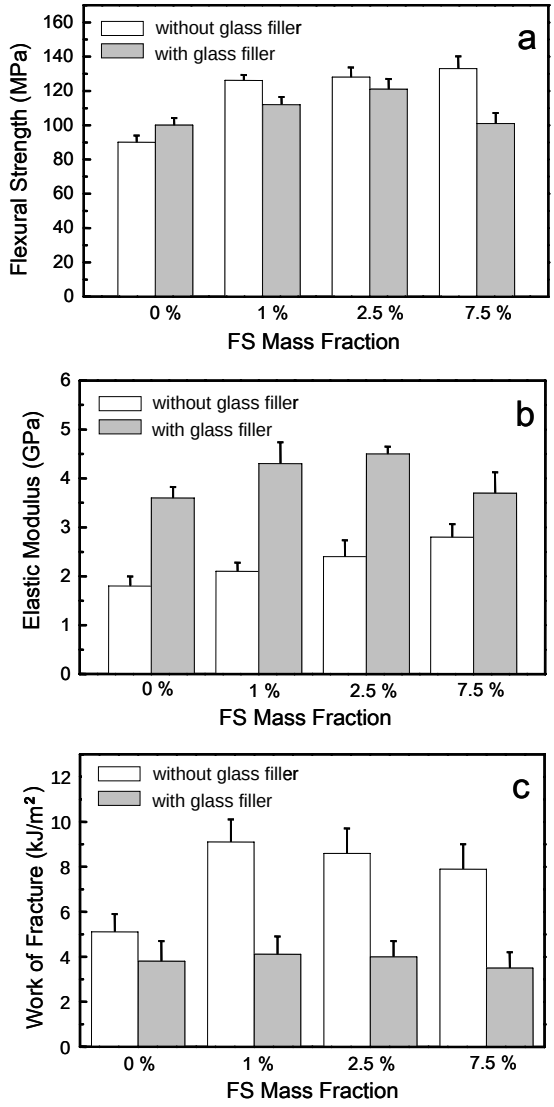


Figure 11. Mechanical properties: (a) flexural strength; (b) elastic modulus; and (c) work-of-fracture, of Bis-GMA/TEGDMA dental resins/composites filled with various mass fractions of nano-FS. Each datum is the mean value of six measurements with error bar representing one standard deviation [5].

The representative fracture surfaces of both neat and nano-FS reinforced Bis-GMA/TEGDMA dental resins can be seen in Figures 12a and 12b. The fracture surface for the neat resin in Figure 12a was smooth and displayed oriented fracture lines due to the extension of crazing, brought forth by the stress concentration points. In contrast, the fracture surface in Figure 12b shows that the nano-FS reinforced resin was rough, but exhibited no clear identifiable fracture lines. This implies that having nano-FS could deflect the micro-crack, thus improving the resistance to the applied force. Once the crack broke off from the nano-FS, it was observed that a rough fracture surface was formed; this suggests that there was energy consumption during the breaking process. In addition, several voids/holes were observed on the fracture surface of the nano-FS reinforced resin. The cause of these voids/holes is from the failure/fallout of the FS agglomerates/particles; thus weakening the filled resin. Two possible results are possible when substituting silanized nano-FS into Bis-GMA/TEGDMA dental resins: (1) high separation and distribution of nano-FS single crystals resulting in a reinforcement effect, and (2) a weakening effect from the formation of FS agglomerates/particles. By uniformly distributing nano-FS into the dental matrices as highly separated nano-scaled single crystals, it is believed that the mechanical properties would be significantly increased compared to the developed composites. The Bis-GMA/TEGDMA dental composites (with conventional glass fillers) that contained small mass fractions of silanized nano-FS gave similar results with improving mechanical properties. The S_F and E_Y from the composites with 1% and 2.5% (mass fractions) nano-FS, were found to be higher compared to the control sample. However, the composites containing 7.5% (mass fractions) silanized nano-FS gave S_F and E_Y values of (101 ± 6) MPa and (3.7 ± 0.4) GPa. These values were found to be virtually the same as the control sample. The WOF values showed no difference among the control sample and the samples containing 1%, 2.5% and 7.5% (mass fractions) nano-FS [4].

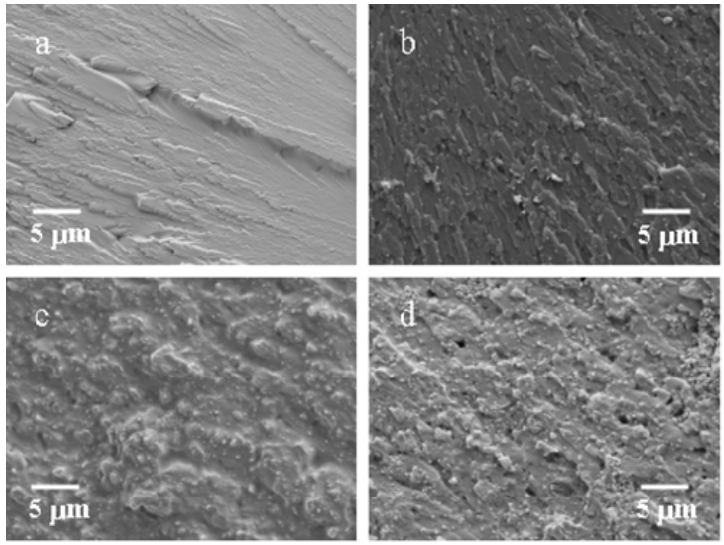


Figure 12. Representative fracture surfaces of three-point flexural specimens: (a) neat/unfilled Bis-GMA/TEGDMA; (b) Bis-GMA/TEGDMA filled with 2.5% (mass fraction) nano-FS; (c) Bis-GMA/TEGDMA filled with 50% (mass fraction) glass filler; (d) Bis-GMA/TEGDMA filled with 2.5% (mass fraction) nano-FS and 50% (mass fraction) glass filler [5].

Electrospun Nano-Scaled Glass Fiber Reinforcement of Bis-GMA/TEGDMA Dental Composites

Electrospun nano-scaled glass fibers are expected to perform more efficiently in the reinforcement of dental composites, compared to micron-scaled glass particles/fibers. Nano-scaled glass fibers contain preferred morphological and mechanical properties such as small fiber diameter, large aspect ratio, and high strength and modulus. If the dental matrix experiences a micro-crack under contact wear, the nano-scaled glass fibers remain intact across the crack planes in a process known as a "bridging" mechanism [22-24]; this in turn supports the applied load. Also, electrospun nano-scaled glass fibers are over 10-times thinner, compared to the micron-scaled glass fibers. The new nano-scaled glass fibers also contain surface silanol (Si-OH) groups which will react with silane coupling agents such as MPTMS. This creates a strong bond between the surface-silanized electrospun nano-scaled glass fiber filler and the dental resin matrix [6].

The process of electrospinning uses electric forces to drive the spinning process, producing fibers that have diameters ranging in the nanometers (~10-1000 nm). Compared to nanotubes, nanowires, and nanorods which are made from a bottom-up method, electrospinning nanofibers are produced through a top-down nano-manufacturing process. The benefits are that it is inexpensive; additionally, the produced continuous nanofibers are fairly easy to process into applications. Over the past few years, a variety of spin doping techniques have been studied. There are basically two main types of spin dopes: 1) aqueous spin dopes originating from alkoxide precursors like tetraethyl orthosilicate (TEOS), and 2) organic spin dopes with alkoxide precursors and carrying polymers like polyvinyl pyrrolidone (PVP). For both types of spin dopes used in electrospinning, the as-electrospun precursor nanofibers need to undergo a high temperature pyrolysis process to burn off and/or remove the organic components to finalize the fabrication of SiO_2 nanofibers [25].

Materials

TEOS, PVP (M_w = 1,300,000), DMF, HAc (glacial), Bis-GMA, TEGDMA, CQ, 4EDMAB, MPTMS, *n*-propylamine, acetone, cyclohexane, and glass powder (with particle sizes ranging from tens of nanometers to several microns) were all acquired from Aldrich Co. (Milwaukee, WI). The commercial dental glass filler used was a finely milled 7% (mass fraction) silanized barium borosilicate glass powder (V-117-2707) purchased from Esstech Co. (Essington, PA) [6].

Surface silanization and preparation of electrospun nano-scaled glass fibers

Initially, the prepared fibers were dispersed in cyclohexane with a mass fraction of 5%. Ultrasonic vibration was performed on the suspension using a 200 W Digital ultrasonic probe, provided by Branson Ultrasonic Corp. (Dansbury, CT). This process was performed three different times for 5 min each. The continuous nano-scaled glass fibers were converted into short fibers using ultrasonic vibrations. Once ultrasonic vibration was complete, the suspension was placed in a rotary evaporator containing MPTMS (mass fraction of 4% to the fibers) and *n*-propylamine (mass fraction of 2% to the fibers), followed by heating the system at 90 °C until dry. Surface silanization was also performed on the Aldrich glass powder for comparison as one of the two samples (the other being the as-received Esstech glass powder

which was already surface silanated). The electospun nano-scaled glass fibers were prepared by using a spin-dope containing 13% (mass fraction) TEOS and 13% PVP in a mixture solvent of DMF/HAc (volume ratio of 15/1) with a pyrolysis temperature set at 800 °C [6].

Preparation of dental composites

Two different dental composite pastes were prepared for this experiment: (1) for the first composite, a variety of mass fractions (0, 1, 2.5, 5, and 7.5%) of surface-silanized electrospun nano-scaled glass fibers were mixed with dental resin systems, consisting of 49.5% Bis-GMA, 49.5% TEGDMA, 0.2% CQ, and 0.8% 4EDMAB. The as-received Esstech glass powder and the surface-silanized Aldrich glass powder were prepared to use for comparison. (2) The second set of dental pastes was prepared by taking the mass fraction of the filler and maintaining it at 70%. The filler contained different mass fractions (0, 1, 2.5, 5, and 7.5%) of surface-silanized nano-scaled glass fibers and the as-received Esstech glass powder [6].

Mechanical properties

In order to test the fracturing strength of the specimens, a standard three-point flexural test (ASTM D 793) with a span of 20 mm and a crosshead speed of 0.5 mm/min was used (QTEST™/10 mechanical testing machine). S_F, E_Y, and WOF were the three mechanical properties tested in this study. The acquired data was statistically analyzed using ANOVA (Winks, TexaSoft, Cedar Hill, TX). The specimens were set in a humidifier at 37 °C for 24 hrs prior to mechanical testing. A total of six specimens were prepared for each measurement; the specimens were hand polished on all four sides with 2400 and 4000 grit silicon carbide paper and water coolant in a longitudinal direction [6].

Results and Discussion

The representative morphologies of the Aldrich glass powder (A), and the Esstech glass powder (B), and the electrospun nano-scaled glass fibers after ultrasonic vibration (C) can be seen in the SEM images of Figure 13. In order to prepare the SEM samples, the silanized Aldrich glass powder, the as-received Esstech glass powder, and the silanized nano-scaled glass fibers were initially isolated in acetone with a mass fraction of ~1%. Using a Heidolph RZR 50 heavy duty stirrer, the suspensions were mechanically stirred for a total of 30 min at 400 rpm. Afterward, small portions of aluminum foil were dipped in the suspensions and then quickly removed. This allowed for any acetone in the suspensions left on the aluminum foil to evaporate; the specimens were then used in SEM examinations. The particle sizes ranged from anywhere to the tens of nanometers all the way to several microns for both the Aldrich and Esstech glass powders. In contrast, the electrospun nano-scaled glass fibers were found to be very consistent with the diameter being ~500 nm and the aspect ratios larger than 100 [6].

Figure 14 shows the mechanical properties (S_F, E_Y, and WOF) for Bis-GMA/TEGDMA (1/1 mass ratio) dental composites containing different mass fractions (0, 1, 2.5, 5, and 7.5%) of silanized Aldrich glass powder (white bars), silanized electrospun nano-scaled glass fibers (gray bars) and the as-received Esstech glass powder (black bars). A total of six measurements were taken with each datum in the plot representing the mean value and the error bars signify one standard deviation. All three mechanical properties experienced higher values for composites reinforced with silanized electrospun nano-scaled glass fibers,

compared to the composites reinforced with silanized Aldrich glass powder. This information suggests that the fiber morphology gives better results on the composite reinforcement, compared to particle morphology; due to the Bridging effect. Still, the composites consisting of silanized fibers had S_F and WOF values that were basically the same as the composites containing the as-received Esstech glass powder (one-way ANOVA, $P > 0.05$). But, the E_Y values from the reinforced fiber composites were substantially higher compared to the Esstech glass powder reinforced composites (one-way ANOVA, $P < 0.05$). The composites mechanical properties (especially the strength) are strongly manipulated by the amount and distribution of structural defects. By including nano-scaled glass fibers into dental composites, there is a possibility that two opposite effects could occur: 1) the reinforcing effect from the "Bridge" mechanism and 2) weakening effect from the formation of structural defects. A way to improve the mechanical properties of the electrospun nano-scaled glass fiber reinforced composites further is by possibly optimizing the chemical composition and the surface treatment method of the fibers [6].

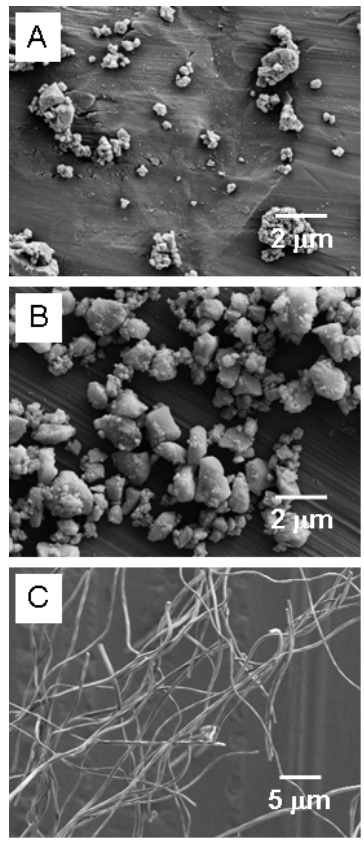


Figure 13. SEM images showing the representative morphologies of the Aldrich glass powder (a), the Esstech glass powder (b), and the electrospun nano-scaled glass fibers after ultrasonic vibration (c) [6].

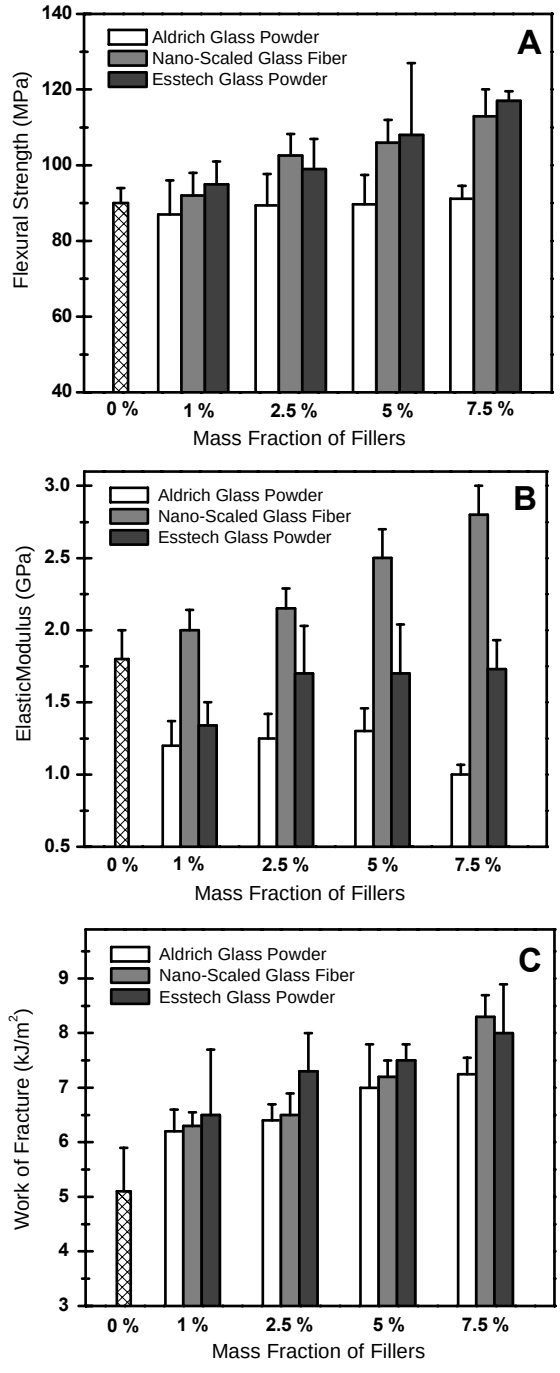


Figure 14. Mechanical properties including (a) flexural strength, (b) elastic modulus, and (c) work-of-fracture of Bis-GMA/TEGDMA (1/1 mass ratio) dental composites containing various mass fractions (0, 1, 2.5, 5, and 7.5%) of the silanized Aldrich glass powder (white bars), the silanized electrospun nano-scaled glass fibers (gray bars), and the as-received Esstech glass powder (black bars). Each datum is the mean value of the six measurements with the error bar representing one standard deviation [6].

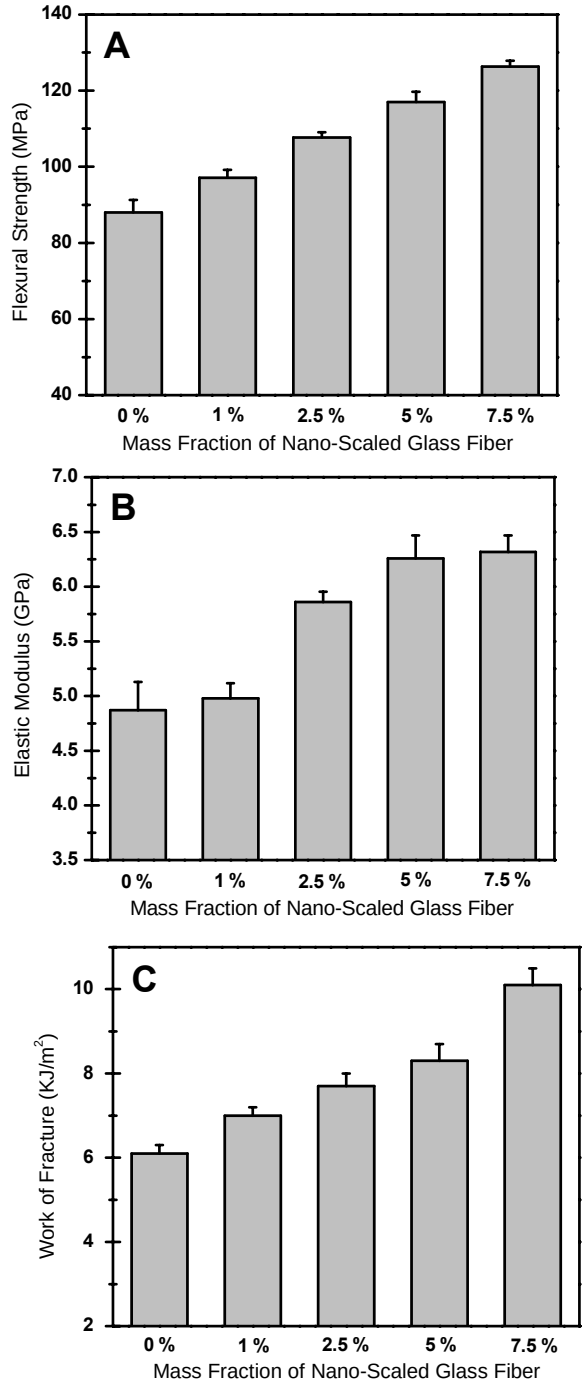


Figure 15. Mechanical properties including (a) flexural strength, (b) elastic modulus, and (c) work-of-fracture of the Bis-GMA/TEGDMA (1/1 mass ratio) dental composites (with the total filler mass fraction at 70%) containing various amounts (0, 1, 2.5, 5 and 7.5%) of the silanized electrospun nano-scaled glass fibers. Each datum is the mean value of six measurements with the error bar representing one standard deviation [6].

The results for the three mechanical properties of Bis-GMA/TEGDMA (1/1 mass ratio) dental composites are in Figure 15. These composites contain different mass fractions (0, 1, 2.5, 5, and 7.5%) of silanized electrospun nano-scaled glass fibers. The filler in the composites had a total mass fraction at 70%; still the filler consisted of a combination of silanized electrospun nano-scaled glass fibers and as-received Esstech glass powder. For instance, the sample containing the 2.5% fibers consisted of 2.5% silanized electrospun nano-scaled glass fibers, 67.5% as-received Esstech glass powder, and 30% Bis-GMA/TEGDMA. By replacing the Esstech glass powder with electrospun nano-scaled glass fibers in dental pastes, the viscosity was raised by a considerable amount. As indicated in Figure 15 the composites (containing a total filler level at 70%) mechanical property values were all increased when substituting small amounts of as-received Esstech glass powder with silanized electrospun nano-scaled glass fibers. In the case of the 7.5% fiber reinforced composite the values for S_F, E_Y and WOF (mean ± standard deviation, n=6) were (127 ± 2) MPa, (6.3 ± 0.2) GPa, and (10.1 ± 0.4) kJ/m^2 respectively. In comparison, the control sample (with 70% Esstech glass powder only) values were (88 ± 3) MPa, (4.9 ± 0.3) GPa, and (6.1 ± 0.2) kJ/m^2. The values improved for all three mechanical properties; with S_F increasing by 44%, E_Y improved by 29 % and WOF increased by 66% [6].

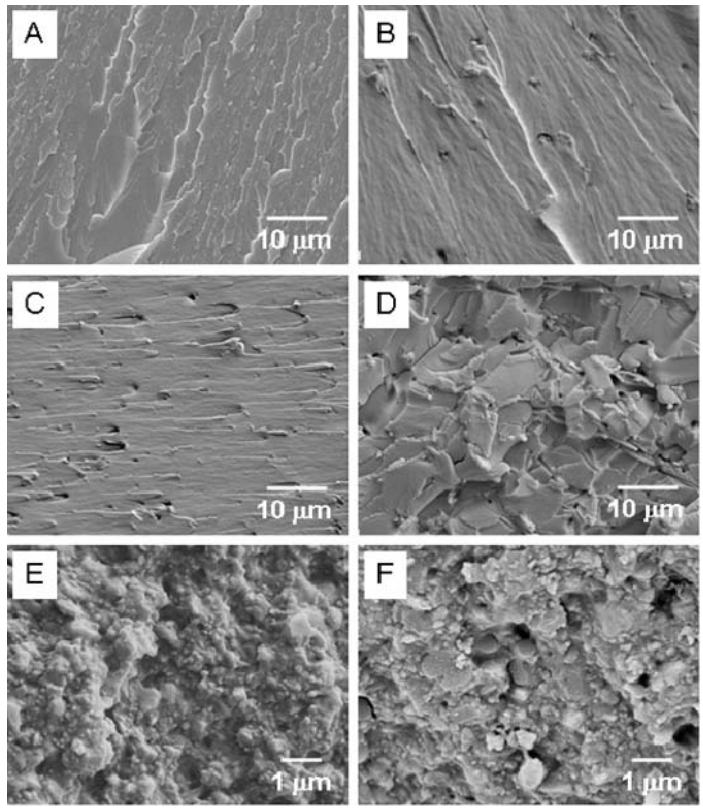


Figure 16. SEM images showing the representative fracture surfaces of the unfilled Bis-GMA/TEGDMA resin (a), the composites filled with 5% silanized Aldrich glass powder (b), as-received Esstech glass powder (c), and silanized electrospun nano-scaled glass fibers (d) as well as the composite containing 70% as-received Esstech glass powder (e), and the composite containing 65% as-received Esstech glass powder and 5% silanized electrospun nano-scaled glass fibers (f) [6].

Figure 16 displays SEM images (A-F) of representative fracture surfaces of unfilled Bis-GMA/TEGDMA resin "A", the composite filled with 5 % silanized Aldrich glass powder "B", the composite containing 5% as-received Esstech glass powder "C", the composite containing 5% silanized electrospun nano-scaled glass fibers "D", the composite filled with 70% of as-received Esstech glass powder "E", and the composite filled with 65% of the as-received Esstech glass powder and 5% silanized electrospun nano-scaled glass fibers "F". In Figure 16A, the unfilled Bis-GMA/TEGDMA fractured in a typical ductile resin manner. The surface of the fracture was smooth, where the fracture lines were found to be oriented, resulting from the extension of crazings set off by the stress concentration points. In contrast, the nano-scaled glass fiber reinforced composites fracture surface (Figure 16D) was rough and had no apparent identifiable fracture lines. This could be due to the fact that the presence of nano-scaled glass fibers could cause deflection of the micro-cracks, thus increasing any resistance to the applied force. Eventually the cracks broke away from the fibers, causing a rough surface on the composite, implying that energy was consumed during the breakage. There were also holes/voids examined on the fracture surfaces of the nano-scaled glass fiber reinforced resin (Figure 16D) and composite (Figure 16F). The voids/holes were caused by the separation of fibers, implying that the interfacial bonding strength can still be improved to attain higher mechanical strength [6].

CONCLUSION

Polyhedral oligomeric silsesquioxane methacrylate monomer (POSS-MA) was studied to determine whether or not it would be beneficial to replace 2,2'-bis-[4-(methacry loxypro poxy)-phenyl]-propane (Bis-GMA) with the POSS-MA monomer to create a novel dental restorative composite. The mechanical properties: flexural strength (S_F), Young's modulus (E_Y), and diametril tensile strength (DTS) were tested in addition to volumetric shrinkage, the degree of methacrylate double bond conversion and the photopolymerization rates. It was found that a small substitution (mass fraction of 10% or less in the resin system) of Bis-GMA with POSS-MA (methacryl-POSS cage mixture) will result in an increase in S_F and E_Y of the composite. However, substituting a large amount of Bis-GMA with POSS-MA (mass fraction of 25% or higher in the resin system) will lead to a decrease in mechanical properties as well as lower conversion of methacrylate double bonds and reduced polymerization rate. It was determined that the maximum S_F can be achieved when 10% (mass fraction) of POSS-MA is used in place of Bis-GMA, and the highest E_Y can reached when 2% (mass fraction) of POSS-MA is used in place of Bis-GMA [1].

Fibrillar silicate (FS) was studied on the basis of whether or not it could be used in the reinforcement of Bis-GMA/TEGDMA dental resins/composites with different mass fractions of nano-FS. It was determined that impregnating small amounts (1% and 2.5%) of silanized nano-FS into Bis-GMA/TEGDMA (50/50 mass ratio) dental resins/composites did indeed improve the mechanical properties (S_F, E_Y, and WOF) by a large amount. However, there was a limit to how much silanized nano-FS can be added to a system to improve its mechanical properties. Mass fraction of 7.5% did not further improve the mechanical properties (one way ANOVA, $P>0.05$) and even could have lowered the mechanical properties. Two opposite effects are possible when impregnating nano-FS into Bis-GMA/TEGDMA dental resins/composites: (1) highly separated and evenly distributed nano-FS single crystals could

cause a reinforcing effect or (2) a weakening effect can occur from the formation of FS agglomerates/particles. Simultaneous completion of uniform dispersion of nano-FS and high degree separation in dental resins/composites is being further studied. Still, because the addition of small mass fractions of nano-FS into dental resins/composites causes a significant increase in mechanical properties, nano-FS may play a role as reinforcing nanofiller for dental composites [4].

The reinforcement of Bis-GMA/TEGDMA dental composites with electrospun nano-scaled glass fibers resulted in the improvement of mechanical properties (S_F, E_Y, and WOF). Photo-cured Bis-GMA/TEGDMA dental composites containing different mass fractions of surface-silanized electrospun nano-scaled glass fibers were thoroughly formulated; a standard three point flexural method was used to measure the mechanical property values. To determine the statistical analysis from the acquired data, the program analysis of variance (ANOVA) was used. Results showed that substitutions of conventional dental filler with small mass fractions (1, 2.5, 5, and 7.5%) of surface silanized electrospun nano-scaled glass fibers dramatically increased the S_F, E_Y, and WOF values of 70% (mass fraction) filled composites by 44%, 29%, and 66% respectively. It is believed that the mechanical properties (especially the strength) of electrospun nano-scaled glass fiber reinforced composites could be enhanced even further by improving the chemical composition as well as the surface treatment of the fibers and fusion of SiO_2 nanoparticles (10-20 nm in size) to the surface of the fibers to obtain optimal physical interlocking among the fiber filler and dental resin matrix. Developing dental composites of the future could be achieved in part of the electrospun nano-scaled glass fibers, especially in large posterior restorations [6].

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Chapter 4

MAPPING THE STRUCTURE, COMPOSITION, PROPERTIES AND DENTAL EROSION IN HUMAN ENAMEL

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ABSTRACT

The structure-property relationship and dental erosion in human dental enamel composites is reviewed. The phase composition, microstructure and mechanical properties as characterized by grazing-incidence synchrotron radiation diffraction, atomic-force microscopy, scanning electron microscopy and Vickers indentation are described and discussed. The existence of distinct graded changes in crystal disorder, phase abundance, crystallite size and hardness within these enamel ceramics is highlighted. The phenomenon of load-dependent hardness in enamel but load-independent hardness in the dentine is highlighted and discussed. An in-situ monitoring technique of dental erosion in tooth enamel ceramics when immersed in soft-drinks is described. Atomic absorption results suggest that the increasing weight loss in tooth enamel during dental erosion in soft drinks can be attributed to the continuous leaching of Ca^{2+} ions, in addition to phosphorus, oxygen, and hydrogen. The effect of dental erosion on the hardness of enamel is also discussed

Keywords: Dental enamel; hydroxyapatite; synchrotron radiation diffraction; graded composition; dental erosion; Vickers indentation

1. INTRODUCTION

Biological materials such as seashell nacre, bones, macadamia nutshells and bamboo are composites that have superior mechanical efficiency in strength, hardness and toughness compared to many man-made composite materials. They exhibit many levels of

hierarchical structures from macroscopic to microscopic length scales, with the smallest building blocks in biological materials being generally designed at the nano-scale with nanometer-sized hard inclusions embedded in a soft protein matrix [1]. These biological composites display graded structures at several levels of hierarchy with length scales that range from micro- to nanometres [1, 2]. For instance, seashells have 2 to 3 orders of lamellar structure [3, 4] whilst bone has 7 orders of hierarchy [5, 6].

Teeth, like other natural biomaterials, are essentially inorganic/organic composites with enviable strength and damage resistant properties. In human teeth, the enamel comprises of ~96% calcium apatite, either as hydroxyapatite (HAP) ($Ca_{10}(PO_4)_6(OH)_2$) or fluorapatite ($Ca_{10}(PO_4)_6F_2$) [2-4]. Such a high mineral content ensures that teeth are the hardest and probably the strongest biological material within the human body. In both the adult and deciduous tooth, enamel is the outer structure that envelops the crown. It is almost fully mineralized with highly organized HAP crystallites, making it mechanically hard and highly resistant to wear. In general, the deciduous teeth are whiter, softer, smaller, and weaker compared to their permanent counterparts. In addition, their enamel is thinner and has a higher organic content. The microstructure of enamel is highly textured with aligned prisms or rods that run approximately perpendicular from the dentin-enamel-junction (DEJ) towards the tooth surface [5]. Each rod consists of tightly packed carbonated hydroxyapatite crystals that are covered by a nanometre-thin layer of enamelin and oriented along the rod axis. However, in deciduous teeth, the outer-most layer is generally devoid of the usual prism structure. It remains unknown whether the HAP crystals and enamel rods are similar in dimension and distribution for both types of teeth. In contrast, dentin is the supporting structure that lies underneath enamel and is primarily composed of ~68% HAP mineralized collagenous matrix surrounding tubular extensions of the dentinoblast cells. This less mineralized tissue provides the tooth with the toughness required to resist catastrophic fracture when subjected to masticatory stresses. The DEJ is the interface region bridging across the enamel and dentine and possesses the desirable capability of arresting crack propagation [6-9]. Hitherto, the influence of age on the structure-property relationships within both types of teeth has been poorly understood.

The purpose of teeth is mastication, the chewing of food. Therefore, teeth are vulnerable to erosion by consumption of acidic food and carbonated drinks. The annual consumption of soft drinks has increased more than five times since 1940s [10]. For instance, in 1947, people consumed approximately two cans of soft drinks per week. In 1997, people consumed approximately two cans per day. In Western countries, more than half of people's personal water requirements come from soft drink consumption. At the same time, scientists have also noticed a connection between soft drink consumption and dental health. Drinking soft drinks between meals was found to increase the number of dental decay and caries in a twenty-year longitudinal study [11]. As a result of an increasing consumption of soft drinks and commercial fruit juices, over the past years, the prevalence of dental erosion and decay has increased dramatically [12]. Acids from the soft drinks have been known to induce the dissolution or erosion of dental enamel as well as caries lesion due to the combined effect of bacteria and degradation of carbohydrates present in the drinks [13]. Therefore, there is a need to ascertain the relationships between exposure to soft-drinks and changes in the structure and mechanical properties of dental tissue.

This chapter reviews the factors affecting dental erosion as well as the structure-property relationships in human enamel. The similarities and differences in the microstructures, properties and dental erosion between adult and baby enamels are highlighted and discussed.

2. HUMAN ENAMEL: STRUCTURE AND COMPOSITION

Human enamel is the most highly mineralised and hardest biological tissue. It is comprised of approximately 96% mineral, 3% water, and 1% organic matter (non-collagenous protein) by weight [14, 15]. The mineral is non-stoichiometric calcium hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$) with carbonate, fluoride, sodium, and magnesium ions frequently found within the structure. These hydroxyapatite (HA) crystallites are laid down as nanorods with cross-sectional dimensions of 50 nm×25 nm and up to 1 mm long [16]. Clusters of these nanorods, known as prisms, contain around 1000 crystallites. They are approximately 5 μm in diameter and may be up to several millimetres long, and the majority are arranged with their long axes at approximately 90° to the enamel-dentine junction (EDJ). The orientation of prisms in enamel has been studied scanning electron microscopy which provide qualitative information on the degree of alignment in different parts of a tooth. X-ray diffraction studies on human enamel has established the space group and lattice parameters as $P6_3/m$ (hexagonal) and a=9.441(2) Å and c=6.878(1) Å respectively [17-19]. X-ray diffraction measurements have also indicated the existence of a higher degree of crystallite alignment in surface enamel compared to enamel close to the EDJ [5, 20].

Dentin and enamel have a more complex hierarchy with their nanostructure being described as staggered mineral platelets (hydroxyapatite, HAp) embedded within a collagen matrix [1]. The smallest dimension of mineral crystals in dentin and bone is on the order of several nanometers. Dentin or enamel is a calcified tissue somewhat similar to bone in which the collagen-rich organic matrix is reinforced by calcium phosphate mineral crystals [21]. At the level of individual mineralized fibrils, the mechanical properties of biomaterials will depend on the precise arrangement of mineral crystals within the fibrils. The orientation and organization of mineral crystals within the protein matrix of bone and dentin have been studied by Fratzl and co-workers [22, 23] using small angle X-ray scattering (SAXS) and by Landis and co-workers [24, 25] using electron microscopy (EM). These experiments have revealed the size and geometry of the basic building crystals of bone and dentin. The shape of the mineral crystals is found to be highly anisometric (platelets) with the anisometry being larger for bone, dentin (platelets 3 nm thick and up to 100 nm long) and enamel (particles 15–20 nm thick, 1000 nm long) than for nacre (platelets 200–500 nm thick and 5–8 μm long).

The size and geometry of the mineral crystals are thought to play important roles in the mechanical properties of biomaterials. For example, the arrangement of mineral platelets in preferred orientations produces intrinsically anisotropic biocomposites. These anisotropic properties facilitate further adaptation to the environmental loading in natural evolution. Stiffness, strength, toughness and hardness are very important in relation to the supporting and protecting functions of bone-like biocomposites. Whilst the protein phase is approximately 3 orders of magnitudes softer and weaker compared to the mineral, the stiffness and strength of biocomposites is not significantly reduced through the presence of protein [1]. Furthermore, since the protein matrix is relatively soft with little capability for the sustaining of an external load, the hard mineral phase must therefore be assumed to carry

most of the external load acting on the composite. However, the brittle mineral phase is very fragile and thus the protein, as the organic constituent in biomaterials, is assumed to play an essential role in achieving and maintaining a high degree of toughness. Qualitatively, the protein matrix behaves like a soft, surrounding layer which protects the mineral platelets from the peak stresses caused by the external load and homogenizes the stress distribution within the composite.

Furthermore, protein ia able to dissipate the dynamic fracture energy more effectively via its intrinsic viscoelastic properties [26] and its hierarchical and adhesive molecular structure [27]. Previous researchers have attempted to address the mechanism of high toughness in biocomposites from various points of view including their hierarchical structures [28-29], the mechanical properties of protein in dissipating fracture energy [27], protein–mineral interface roughness [28] and the reduction of stress concentration at a crack tip [30]. However, the co-relationship and complex interplay between mechanical properties and the nanostructure of biological materials are hitherto still poorly understood.

3. HUMAN ENAMEL: DENTAL EROSION

3.1. Effect of Soft Drinks

West *et al*. [31] found that between 1990 and 2000, 56% more soft drinks had been sold, and moreover that the number is increasing at 3% annually. In the past 50 years, soft drink consumption has increased more than five times [10]. In the 1940s, scientists started to notice a connection between soft drink consumption and dental health. Although initial studies with army recruits showed no relation, later studies were more conclusive [32]. Drinking soft drinks between meals was found to increase the number of dental caries in a twenty year longitudinal study. Also, there have been a large number of anecdotal stories linking soft drink consumption with dental decay. It seems the more acidic soft drinks cause more dental decay. Thus, lemonade is most lethal, followed by energy drinks and sports drinks, and then followed by supplemented water drinks, iced tea, and cola beverages. In a study by Hemingway and coworkers [33], cranberry juice was found to be the most harmful of all the soft drinks investigated. In a similar study, Lupi-Pegurier *et al*. [34] found that while fruit juices and carbonated drinks had an effect on dental health in a reasonably short amount of time, wine, appeared to have little erosive effect.

As a result of an increasing consumption of soft drinks and commercial fruit juices, over the past years, the prevalence of dental erosion seems to have increased [35]. Acids from dietary, environmental and gastric sources are known to induce the dissolution and erosion of dental enamel. When enamel dissolves, this dissolution causes either a caries lesion or erosion. Therefore, soft drink consumption can lead to both caries lesion from the degradation of carbohydrates, and erosion from acids. Dental erosion can lead to an irreversible loss of dental hard tissues due to a chemical process without the involvement of microorganisms, caused by either extrinsic or intrinsic agents. It is thus important to understand how soft drink consumption can lead to dental erosion.

3.2. Factors Affecting Dental Erosion

Several parameters are known to affect the severity of dental erosion. Firstly, the erosive medium itself may be highly abrasive or less abrasive. Secondly, the contact with the tooth is a factor. Frequency of contact and method of contact are influential. Thirdly, natural protective devices, such as salivary composition, flow rate, buffering capacity, pellicle formation, clearance rates, and unique dental anatomy and physiology affect the onset of erosion [33]. In addition, a range of associated behaviors also incite erosion in enamel. People who partake in these activities are said to be individuals at risk of dental erosion. Anorexia and bulimia as well as other conditions which involve frequent vomiting such as binge drinking or gastro-oesophagal reflux disease are prone to greater erosion [36]. Some occupations involve working with acidic industry fumes and even professional swimming can place people at risk of dental erosion as these settings have higher acidity. Consumption of illegal drugs such as ecstacy and cocaine also leads to erosion of dental tissue. However, the major factors affecting dental erosion are:

(a) Exposure time and frequency

The first is the frequency of soft drink exposure; the more the teeth are exposed to soft drinks, the more erosion is expected. The factor here is the exposure time. The longer the teeth are exposed to soft drink, the longer the teeth are interacting with the erosive acids and thus the greater weight loss in dental issue due to dissolution of calcium ions within the enamel [37]. In addition, soft drinks consumed on their own are more corrosive than soft drinks consumed with meals. This is because soft drinks consumed alone tend to stick on the surfaces of the teeth and are not washed off by the body mastication system including saliva.

(b) The pH of soft drinks

The acidity of the soft drink is considered to be the main factor affecting erosion [38]. However, there is controversy over how the acids affect the teeth material. Some researchers have said that when the pH level gets as low as 5.5 or less, erosion can occur particularly to the enamel parts of the teeth [32]. However, according to other researchers [39-40], it is not the pH of the solution, but actually the amount of acid, which makes the soft drink more or less erosive. Nonetheless, it is noticeable that enamel dissolution occurs more at a lower pH, a more acidic environment. While some of the acids result in more or less erosion at different levels, hydrochloric acid and phosphoric acid erode heavily at low pHs and then drop away quickly. In contrast, the erosive action of citric acid drops more steadily as the pH increases. Hydrochloric acid is highly erosive when it is strongly acidic and not erosive when it is slightly acidic. Citric acid in soft drinks erodes at almost the same amount from pH 2.0 to pH 6.0 which suggests that the nature of the acid is also important along with the pH.

The majority of the soft drinks contain two common acids, phosphoric acid and citric acid, although malic acid or tartaric acid can also be present. Phosphoric acid is very erosive at pH 2.5 but much less so at pH 3.3 which stresses the role of the amount of acid, rather than the pH. It should be noted that malic and tartaric acid are especially erosive because of their ability to chelate calcium at higher pH.

(c) The ability to chelate calcium

The ability to chelate calcium is an important factor in determining the extent of erosion. Certain acids such as citric acid which are commonly found in soft-drink due to their preservation abilities are chelating agents. Forming a chemical claw around a metal ion, chelating agents are able to quickly drain calcium ions from the teeth, thus changing the charge of metal ions and form new molecules.

While most soft drinks contain phosphoric, citric, malic, and tartaric acid, other organic acids may be present. If these acids are polybasic, they will have an ability to chelate calcium in environments which are not so acidic [10]. Another concern of the polybasic acids is that they are able to maintain a low pH or acidity of the soft drink.

(d) Temperature

Another influence is the effect of the temperature of the soft drinks which are being consumed. This is important because drinks are served at a range of temperatures in real life from iced-drinks to boiling drinks. Most commonly, it is accepted that the rate of any chemical reaction is faster as the temperature is increased. Therefore, it seems soft drinks at a higher temperature will be more erosive. Moreover, a solution of weak acid will attain a lower pH if the temperature is increased. Therefore, a higher temperature will result in more erosion. For instance, there was a 2.5 times increase in the amount of material loss in enamel and dentin samples when the temperature was increased from 5°C to 60°C [41]. Similarly, orange juice erodes enamel at 37°C twice as much than at 4°C. Hence, temperature has a strong influence on the amount of erosion.

4. EXPERIMENTAL PROCEDURE

4.1. Specimen Preparation

Thin slices of adult and baby human canine or molar teeth were used for the study. A precision diamond blade cutter was used to cut each tooth into two 1.0 mm thick slices that were either parallel ("occlusal-section") or perpendicular ("axial-section") to the occlusal surface. Prior to the diffraction measurement, the buccal and lingual sides were ground with SiC paper in order to obtain a plano parallel flat plate. Both atomic-force microscopy (AFM) and optical microscopy were used to reveal the surface topography and microstructure of polished and etched tooth samples. A Nikon optical microscope and a Digital Instrument Dimension 3000 scanning probe microscope were used for the imaging study. The AFM experiments were carried out in contact mode in air with a gold-coated cantilever and a tip of Si_3N_4, with a spring constant of 0.58 Nm^{-1}.

4.2. Synchrotron Radiation Diffraction (SRD)

Depth-profiling of the near-surface composition and texture of flat-plate specimens was conducted at the Photon Factory in Japan using grazing-incidence SRD. Imaging plates were used to record the diffraction patterns at a wavelength of 0.7 Å and grazing angles, α, of 0.2,

0.4, 0.8, 1.0, 3.0, 5.0 and 10.0°, with the depth of x-ray penetration into the sample depending on the value of α. In general, the depth of penetration, d, increases linearly with α [5], *i.e.*, $d = 2\alpha./\mu$, where μ is the linear attenuation coefficient of enamel. The degrees of cellulose crystallinity and abundance were calculated from the ratios of peak intensity and integrated intensity, respectively, for reflection (112).

4.3. Scanning Small-Angle X-ray Scattering Analyses

Scanning small angle x-ray scattering experiments was performed at room temperature on both adult tooth and baby tooth. Diamond blade-produced thin sections (~200-300 μm thick) of tooth specimens were mounted on a movable x-y sample holder (movement precision of 5 μm) at the SAXS instrument on Beamline 15-ID-D at the Advanced Photon Source in Chicago. Radiation of wavelength 1.0 Å was used and the distance from specimen to detector (CCD) was set so as to cover a q-range of 0.002 – 2.8 Å. Measurements was performed with the primary beam (beam diameter 100-200 μm) being either parallel or perpendicular to the fibre or plate direction of the mineral crystals and the scattering being measured along a direction normal to the layered planes in the microstructure. Mapping of the nanostructure within each sample was performed through both vertical and horizontal shifts using a high precision x-y sample stage at fine scanning steps of 10 μm.

4.4. Vickers Indentation

Both adult and baby tooth samples were used for the measurement of indentation responses. The teeth were cut either parallel or perpendicular to the occlusal surface using a precision diamond blade. The cut specimens were then cold mounted in epoxy resin and polished to a 1 μm surface finish. Indentation responses of polished samples as a function of: (a) load (2-100 N), (b) loading time (0-24 h), and (c) depth-profile were measured using a Zwick microhardness tester. Test (a) was designed to evaluate whether or not the hardness was load-dependent due to an indentation-size effect. The viscoelastic flow or creep and graded characteristics were evaluated in test (b) and (c), respectively. The diagonal lengths of the indent, $2a$, were used to calculate the hardness, determined here as $H_v = P/2a^2$, where P is the load. Values of fracture toughness, K_{1c}, were calculated as $K_{1c} = 0.025P/c^{1.5}$ where c is the average crack length in *mm*.

Vickers indentation was also used to investigate the effect of erosion by soft drinks on the hardness of enamel surface. Since the erosive process involves enamel dissolution, which is associated with a softening of the surface due to weakening of the enamel structure, measurements of hardness would provide a simple method of observing the early stages of enamel erosion.

4.5. In-situ Weight Loss Measurements of Dental Erosion in Soft Drinks

Clean and caries-free adult human molars and premolars were used for the study of dental erosion in soft drinks. Each tooth specimen was weighed to 0.1 mg on an electronic balance. The test beverages used for the erosion study were Mountain Dew, Diet Coke and Sprite Zero with pH values of 3.1, 3.2 and 3.3 respectively [10]. The experimental set-up for the in-situ weight loss measurements is shown in Figure 1. The set-up is very similar to the Archimedes method for the measurement of porosity and density in porous ceramic materials. The weight loss measurements were conducted both with and without stirring. The purpose of stirring with a magnetic rod at 300 revolutions per minute (rpm) was designed to simulate the action of turbulent flow during drinking in a mouth. A tooth sample was used continuously throughout each experiment by immersing it in a plastic container containing 200 mL of soft drink. A new tooth sample was used for each of the three soft drinks tested. Tap water was also used as a control.

The apparent weight change of tooth sample immersed in a soft drink was measured continuously using an electronic scale (Denver Instrument,AA-200) at 0.5 h intervals for the first 6 h and thereafter at 12–36 h intervals for a total of 7 days or 168 h. The stirring was stopped for 2 min to allow equilibration before the new apparent weight was read from the scale. This new technique avoids the error-prone step in the conventional method of removing the tooth sample from the drink, followed by drying before weighing it. This allows the weight change of tooth sample due to erosion by the soft drink to be continuously measured with good precision. In this technique, the apparent weight (W_a) of the tooth sample immersed in a solution is governed by the Archimedes principle as follows:

$$W_a(t) = W - B \tag{1}$$

where W is the weight of the sample in air and B is the buoyancy of the solution. Since the value of buoyancy remains the same or time-independent, the continuous change of the apparent weight is related to the change of the weight of the sample as follows:

$$W_a(t) = W(t) - B \tag{2}$$

Thus the weight loss of tooth enamel in wt % at a particular time (t) can be calculated as follows:

$$\text{Weight loss (wt\%)} = \frac{w_t - w_o}{w_o} \times 100 \tag{3}$$

where W_o is the initial apparent weight of tooth sample at the start of measurement and W_t is the apparent weight of tooth sample at a specific time, t.

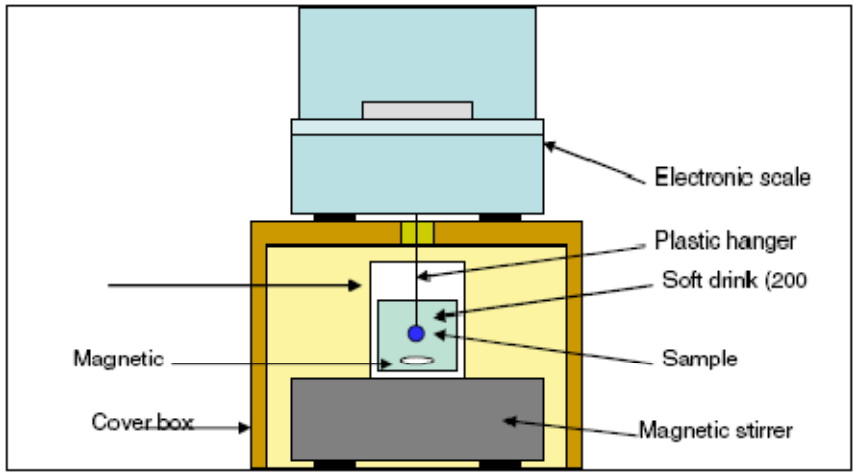


Figure 1. Schematic diagrams showing the experimental set-up for the in-situ measurement of weight loss in tooth enamel when immersed in a soft drink over 7 days.

4.6. Atomic Absorption Spectroscopy

The experimental set-up for the dental erosion of tooth enamel in Sprite Zero over 7 days or 168 h is shown in Figure 2. A tooth sample was used continuously throughout this experiment by immersing it in a plastic container containing 40 mL Sprite Zero. The concentration of Ca2+ leached out from the enamel was measured using atomic absorption spectroscopy at 0.5 h intervals for the first 3 h and thereafter at 12–36 hour intervals for a total of 7 days or 168 h. A fresh 40 mL of Sprite Zero was used for each time interval measurement. Calcium ions determination was performed using atomic absorption spectroscopy on a SpectrAA-50. Calcium standards of concentrations 10, 20, 40, 50, 100 and 150 mg/L were used to calibrate the instrument.

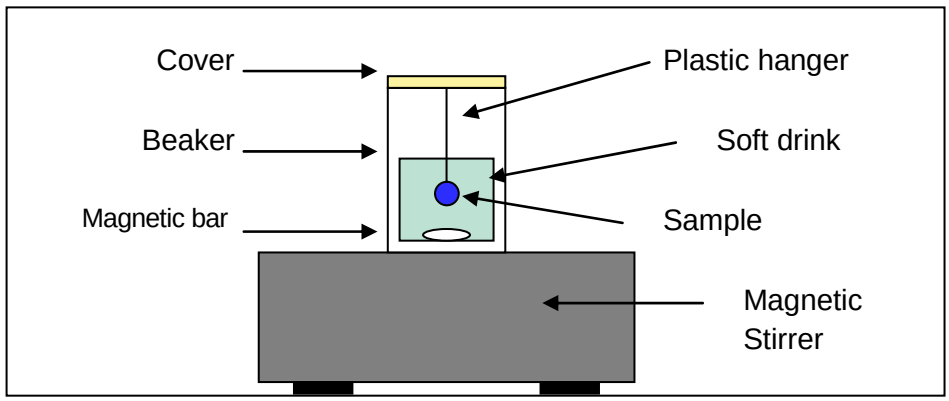


Figure 2. Schematic diagrams showing the experimental set-up for the measurement of dental erosion of tooth enamel in Sprite Zero for subsequent analysis of leached Ca2+ concentration by atomic absorption spectroscopy.

5. RESULTS AND DISCUSSION

5.1. Structural, Compositional and Microstructural Characteristics

Analysis of the structure-property relationships in both baby and adult enamel teeth have revealed several distinct similarities and differences (Figures 3-6). Firstly, phase analysis of the SRD patterns (Figure 3) indicated HAP to be the predominant phase present in both types of teeth as indexed according to the Powder Diffraction File (PDF) 74-0566 [43]. Secondly, SRD depth-profiles of canine teeth in both age groups indicated contrasting but distinct depth-dependent variations in crystallinity (Figure 4), phase abundance (Figure 5) and (112) peak-broadening (Figure 6) of HAP, thus indicating the presence of a graded microstructure within the tooth canine enamel. Similar graded structures have also been observed in adult molar enamel [5]. Thirdly, from the Scherer equation, $D = \kappa\lambda / (\beta \cos\theta)$, the value of crystal size, D, is inversely proportional to the Full Width Half Maximum (FWHM), β, of the respective peak. This relationship suggests the crystal size of HAP to be smaller in the adult when compared to that of the baby. Indeed, this surprising size difference has also been confirmed by atomic-force microscopy (Figures 7 & 8) with the average grain sizes for the adult and baby enamel being 94 and 185 nm, respectively [44-45]. In addition, elongated HAP grains are observed in the adult enamel whereas more equiaxed grains are found in the baby enamel.

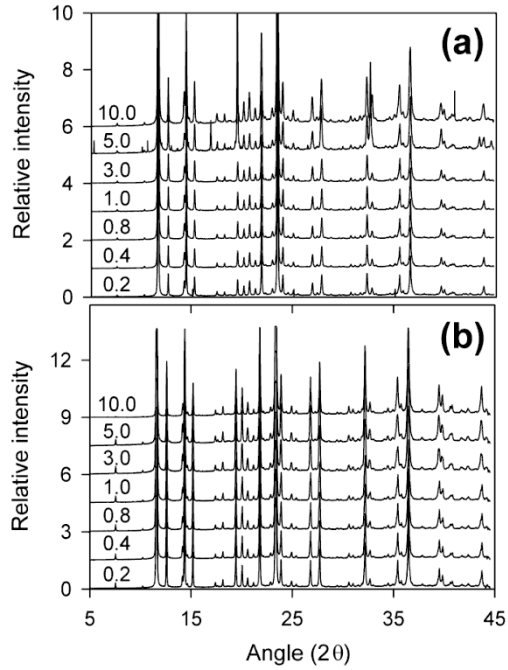


Figure 3. SRD plots of canine enamel as a function of 2θ: (a) baby, and (b) adult.

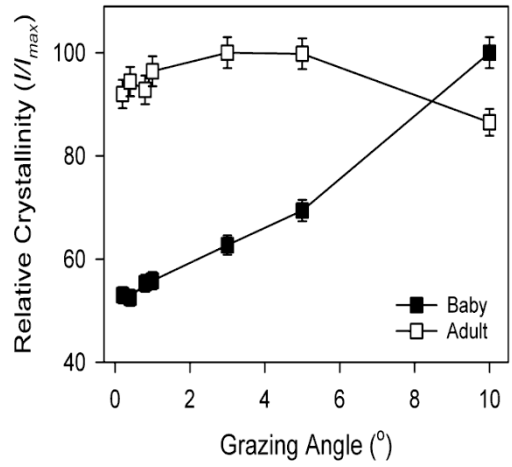


Figure 4. Comparison of relative crystallinity in the baby and adult canine tooth.

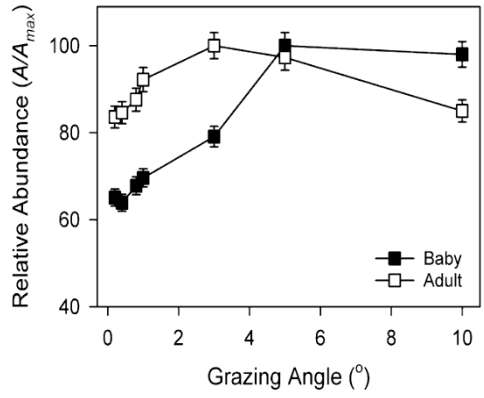


Figure 5. Comparison of relative abundance of HAP in the baby and adult canine tooth.

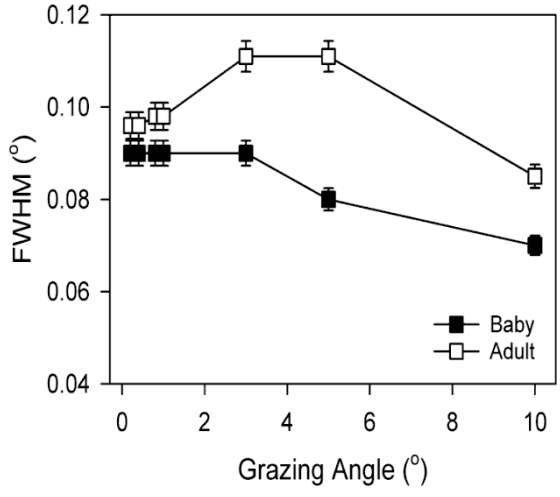


Figure 6. Comparison of (112) peak broadening in the baby and adult canine tooth.

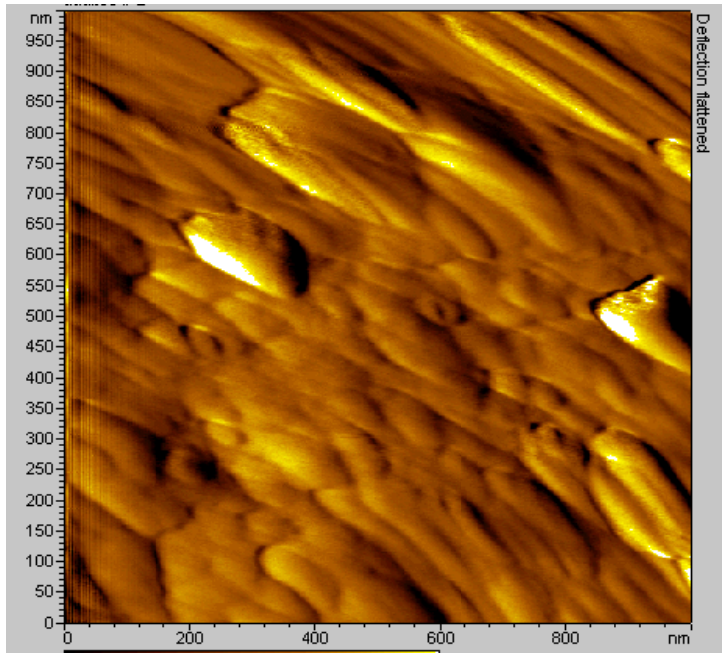


Figure 7. AFM showing the HAP grains of an adult enamel.

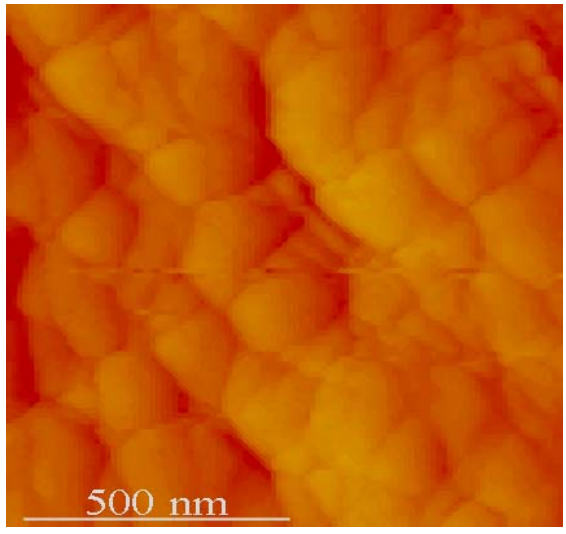


Figure 8. AFM showing the HAP grains of a baby enamel.

Finally, the preferred grain orientation or texture in both age groups was similar with the HAP grains being aligned approximately orthogonal to each other between the occlusal- and axial-sections. These highly textured microstructures have been indicated by the SRD patterns (Figures 9 & 10) and verified by optical and electron micrographs with elongated enamel rods being seen in the axial-section whereas key-hole shaped enamel rods were noted in the occlusal-section [2, 5]. HA crystallites are most aligned in the cuspal regions: on both sides of the buccal cusp and on the inner side of the lingual cusp HA crystallites are highly aligned [5, 14]. Conversely, along the sides of the tooth away from the cusps generally the crystallites are

less ordered. A gradation in texture within the enamel has also been observed by grazing-incidence SRD [5]. 2D images from SAXS (Figures 11 and 12) clearly demonstrate the existence of texture variations within the baby and adult tooth enamel. Contour maps of the texture [14] and hardness [42] variations within the tooth enamel have also been obtained to highlight the anisotropic and heterogeneity nature of human teeth. This display of gradation in texture within the enamel suggests that the presence of a three-dimensional interlocking HAP structure has been designed by nature to impart strength and hardness for wear resistance and stress-bearing capability.

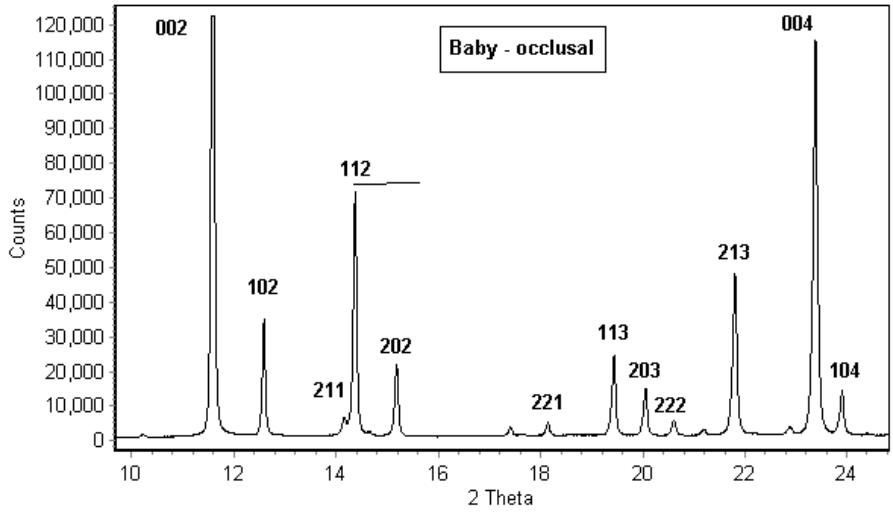


Figure 9. SRD plot of the occlusal surface of baby tooth.

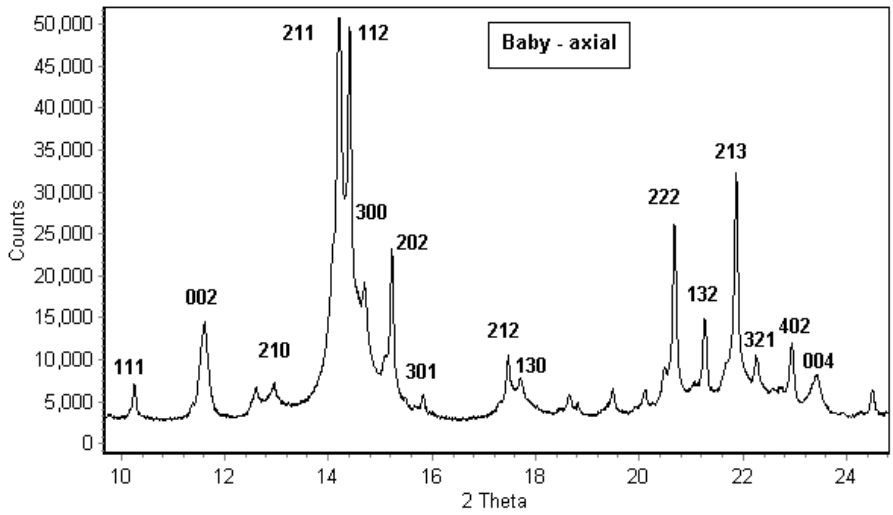


Figure 10. SRD plot of the axial-section of baby tooth.

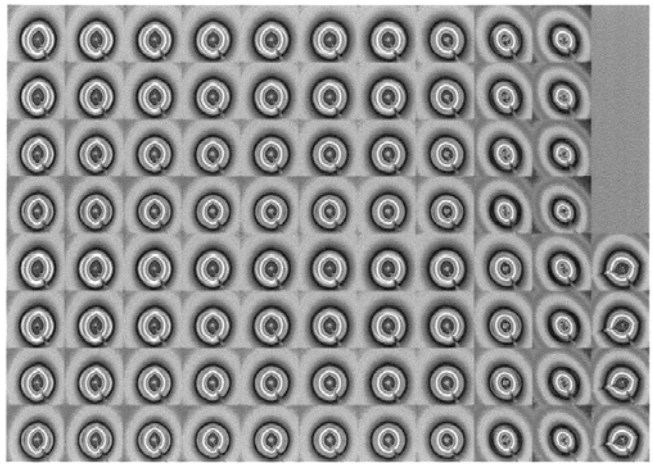


Figure 11. SAXS data showing the 2-D mosaic images of baby tooth highlighting the gradation in texture or preferred grain orientation along the cross-section from enamel to dentin.

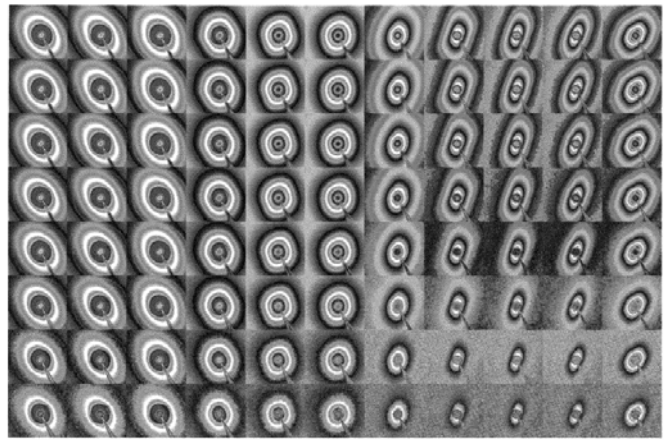


Figure 12. SAXS data showing the 2-D mosaic images of adult tooth highlighting the gradation in texture or preferred grain orientation along the cross-section from enamel to dentin.

5.2. Indentation Responses and Damage

A pronounced load-dependent hardness behaviour was evident within the enamel layer for both baby and adult teeth (Figure 13). In contrast, this phenomenon is not displayed by the dentin layer [8]. Such a phenomenon is well-known in coarse-grained metals and ceramics such as Ti_3SiC_2 and can be attributed to a grain-size effect [47-48]. At small loads, the contact diagonal, $2a$, of the indentation is less than the grain size and, as such, the hardness measures properties of single grains; when $2a$ becomes much larger than the grain size at high loads, the hardness measures polycrystalline properties, with more grains being oriented for deformation by slip. This indentation-size effect can be ruled out for human enamel by virtue of its fine sub-micrometre microstructure. Instead, the origin of load-dependent hardness in human enamel may be attributed to its highly textured microstructure which favours the stochastic nature of deformation damage by virtue of a statistical variation in crystallographic

orientation of individual grains. Only those grains of correct orientation will favour the occurrence of intergrain deformation along certain specified grain-boundaries. The expansion of this deformation zone is a result of the activation of additional grain-boundaries as the pressure intensifies within the Vickers compression-shear zone. However, it is also quite probable that at higher loads the indentor may have partially penetrated into the dentin layer and thus accounted for the lower hardness observed.

The non-viscoelastic nature of both dentin and enamel was indicated by the absence of reduction in hardness with prolonged indentation time (Figure 14). In contrast to most viscoelastic polymers which exhibit indentation creep as a result of relaxation processes and viscoelastic flow [49-50], both enamel and dentine were found to be creep resistant, which might otherwise lead to undesirable permanent deformation.

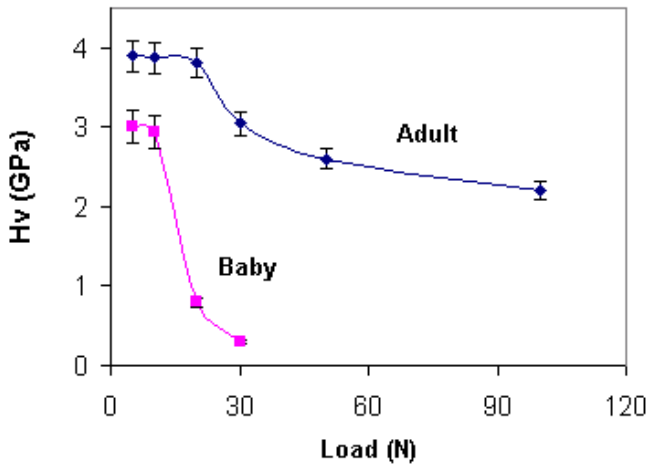


Figure 13. Variation of hardness as a function of load for baby and adult enamel.

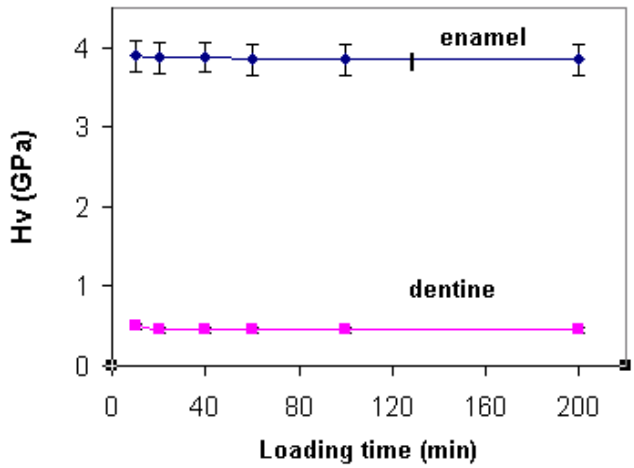


Figure 14. Variation of hardness as a function of loading time for adult enamel and dentin.

Vickers microhardness for the baby tooth was found to be lower than for the adult, indicating the adult tooth enamel to be harder in order to provide a greater capacity for stress-bearing and wear resistance (Figure 15). In all cases, hardness decreased progressively from the enamel to the dentine by virtue of a decreasing content of HAP. This graded nature of teeth has also been previously observed [5, 42, 51]. When compared to the adult tooth, the baby tooth has lower fracture toughness and is thus more vulnerable to fracture (Figure 16). The lower toughness may be related to a coarser HAP grain microstructure in the baby enamel. However, it should be pointed out here that the mechanical properties of biological materials such as human teeth are dependent not only on differences in structure and microstructure, but also the moisture content present. Indeed, the strength of bamboo has been observed to be dependent on its moisture content [46]. Hence, it is quite possible that the observed baby-adult differences in hardness and fracture toughness may also be related to the different degree of dryness or amount of moisture present.

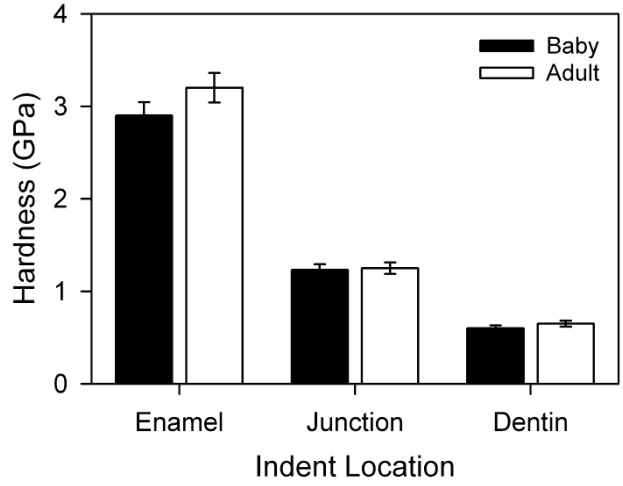


Figure 15. Comparison of hardness profiles in the baby and adult tooth.

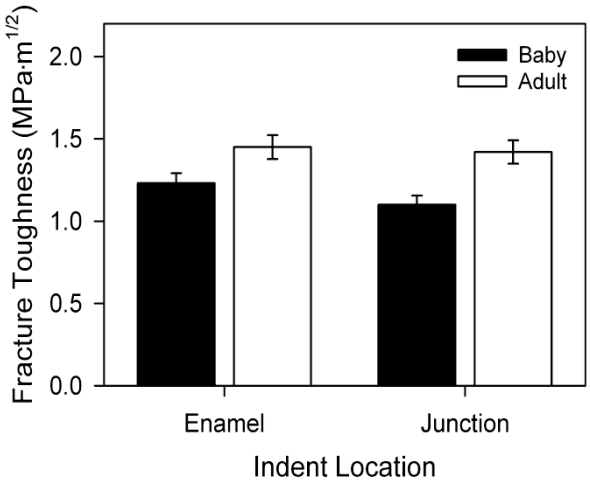


Figure 16. Comparison of fracture toughness in the baby and adult tooth.

Extensive damage was observed within the vicinity of a distorted indent at small loads in adult enamel, albeit with no indication of cracks (Figure 17). Indentation cracks were seen to form in enamel for loads greater than 50 N but not in dentine. In the former case, extensive damage in the vicinity of the indent was also observed on the axial surface but not on the occlusal surface. In both cases, a pronounced display of anisotropy in the cracking pattern was noted which suggests a highly heterogeneous or textured microstructure. It is also interesting to note that the sides of the indent appeared to be covered with a thin layer of enamel, a characteristic exclusive to biomaterials. In contrast to this, cracks readily formed in the baby enamel even at low loads (Figure 18) which indicates the poor fracture resistance of baby teeth.

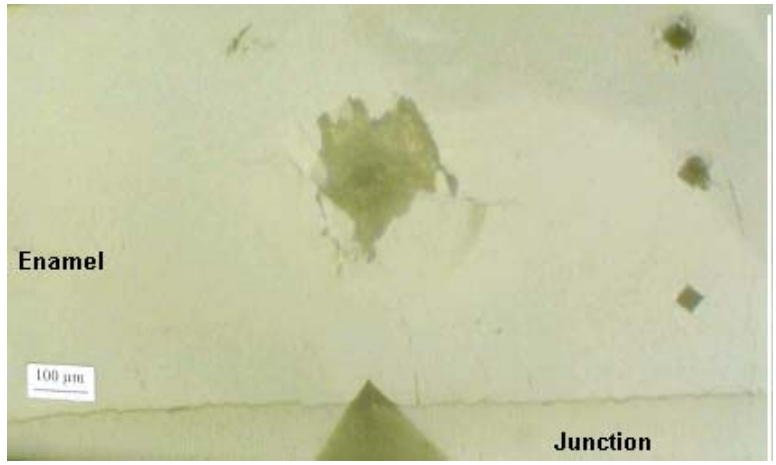


Figure 17. Characteristics of damage in the vicinity of indents for the adult tooth.

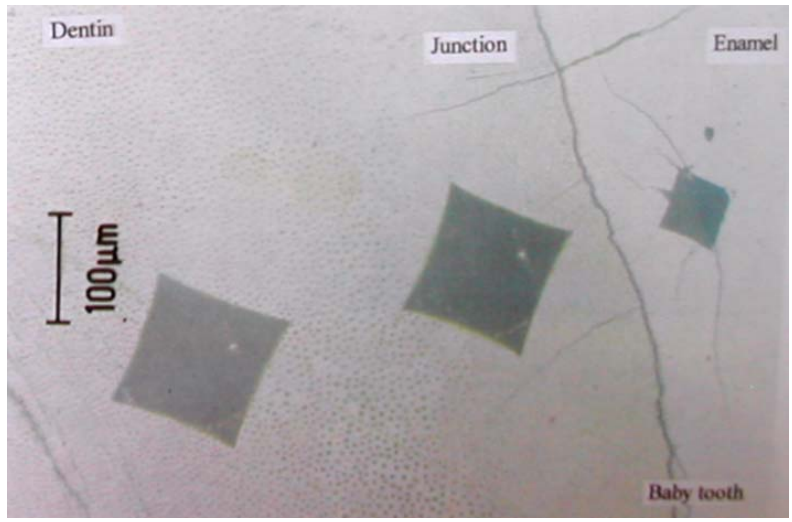


Figure 18. Characteristics of damage in the vicinity of indents for the baby tooth.

5.3. Effect of Soft Drinks on Dental Erosion

The effect of stirring on the rate of dental erosion have been investigated. Figure 19 shows the effect of Mountain Dew and Diet Coke on weight loss of tooth enamel in the absence of any stirring. Up to 3.8 and 2.9% weight loss was observed after soaking continuously for 166 hours which are more than three times than those reported by Fraunhofer and Rogers [10]. Although Mountain Dew dissolves the enamel material more readily than Diet Coke, in terms of acidity there is no significant difference between Diet Coke (3.22 pH) and Mountain Dew (3.14 pH). This indicates that other chemical parameters such as titratable acidity, Calcium and Phosphate concentrations may determine erosion in addition to the pH [32-33].

As might be expected, a greater weight loss of tooth enamel was observed when stirring was used where the weight loss increased four times to 13.8 and 9.2% (Figure 20). The results of this study provide empirical evidence for people not to swish soft-drink in their mouths. Instead, people should swallow soft-drink immediately or use a straw. The results for stirring illuminate the dramatic role of saliva and the pellicle, an anti-erosion mechanism, which surrounds the enamel. The pellicle protects the enamel from the erosion. The acquired pellicle can be formed in 3 minutes and protects the teeth from erosive oral fluids [52-53]. Not only can the pellicle be formed in three minutes, but this three minute protective device has been shown to have a significant effect on the reduction of erosion from citric acid. When soft-drink is swished around the mouth, stimulated by stirring in this experiment, the acquired pellicle is unable to form. Therefore, the teeth are more likely to erode without this protection as shown in the results. Nonetheless, while these findings seem alarming, in real life saliva would play a role in re-mineralization of the affected enamel sites. As a controlled experiment, saliva was not included into the system; therefore, this is a limitation of the study. Nonetheless, the results still show a clear relation between the movement of soft-drinks and their effect on the tooth erosion. Specifically, in laboratory experiments, stirring has a significant impact on the amount of erosion.

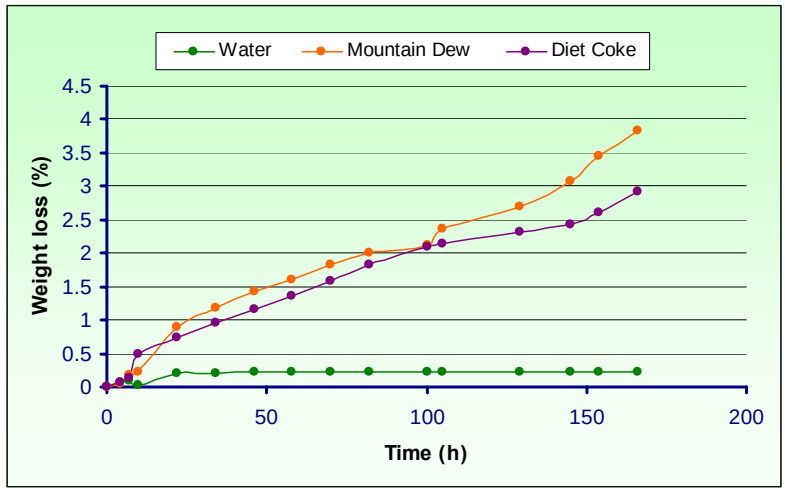


Figure 19. Comparison of the amount of erosion, as percentage of total weight loss, between Mountain Dew and Diet Coke for 166 hours without stirring.

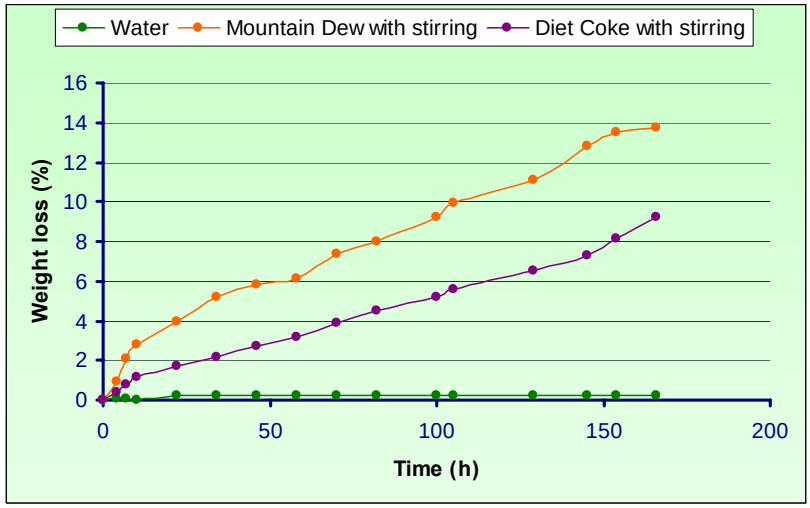


Figure 20. Comparison of the amount of erosion, as percentage of total weight loss, between Mountain Dew with stirring and Diet Coke with stirring for 166 hours.

The erosive nature of Sprite Zero on tooth enamel is highlighted in Figure 21. This finding of Sprite Zero being the more erosive than Coca-Cola supports the results reported in previous studies [10, 54]. The important information here is that not only pH of a drink important, the pH of Mountain Dew is 3.14 more acidic than Sprite Zero at 3.34, however, the drink's ability to maintain or 'buffer" its on pH is what is important. This is known as a soft-drinks buffering capacity. It is expected that Sprite Zero has a high buffering capacity, which means that when the saliva enters the dental area and attempts to change the pH, the acids in the soft drinks, namely, phosphoric and citric acid can resist the change [54].

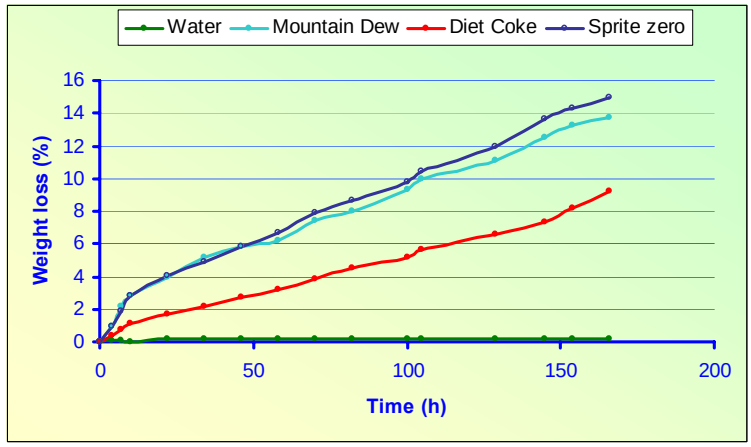


Figure 21. Comparison of the amount of erosion, as percentage of total weight loss of child enamel samples, between three soft drinks, Mountain Dew, Diet Coke, and Sprite Zero with stirring for 166 hours.

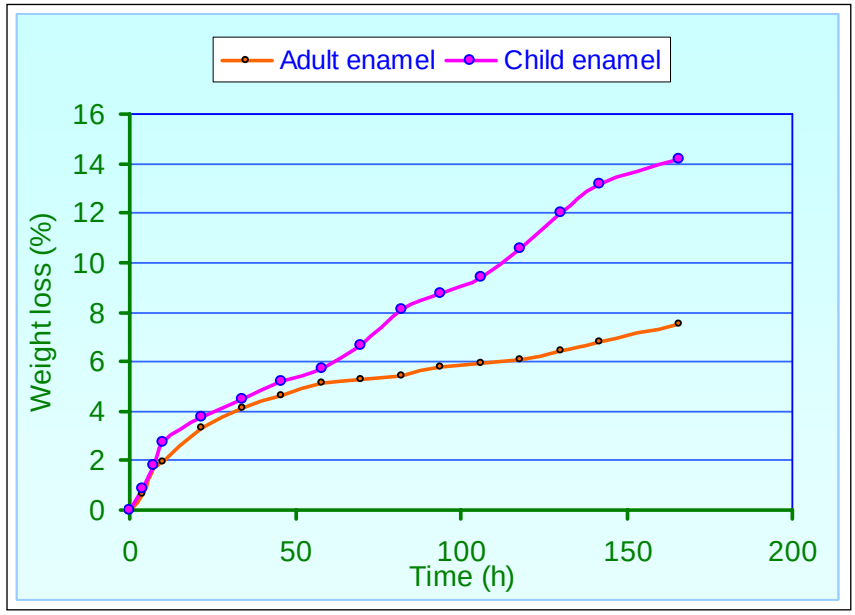


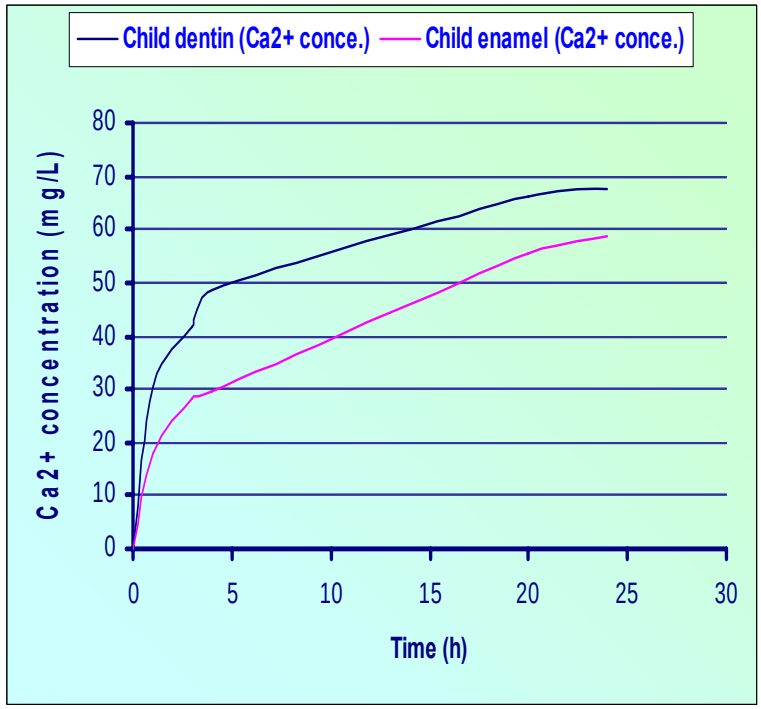
Figure 22. Comparison of the amount of erosion as a percentage of total weight loss from child enamel and Adult tooth enamel samples by Sprit Zero.

Figure 22 shows the difference in weight loss between baby enamel and adult enamel due to dental erosion by Sprite Zero in 166 hours. The child enamel was much susceptible to erosion with 14.92% of the total weight eroded when compared to only 7.5% for the adult enamel.

Atomic absorption spectroscopy (AAS) was used to monitor the amount of Calcium ion concentration as leached from tooth enamel and tooth dentin. For the baby tooth, it was found that Sprite Zero leached a higher concentration of Calcium from the dentine sample at 67.4 mg/L and just 58.9 mg/L in 24 hours from the enamel sample (Figure 23). The process of calcium leaching in an acidic solution can be described as follows:

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^-$$

For the adult samples, the same was found with the enamel less likely to have Calcium leached compared with the dentin. The results showed 196.6 mg/L was leached from the dentin whereas just 179.6 mg/L was leached from the enamel in 72 hours (Figure 24). As the dental tissue is demineralized due to dental erosion, it becomes weaker. This demineralization causes a change in the mechanical properties of the dental tissue, such as hardness and elastic modulus. This degradation in mechanical properties (i.e. hardness and elastic modulus) as a result of dental erosion has been reported in a number of studies. For instance, Barbour et al. [55-56], reported that hardness of tooth enamel decreased as the exposure time to soft drinks increased. As the exposure time increases, the amount of Calcium ions leached out of the dental tissue increases. As the amount of Calcium ions leached out increases, the dental tissue becomes softer. In fact, there appears to be a determinable relationship between the hardness and the amount of Calcium ions leached out of dental tissue.



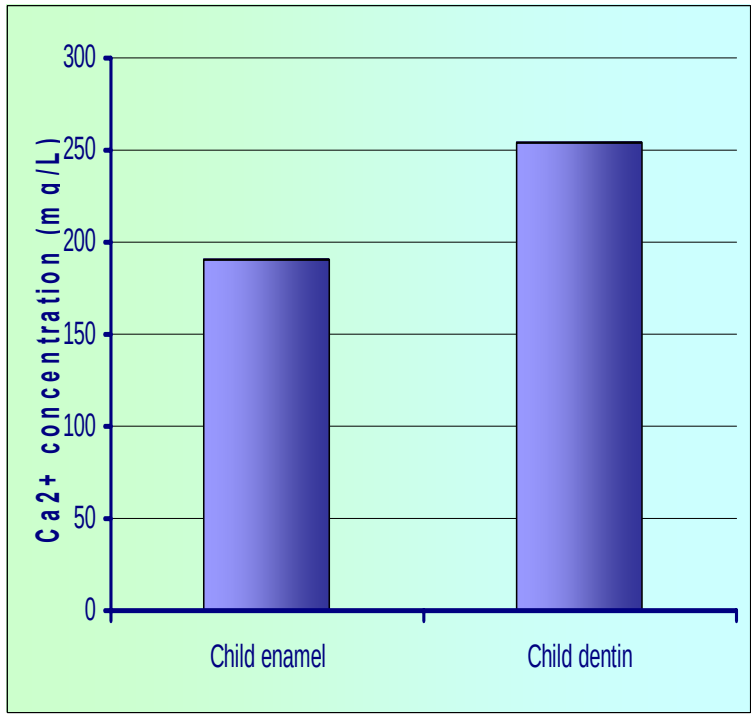


Figure 23. Comparison between Ca+2 concentrations leached from a child enamel sample and a child dentine sample by Sprit Zero.

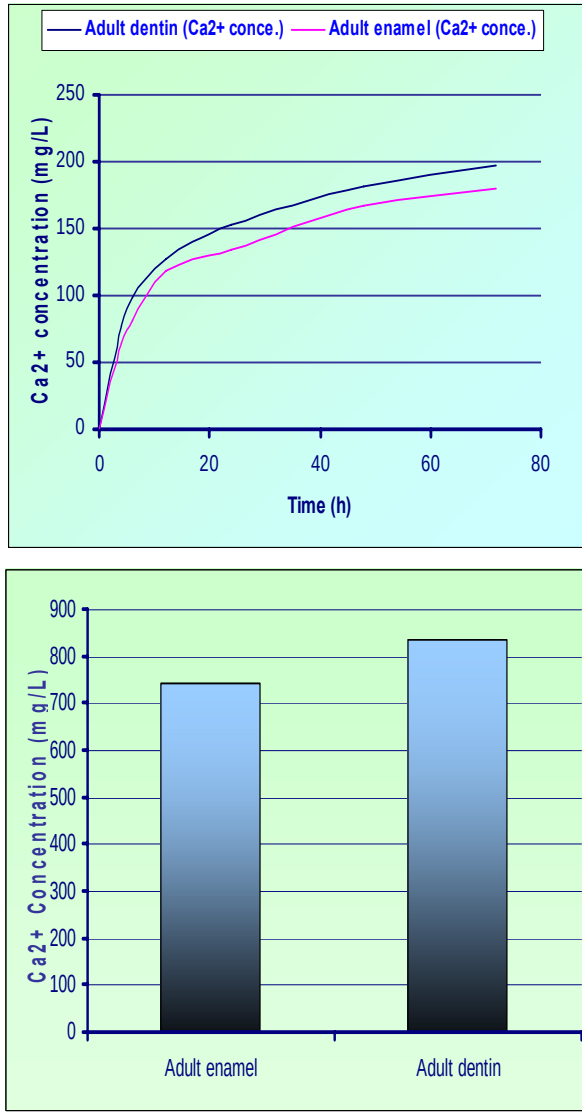


Figure 24. Comparison between Ca+2 concentrations leached from adult enamel and adult dentine by Sprit Zero.

5.4. Effect of Dental Erosion on Hardness of Human Enamel

The effect of soft drinks and alcoholic drinks on the hardness of tooth enamel has been investigated. Several features of erosion-induced enamel softening are worth-noting. Firstly, rapid softening of enamel occurred in the first 3 h when it was exposed to Coca-Cola for up to 24 h (Figure 25). This result concurs with the work of Van Eygen and coworkers [57] where they reported that even a short period of soft drink intake can cause reductions in mechanical properties such as microhardness. Furthermore, it has been shown that hardness decreases proportionately with an increased time of immersion to erosive agents. Secondly, the

softening effect of the soft drink was greater in baby enamel than in adult enamel when they were both exposed to orange juice for up to 24 h (Figure 26). A similar reduction in hardness was reported for baby enamels when they were immersed in an acidic solution for up to 30 minutes [58]. Thirdly, Coca-Cola has a greater erosive power than orange juice on the baby enamel (Figure 27). Fourthly, alcoholic drinks are less erosive when compared to soft drinks probably because of acids present in the latter (Figure 28). This observation has also been reported by Barbour *et al*. [59] where they found that mineral water, had negligible change in both hardness and reduced elastic modulus but both citric acid and citric acid with added calcium and phosphate caused a significant drop in hardness accompanied by a marked drop in reduced elastic modulus. Lastly, carbonated drinks have a greater erosive power on human enamel when compared to fruit juices (Figure 29).

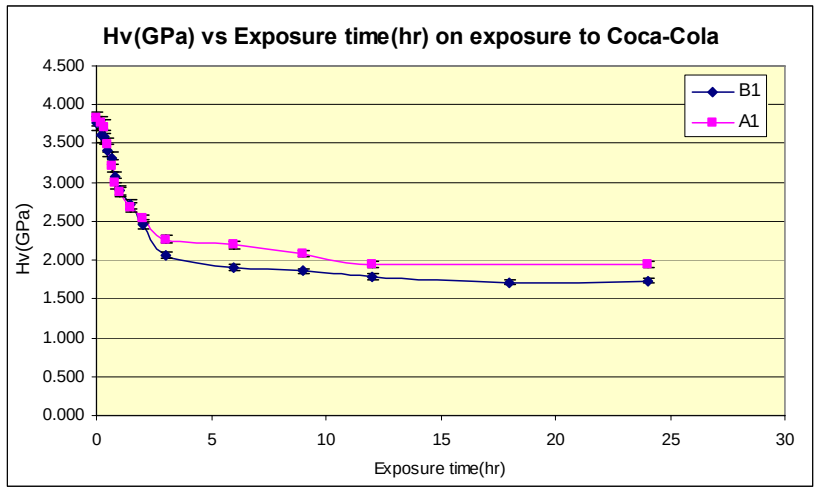


Figure 25. Graph showing Vickers hardness comparison between baby and adult enamel exposed to Coca-Cola. [Legend: A2 = adult enamel; B2 = baby enamel].

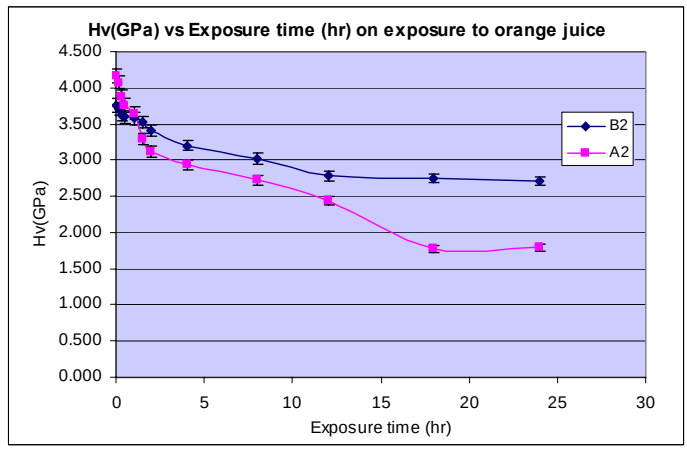


Figure 26. Graph showing Vickers hardness comparison between baby and adult enamel exposed to Squeeze orange juice. [Legend: A2 = adult enamel; B2 = baby enamel].

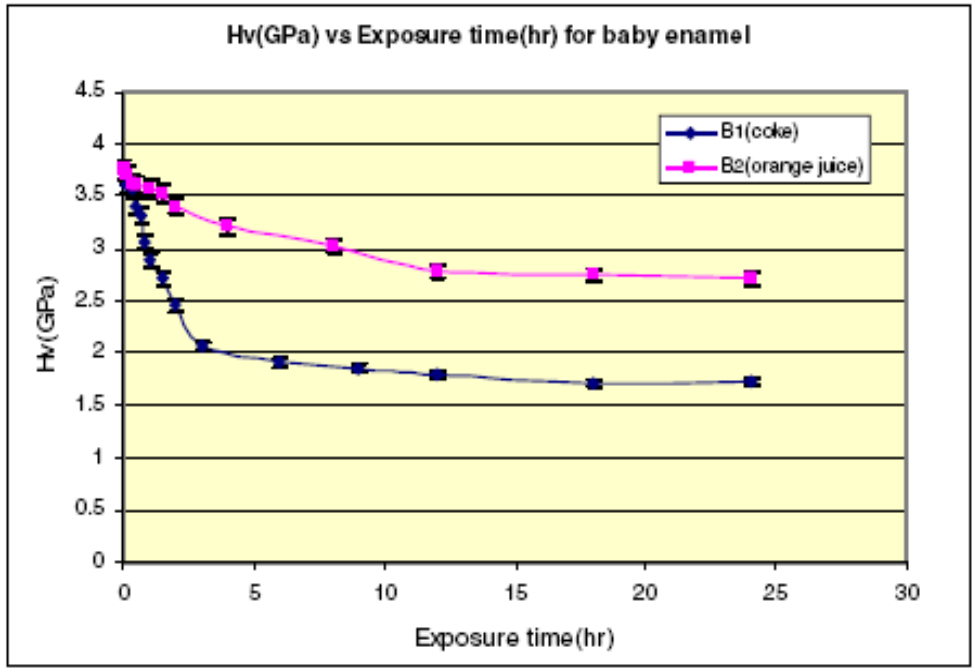


Figure 27. Graph showing Vickers hardness comparison for baby enamel exposed to the non-alcoholic drinks.

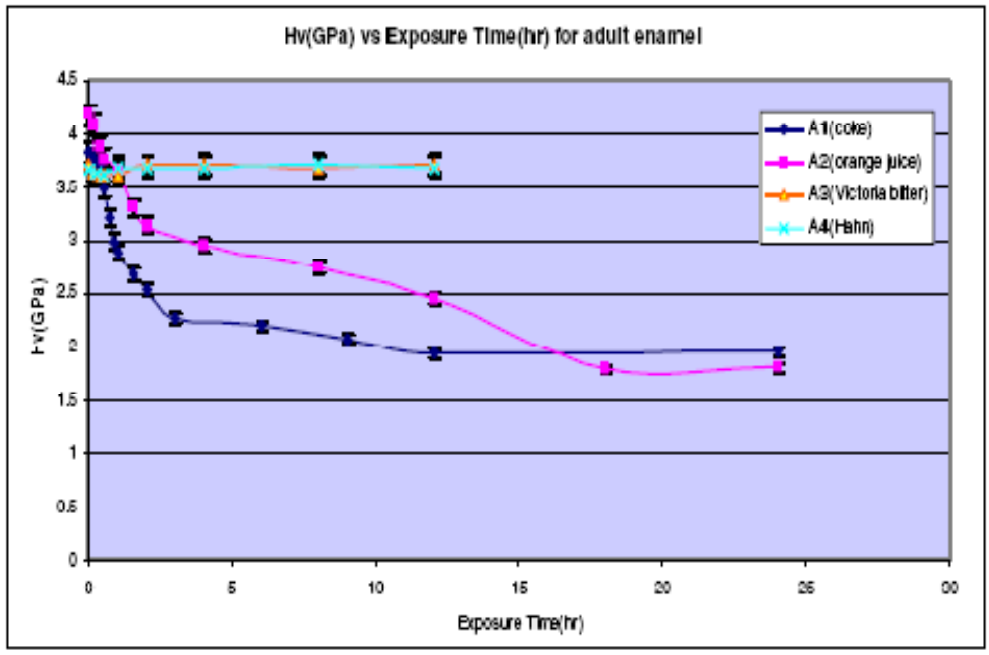


Figure 28. Graph showing Vickers hardness comparison for baby enamel exposed to the non-alcoholic drinks.

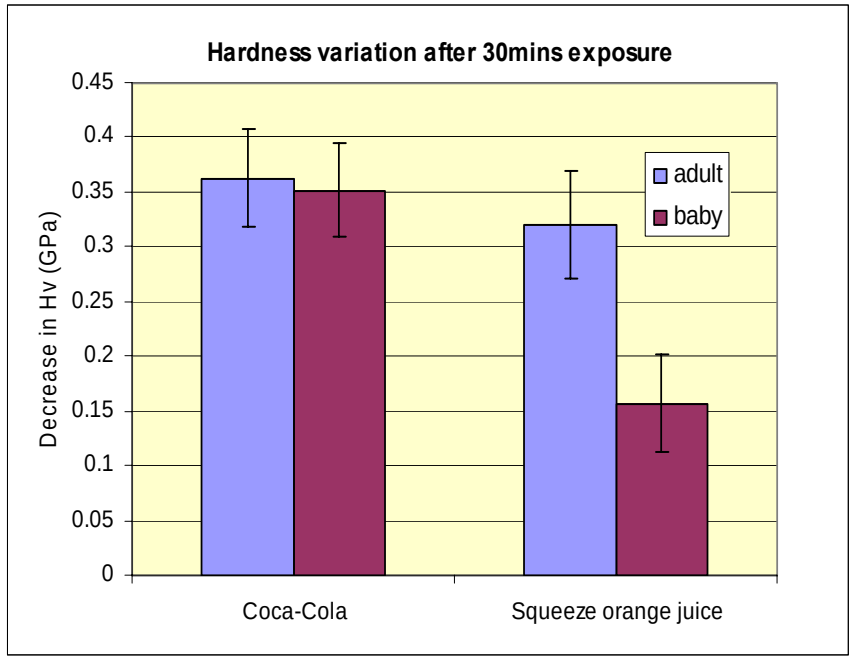


Figure 29. Graph showing Vickers hardness variation comparison for both baby and adult enamel samples exposed to the non-alcoholic drinks within a period of 30 minutes.

CONCLUSION

The mechanical properties and dental erosion in human enamel are strongly dependent on its composition, texture and microstructure. The existence of distinct graded changes in crystal disorder, phase abundance, crystallite size and hardness within these enamel ceramics has been characterized grazing-incidence synchrotron radiation diffraction, atomic-force microscopy, scanning electron microscopy and Vickers indentation. The hardness of tooth enamel is load-dependent but load-independent hardness in the dentin. Both baby and adult tooth enamel undergo dental erosion when they are exposed to carbonated soft drinks with the former being more susceptible. Alcoholic drinks do not cause any significant erosion and softening of the tooth enamel. Atomic absorption results suggest that the increasing weight loss in tooth enamel during dental erosion in soft drinks can be attributed to the continuous leaching of Ca^{2+} ions, in addition to phosphorus, oxygen, and hydrogen. As the exposure time to erosive soft-drinks increased, the amount of Calcium ions leached increased with the concomitant increased softening of the dental tissue.

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Chapter 5

EFFECT OF FILLER CONTENT AND SIZE ON MECHANICAL PROPERTIES OF DENTAL RESIN COMPOSITES: EXPERIMENTAL AND COMPUTATIONAL INVESTIGATION

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ABSTRACT

Dental resin composites have been commonly used as restorative material for dental treatment. A resin composite is a dispersion-strengthened material composed of silica glass and dimethacrylate resin. In order to enhance the chemical bonding between the silica and matrix resin, the silica glass is treated with a silane coupling agent, which has a methacryloyl group at its terminal end. As a consequence of the bonded filler phase, these materials have much better mechanical properties than did unfilled resins.

The mechanical properties of dental resin composites such as compressive strength, diametral tensile strength, flexural strength, and fracture toughness have been experimentally studied in relation to the filler content and particle size. On the other hand, several computational studies on failure behavior of dental resin composites as determined by finite element analysis have been published.

This article provides an overview of the relationship between filler loading condition and mechanical properties of dental resin composites from experimental and computational points of view.

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Keywords: Dental resin composites; Mechanical properties; Filler content; Filler particle size; Finite element analysis.

INTRODUCTION

Generally, a composite material is formed by combining two or more kinds of materials, with properties superior to any one component. A resin composite is one of the most famous composite materials in dentistry and is a dispersion-strengthened composite material composed of silica glass and dimethacrylate [1,2]. Dental resin composites have been commonly used as restorative material for dental treatment. In order to enhance the chemical bonding between the silica and matrix resin, the silica glass is treated with a silane coupling agent, which has a methacryloyl group at its terminal end. As a consequence of the bonded filler phase, these materials have much better mechanical properties than did unfilled resins [3,4].

Dental resin composites are classified by the particle size and distribution of the filler [1,2], as follows: 1) Microfilled resin composites contain spheric colloidal silica with an average particle size of 0.04 μm. It is difficult to load a large volume of microfillers in the resin matrix because of the large surface area. Filler content in microfilled resin composites is therefore limited to about 30% to 55% by volume. A lower filler volume results in this composite with inferior mechanical properties [5]. 2) Hybrid resin composites contain large filler particles of an average size of 15-20 μm, and also a small amount of colloidal silica, which has a particle size of 0.04 μm. The filler content in hybrid resin composites is 60% to 65% by volume. 3) Microhybrid resin composites contain a mixture of small particles of 0.5-3.0 μm and colloidal silica of 0.04 μm. They can contain a high filler content of 70% by volume, because colloidal silica particles fill in spaces between small particles.

As is well-known, filler content, filler size, and matrix viscosity influence the mechanical properties of hybrid composites [6,7]. Additionally, the handling characteristics of resin composites are important in restorative procedures. Thus, two types of dental composite were developed - namely, packable [8,9] and flowable [10,11] resin composites. The introduction of these materials necessitates a new classification method, based on the viscosity of the composites. Packable composites, also called condensable composites sometimes, were introduced to the market with high expectations as an alternative to amalgam. They are characterized by a high filler load and a filler distribution that gives them a different consistency when compared with traditional hybrid composites. As for flowable composites, they build on the chemistry of traditional hybrid composites but contain smaller filler concentrations and in some instances, modified resin formulations. They are characterized by low elastic modulus and low viscosity, and that they improve the wettability of tooth structure. In addition, dental composites based on nanotechnology were recently developed [12-14]. By leveraging recent advances in nanotechnology, nanofill composites that contain a unique combination of nanofillers and nanoclusters embedded in an organic polymer matrix are produced. They feature excellent strength and wear properties when compared with conventional hybrid composites [15,16].

With these points as background, the filler distribution and particle size are important features since, by manipulating them, the properties of composite materials can be varied widely; especially, the mechanical properties of dental resin composites are controlled by

these filler distribution and particle size. Numerous experimental studies have reported relationships between filler loading condition and mechanical properties of dental resin composites (as described later). Additionally, experimental analysis of the fracture mechanism of dental resin composites has also been performed by the acoustic emission (AE) analysis [17], the scanning electron microscopy analysis [18] and others [19]. These experimental methods have been used as an effective means to characterize the microscopic fracture process of composites. However, experimental research in this field seems inefficient, laborious, tedious, and expensive, and is probably ineffectual. Therefore, in addition to experimental approach, a convenient systematic approach for analyzing composites is need, because that will be helpful in reducing the number of experiments.

Computationally, the finite element analysis (FEA) is a powerful and effective tool for analyzing stress distribution of structures in industrial environments [20]. FEA is a technique in which a complex structure is subdivided into a number of small elements of simple shapes. When deformations of all small elements are simultaneously calculated, total deformation of the overall structure can be reconstructed. For dental applications, FEA is employed to evaluate dental restorations [21], fixed partial dentures [22], dental posts [23], dental implants [24], and implant-supported prostheses [25]. Geng et al. [24] explain that FEA is an effective computational tool that has been adapted from the engineering arena to dental implant biomechanics. Accordingly, stress distribution that occurred in the composite inlays and onlays and in the tooth structures has been analyzed by means of FEA [26-31]. Furthermore, several studies on failure behavior of dental resin composites as determined by FEA and failure criterion have been published [32-34].

From these above-mentioned points, this article provides an overview of the relationship between filler loading condition and mechanical properties of dental resin composites from experimental and computational points of view.

EXPERIMENTAL INVESTIGATION

Strength is the maximal stress required to fracture a structure. Various strengths of resin composites have been evaluated by different methods. It is called compressive strength, diametral tensile strength, and flexural strength depending upon the predominant type of stress present. Generally, high compressive strength of dental composites does not indicate high tensile strength, reflecting the somewhat brittle behavior of them [35]. Thus, the measurement of the tensile strengths of brittle materials such as resin composites is extremely difficult. The diametral tensile test is an alternative method for measuring the tensile strength of brittle materials, and the diametral tensile strength derived from this method is often quoted for dental composites. The flexural strength represents the maximal stress to bending load, and flexural modulus (or elastic modulus) describes material's stiffness, a measure of the resistance to deformation under load of the material, with a high number indicating greater stiffness. Many researchers have reported the experimentally derived influence of filler loading condition on the mechanical behaviors of dental composites, including compressive strength, diametral tensile strength, flexural strength, elastic modulus, and fracture toughness [36-64]. Here, experimental studies on the effect of filler content and particle size on mechanical properties of dental resin composites are shown in Table 1.

As the effect of filler content on mechanical properties of resin composites, the higher strength of hybrid resin composites, as compared with microfilled composites, reflect the higher volume fraction of the high-strength filler component [56]. For example, Lu et al. [5] evaluated the mechanical properties of six commercially available resin composites; a submicron filled composite, a nanofilled composite, two microfilled composites, and two microhybrid composites. As a result, the microhybrid composites had higher strength for diametral tensile strength, flexural strength, and flexural modulus than the microfilled composites. Cobb et al. [65] reported that packable composites have mechanical properties such as compressive, diametral tensile, and flexural strengths superior to those of microfilled composite. They explain that the higher properties of hybrid and packable composites in comparison with microfilled composites are due to their higher filler loadings. In addition, the flexural modulus of microfilled and flowable composites are about 50% lower than values for multipurpose hybrids and packable composites, which reflects the lower volume percent filler present in the microfilled and flowable composites [66]. Kim et al. evaluated the effects of filler content on flexural properties of both commercially [40] and laboratory [50] resin composites. These papers report that the flexural properties of both resin composites increases with filler content. To summarize above-mentioned studies on filler content, many mechanical properties change progressively as filler content is increased. On the other hand, the composites with high filler content show a greater decreasing ratio of flexural strength than those with low filler content, after immersion in water [51]. Dental resin composites consist of matrix, filler, and coupling agent, and the properties are derived from the respective properties of matrix and filler, and the connective status between matrix and filler. In other words, high mechanical properties resulted from the good stress-transfer ability between the filler and the resin matrix within dental composites. Filler silanation is an important factor for determining composite material strength. Thus, this result may be related to a bond failure between the filler and the matrix caused by the hydrolysis of the silane coupling agent. The mechanical properties of aged-resin composites can be greatly influenced by silanization and the filler content [51,55,56].

The filler particle size, as well as filler content, affects mechanical properties of resin composites. There are reports that the compressive and diametral tensile strengths of resin composites decrease with filler particle size but constant filler content [62,63]. Miyasaka [62] investigated effects of sizes of fillers on mechanical properties of hybrid resin composites. The author concluded that compressive strength and diametral tensile strength of resin composites tended to decrease as filler size increased. Thus, it was suggested that there were differences in stress concentration between the filler and the matrix resin interface, which resulted from different particle sizes of fillers. Dental resin composites are composed of silica filler with rigidity and matrix resin with elasticity. When filler content of composites is constant, smaller silica particles have higher surface energy at interface between filler and matrix in comparison with larger silica particles, because the surface area of the silica particles increases with decreasing particle size of the filler. For example, Hosseinalipour et al. [59] reported that reinforcement of dental resin composites with silica nanoparticles resulted in a significant increase in the evaluated mechanical properties in comparison with the conventional composite. Likewise, Beun et al. [44] indicated that the nanofilled resin composites showed higher elastic moduli than those of universal and microfilled composites. These indicate that nanofillers have higher contact surfaces with the organic matrix than do microparticles of conventional composites.

Nowadays, the use of resin composites has been extended to posterior teeth. However, their use on a high-stress-bearing site has been limited by occasional catastrophic failure due to their inherent brittleness. Besides the compressive, tensile, and flexural strength, it is important that the microscopic fracture process (i.e. initiation and propagation) be understood [67]. Thus, fracture toughness is an intrinsic characteristic of a material concerning resistance to crack propagation. Ferracane et al. [37] revealed that crack propagation in dental resin composites is mainly through the matrix, but that occasional cleavage or debonding of the larger filler particles in the composite occurs as well. Kim et al. [50] monitored the fracture mode of dental resin composites by AE method. The authors indicated, by the AE detection method, that the microcracks generated during the fracture toughness were distributed more widely with an increase in the filler content. Davis & Waters [47] explained that failure stress increases with filler content, and that the failure behavior is clearly related to the stress-intensity factor for crack initiation. Also, Kim et al. [40] observed that the composites with the highest filler content exhibited the highest flexural strength, flexural modulus and hardness, but that the maximum fracture toughness was obtained at approximately 55 % of filler content. On the other hand, Bonilla et al. [42] reported that there was no correlation between the filler content and the fracture toughness of these flowable composites. According to Bonilla et al. [42], the composite with the methacrylates (32.5% by volume) had higher fracture toughness than the composite having urethane dimethacrylate (35% by volume) as the major component of the resin matrix. This is because flowable composites have a large resin matrix component, and indicates that the fracture toughness of flowable composites is dependent on resin matrix. Therefore, property of resin matrix also may contribute to the fracture toughness in case of flowable composites with low filler content, though the filler content has the effect on fracture toughness in case of conventional composites.

COMPUTATIONAL INVESTIGATION

It is well known that the optimization of the mechanical properties of composites is based on the knowledge of the relationship between the microstructure and the macroscopic response. FEA has potential to predict the mechanical behavior of dental composites which can be microscopically divided into the filler, matrix, and interphase. Several numerical models have been developed to help predict levels of elastic modulus in composite materials [68-71]. These most numerical models applied a cubic shape as unit cells which were constructed with filler, matrix, and interface, in composite modeling. As a result, it was indicated that the predicted elastic modulus increase with filler content in agreement with experimental results of elastic modulus. Meanwhile, the effect of filler particle size on flexural properties of laboratory resin composites from experimental and computational points of view was evaluated in our laboratory [64]. As the experimental results, flexural strength decreased with increasing particle size of the filler of resin composites. Furthermore, the results of FEA indicated that stress concentration increased with increasing filler particle size in agreement with experimental results of flexural strength. In addition to this fact, the results of our previous study agreed with Miyasaka's experimental study [62].

Mechanical properties of composites depend on stress transmission at interphase between the silica filler and the resin matrix in resin composites [53]. In composites, silica glass is treated with a silane coupling agent in order to reinforce bonding between the silica and resin

matrix. The initial fracture of resin composites generally occurs at the filler/matrix interphase because rigidities of the silica filler and matrix resin are different. Therefore, interphase between filler and matrix is considered to be one of the most important factors in optimum design, and the estimation of fracture mechanisms of composites is very important. In view of this, the fracture toughness of composites can be enhanced by silanation to increase the interface toughness and the energy dissipated during the fracture process [72]. Accordingly, FEA was utilized to analyze the influence of interphase stiffness [68], imperfect interphase [69], and interphase thickness [68,69,73]. As already mentioned, composite is material composed of silica filler and matrix resin. To analyze the composites by using FEA, the material properties of components such as filler reinforcement, resin matrix resin, and interphase are need. In particular, elastic moduli and Poisson's ratio are basic input parameters for all components in computational FEA of dental structures. Accordingly, material properties used in FEA by several studiess are shown in Table 2.

Table 1. Experimental studies on the effect of filler content and particle size on the mechanical properties of resin composites

Effect	Sample	Reference
Filler content	Commercially available composites	[5, 8, 10, 14, 36-46]
	Laboratory composites	[7, 47-59]
Filler particle size	Commercially available composites	[60, 61]
	Laboratory composites	[62-64]

Table 2. Material properties used in FEA studies of resin composites composed of filler, resin matrix, and interphase

Study	Component	Elastic Modulus (GPa)	Poisson's ratio (-)
Tsui et al. [68]	Filler (Glass bead)	70.0	0.22
	Matrix (Polycarbonate)	2.28	0.38
	Interphase	0.05-2.28	0.3 or 0.48
Wu et al. [69]	Filler (Borosilicate glass)	75.0	0.24
	Matrix (Dimethacrylate resin)	3.2	0.35
	Interphase	1.6 or 6.4	0.35
Wang et al. [73]	Filler (E-glass)	75.0	0.24
	Matrix (Dimethacrylate resin)	1.7	0.35
	Interphase	1.7 or 3.12	0.32

With regard to the effect of interphase properties, Tsui et al. [68] explored the effect of different interphase thickness on elastic modulus and stress distribution in composites with various filler content. The authors concluded that elastic modulus of the resulting composite decreases with increase in the interphase thickness. Likewise, Wu et al. [69] reported that the elastic moduli of composites vary approximately linearly with a change in interphase thickness for any filler content. Wang et al. [73] proposed numerical model that the effect of particle-particle interactions and filler content on fracture toughness of composite can be considered. The authors showed that the existence of interphase material not only provides

good stress transfer between particle and matrix, but also affects the crack propagation direction as the crack interacts with the particle. Additionally, the authors concluded that crack propagation and crack interaction were simulated by their proposed numerical model.

Linear static and homogeneous models for dental restorations have been employed extensively in FEA studies [74,75]. This is chiefly because linear analysis is useful for analyzing the stress distribution of homogeneous materials. However, dental resin composite is a heterogeneous material composed of reinforcement silica filler and resin matrix, and their mechanical behavior involves various types of local failure. Conventional linear analysis is not adequate for analyzing local failures, which exert drastically different effects on overall mechanical properties of a resin composite. Against this background, non-linear analysis [76] has become an increasingly powerful approach to predict stress and strain within composites in realistic situation that cannot be solved by a linear static model. Nishiwaki et al. [77,78] proposed the quasi-three-dimensional model that can consider the heterogeneity of composites, and indicated this numerical model was effective in predicting the nolinear mechanical behavior of heterogeneous composites. In our laboratory, we proposed the numerical model [79,80] for dental composites, according to the quasi-three-dimensional model proposed by Nishiwaki et al. The proposed numerical model is constructed independently for silica filler and matrix resin, and can address failure, applying von Mises criterion and maximum stress criterion. In the previous study, the effect of filler particle size of composites on the flexural properties of composites using the FEA has been validated by experimental results. [80]. Also, the proposed method of failure progression analysis could better simulate the failure process of composites under three-point bending conditions. The above results indicate that the proposed numerical model is effective for evaluating the flexural properties of dental filler composites of any content range.

From these computational investigations, it was confirmed that strength of composites is affected by the filler content and particle size. FEA has great advantages, because it can detect the fracture mechanisms and the stress distribution at the filler/matrix interphase in composite resins under loading that usually can not be obtained from experiments. Thus, the precise prediction of mechanical properties and fracture mechanisms using FEA will become an important guide to composite design.

Finally, FEA has some limitations, derived from its status as a simplified numerical model for composites under limited condition *in vitro*. In the case where resin composite is applied to restorative materials for different types of cavity, the mechanical properties under practical conditions must be estimated in advance. Therefore, application of the numerical model which can consider the effect of filler loading condition such as filler content and particle size to clinical situations seems inevitably necessary.

CONCLUSION

This article reviewed the relationship filler loading condition and mechanical properties of dental resin composites. Filler content and particle size were determined to highly influence the mechanical properties of dental resin composites, from both experimental and computational approaches. Although the investigation on mechanical properties has been summarized as above, more research is required to further examine the relationship between

the clinical and handling characteristics of dental restorative applications and the effect of filler loading condition determined by experimental and computational investigations.

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Chapter 6

INHIBITION OF TELOMERASE ACTIVITY IN HL-60 CELL LINE BY METHACRYLIC MONOMERS

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ABSTRACT

Methacrylic compounds, like bis-phenol A glycerolate dimethacrylate (Bis-GMA), triethyleneglycol-dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), urethane-dimethacrylate (UDMA) and 1,4-butanediol dimethacrylate (BDDMA) are used as polymerizable components of composite resins and some cements utilized in dentistry and in other medical fields.

After performing dental restorations, amounts of uncured monomers are released either into the oral cavity or in pulpal tissues whence they can leach into the blood circulation causing, or contributing to, local or systemic adverse effects. Since the intracellular mechanisms of the aforesaid effects are still not completely clear, many *in vitro* studies with methacrylic monomers have been performed in the attempt to explain them. These studies have underlined that monomers display genotoxic, allergenic, cytotoxic, estrogenetic and mutagenic activity. Moreover, these monomers alter lipid metabolism, glutathione concentration, reactive oxygen species production, energy metabolism cell cycle and behave as differentiating agents on human promyelocytic HL-60 cell line. The last property was especially intriguing because HL-60 cells possess high telomerase activity, a phenotype related to their immortalized status. Telomerase, adding telomeric repeats to the 3'-end of telomeres, protects chromosomes from the telomeric attrition associated with the 'end-replication problem'. Telomerase activity is present in human stem cells, progenitor cells, and germ cells but is undetectable in the vast majority

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of adult somatic tissues. During cell differentiation telomerase looses its function of synthesis and maintenance of the telomeric units.

In this work, we verified whether the differentiation of HL-60 cells, induced by Bis-GMA, HEMA, TEGDMA, UDMA or BDDMA, is also accompanied by a decrease of telomerase activity.

The results show that all monomers and all-*trans*-retinoic acid (ATRA) – used as positive control – induce cell differentiation.

Moreover, cells treated with TEGDMA, HEMA, UDMA, BDDMA or ATRA display a decrease of telomerase activity (about 50%) in respect to untreated cells. On the contrary, Bis-GMA does not provoke any alteration of enzymatic activity.

These observations suggest that the ability of some methacrylic monomers to induce differentiation of promyelocytic leukemia cells may be mediated by their capacity to determine a down-regulation of telomerase activity.

Keywords: methacrylic monomers, cell differentiation, telomerase.

1. INTRODUCTION

Composite resins – largely utilized in some medical fields, for example in dental restorations and in orthopaedic prostheses [1, 2] – are complex materials constituted by an organic polymerizable matrix and an inorganic reinforcing filler joined by a coupling silanic agent [3, 4]. The organic matrix is frequently composed by bis-phenol A glycerolate dimethacrylate (Bis-GMA) with addition of other less viscous methacrylic monomers like triethyleneglycol-dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), urethane-dimethacrylate (UDMA) or 1,4-butanediol dimethacrylate (BDDMA) (Figure 1) the main function of which is to improve material handling and filler incorporation.

After performing dental restorations with composite resins, small amounts of monomers – residual from inevitably incomplete polymerization – are released either into the oral cavity and, through dentinal diffusion [5-7], into the pulpal tissues from where they spillover into the blood circulation [5]. Subsequently, the monomers may cause, or at least contribute to, adverse biological effects, [8] *i.e.* damage to the oral soft tissues, as already observed *in vivo* [9], and a remarkable *in vitro* cytotoxicity in primary and immortalized cultures [5, 6]. Although *in vitro* experiments with cells are not able to reproduce closely the *in vivo* conditions, they have been largely utilized because of the possibility to investigate separately the action of xenobiotics on different cell populations.

In particular, *in vitro* studies carried out utilizing the above-mentioned molecules have underlined their genotoxic, allergenic [10], cytotoxic and mutagenic activity [9]. Moreover, methacrylates alter lipid metabolism, glutathione (GSH) concentration, reactive oxygen species (ROS) production, cell cycle, energy metabolism and mitochondrial activity [11-18]. Nevertheless, recent *in vitro* studies [17, 18] have shown that TEGDMA, Bis-GMA, HEMA, UDMA and BDDMA are able to induce differentiation in HL-60 cells, a human promyelocytic leukemia cellular line derived from peripheral blood leukocytes blocked at the promyelocytic stage [19]. Such effect is evidenced by the recovery of the oxidative burst caused by the activity of membrane NADPH oxidase, an enzyme constituted by several proteins shared between membranes and cytosol in resting cells [20-22]. The cytosolic proteins p47phox (phox: pha-gocyte oxidase), p67phox, and p40phox interact with each other

to form a complex joining the small G-proteins, Rac1 (in monocytes) or Rac2 (in neutrophils) respectively [23]. The membrane associated components of the NADPH oxidase are constituted by a glycosylated 91-kDa protein (gp91phox or NOX2) and a 22-kDa subunit (p22phox), forming the flavocytochrome b558 [24]. The spatial separation of the NADPH oxidase components ensures that the enzyme is dormant in resting cells. However, in response to stimulation, the cytosolic components migrate almost instantly to the membrane and bind to the flavocytochrome b558 to form the active enzyme, the whole process being tightly regulated by protein-protein interactions and by phosphorylation of p47phox [23, 25-27].

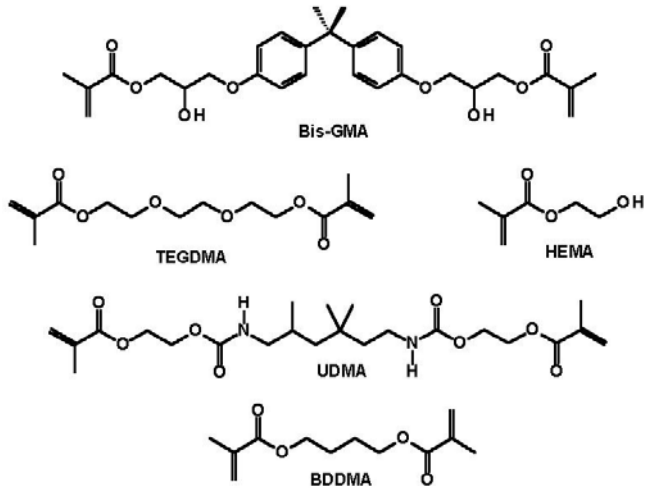

Figure 1. Chemical structure of Bis-GMA, TEGDMA, HEMA, UDMA, and BDDMA.

Cellular differentiation is related to the activity of telomerase, a ribonucleoprotein with reverse transcriptase capability that adds telomeric repeats to the 3'-end of the G-rich strand of telomeres, thus protecting chromosomes from the telomeric attrition associated with the 'end replication problem'. In fact, telomeres are constituted by 5'-TTAGGG-3' nucleotide repeats that cap the ends of linear chromosomes maintaining chromosomal stability [28,29]. Since tens of nucleotides of telomeres are lost in each mitotic division [30], the progressive telomeric attrition can result in an end-to-end fusion, likely a possible signal for senescence and oncogenic mutations [29].

Telomerase during cell differentiation loses the function of synthesis and maintenance of the telomeric units, while such activity can in fact be detected in most cells with increased ability to replicate (cancerous, germinal and some stem cells), [31-34], but not in somatic ones [35-38], suggesting that telomerase plays a crucial role in proliferation, cellular immortality and carcinogenesis. Moreover, forced expression of telomerase gene in non expressing cells results in their immortalization [39] or in conversion into cancer cells [40].

It has been discovered that telomerase enzyme needs three components for its function: the hTR (ubiquitously expressed RNA subunit), the hTERT (catalytic portion over-expressed in ~90% of cancers) and finally the dyskerin, considered an hTR stabilizer [41]. The mRNA level of hTERT is considered the major determinant of telomerase activity because it is often positively correlated with this parameter [32, 42, 43].

High telomerase activity has been already observed in HL-60 cells, a phenotype related to their immortal status [32, 44]. Previous studies showed that HL-60 cells are able to differentiate into monocytes and macrophages in presence of compounds like dimethyl sulfoxide (DMSO) and 1,25 α-dihydroxy vitamin D3 [45-47]. On the other hand, addition of all-*trans* retinoic acid (ATRA) to the growth medium induces differentiation of HL-60 cells into neutrophils [**48**] and causes a remarkable decrement of hTERT activity in these cells. It is well known that ATRA freely enters the cell membrane and is escorted by cytoplasmatic proteins to the nucleus where it binds to a class of nuclear receptors related to vitamin D and thyroid hormone receptors. Although the mechanisms of the anti-cancer effects of retinoids like ATRA are not fully understood, it can be hypothesized that the reversal of the immortalized phenotype involves the inhibition of telomerase activity, the reduction of cell proliferation, the expression of differentiation markers and, finally, the capability to become susceptible to apoptosis [49].

The purpose of this work was to verify if the differentiation of HL-60 cells, induced by the above mentioned methacrylic monomers, could be accompanied by a decrease of the telomerase enzymatic activity, contributing to a better understanding of the mechanism of action of molecules present in dental composite resins so largely utilized in medical and odontoiatric fields.

2. MATERIALS AND METHODS

All chemicals and reagents were obtained from Sigma-Aldrich Srl, Milan, Italy, unless otherwise indicated. Human leukemic HL-60 cell line (Istituto Zooprofilattico, Brescia, Italy) was maintained under a CO_2 humidified atmosphere (5 %, 37 °C) in RPMI 1640 with heat inactivated Fetal Calf Serum (10% v/v), penicillin (100 units/mL), streptomycin (100.0 μg/mL) and glutamine (2.00 mmol/L).

Experimental Conditions

In previous works [17,18] we evaluated the monomer concentrations causing HL-60 differentiation in absence of cytotoxicity (with exception of UDMA and BDDMA). Therefore, all the experiments described below were performed with either ATRA (1.0×10^{-3} mmol/L), HEMA (1.10 mmol/L), TEGDMA (0.4 mmol/L), Bis-GMA (1.6×10^{-2} mmol/L), UDMA (55.0×10^{-3} mmol/L) or BDDMA (0.4 mmol/L) at the concentrations specified and the incubations were performed in a 5 % CO_2 humidified atmosphere.

Preparation of solutions of All-Trans Retinoic Acid and Methacrylates

Stocked DMSO solutions of TEGDMA (0.400 mol/L), Bis-GMA (0.016 mol/L), UDMA (0.055 mol/L), BDDMA (0.400 mol/L) and ATRA (1.000 mmol/L) were prepared immediately before use. As a general procedure, an aliquot (1.0 μL) of the above described solutions was added to HL-60 cells about (200,000 cells/mL) in RPMI 1640 medium (1.0

mL); pure HEMA was on the contrary added to the cells to reach a final concentration of 1.10 mmol/L. Except for HEMA-treated cells, DMSO final concentration (0.1% v/v) was utilized in all samples because preliminary investigations showed that this concentration did not induce any alteration in the parameters under study [17,18].

Cell Viability

Exponentially growing HL-60 cells (2 x 10^5 cells/ mL) in RPMI 1640 were incubated with ATRA, HEMA, Bis-GMA, TEGDMA, UDMA, BDDMA or DMSO for 96 hrs. Total cell number was then determined by the NucleoCounter (ChemoMetec A/S Allerød, Denmark). Both cellular proliferation and cellular mortality were calculated as already described [17,18].

Differentiation Assay

The respiratory burst of methacrylate-treated cells was assessed through chemiluminescence technique (CL) [**50**]. Both untreated and treated HL-60 cells (1 x 10^5), either unstimulated or stimulated with phorbol 12-myristate 13-acetate (1.5 nmol), were treated with luminol (100.0 nmoles) to prepare CL specimens. All samples were adjusted to final volume (1.0 mL) by adding Krebs-Ringer-phosphate (KRP) buffer solution and measurements were performed using an automatic luminometer (Autolumat LB 953, EG&G, Turku, Finland) for 120 min. at 25 °C and results were expressed as signal of treated *vs* untreated cells, both corrected by subtracting background luminescence.

RNA Extraction, cDNA Synthesis, and PCR Amplification

All the kits and reagents used in these experiments were obtained by Promega Italia s.r.l., Milan, Italy.

Total cellular RNA was purified using the SV total RNA Isolation System. The Reverse transcription-PCR (RT-PCR) was performed as follows: total RNA (2 µg), oligo dT (1 µg), 10 mmol/L dNTPs (3 µL), Recombinant RNasin Ribonuclease Inhibitor (25 units), M-MULV 5x Reaction Buffer (5 µL) and M-MULV RT (200 units) were incubated for 1 hr at 42 °C. The obtained cDNA was used as template for PCR reactions; the sequences of the PCR primers and PCR conditions are listed in Table 1. After 40 cycles of amplification, PCR products were resolved by electrophoresis using 1 % agarose gel.

Table 1. Sequences of the PCR primers and PCR conditions

PRIMER	SEQUENCE	Pre-PCR incubation	Denature	Annealing	Extension	Cycle
hTERT-f	ACGGCGAC ATGGAAGA ACAA	95°C	95°C 15"	62°C 30"	72°C 30"	40
hTERT-r	CACTGTCT TCCGCAAG TTCAC	95°C	95°C 15"	62°C 30"	72°C 30"	40
GAPDH-f	GAAGGTGA AGGTCGGA GT	95°C	95°C 15"	62°C 30"	72°C 30"	40
GAPDH-r	GAAGATGG TGATGGGA TTTC	95°C	95°C 15"	62°C 30"	72°C 30"	40

Telomeric Repeat Amplification Protocol Assay

The telomerase activity was tested by Telomeric Repeat Amplification Protocol (TRAP) method [32]. Cells were harvested and lysed in CHAPS lysis buffer. The lysate was centrifuged for 20 min. at 12,000 x g at 4 °C, and the supernatant (2 µL) was used in PCR amplification according to the manufacturer's protocol (Intergen, Burlington, MA). In addition, supernatant (10 µL) from each sample was incubated at 94 °C for 10 min. to serve as a heat inactivation control. PCR was performed at 30 °C for 30 min. followed by 38 cycles at 94 °C for 30 sec., 59 °C for 30 sec. and 72 °C for 1 min. The last extension step is performed at 55 °C for 25 min. An internal control together with a specific primer was added to the reaction mixture to monitor the efficiency of PCR. To determine if ATRA directly inhibits telomerase enzyme activity in cell-free system, protein extracts from control HL-60 cells were incubated with various concentrations of ATRA (0.2, 1, and 5 µmol/L) at 37 °C for 10 min. before the standard TRAP assay. The PCR products were analyzed by electrophoresis on a 10% non-denaturing polyacrylamide gel and stained with SYBR Green (Molecular Probes, Eugene, OR).

Statistical Analysis

Data are expressed as the mean ± statistical error of the mean (SEM) of at least three different experiments performed in duplicate. The means were compared by analysis of

variance (ANOVA) followed, when appropriate, by a multiple comparison of means by Student-Newman-Keuls test: $p < 0.05$ was considered significant.

3. RESULTS

Effects of Methacrylic Monomers on Cells Proliferation and Differentiation

As summarized in Table 2 a cytostatic effect on HL-60 cells is shown, at the concentrations here employed, by all monomers except HEMA. Such effect matches cell mortality rate only in the case of UDDMA and BDDMA (Figure 2). On the other hand, all the tested molecules show a differentiating action (data not shown). As already reported [17, 18], DMSO (0.1 %) did not induce any effect on the cells (data not shown).

Table 2. Effects of methacrylic monomers on cells proliferation, differentiation and hTERT activity

	Cytostatic effect	Cell Mortality	Differentiating effect	Telomerase activity
HEMA	–	–	+	+(p<0.05)
TEGDMA	+(p<0.01)	–	+	+(p<0.001)
Bis-GMA	+(p<0.01)	–	+	–
UDMA	+(p<0.001)	+(p<0.01)	+	+(p<0.01)
BDDMA	+(p<0.01)	+(p<0.01)	+	±
ATRA	+(p<0.01)	–	+	+(p<0.01)
DMSO	–	–	–	–

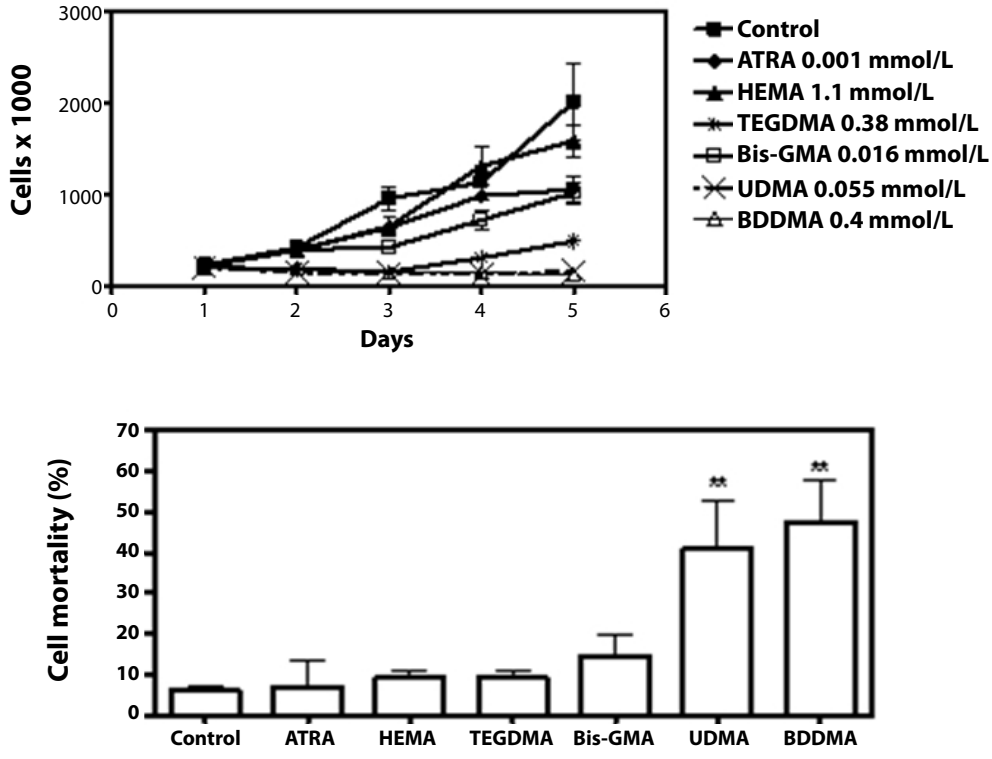

Figure 2. Proliferation curves and mortality of HL-60 cells, untreated or treated with ATRA or methacrylates; the total cell number was determined everyday using NucleoCounter. ** $p<0.01$.

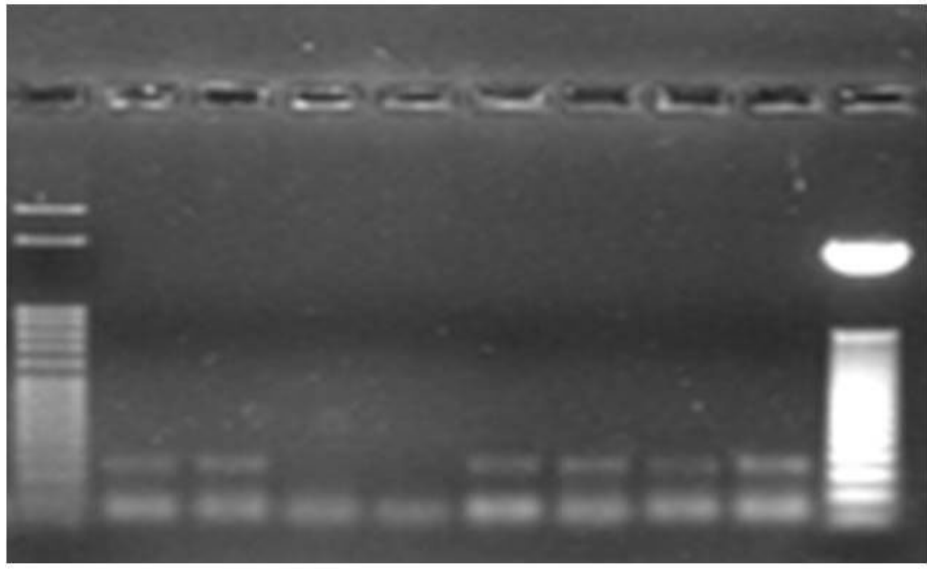

Figure 3. Gel electrophoretic pattern of MW marker, control, HEMA, negative control, negative control, TEGDMA, Bis-GMA, UDMA and BDDMA respectively.

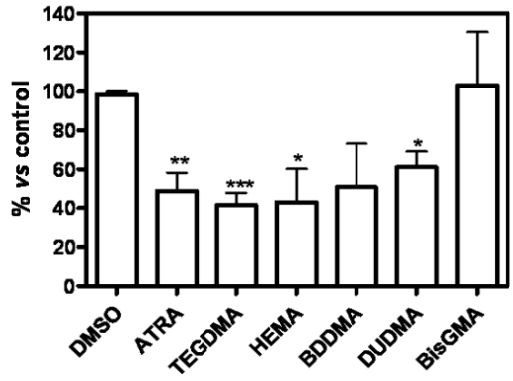

Figure 4. Telomerase activity in HL-60 cells, untreated or treated with ATRA or methacrylates. Results as expressed as percentage *vs* control. * $p<0.05$; $p<0.01$; **$p<0.001$.

Effects of Methacrylic Monomers on Htert mRNA Production in HL-60 Cells

mRNA production from hTERT and GAPDH genes was qualitatively evaluated and results reported in Figure 3 showing that both mRNAs are present in HL-60 cells independently from treatment with monomers.

Effects of Methacrylic Monomers on Htert Activity in HL-60 Cells

Enzyme activity decreased significantly in HL-60 cells treated with ATRA, TEGDMA, HEMA and UDMA (about 50%) and much less significantly (about 30 %) in those treated with BDDMA. In presence of Bis-GMA, HL-60 cells showed no change of telomerase activity (Figure 4 and Table 2).

4. DISCUSSION

In the last decades the widespread use of composite resins in dentistry and other medical fields both for young and adult patients drove many toxicological researchers to make major efforts to elucidate the biological interactions between released methacrylic monomers and cells.

Here we confirm the effects of HEMA, TEGDMA, Bis-GMA, BDDMA and UDMA on HL-60 proliferation and differentiation, already observed in previous studies [17, 18]. As expected, the above mentioned monomers cause a reduction of cell proliferation rate coupled with an increase of cell mortality induced – in these experimental conditions – only by BDDMA and UDMA. Furthermore, the recovery of the oxidative burst in HL-60 cells treated with the monomers proves that such molecules can provoke cellular differentiation. These interesting results – together with the consideration that telomerase is an enzyme particularly active in stem and cancer cells but virtually inactive in the differentiated ones – prompted us

to start investigations about the telomerase activity with the aim to better explain the mechanism of the cellular differentiation induced by methacrylates in HL-60 cells.

We have thus verified, in the first instance – using the rt-PCR technique – the presence of telomerase mRNA in HL-60 cells treated with monomers and then assayed the enzyme activity by the particularly suitable TRAP assay.

The obtained results showed a different behaviour of the five examined monomers: the effects of TEGDMA, HEMA and UDMA on telomerase activity were similar to that obtained with ATRA, halving the enzyme functionality. A different behaviour was instead showed by BDDMA, because this compound did not induce a very significant reduction of the enzyme activity. Lastly, Bis-GMA did not induce any change in the function of telomerase in respect to control. These differences are difficult to explain, because Bis-GMA caused the same biological effects regarding proliferation and cellular differentiation as the other examined molecules. On the other hand, the structural differences of Bis-GMA in respect to the other methacrylic monomers – for example in terms of stiffness of the aromatic moiety (see again Figure 1) – are seemingly responsible for this particular result. Moreover, we have reported in previous works [17, 18] that – at the same concentrations used in this study – Bis-GMA did not induce in HL-60 any depletion of GSH, a modulating agent of telomerase activity [51]. This behaviour could therefore explain the lack of alteration of the enzyme activity by Bis-GMA monomer. However, the previous observation raises still more questions because UDMA, which does not modify GSH concentration just like Bis-GMA, reduces instead telomerase activity and BDDMA, which conversely reduces GSH concentration, does not affect at all enzyme functionality.

From the obtained results it emerges for certain that a decrease of telomerase activity is clearly not a strict requirement for cell differentiation, because cells treated with Bis-GMA and BDDMA differentiate normally, as already confirmed [52].

Lastly another intriguing observation is that the oxidative burst occurred in HEMA treated cells while telomerase activity declined, in apparent contradiction with the continuing cellular grow.

CONCLUSION

The reported data suggest that each monomer here examined could down regulate cell proliferation with a different mechanism and this consideration is supported by the findings that not always is present a linkage between proliferation and telomerase activity.

Given that the major transcription factors regulating hTERT expression are redox sensitive, hTERT is inevitably affected by the presence of methacrylates which alter in different way the cellular redox status [53, 54].

Further studies are therefore needed to rationalize the relationship between the HL-60 cells differentiation induced by methacrylates and the hTERT activity.

In the light of the above discussion and considering the widespread use of the composite resins in medicine, detailed studies about the biochemical interactions between cells and each methacrylic monomer are clearly an important prerequisite that can aid to design materials with considerably improved characteristics of biocompatibility.

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Chapter 7

SCALE DEVELOPMENT TO ASSESS PSYCHOLOGICAL STATES IN DENTAL PATIENTS

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ABSTRACT

The authors have developed psychological scales to assess the states and behavior of dental patients with a particular focus on self-efficacy theory. When developing such scales, it is essential to verify their reliability and validity. We describe classic methods for developing scales to assess the psychological states of dental patients. These methods can also be applied to other psychological theories. Reliability factors, consistency, and stability are discussed, and validity factors—including content validity, criterion-related validity, and construct validity—are explored. In our past studies, we developed a self-efficacy scale for self-care among periodontal disease patients, and proved its effectiveness in predicting loss to follow-up for long-term periodontal treatment. Here, we also show the clinical applications of the psychological scales, whose reliability and validity has been confirmed.

PART 1. METHODS FOR SCALE DEVELOPMENT

1. What is a Psychological Scale?

In the field of psychology, *psychological scales* are widely used to examine people's consideration and behaviors. A psychological scale is generally composed of two or more questions, and enables researchers to measure psychological constructs (character, affective state, interpersonal relationships, adaptive state, etc.). In other words, a psychological scale is a tool that can convert a person's subjective judgment into an objective measurable value. If we use a psychological scale whose correctness of measurement is sufficiently verified, we can perform a variety of applied research (Murakami, 2006).

2. What Makes for a Good Psychological Scale?

A psychological scale is generally evaluated from the viewpoint of its *reliability* and *validity*. We'll discuss reliability and validity in detail later; in brief, *reliability* measures consistency between items or stability across time in the measurement, while *validity* measures how accurately a scale's score can be measured. A good psychological scale should be both highly reliable and highly valid.

3. Making a Draft Scale

If you plan on developing a psychological scale, follow these procedures.

3.1. Clarify the subject to be measured

The first process of scale development is to clarify what the questionnaire measures. The subject which the psychological scale measures should be made as concrete as possible (Murakami, 2006). Scale developers should ask themselves if the construct they wish to measure is distinct from other constructs. Moreover, while there are many technical aspects involved in developing and validating a scale, one should not overlook the importance of being well grounded in the established theories related to the phenomenon to be measured (DeVellis, 2003).

3.2. Make an item pool

There are a number of methods for making and collecting the items that will be part of your scale:

1. Cast a wide net to collect previous research and related articles that treat the concepts you'll be measuring and trying to work with.
2. Work with other researchers to generate the list of items.
3. Interview the people and the patients who will be the subject of measurement. You can also conduct the questionnaire survey using the free description form.

3.3. Determine the format for questions

You can choose from among several question formats. Typical formats include the Thurstone scale (Thurstone, 1928), the Guttman scale (Guttman scale, 1950), the Likert scale (Likert, 1952), and the Semantic Differential scale (Osgood & Tannenbaum, 1955). We'll look at the Likert scale in more detail as it is the one most commonly used.

Each item in a questionnaire based on a Likert scale is presented as a declarative sentence, followed by response options that indicate varying degrees of agreement with or endorsement of the statement. Likert scaling is widely used in instruments measuring opinions, beliefs, and attitudes. The response options should be worded so as to have roughly equal intervals with respect to agreement (DeVellis, 2003). This is the format of a typical five-point Likert item:

1. Strongly disagree
2. Disagree
3. Neutral
4. Agree
5. Strongly agree

This example scale has five points, but three-, seven-, and nine-point scales are also common.

4. Item Analyses: Determining the Final Version of the Scale

In order to analyze the individual items used in a scale, it is necessary to conduct a preliminary examination with the draft scale. To select the items adopted for the final version of the scale, you'll need to perform an item analysis. There are several ways to do so.

4.1. Item-total correlation analysis

If a participant has a high total score on the scale, you can expect that participant's scores for each individual item on the scale to also be high. Therefore, an item with a low correlation coefficient with the total scale score should be excluded from the draft scale, because it is apparently unrelated to total scale score (Kamahara et al, 1998).

4.2. Coefficient α (Cronbach, 1951)

One of the most important indicators of a scale's quality is the reliability coefficient, or *alpha.* Non-central mean, poor variability, negative correlations among items, low item-scale correlations, and weak inter-item correlations will tend to reduce alpha. Therefore, after we have selected our items, weeding out the poor ones and retaining the good ones, alpha is one way of evaluating how successful we have been (DeVellis, 2003).

Theoretically, alpha can take any value from 0 to 1. Nunnally (1978) suggests a value of 0.70 as a lower alpha. Different methodologists and investigators prefer different levels of alpha. According to DeVellis (2003), his personal comfort ranges for research scales are as follows: below 0.60, unacceptable; between 0.60 and 0.65, undesirable; between 0.65 and

0.70, minimally acceptable; between 0.70 and 0.80, respectable; between 0.80 and 0.90, very good; much above 0.90, one should consider shortening the scale.

4.3. Ceiling and floor effects

You should calculate the mean value and the standard deviation of each item. Then, delete an item if it demonstrates the *ceiling effect* (mean value ± standard deviation > the highest scale score) or the *floor effect* (mean value ± standard deviation < the lowest scale score) are found (Scott et al. 2008).

4.4. Factor analysis

You can use *factor analysis* to determine if the selection process has succeeded. This can help you determine empirically how many constructs and factors underlie scale items. There are two major guidelines for judging when enough factors have been extracted (DeVellis, 2003). One is the *eigenvalue rule* (Kaiser, 1960), and the other is the *scree test* (Cattell, 1966).

An eigenvalue represents the amount of information captured by a factor. For certain types of factor analytic methods (specifically, *principal components analysis*), the total amount of information in a set of items is equal to the number of items. Accordingly, the eigenvalue rule asserts that factors that have eigenvalues less than 1.0 (and thus contain less information than the average item) should not be retained.

The scree test is also based on eigenvalues, but uses their relative rather than absolute values as a criterion. It is based on a plot of the eigenvalues associated with successive factors. Cattell (1966) suggested that the right number of factors can be determined by looking at the drop in the amount of information across successive factors.

A statistical indication of the extent to which each item is correlated with each factor is given by the factor loading. In other words, the higher the factor loading, the more the particular item contributes to the given factor. Thus, factor analysis also explicitly takes into consideration the fact that the items measure a factor unequally (Carmines & Zeller, 1979).

5. Reliability Examination

Fundamentally, reliability concerns the extent to which an experiment, test, or measuring procedure yields the same results on repeated trials. Reliability can be examined from a number of perspectives.

5.1. Internal consistency

Internal consistency, as the name implies, is concerned with the homogeneity of the items within a scale. Scales based on classical measurement models are intended to measure a single phenomenon. Measurement theory suggests that the relationships among items are logically connected to the relationships of items to the latent variable. Although we cannot directly observe the linkage between items and the latent variable, we can certainly determine whether the items are corrected to one another (DeVellis, 2003). Typical methods of examining the internal consistency are the *coefficient α* and *split-half correlations.* As mentioned in section 4.2 above, we omit the explanation of coefficient α in this section. A

split-half correlation is a correlation coefficient calculated between scores on two halves of a test. For example, the scale items may be divided into two groups, based on whether the item number is odd or even. Then, the relation between the scores in the two groups is examined. These methods show whether the participant reacted similarly to each item, and verify the internal consistency of the scale.

5.2. Stability

The *retest method* is the method most widely used to assess stability. In this method, the same tests are administered to a sample twice; the correlation coefficient is calculated; and this offers a coefficient of reliability. When the test is repeatedly conducted to the same object, it can be deemed stable if the result is steady. Because in the retest method the same test is conducted twice within a certain time interval, the participants' characteristics shouldn't change between the two tests.

The appropriate length for the interval between the first and second test is controversial, and likely to be a source of worry for researchers. If it is too long, things may have changed; if it is too short, participants may remember the answers they gave on the first test. Typical intervals range from one month from one week. This is a relatively easy method of presuming the coefficient of reliability (Murakami, 2006).

6. Validity

Validity is the extent to which any measurement instrument measures what is intended to measure. According to the conventional interpretation, validity is inferred from the manner in which a scale has been constructed, its ability to predict specific events, or its relationship to measures of other constructs. There are essentially three types of validity that correspond to these operations (DeVellis, 2003): *content validity, criterion-related validity,* and *construct validity.*

6.1. Content validity

Content validity has played a major role in the development and assessment of various types of tests used in psychology and (to an even greater extent) education. Fundamentally, content validity depends on the extent to which an empirical measurement reflects a specific domain of content (Carmines & Zeller, 1979).

To assess content validity, two or more specialists should read a test item thoroughly to evaluate it. Content validity can also be assessed by the agreement rate and the correlation coefficient between the specialists' judgments.

6.2. Criterion-related validity

The traditional definition of criterion-related validity is the correlation of a scale with some other measure of the trait or disorder under study, ideally, a "gold standard" that has been long used and accepted in the field (Streiner & Norman, 2008). There are two types of criterion-related validity. If the criterion already exists, then concurrent validity is assessed by correlating a measure and the criterion at the same point in time. Predictive validity, on the other hand, concerns a future criterion that is correlated with the relevant measure (Carmines

& Zeller, 1979). This latter form of criterion validation is often used in college admission tests, where the ultimate outcome is the testees' graduation rate four years after testing; it is also used in diagnostic tests, where researchers must await the outcome of an autopsy or the further progression of a disease to confirm or disconfirm their predictions (Streiner & Norman, 2008).

6.3. Construct validity

Both criterion validity and content validity have limited usefulness for assessing the validity of empirical measures of theoretical concepts employed in the social sciences. It is partly for this reason that primary attention has been focused on construct validity (Carmines & Zeller, 1979). Construct validity is directly concerned with the theoretical relationship of a variable to other variables (Cronbach & Meehl, 1955). Fundamentally, construct validity is concerned with the extent to which a particular measure relates to other measures consistent with theoretically derived hypotheses concerning the concepts (or constructs) that are being measured (Carmines & Zeller, 1979). In other words, a hypothesis has construct validity if the theory from which it is derived can be proven without theoretical contradiction by the result of scale scores.

PART 2. APPLYING PSYCHOLOGICAL SCALES TO DENTAL RESEARCH

Now that you've seen how to construct a psychological scale, it's time to take a look at a practical example. We'll discuss a psychological scale that we developed for applied research in the field of dentistry.

1. Self-Efficacy Theory

In 1977, Bandura advocated the concept of self-efficacy within the framework of social learning theory, asserting that the confidence of an individual determines "how well he/she can take the actions necessary for producing certain results." In clinical practice, self-efficacy refers to how certain a patient feels about his or her ability to take the actions necessary to improve symptoms and maintain his or her health (Kakudate et al. 2010).

Focus in clinical medicine has been placed on the function of self-efficacy as an antecedent factor for behavior modification; for instance, symptoms of diabetes and other chronic diseases can be improved by enhancing patient self-efficacy (Grossman et al. 1987; Smarr et al. 1997). However, there has been little investigation into an oral health care–specific self-efficacy scale.

2. Development of the Self-Efficacy Scale for Self-Care (SESS) among Periodontal Patients (Kakudate et al., 2007, 2008, 2010)

The effectiveness of patient self-care and regular professional care in the treatment and prevention of periodontal diseases has been reported in some detail (Douglass, 2006; Kressin et al., 2003; Axelsson et al, 2004). The ability of periodontal disease patients to adhere properly to such health-promoting actions is vital to the successful prevention and treatment of periodontal disease. We developed the self-efficacy scale for self-care (SESS) among periodontal disease patients (Kakudate et al., 2007, 2008). As shown in Table 1, the SESS is composed of three subscales: self-efficacy for dentist consultations (SE-DC, 5 items), self-efficacy for toothbrushing (SE-B, 5 items), and self-efficacy for dietary habits (SE-DH, 5 items).

3. Creating the Draft SESS

3.1. Clarify the subject for the measurement

A total of 250 participants were sampled: Japanese patients who visited a private dental clinic in Sapporo, Japan, for periodontal treatment. Patients were diagnosed with mild to moderate chronic periodontitis.

3.2. Make an item pool

An experienced team of six dentists and one dental hygienist, all experts in periodontal dental practice, collected statements and made the draft 43-item pool, referring to past literature (Champion et al., 2005; George et al., 2007; Resnick et al., 2000; Rossen & Gruber, 2007; Syrjälä et al., 1999; Travess et al., 2004). One investigator interviewed periodontal patients. Lastly, the team judged the content validity of the collected items based on their clinical experience.

3.3. Determine format for questions

The answers were scored using a five-point Likert scale (Tarini et al., 2007) for each item, ranging from 1 (not confident) to 5 (completely confident). The SESS score for each participant was expressed as the sum of the scores assigned for all 15 items, and the possible scores ranged from 15 to 75.

4. Item Analyses of the SESS in the Pilot Study

Preliminarily, the draft scale consisting of 43 items was designed and applied to 61 periodontal disease patients who visited the Sapporo clinic for periodontal treatment. Each patient was asked to fill in a questionnaire of the draft the self-efficacy scale for self-care (SESS) and general self-efficacy scale (GSES) (Sakano et al., 1986). All patients in this study gave informed consent for participation in the study.

First of all, because the ratio of non-responding was high, one item was deleted. Next, we deleted 15 items because the ceiling effect and the floor effect had been confirmed. In

addition, four items were deleted because of low item-total correlation. Items with correlations greater than 0.4 were selected in the final questionnaire. Furthermore, a factor analysis by the principal factor method combined with a Varimax rotation was performed, and the eight items with factor loadings of less than 0.4 were deleted. The remaining items were selected as the standard 15 items of the SESS.

5. Second Study

We conducted a second study to verify factor confirmation, reliability, and validity. The participants in the second study consisted of 189 randomly selected patients (mean age 50.9 ± 15.2) who are different from the participants of pilot study and visited the Sapporo clinic for periodontal treatment. Participants were split into the two groups. Group 1 consisted of 129 patients who were diagnosed with mild to moderate chronic periodontitis at the initial dental visit. Following the baseline oral examination, the patients were informed of the results, diagnosis, the causes of periodontal disease, and treatment procedures. Group 2 was composed of 60 patients who had completed periodontal treatment and continued regular maintenance care every two to six months.

Each patient was asked to fill in a questionnaire of the draft SESS and GSES after receiving oral hygiene instruction from a dental hygienist who was not given an explanation about this study.

6. Factor Confirmation of the SESS: Determining the Final Version of the SESS

The factor structure of the 15-item SESS was confirmed by factor analysis using the principal factor method and Varimax rotation. The factors with eigenvalues of 1.0 or more were selected, and the items with factor loadings of less than 0.4 were deleted. A name was given to each extracted factor. The factor analysis extracted three factors: self-efficacy for dentist consultations, self-efficacy for brushing teeth, and self-efficacy for dietary habits (Table 1).

7. Reliability of the SESS

The scale had a previously estimated reliability for both internal consistency (Cronbach's alpha = 0.86) and retest stability (Spearman's rank correlation coefficient = 0.73; $p < 0.001$). Similarly, the internal consistency (Cronbach's alpha) of SE-DC, SE-B, and SE-DH were 0.90, 0.86, and 0.76, respectively, while the retest stability scores according to Spearman's rank correlation coefficient were 0.57 ($p < 0.01$), 0.39 ($p < 0.05$), and 0.53 ($p < 0.01$), respectively. These results suggest that the scale is highly reliable.

Table 1. Self-efficacy scale for self-care (SESS)

Self-efficacy for dentist consultations (SE-DC)
Q1: I go to the dentist for treatment of periodontal disease. Q2: I cooperate with my dentist and hygienist for treatment of periodontal disease. Q3: I visit my dentist regularly, even after treatment is completed, to prevent recurrence. Q4: I have regular checkups even when I am busy with work or housework. Q5: I have regular checkups even when my mind is not relaxed.
Self-efficacy for brushing of the teeth (SE-B)
Q6: I brush my teeth as instructed. Q7: I brush my teeth carefully and thoroughly. Q8: I brush the border between the teeth and gums. Q9: I move the toothbrush with a short, quick motion. Q10: I take time to brush my teeth carefully.
Self-efficacy for dietary habits (SE-DH)
Q11: I try not to spend too much time eating. Q12: I eat my meals at fixed times during the day. Q13: I try to eat a well-balanced diet. Q14: I try not to drink right before bed. Q15: I try not to eat too many sweets.

Information for each of the 15 items was scored according to a 5-point Likert scale ranging from 1 (not confident) to 5 (completely confident).

Source: (Kakudate et al., 2008; Morita et al., 2010)

8. Validity of the SESS

We had already examined content validity when we created the draft of version of the SESS, as described in section 3.2; next, we looked at criterion-related validity and concurrent validity.

8.1. Criterion-related validity

Concurrent validity. As for concurrent validity, the correlations between the SESS and the GSES scores were calculated. The SESS points showed a significant correlation with the GSES points ($r = 0.44$), indicating that our scale had concurrent validity.

Predictive validity. We examined the predictive validity of the SESS to predict loss to follow-up from long-term periodontal treatment in patients with mild to moderate chronic periodontitis in a 30-month long longitudinal prospective cohort study (Kakudate et al., 2010). The study showed that, when compared with the high-scoring SESS group (60–75), the odds ratios of loss to follow-up for the middle- (54–59) and low-scoring (15–53) groups were 1.05 (95% confidence interval: 0.36–3.07) and 4.56 (95% confidence interval: 1.11–18.74), respectively. Therefore, the predictive validity of the SESS was confirmed.

8.2. Construct validity

The construct validity was examined by hypothesis testing in the cross-sectional study. The hypothesis tested was as follows:

The SESS score of patients with successful maintenance therapy is higher than that of initial-visit patients who had yet to receive periodontal treatment.

Mean values of the SESS score were compared between the groups. The periodontal patients with successful maintenance therapy had a significantly higher SESS score (mean = 60.90 ± 6.64; n = 60) than initial-visit patients did (mean = 56.86 ± 7.56; n = 129) ($p < 0.001$). Therefore, construct validity was also confirmed.

CONCLUSION

This chapter explained methods of scale development to assess psychological states, and applied the same in the case of dental patients. The content of this chapter will contribute to the development of better psychological scales for use in dentistry.

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Chapter 8

TRENDS IN DENTAL POLYMER NANOCOMPOSITES

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BACKGROUND

Nanotechnology is offering us the ability to design materials with totally new characteristics. Nanotechnology is also known as molecular nanotechnology or molecular engineering is the production of functional materials and structures in the range of 0.1 to 100 nanometers-the nanoscale-by various physical or chemical methods [1].Today the revolutionary development of nanotechnology has become the most highly energized discipline in science and technology [2]. The intense interest in using nanomaterials stems from the idea that they may be used to manipulate the structure of materials to provide dramatic improvements in electrical, chemical, mechanical and optical properties [3].

A nanometer is 1/1,000,000,000 (one-billionth) of a meter or 1/1,000 of a micron. This is about 10 times the diameter of a hydrogen atom, 1/1,000 the size of a small bacterium or 1/80,000 of a human hair. Frequently, nanotechnology is used to describe research or products where critical component dimensions are in the range of 0.1 to 100 nanomers. In theory nanotechnology can be used to make products lighter, stronger, cheaper and more precise. If this type of material was used to make an airplane instead of metal the airplane could weigh 50 times less but be just as strong. A servey in 1998 revealed that industries where micro-engineered products are the most desirable are in electronics and biomedicine. The current status of this technology is in creating value-added products. At a nanotechnology fair held in Hanover, Germany (April 2002) some of the products that were displayed, included a lacquer that can be applied over automobile paint to make it a scratch-resistant and transparent coating for glass that shields against UV rays. Other areas currently being commercialized include a nanofiber mesh to prevent body tissues from sticking together preventing the formation of scar tissue, chips-the size of a compact disc-could replace central air conditioning units, a ultra thin monitor that looks like paper but can change what it displays like a computer screen or LCD and batteries that last longer.

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Nanocomposites are a new class of composites that are particle-filled polymers for which at least one dimension of the dispersed particles is in the nanometer range. A large amount of research is being devoted to development of nanocomposites of different types for various applications, including structural materials, high performance coatings, catalysts, electronics, photonics and biomedical systems [4]. Every property has a critical length scale and by using building blocks smaller than the critical length scale –such as nanoparticles one can capitalize on the manifestations of physics at small sizes. An example of this is in light scattering. When a particle shrinks to a fraction of the wavelength of visible light (0.4-0.8 μm) then it would not scatter that particular light resulting in the human eye's inability to detect the particles. This has tremendous implications for the optical properties of materials [5].

One of the most significant contributions to dentistry has been the development of polymer based composite technology. Adhesively bonded composites have the advantage of conserving sound tooth structure with the potential for tooth reinforcement, while at the same time providing a cosmetically acceptable restoration. .

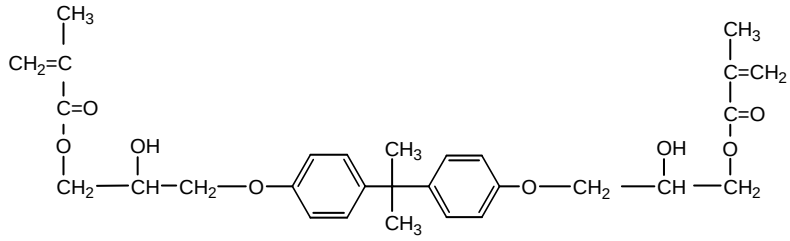
Dental Polymer Composites

Dental polymer composites are interconnected heterogeneous materials that generally have three discernable phases: (1) a polymeric matrix or continuous phase formed by polymerization of one or more monomer/oligomers (2) a higher modulus dispersed phase consisting of fillers of various types (silica, ceramic etc) sizes, shapes and morphologies and (3) an interfacial or interphasial phase that bonds to both the continuous and dispersed phases, thereby enhancing the moduli and mechanical properties of the weaker polymer phase and also facilitating stress transfer between these phases by forming a unitary material. Adhesion of lower moduli polymer matrices to higher moduli inorganic fillers can occur as a result of van der Waals forces, ionic interactions, hydrogen bonding, ionic or covalent bonding, interpenetrating polymer network formation and for certain types of fillers by micromechanical interlocking mechanisms. For most mineral reinforced dental composites the primary interphasial linkage between the polymer matrix and the filler is by chemical bond formation mediated by a silane coupling agent [6].

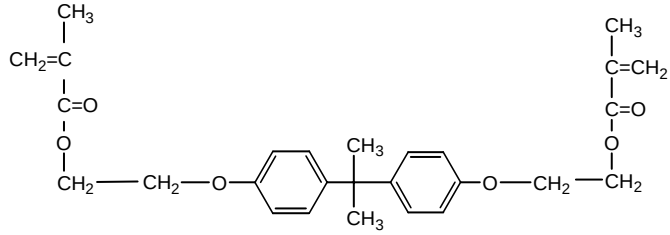
Usually the organic matrix is based on methacrylate chemistry, especially cross-linking dimethacrylates like :

(a) 2,2-bis[4,2-hydroxy-3-methacryloyloxypropyl)phenyl]propane (Bis-GMA)
(b) ethoxylated Bis-GMA (Bis-EMA),
(c) 1,6-bis[2-methacryloyloxyethoxycarbonylamino]-2,4,4-trimetylhexane (UDMA), (d) dodecanediol dimethacrylate (D_3MA) or
(e) triethylene glycol dimethacrylate (TEGDMA) (Figure 1) [7-10].

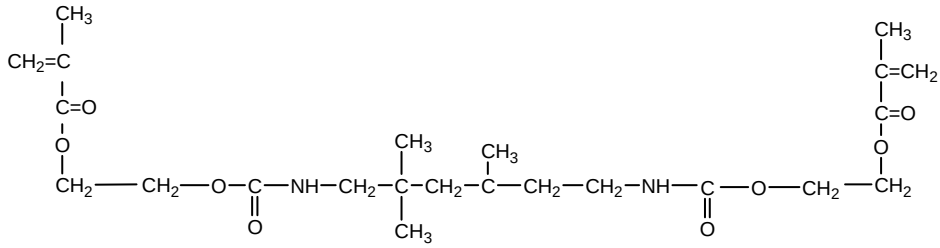
The first three monomers are used as base monomers and the last two as diluents which are added in order to control the viscosity of the organic matrix to be suitable to accept a large amount of the inorganic filler.



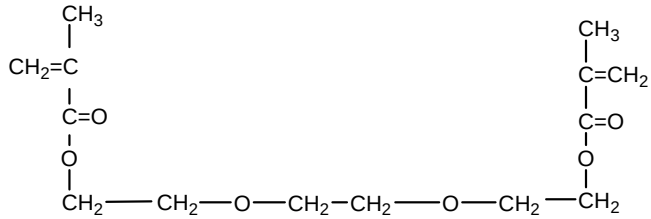
(a) Bis-GMA



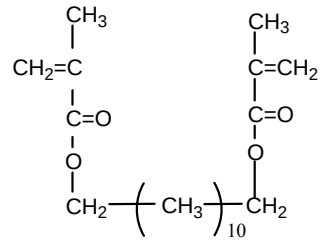
(b) Bis-EMA



(c) UDMA



(d) TEGDMA



(e) D_3MA

Figure 1. Dimethacrylates mostly used in dental polymer composites for the preparation of the organic polymeric matrix; (a)-(c): base monomers. (d) and (e): diluents.

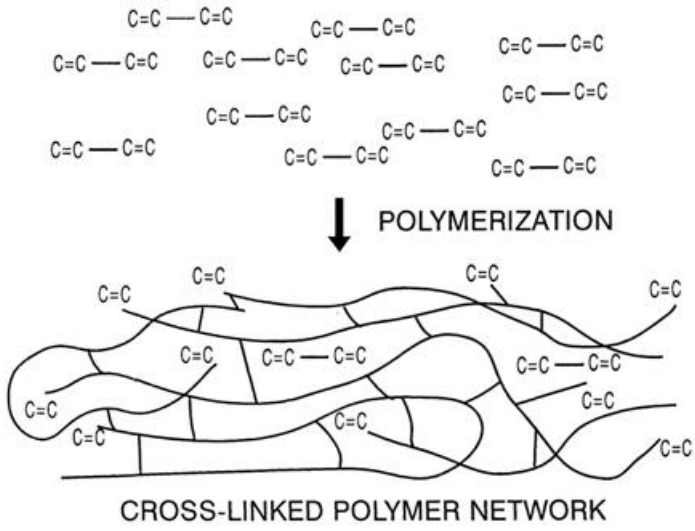


Figure 2. Schematic representation of the polymerization of dimethacrylate monomers to form the cross-linked polymer network of dental composites containing many unreacted pendant methacrylate groups (-C=C-) [11].

The free radical polymerization of the matrix monomers leads to a three-dimensional network (Figure 2) [11]. Most of the contemporary dental polymer composites are light-curing composites which polymerize, harden by irradiation with visible light in the wavelength range 400-500nm. Nearly all composite manufacturers are using camphorquinone as the photoinitiator. The absorption maximum of camphorquinone is at 468 nm. Amines are the most frequently used co-initiators [12].

The fillers used in dental composites directly influence the radiopacity, mechanical properties such as hardness, flexural and compressive strength and thermal coefficient of expansion. The use of heavy metals such as barium and strontium incorporated in the glass provide radiopacity. Dimethacrylate monomers have a high coefficient of thermal expansion. This coefficient is reduced by the addition of fillers and ideally dental composites should have similar coefficients of thermal expansion to enamel and dentine of tooth, which is $17x10^{-6}/^{o}C$ and about $11x10^{-6}/^{o}C$ respectively [17]. The fillers provide the ideal means of controlling various aesthetic features such as color, translucency and fluorenscene. Polymerization shrinkage largely correlates with the volumetric amount of the filler in the composite. By incorporating large amount of fillers the shrinkage is mauch reduced because the amount of resin used is reduced and the filler does not take part in the polymerization process. However, shrinkage is not totally eliminated and will depend on the structure of monomers used and the amount of filler incorporated.

One of the most important considerations in the selection of filler is the optical characteristics of the composite. The monomers used in dental composites have a refractive index of approximately 1.55. Fillers with refractive indices which differ greatly from this value will cause the composite to appear optically opaque, creating an esthetic and curing problem. Because glasses can have refractive indices ranging from 1.4 to 1.9 the selection of the appropriate filler for dental composites must be guided by a consideration of this important variable. The filler most used until quite recently was fused or crystalline quartz

and various borosilicate or lithium aluminosilicate glasses. The glass or quartz was ground or milled into particles of various sizes ranging from approximately 0.1μm to 100 μm. The major advantage to using quartz was that it is readily available and has an excellent optical match to the polymer matrix. However quartz has drawbacks in that it is not radiopaque and can be very abrasive to enamel. These characteristics ensured that as the surface of the composite was abraded the polymer would wear away more quickly than the fillers leaving them raised and exposed from the surface. This made the surface of the restoration rough and less enamel-like due to appreciable scattering of incident light. Thus polishability and esthetics were compromised. Most current composites are filled with radiopaque silicate particles based on oxide of barium, strontium, zinc, aluminum or zirconium [11].

The average particle size and particle size distribution of the filler is important as it determines the amount of the filler that can be added to the monomers, without the necessary handling characteristics being lost. Particle size also has a pronounced effect on the final surface finish of the dental composite in that the smaller the filler particle size the smoother the composite will be [13].

The composites have been classified according to the type of filler employed into three main groups, the traditional or macrofilled composites the hybrid or blended composites and the microfilled composites [9,11,13,14-16]. The macrofilled composites contain glass filler particles with a mean particle size of 10-20 μm and a largest particle size of 40 μm. These composites had the disadvantage that the surface finish was very poor with the surface having a dull appearance due to the filler particles produting from the surface as the resin preferentaially removed around them. These composites are significantly less frequently used nowdays because of esthetic reasons. The hybrid composites contain large filler particles of an average size of 15-20 μm and also a small amount of colloidal silica which has a particle size of 0.01-0.05 μm. It should be noted that virtually all composites now contain small amounts of colloidal silica but their behavior is very much dteremined by the size of the larger filler particles [13]. Microfilled composites containing amorphous silica were developed to address the polishing requirements of anterior restorations. Thses silicon dioxide particles are submicroscopic, averaging approximately 0.04 μm in diameter, though the size varies among materials. Because the filler particles in a microfilled composite are so small, they have from 1,000 to 10,000 times as much surface area as filler particles in conventional composites. The increased surface area must be wetted by the monomer matrix and which results in a significant increase in viscosity. This increase in viscosity limits the percentage filler content of the composite to approximately 35 wt%, which in turn limits the strength and stiffness of the composite.

During the initial development of dental composites it was shown that the acquisition of good properties in the composite was dependent upon the formation of a strong bond between the inorganic filler particles and the organic polymer matrix. If there is a breakdown of this interface, the stresses developed under load will not be effectively distributed through out the composite. The interface will act as a primary source for fracture leading to the subsequent disintegration of the composite. In most mineral reinforced dental composites the primary interphasial likage between the polymer matrix and the filler phase is by chemical bond formation mediated by a dual functional organosilane, termed a silane coupling agent [6,17-19]. In dental composites based on dimethacrylates, adhesion between the polymeric matrix and the reinforcing filler is usually achieved by the used of the silane coupling agent 3-methacryloxypropyltrimethoxysilane or γ-MPS. This is a bifunctional molecule capable of

reacting via its alkoxysilane groups with the filler and itself and with the polymer matrix by virtue of its methacrylate functional group. The overall degrees of reaction of the silane with the glass filler (oxane bond formation) with itself (by siloxane formation) and with the polymer matrix (by graft copolymerization) determine the efficacy of the coupling agent.

Dental Polymer Nanocomposites

No one composite material has been able until now to meet both the functional needs of a posterior Class I and II restoration and the superior esthetics required for anterior restorations. Many attempts are made to develop a composite filling material that could be used in all areas of the mouth with high initial polish and superior polish retention (typical of microfills) as well excellent mechanical properties suitable for high stress-bearing restorations (typical of hybrid composites). To this end novel nanofillers were developed and then nanocomposites using advanced methacrylate resins and curing technologies [5].

Nanofillers are very different from traditional fillers and require a shift from a top-down to a bottom-up manufacturing approach. To make filler particles of the mechanically strong composites of today (such as macrofills, hybrids and microfills) one starts from dense, large particles (mined quartz, melt glasses, ceramics) and comminutes them to small particle size. However these milling procedures usually cannot reduce the filler particle size below 100 nm (1nm =1/1000μm). To circumvent this roadblock, synthetic chemical processes were used to produce building blocks on a molecular scale. These materials then were assembled into progressively larger structures and transformed into nanosized fillers suitable for a dental composite. This attempt was made toward the development of a new dental commercial nanocomposite Filtek Supreme Universal Restorative (3M ESPE, Dental Products, St. Paul, Minn.) that has the esthetic properties required for cosmetic restorations and the mechanical properties necessary for posterior restorations [5].

In this study two new types of nanofiller particles were synthesized: nanomeric, or NM, particles and nanoclusters, NCs. The NM particles are monodisperse nonaggregated and nonagglomerated silica nanopartcles. Aqueous colloidal silica sols were used to synthesize dry dowders of nanosized silica particles 20 and 75 nm in diameter. These particles were treated with 3-methacryloxypropyltrimethoxysilane (γ-MPS). γ-MPS makes the filler compatible with the resin before curing to prevent any agglomeration or aggregation. Also two types of NC were synthesized using proprietary processes. The first type consists of zirconia-silica particles synthesized from a colloidal solution of silica and a zirconyl salt. The primary particle size of this NC filler ranges from 2 to 20 nm while the spheroidal agglomerated particles have a broad size distribution with an average particle size of 0.6 μm. The second type of NC filler which were synthesized from 75-nm primary particles of silica has a broad secondary particle size contribution with a 0.6 μm average. The surface of both types of nanocluster filler particles were treated with γ-MPS. The polymer matrix used in this work was consisted of Bis-GMA, Bis-EMA, UDMA and TEGDMA containing photoinitiators for light curing and stabilizers. Using statistically designed experimentation methodology many combinations of NC and NM fillers were studied to determine an optimal formulation for the Filtek Supreme Universal Restorative dental nanocomposite. The formulation for the dentine, body and enamel shades of Filtek Supreme Standard pastes

contain zirconia-silica NCs and silica NPs. The effective primary particle size is 20 nm. The formulations of Filtek transluscent shades contain a filler predomentally composed of individual NM particles 75 nm in diameter and a minor amount of silica NCs [5]

Figure 3 shows three transmission electron micrographs: (A) of a nanocomposite filled with 75-nm diameter NM particles only (B) of a nanocomposite filled with NCs alone (C) of a commercial composite made with large particle-size dense hybrid filler (particle size approximately 1 μm)

The compressive and diametral strengths and the fracture resistance of the nnocomposites were equivalent to or higher than those of other commercial composites tested. The three body wear results of the nanocomposites were also statistically better than those of all other composites tested (microfill, hybrid and microhybrid).

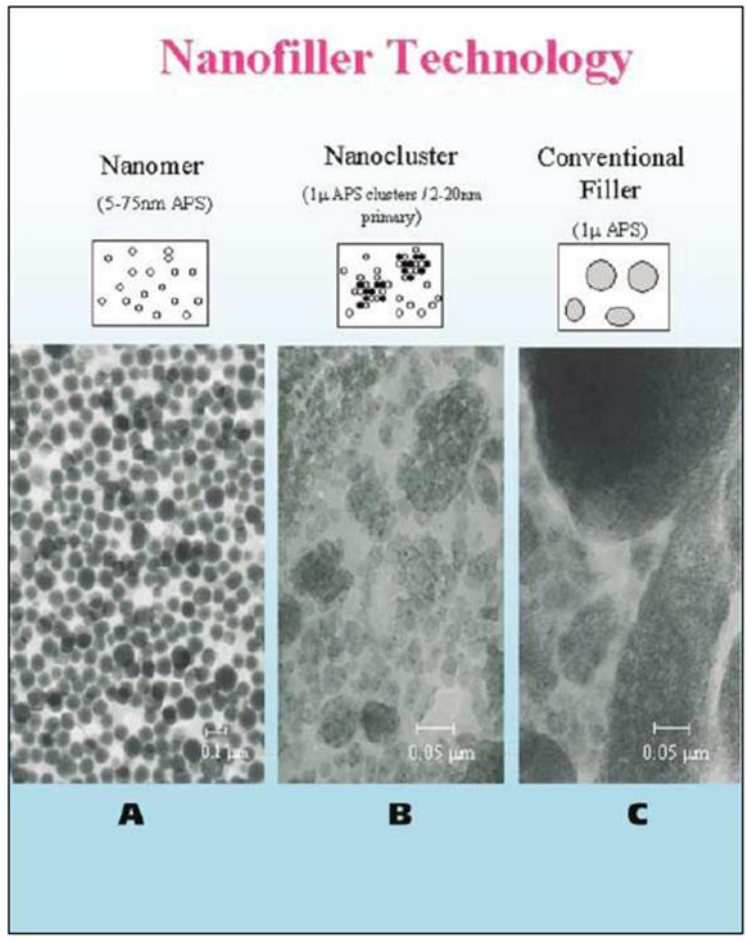


Figure 3. Schematics and transmission electron microscopic images of composites. (A): composite with nanometric particles (x 60,000 magnification) (B) composite with nanocluster particles (x 300,000 magnification) (C) composite with hybrid fillers (x 300,000 magnification). nm : nanometers ; APS Average Particle Size; μm micrometer [5].

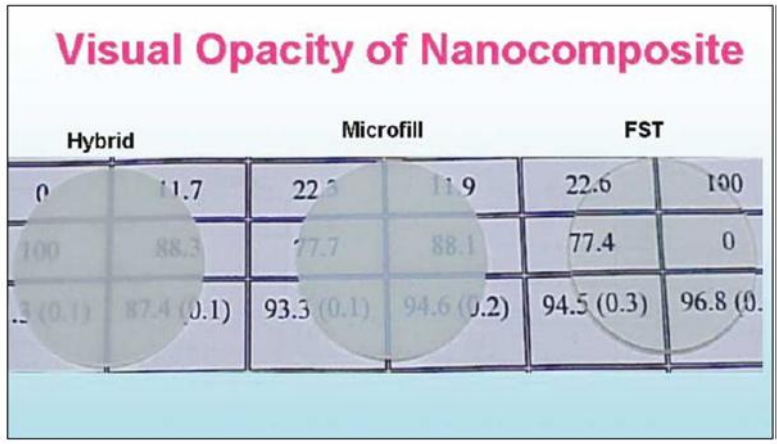


Figure 4. Optical effect of nanocomposite material versus that of the other types of composites. (FST: Filtek Supreme Transluscent formulation) [5].

Nano-fillers also offer advantages in optical properties. In general it is desirable to provide low visual opacity in un-pigmented dental composites. This allows the clinician to construct a wide range of shades and opacities and thus provide highly esthetic restorations. In hybrid materials, fillers consist of particles averaging 1 mm in size. When particles and resin are mismatched in the refractive index which measures the ability of the material to transmit light, the particles will scatter light and produce opaque materials. In NM-particle materials the size of the particles is far below the wavelength of light, making them un-measurable by the refractive index. When light comes in, long-wavelenght light passes directly through and materials show high translucency. As shown in Figure 4 the disks made with hybrid and microfill filler are rather opaque. The nanocomposite made predominantly with the NM particle filler (FST) is very clear as the background can be seen through the composite. In addition when placed on a black background the nanoparticles preferentially scatter blue light, giving the composite an opalescent effect. The ability to create a nanocomposite with a very low opacity provides the ability to formulate a vast range of shade and opacity options from the very transluscent shades needed for the incisal edge and for the final layer in multilayred restorations to the more opaque shades desired in the enamel, body and dentin shades [5].

Palin et al [20] studied the influence of short and medium-term immersion on water uptake and mechanical properties of a nanofilled composite (Filtek^{TM} Supreme in body and translucent shades) compared with a conventional microhybrid composite (Filtek^{TM} Z250). Strength degradation occurred at different rates between material types. Water uptake and mechanical properties of the test materials were influenced by the size and the morphology of the reinforcing particulate phase. The use of nanoparticles and associated agglomerates in modern nanocomposite exhibit distinct mechanical and physical properties compared with the conventional microhybrid composite. In a next work the authors studied composites with microhybrid (Filtek^{TM} Z250) nanohybrid (Grandio) and nanofilled (Filtek^{TM} Supreme) filler particle morphologies. Filler particles were provided by the manufacturer or separated from the unpolymerized resin using a dissolution technique. Filler particles were subjected to compression using a micromanipulation technique between a descending glass probe and a

glass slide. The number of distinct fractures particles underwent was determined from force/displacement and stress/deformation curves and the force at fracture and pseudo-modulus of stress was calculated. [21]. It was found that the "nanoclusters" of Filtek™ Supreme exhibited multiple fractures and a higher force at fracture compared with the spheroidal and irregular filler technologies. This was attributed to the ability of the "nanoclusters" to deform and collapse into pre-existing cluster porosities and through progressive fragmentation of the main cluster structure, which subsequently acted to absorb and dissipate propagating cracks. The authors suggested that incorporation of the "nanocluster" particles into the resin matrix as a complete system would have the potential to produce unique mechanical properties since deformation of the particle may enhance the resistance to crack propagation of the composite and subsequently improve the longevity of the restoration. Consequently as a continuation of this work a hypothesis was proposed where the bi-axial flexure strength (BFS) of a nanocluster-containing composite would differ markedly from microfill, microhybrid and nanohybrid composites following dry and wet cyclic pre-loading regimes. The nanocluster system exhibited distinctive properties in response to the cyclic fatigue pre-loading regimes, such as increased resistance to fracture and improved reliability in strength irrespective of environmental conditions. Consequently the hypothesis was accepted since nanoclusters provided a distinct reinforcement mechanism to the resin matrix. The eagglomerated nanoparticles produced an interconnected network wher the interstices were infiltrated with the silane coupling agent producing an interpenetrating phase composite structure. The combination of unique reinforcement and silane infiltration of structural porosities improved the damage tolerance and may enhance the clinical longevity of nanocluster composite restorations [22].

Characterization of three nanofilled composites (Supreme, Grandio and Grandio Flow) four universal hybrid (Point-4, Tetric Ceram, Venus, Z-100) and two microfilled (A110, Durafill VS) composites showed that nanofilled resin composites exhibit mechanical properties at least as good as those of universal hybrids and could thus be used for the same clinical indications as well as anterior restorations due to their high aesthetic properties [23].

In an effort to improve the mechanical properties of dental resin-based composites TiO_2 nanoparticles (with diameter <20nm) treated with the organosilane allytriethoxysilane (ATES) were used. TiO_2 nanoparticles were sonically dispersed in an ethanol solution containing ATES. The modified particles were washed in pure ethanol and dried before being used as filler. By spatulation TiO2 particles were manually blended with a resin monomer consisting mainly of UDMA. The particles were then manually added to Z100 dental composite and the mixture thoroughly blended. Surface modification by the organosilane ATES influences the dispersion and linkage of TiO_2 nanoparticles within a resin matrix and the modified particles were found to improve the microhardness anf flexural strength of dental composites [24].

A low shrinkage dental light curable nanocomposite was prepared by using an epoxy resin 3,4-epoxycyclohexylmethyl-(3,5-epoxy)cyclohexane carboxylate matrix with 55 wt% of 70-100nm nanosilica fillers through ring opening polymerization. γ-glycidoxypropyl trimethoxysilane was used to midify the surfaces of silica nanoparticles. The developed epoxy resin based nanocomposite demonstrated low shrinkage and high strength and is suitable for dental restorative material applications [25].

Fong at al [26] studied the reinforcement of electrospun nylon 6/fibrillar silicate nanocomposite nanofibers on Bis-GMA/TEGDMA dental composites. The hypothesis was

that uniform distribution of nano-scaled and highly aligned fibrillar silicate single crystals in electrospun nylon 6 nonofibers would improve the mechanical properties of the resulting nanocomposite nanofibers and would lead to the effective reinforcement of dental composites. The nylon 6/fibrillar silicate nanocomposite nanofibers were crystalline, structurally oriented and had an average diameter of approximately 250 nm. To relatively well distribute nanofibers in dental composites the nanofiber containing composite powders with a particle structure similar to that in interpenetration networks were prepared first and then used to make the dental composites. The results indicated that small mass fractions (1% and 2%) of nanofiber impregnation improved the mechanical properties substantially while larger mass fractins (4% and 8%) of nanofiber impregnation resulted in less desired mechanical properties.

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Chapter 9

DENTAL NANOCOMPOSITES

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INTRODUCTION

Developed over 40 years ago, dental composites have been widely adopted by the profession to replace traditional dental amalgams. These composites as restorative materials are extensively used in dentistry because they are safer and easier to use. Today, dental nanocomposites have wide spread applications, due to their physical and mechanical properties, excellent aesthetic and lack of side effects for patient and dentistry. When the hard tissue of teeth is damaged by dental caries and cavity preparation, the use of dental nanocomposites is the best treatment [1].

Dental composites consist of hard inorganic particles dispersed in a soft organic resin matrix. Properties of the composites are greatly influenced not only by the properties of their fillers but also by the chemical structure of the monomers, which are used in the matrix phase. Basically, a dental composite is a mixture of particles within an acrylic monomer that is polymerized during application [2]. In more detail dental composites consist of four major components which are an organic polymer matrix, inorganic filler particles, coupling agent, and the initiator-accelerator system. The resin forms the matrix of the composite material, binding the individual filler particles together through the coupling agent. Development of 2, 2-bis-[4-(methacryl-oxypropoxy)-phenyl]-propane (Bis-GMA) and dental composites by Bowen and their introduction to restorative dentistry was so successful that they were soon accepted as an esthetic filling material. The most widely used resin in dental composites is based on the copolymer prepared from a combination of Bis-GMA and triethylene glycol dimethacrylate (TEGDMA) (Fig. 1). TEGDMA is usually added to Bis-GMA in order to achieve workable viscosity limits since the latter monomer possesses very high viscosity ($>10^6$cP) due to the intermolecular hydrogen bonding. In Bis-GMA/TEGDMA dental resin

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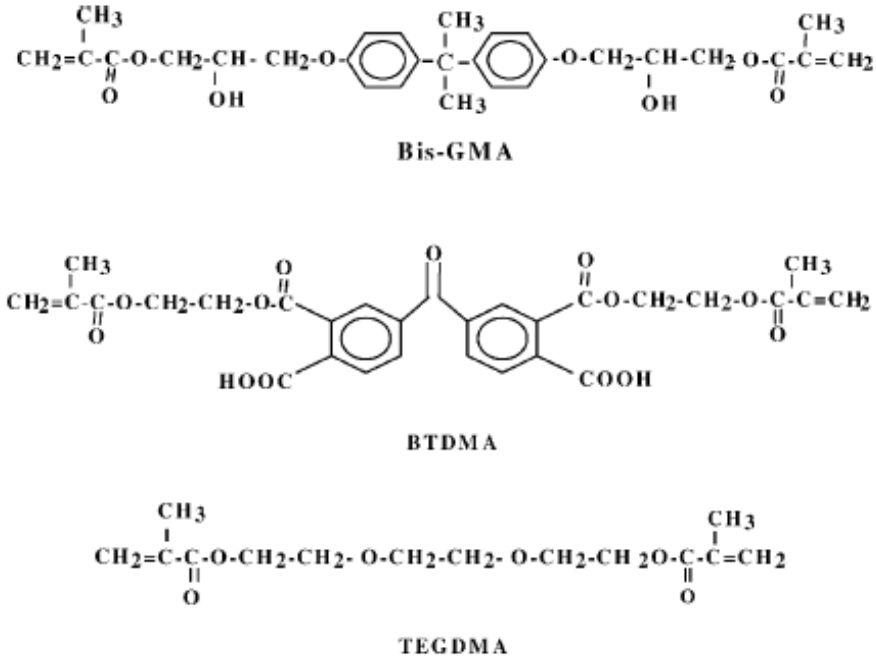
systems, Bis-GMA functions to limit the polymerization induced volumetric shrinkage and to enhance resin reactivity, while TEGDMA provides for increased vinyl double bond conversion [3].

Although Bis-GMA has widely been used as the main monomer in most resin composite systems due to its superior aesthetic quality, simple operation technique, enhanced mechanical strength, less shrinkage, higher modulus, and reduced toxicity because of its lower volatility and diffusivity into the tissue, and the composites have undergone significant development since their advent, they still have shortcomings limiting their application. Lack of good adhesion to the tooth structure and polymerization shrinkage are the most important problems. Considerable interest has been devoted to synthesizing new monomers to provide alternative monomers to overcome the problems [4].

BTDMA as a dimethacrylate monomer containing carboxylic acid groups in its structure has been shown that can interact with the Ca^{2+} ions of the tooth structure so it has the potential to provide better adhesion properties in dental composites (Fig. 1) [5].



Figuere 1. Chemical structures of Bis-GMA, BTDMA and TEGDMA.

LIGHT-CURED DENTAL NANOCOMPOSITES

Dental composites are usually cured (hardened) by photo-initiated free radical polymerization (photopolymerization). Camphorquinone (CQ) is extensively used as a photosensitizer for the visible light cured composite resin and ethyl-4-(N,N-dimethylamino)benzoate (4EDMAB) is a commonly used co-initiator [6].

Since the introduction of dental restorative resins, several energy sources, such as UV, halogen lamps and LED (light emitting diodes) curing units, have been used to initiate the

photopolymerization process of the resins. UV curing units is no longer used in dental applications because of its side effects on the oral mucous [7]. But lasers such as argon laser with its inherent optical characteristics like low beam divergence, monochoromacity, collimation, coherency, absorption selectivity because of wavelength tunability, and fiber delivery capability can all make it to be practically a better candidate, which effectively can reduce curing time, provide a larger degree of conversion (DC) of monomers, and enhance physical properties of cured composites. On the other hand, the argon laser appeared as a suitable alternative polymerization source of composite resins, particularly when the camphoroquinone, with broad peak activity in the 470 nm range (Fig. 2), is used in dental composite as a photoinitiator [3]. Since the early 1980s, one research focus has been the use of the argon laser for photopolymerization of composite resin restorative materials [8].

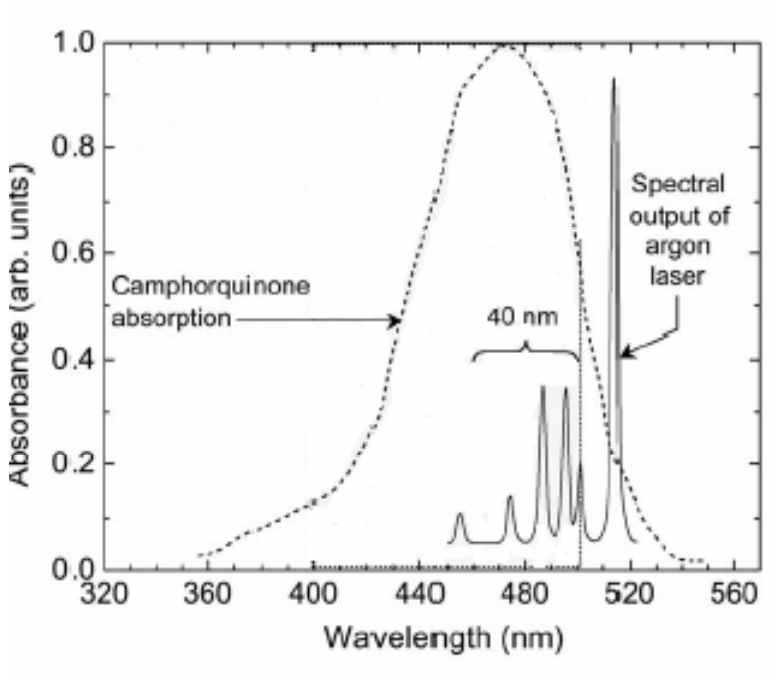


Figure 2. Absorption spectrum of camphorquinone [9].

The physical and mechanical properties of composites depend on their resin compositions, filler type, size and content, degree of conversion, flexural strength, flexural modulus, water sorption, solubility and reaction temperature. These parameters were studied systematically. For nanocomposite, an improvement in physical properties is expected due to the increased interfacial interactions between resin and fillers [10].

Among the other factors such as light intensity, initiator system, light irradiation method and polymerization temperature, varying the relative amounts of the matrix monomers has a significant effect on the mechanical properties of the resin composites [11].

PARTICLES

As dental composites cannot withstand heavy occlusal forces, many ways have been introduced to reinforce them, such as using fibers and whiskers as reinforcing agents. Some of dental resins are reinforced with inorganic fillers, and the mass faction of inorganic fillers in commercial dental composites is as high as 75% [12]. A wide variety of fillers have been employed in composites to improve the properties and developments in filler technology are responsible for many improvements in composites which are used today [2]. Despite many improvements in this field, dental composites do not have enough toughness, strength and durability in order to be used in stress bearing areas. There are numerous types of inorganic fillers. Fillers such as SiO_2, ZrO_2 and Al_2O_3 of micron or nano particle size are usually used. Most of the fillers which are used to reinforce dental composites are silicate glasses [13].

The relatively low strength and durability of the composites, however, have limited their uses. The strength of the inorganic filler reinforced dental composites is usually in the range from 80 to 120 MPa, and the average life time is 5 years or less. By comparison, dental amalgams have strength over 400 MPa and have a life time of more than 15 years. Investigations of the reasons for failure revealed that, among other things, inorganic filler was a major contributor. Ironically, the inorganic fillers which are added for the purpose of fortifying the dental composites are actually responsible, at least in part, for the demise. Stresses are transmitted onto the filler particles projecting from the occlusal surfaces through the boluses of foods during chewing [14].

Glass–ceramics are polycrystalline materials which consist of a glass matrix and one or more crystalline phases. The glass fillers are not strong enough and exhibit cracks that either cut through the glass fillers or propagate around the filler particles. To overcome the problem, much effort has been made into the use of glass fibers, nanoporous fillers, branched fibers or even ceramic whiskers [2].

Since the inorganic filler particles are considerably harder than the dental resin matrices, the stresses are transmitted through the filler to the resin. Wherever the submerged portions of the filler particles are angulated or irregular in shape, the stress concentration may become excessively high. Such a condition tends to generate small cracks around the filler particles, thereby weakening the matrices locally. Reinforcement with nanofibers was shown substantial improvements on mechanical properties of dental composites, such as flexural strength, elastic modulus and the work-of-fracture. The small diameter of nanofibers also provides for a large ratio of surface area to volume, which can enhance the intermolecular hydrogen bonding between nanofiber filler and the resin matrix. Furthermore, nanofibers are continuous. If a micro-crack is initiated in a matrix under contact wear and/or other stresses, the nanofibers remain intact across the crack planes and support the applied load. Therefore, crack opening is resisted by the nanofibers and the matrix is reinforced [15].

The uniform distribution of nanofibers improved the strength and modulus of the resulting nanocomposite nanofiber. The silanized single crystals on the surface of nanofibers also enhance the intermolecular interaction/bonding between the nanofiber filler and the resin matrix [12].

Apart from fillers, a good bond between fillers and the resin matrix is essential in dental composites. Silane coupling agents provide the bond between two components in dental composites, but this bond can be degraded by water absorbed by the composites. The idea of

increasing the micro-mechanical retention between fillers and resin in order to reinforce the coupling agent was first described by Bowen et al. in 1976. Their strategy was to use multi-phase glasses which can be etched and produce porous fillers [16].

Recently, nanosized particles dispersed in the organic matrix to give high strength, hardness and toughness.

DENTAL NANOCOMPOSITES CHARACTERIZATIONS

Degree of Conversion

The most important dental composite characteristics are the degree of conversion (DC) and the depth of polymerization. These are great importance for the clinical longevity of the restorations. The DC of resins is a major factor influencing their bulk physical properties. In general, the higher conversion of double bonds, the greater mechanical strength. The DC is the measurement of the percentage of consumed double bonds. Conversion of the monomer to the polymer in light-activated composites is dependant on several factors, such as light source, power density, light wavelength, resin composition, transmission of light through the material and amount of activator-initiator and inhibitor present [17].

Low DC of dental composite may give inadequate wear resistance and a low bonding stability to tooth surface. This clinical problem can result in marginal shrinkage, subsequent loss of anatomic form and fractures in the restorations. Several methods have been used to investigate the effectiveness of irradiation source on the polymerization of dental composite, such as micro hardness, optimal microscopy and vibration methods including infrared spectroscopy (FTIR) and Raman spectroscopy (RS) [18].

Vibration methods allow precise assessment of the depth of polymerization and DC (i.e., the percentage of vinyl group converted to aliphatic functions) of methacrylate composite resins.

METHOD OF TEST

To measure the degree of conversion, the uncured composite is placed between two polyethylene films, pressed to form a very thin film and absorbance peak obtained by transmission mode of FTIR. Then the sample is cured and the absorbance peak recorded for the cured sample. The quantity of the remaining double bonds is determined by a method described by Ruyter and Gyorosi. The percentage of unreacted carbon-carbon double bonds is determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1638 cm^{-1})/aromatic C….C (peak at 1608 cm^{-1}), which is used as an internal standard, since it doesn't change during the polymerization reaction, before and after curing of the specimen. The percentage degree of conversion is obtained as follows [19]:

$$DC\% = (1 - \frac{\left[\frac{abs(aliphatic:C=C)_{1638cm^{-1}}}{abs(aromatic:C.....C)_{1608cm^{-1}}}\right]_{Cured}}{\left[\frac{abs(aliphatic:C=C)_{1638cm^{-1}}}{abs(aromatic:C....C)_{1608cm^{-1}}}\right]_{Uncured}}) \times 100 \quad (1)$$

Commercial dental restorative materials are usually polymerized in less than 60 s with a degree of conversion from 40 to 75%. Our previous research on preparation of dental nanocomposites based on Bis-GMA, TEGDMA and nano SiO_2 indicated that higher resin concentration and power density resulted in a higher degree of conversion. The relationship between degree of conversion and irradiation time for specimens cured at 260 mW/cm^2 and 340 mW/cm^2 and composed of 20% and 25% nanofiller is shown in Fig. 3 [20].

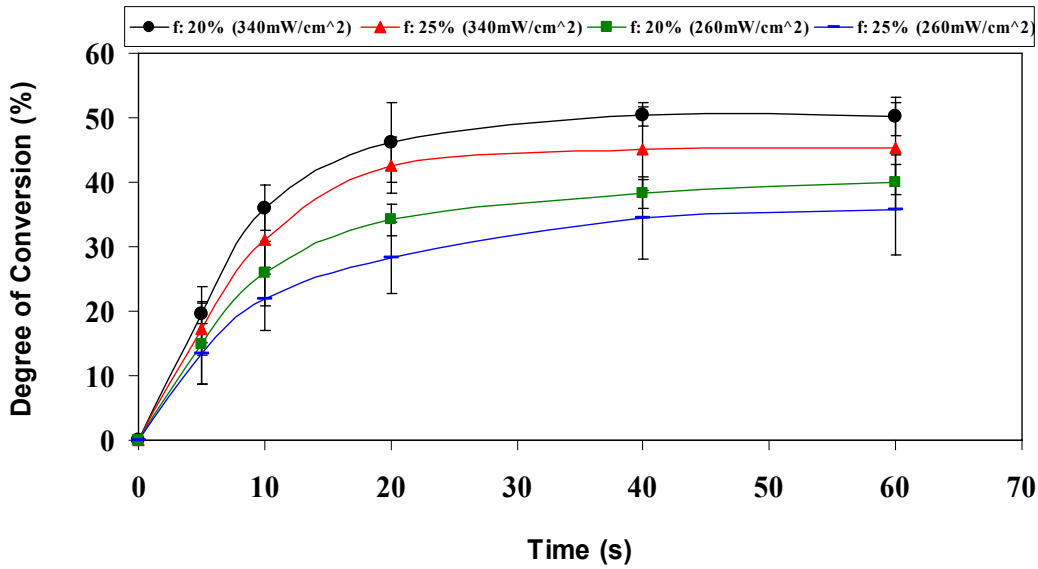


Figure 3. Percent degree of conversion vs irradiation time for cured nanocomposites with different power densities and filler loading.

FLEXURAL STRENGTH AND FLEXURAL MODULUS

Other important parameters affecting the physico-mechanical properties of dental nanocomposites are flexural strength (FS) and flexural modulus (FM). If the amalgams are to be replaced by composites as restorative materials, they must possess some acceptable mechanical characteristics such as high strength and high wear resistance to stand a normal biting or chewing force [20].

METHOD OF TEST

The flexural strength and flexural modulus of the polymerized composites are measured with three-point bending test according to the ISO4049:2000 (Fig. 4). The samples for measuring both flexural strength and flexural modulus are prepared in a rectangular brass mold (length= 25 mm, width= 2 mm, height= 2 mm) (Fig. 5). Five specimens for each experimental group are required and their mean value is determined.

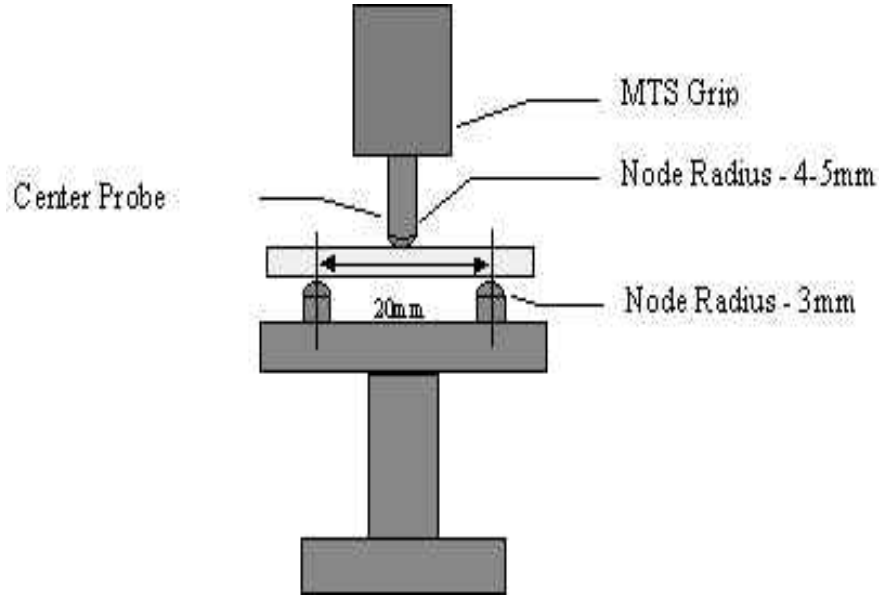


Figure 4. Method of three-point bending test.

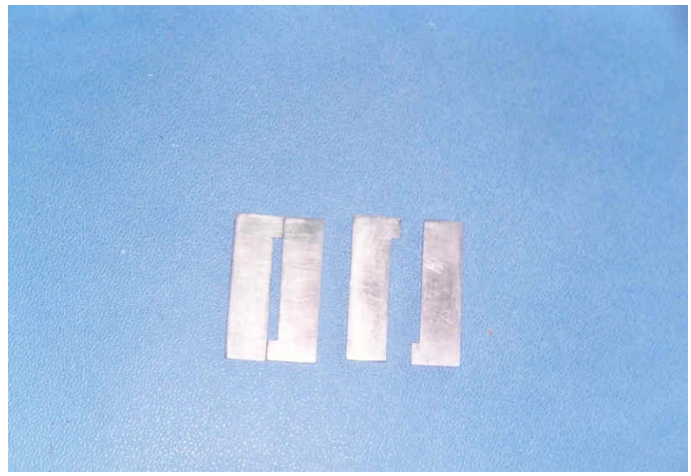


Figure 5. Mold of flexural strength and flexural modulus tests.

The flexural strength and flexural modulus were calculated from the following equation [19]:

$$FS = \frac{3fl}{2bh^2} \tag{2}$$

$$FM = \frac{f\ l^3}{4bh^3d} \tag{3}$$

where *f* is the maximum force (N), l the distance between the supports (mm), b the specimen width, h the height of the bar (mm) and d rate of curvature under load (mm).

Investigations show that maximum flexural strength and flexural modulus of the nanocomposites can be achieved by increasing the intensity and percentage of filler [20].

SOLUBILITY AND WATER SORPTION

One of the issues raised in the application of dental composites is that in aqueous environment they can absorb water and elute unreacted monomers. The release of unreacted monomers from resin composite may stimulate the growth of bacteria and promote allergic reactions. The water intrusion in the dental material can lead in a deterioration of the physical/mechanical properties, decreasing the life of resin composites mainly by silane hydrolysis and microcrack formation. Water sorption can affect the physical and mechanical properties of dental resins. Properties such as tensile strength, flexural strength, modulus of elasticity and wear resistance have been shown that may negatively be affected by water sorption. Excessive water uptake can promote breakdown causing a filler–matrix debonding. The water ingress may have, however, some beneficial effects on the expansion of the composite. Thus compensating the polymerization shrinkage with improved marginal sealing and relaxation of the stresses set up within the matrix during shrinkage [21].

Studies have shown that water is absorbed predominantly within the matrix resin and is most affected by the structure and the amount of this phase; so the study of the water sorption and solubility of polydimethacrylate resins made from neat monomers is important to understand their behavior in the composites.

Polydimethacrylate resins are glassy polymers. The sorption of water in glassy polymers is generally described by a dual-mode theory, which assumes that the amount of the sorbed molecules consists of two populations. One is held by ordinary molecular dissolution in the polymer matrix according to the Henry's law and the second is trapped in polymer micro-voids following the Langmuir isotherm. A clear physical picture of this behavior is described by the free volume theory, which suggests that glassy polymers generally have a non-equilibrium liquid structure, containing an equilibrium hole-free volume responsible for Henry's sorption and an extra nonequilibrium hole-free volume, frozen into the polymer (micro-voids) responsible for Langmuir's sorption. The total hole-free volume effective for water diffusion depends on the macromolecular packing density. Flexible polymer chains with polar groups, especially those forming hydrogen bonds, which increase the intermolecular attractions, favor high packing density. The sorbed water which is molecularly dispersed into the polymer matrix acts as plasticizer, causing the swelling of polymer. The

quantity of thus sorbed water depends on the available equilibrium hole-free volume, the physicochemical affinity of polymer groups to water, and the resistance of polymer chains to a swelling deformation stress.

On the contrary, the water molecules which are accommodated in micro-voids are hydrogenbonded, form clusters and do not cause swelling of polymer but act rather as filler particles [22].

Polydimethacrylates are cross-linked glassy polymers. The presence of cross-links between polymer chains generally results in a significant decrease in the solvent permeability of polymer because they decrease the hole-free volume and the ability of polymer chains for swelling.

The water sorption in dental composites is dependent on the monomers, composition and degree of polymerization. Other factors influencing water sorption are immersion time, temperature and surface condition.

The dissolution of the resin components is also influenced by the polymerization, immersion time, temperature, water sorption and environmental stress.

According to ISO 9000s standard for dental restorative resins, a resin in order to be suitable for use as dental material must show water sorption lower than 50 $\mu g/mm^3$ and solubility lower than 7.5 $\mu g/mm^3$ [23].

METHOD OF TEST

Solubility and water sorption are measured according to ISO 4049. Composite is inserted into stainless steel mold, with 6mm diameter and 1mm thickness, between two glass slides. The composite then cured on each side to form disk shape specimen. Five specimens are required for each test. After curing, the weight of the specimen (m1) was measured. Then, the disc is immersed in distilled water for a week at room temperature so that unreacted monomers would be eliminated.

After that, the specimen is removed, dried and stored in an oven at 37°C until a constant mass (m2) had been achieved. The solubility is determined from the following equation [19]:

$$SL\% = ((m_1 - m_2) / m_2) \times 100 \quad (4)$$

The specimen is dipped again in distilled water at room temperature so that the water sorption could be measured and after different time periods, it is removed and blotted dry according to the ISO 4049 standard to remove excess water and is then weighed. Its weight is recorded until there is no significant change in weight.

The value of water sorption is calculated for each specimen from the following equation:

$$WS_t\% = ((m_t - m_2) / m_2) \times 100 \quad (5)$$

Where, WS_t denotes water sorption at time t, m_t weight at time t and m_2 initial weight.

Meanwhile, there is another method for calculation of SL and WS. The diameter and the thickness of the specimen is measured at five points and the volume (V) is calculated in cubic millimeters. The values of solubility and water sorption are obtained for each disc using the following formulae [19]:

$$SL= (m_1-m_2)/V \quad (6)$$

$$WS_t= (m_t-m_2)/V \quad (7)$$

TEMPERATURE RISE

Since, polymerization is an exothermic reaction, analysis of the temperature rise during the irradiation process is very important. The amount of temperature change was related to factors such as percentage of resin used in the composition, power density, curing time and degree of conversion. The power density, the exposure time and the temperature of the exposed tooth are closely interconnected. The larger the intensity, the larger the heat production and, consequently, higher the temperature of the exposed surface. According to Zach et all. the temperature of the tooth can induce irreversible pulpal lesion, when higher than 42.5°C.

Decreasing in curing time for dental composites and adhesives is an important aspect of clinical success. Recently, laser has been marked as an alternative to conventional light-curing units for quick, safe and effective polymerization of dental composites. Laser light has a single, narrow band of wavelength that emits in parallel waves that are in phase spatially and temporally [20].

METHOD OF TEST

Five specimens for each case must prepare under the same condition as solubility sample and then cure. For measuring the temperature change a K-type thermocouple, whose output is connected to a computer to continuously monitor and plot the thermal behavior, can be used. The temperature rise is then obtained by subtraction of the ambient temperature from the total temperature, that is equal to the sum of irradiation and chemical reaction temperature.

MICROHARDNESS

Five specimens for the measurement of Vickers hardness testing are required in a cylindrical aluminum mold (height= 2 mm, diameter= 6 mm). The hardness is then measured at three different sites of the specimens by a Microhardness tester applying a load of 10 g in each case, and the mean value is calculated. Vickers hardness number (VHN) can be determined from the following equation [24]:

$$VHN= 2P\sin(\theta/2) / L^2 = 1.854\ P / L^2$$

Where P is the load (kg), θ is the angle between each plane of diamond pellet (136°), and L the length of diagonal indentation (mm).

SHRINKAGE

A major drawback with dental composites, however, is polymerization shrinkage of these restorative materials. Polymerization shrinkage in dental composites is the result of conversion of intermolecular van der Waals distances of the resin-monomers to the covalent bond-lengths during light-curing. As shrinkage is a consequence of the polymerization reaction it should follow the polymerization reaction pattern. Molecular weight of monomers is one of the most parameters in shrinkage. High molecular weight monomers results in lower shrinkage as well as higher mechanical properties owing to the monomers structure. Polymerization shrinkage may lead to stress development on the cavity walls resulting in further marginal gaps, secondary caries and clinical failure of the restoration. The reduction of shrinkage has been the subject of numerous studies which can be summarized in two categories: (I) materials development reduction of the shrinkage by introducing new low-shrinking monomers, monomers with liquid crystalline structure which can reduce the shrinkage due to phase change during polymerization, reducing the shrinkage-induced stress using non-bonded fillers [11], and incorporation of more fillers into the matrix phase using multimodal filler particle size distributions; and (II) methods development using different light irradiation regimes to reduce the shrinkage-induced stress and various placement techniques aiming to relieve the stress [10].

Despite the extensive studies on the shrinkage-related subjects, the complex behavior of the materials during the light-curing process is still not completely clear.

The photopolymerization process of multifunctional monomers and dental resin composites has mainly been studied using thermal analysis techniques in which the exothermic reaction of the polymerization is monitored to determine the curing kinetics of the materials. Shrinkage is another phenomenon which can be considered as a method to study the polymerization kinetics of the dental resin-monomers. Researchers in their works studied the photo-curing behavior of dental resin-monomers by monitoring the polymerization shrinkage-strain of the materials [25].

Although researches indicated that the nanofiller content of dental composites plays an important role in the physical and mechanical properties of the materials, there are few systematic investigations have been made on the effect of filler on the shrinkage-strain kinetics. Studies show the shrinkage-strain and shrinkage-strain rate in light-cured dental nanocomposites are increased with increasing temperature. The shrinkage-strain and the maximum shrinkage-strain rate progressively decreased with the increase of filler loading. The linear shrinkage of microfilled composites ranged from 2 to 3% after curing. Hybrid composites and microhybrid composites shrink from 0.6 to 1.4%. Such shrinkage causes micro leakage, a well-known effect of contraction gaps on the interface of resin and tooth. Saliva, fluid, food residue and microorganisms trapped in the gaps lead to decayed teeth and damaged enamel, which is a major problem in current restorative and esthetic dentistry [26].

Composites with epoxy resin and nanosilica fillers provide materials with high mechanical properties and low polymerization shrinkage.

SUMMARY

The ultimate goal of advanced dental composite research is to produce a material that can be used in all circumstances as an amalgam replacement material. The composites are used to replace missing tooth structure and modify the color and contour of the teeth in order to enhance esthetics. Two major components of dental composites are polymer matrix and filler particles. Changes in composition and chemistry of the constituent monomers and filler can alter the physical properties of the materials.

Owing to its high mechanical strength, low volatility, and relatively low polymerization shrinkage, Bis-GMA is the primary component in a majority of commercial dental resins. However, Bis-GMA is also characterized by a very high viscosity and low polymerization conversion. Hence, 20-50 wt% of less viscous dimethacrylates such as TEGDMA are added to improve handling of the dental formulations, as well as to achieve higher DC due to the increase in the mobility of the molecules, as mentioned before. Increasing the ratio of TEGDMA to Bis-GMA in a polymethacrylate network was shown to increase water uptake and alter the strength and hardness of the material [27-29].

Recently, nano particles used in the organic matrix to give excellent physical and mechanical properties due to high strength, hardness and toughness of this component.

Researches confirmed that the values of solubility depend on percentage of degree of conversion, i.e., the low amount of the unreacted monomer and the low solubility values are due to the high DC%. The absorbed water in the composites can act as a plasticizer, and, hence, it reduces their hardness. High degree of conversion and filler loading can improve the mechanical properties. An increase in total temperature rise during polymerization, which is partly due to radiation and partly due to the reaction, can be seen when the intensity is increased [30].

Finally, the application of laser with optimum power density for the curing of dental composites is very suitable for dentistry due to the lack of side effects for patient and dentist, access to appropriate degree of conversion in a short time, and reduction of the polymerization temperature that can decrease pulp damage.

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Chapter 10

ON THE CRITICAL PARAMETERS THAT REGULATE SELF ORGANIZED BIOCOMPOSITES

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ABSTRACT

Inherent donor-site limitations with respect to tissue rejection and disease transfer are shortcomings of autografting and allografting. The development of bone-like biocomposites that induces and promotes new tissue formation at the required site would therefore be desirable.

The self organized calcium phosphate/collagen composite has been drawn attention in tissue reconstruction. The mechanical properties and biodegradability of the biocomposites need to be managed in parallel. The cross-linking between calcium phosphate particles and collagen molecules is a critical parameter in this regard. The improved cross-linking properties of the bone-like collagen composites with or without cross-liking reagents have been explored in past decade. The size distribution, composition, and crystallization of the particles may have another important role in the self organization. This review describes current strategies in development of artificial bon-like biocomposites for use in craniofacial orthopedics and periodontal repair.

Keywords: bone, calcium phosphate, collagen, cross-linking

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INTRODUCTION

The use of allograft has become widespread for the potential applications in trauma setting that include reconstruction of skeletal defects, augmentation, fracture repair, and treatment of nonunion whereas the autografts include additional expense and trauma, possibility of donor site morbidity, and limited availability. The primary application of allografts in trauma surgery consists of cancellous or corticocancellous chip as an osteoconductive filler for metaphyseal defects such as occur with tibial plateau fractures [1]. Approx. one-third of the bone grafts used in U.S. are allografts [2-5]. Allograft bone is an attractive, alternative to autogenous one as it avoids donor site morbidity.

In the case of allografts, in addition to limited supply, potential viral transmission and immunogenicity are of particular concern [6]. Disease transmission is the major disadvantage of the allograft materials and also the risk is increased when fresh allografts are used. The risk of disease transmission with fresh allografts, the difficulty with the storage and distribution of these grafts have led to predominant used of fresh-frozen and freeze dried allografts.

Frozen allografts are stored at temperatures below -60 °C which decreases enzyme degradation and host immune responses [2]. Freeze-drying involves removal of water from the tissue with subsequent vacuum packing and storage at room temperature. The host immune response is less robust than that of fresh allograft. However, this destroys all osteogenic cells and leaves only limited osteoinductive capabilities.

Though autografts and allografts remain the gold standard for bone repair, these suffer from those disadvantages. Because of the limitation within the autografts and allografts, there is a great need to develop synthetic biomaterials for bone repair.

Bone is a mineralized natural biocomposite which mainly composed by biominerals and biopolymer, namely, calcium phosphate and collagen fibers [7-10].

Calcium phosphate/collagen biocomposites have been expected as their similarity in some properties of bone that include biodegradability and osteocondcuctivity [11, 12].

However, the development of such biocomposites has generally been restricted due to antigenicity and some of difficulties associated with the formulations [13].

Bone Properties

Bone is a complex biocomposite with biopolymer and biominerals. The biopolymer consists of matrix proteins, mostly type-I collagen with some minor non-collagenous proteins [14, 15]. Bone is formed by a series of complex events rigorously orchestrated by different types of bone cells interacting with each other and with the extra cellular matrix proteins.

Osteoblasts are responsible for production and mineralization of the bone matrix while osteoclasts maintain bone homeostasis with bone resorption [16-18].

Important physicochemical properties of bone include interconnecting porosity, biodegradability, bioactivity, osteoconductivity and osteoinductivity.

The bone and tooth mineral was identified as hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) based on its similarity to the X-ray diffraction profiles of mineral apatite in comparison with each other [19-21]. Bone apatite contains minor and trace elements such as carbonate, magnesium, and sodium. The bone apatite has been therefore concluded as a carbonate hydroxyapatite (CHA).

Bone apatite crystals are irregularly shaped platelets of variable length (30-50 nm) and thickness oriented with their *c* axis parallel to one another and also lies along the collagen fibrils [22].

The organic matrix of bone consists of collagen and series of non-collagenous proteins and lipids [23]. Some 85-90 % of the total bone protein consists of collgen fibers. Type I collagen, the principal component of the organic matrix of bone, as well as other connective tissues, is a large fibrous protein with a highly repetitive amino acid sequence[Gly (glycine)–X–Y]n (often X is proline and Y is hydroxyproline) [23].This repetitive sequence allows three polypeptide chains (called a chains; type I collagen is composed of two α1 and one α2 chains) to fold into a unique triple-helical structure. It consists of three domains: the –NH2 terminal nontriple helical, the triple helical, and the –COOH terminal non-triple helical domains. The single uninterrupted triple helical domain represents more than 95% of the molecule. The structure of collagen has been studied in great detail and the main results concern the details of the packing of collagen molecules in the fibril.

The most distinct feature of type I collagen in mineralized tissues can be seen in its cross-linking chemistry and molecular packing structure. The intermolecular cross-linking provides the fibrillar matrices with various mechanical properties such as tensile strength and viscoelasticity of bone [2, 24].

Synthetic Bone-like Biocomposites

Calcium phosphate ceramic can form a suitable scaffold for cells and serve as a delivery vehicle of osteogenic promoter [22, 25-28].

There are however some issues in poor degradability of the materials and also mechanical properties such as tensile strength and brittleness [9, 10, 29].

The addition of biopolymer in calcium phosphate ceramics can improve the degradability of the ceramics and alter their mechanical properties. Also the drug release properties can be managed because there is a wide range of different polymers [6, 11, 19, 25, 30, 31] which show different degradation range and mechanisms have been available (Table 1).

Because collagen is one of the most abundant biopolymer in the human body, the collagen based biomaterials have been intensively investigated [7, 8, 12, 13, 32-36].

The importance of collagen in extracellular matrix and its role in the developmental cascade leading to new bone or cartilage form progenitors implicates the collagen as a strong candidate material in a biomeimetic approach to bone repair [24]. Since collagen experiences loss of biological stability during application [12], it is often reinforced with HA particles in a collagen composite. HA/collagen composites in particular have recently received much attention, as human bone is mainly composed of HA and collagen fibers. The addition of a calcium phosphate compound to collagen sheet gave higher stability, increased the resistance to three-dimensional swelling compared to collagen alone [33]. These HA/collagen composite behaved mechanically in a superior way to the individual components. The ductile properties of collagen help to increase poor fracture toughness of HA [37].

Table 1. Calcium phosphate-based biomaterials: Commercial

calcium deficient apatite (CDA)	**Osteogen (Impladent, NY)**
hydroxyapatite (HA) $Ca_{10}(PO4)_6(OH)_2$	**Calcitite**
	Ostegraf (Ceramed,CO)
	Bioroc (Depuy-Bioland,France)
HA/polyethylene	**HAPEX (Gyrus, TN)**
HA/$CaSO_4$	**Hapset (LifeCore, MINN)**
coralline HA (derived from coral) (Interpore, CA)	**Interpore, ProOsteon**
bovine bone Ap (unsintered)	**BioOss(EdGeitslich, Switzerland)**
bovine bone apatite (sintered)	**Endobon (Merck, Germany)**
	Osteograf-N (Ceramed, CO)
tricalcium phosphate (β-TCP) $Ca_3(PO_4)_2$	**Vitoss (Orthovita, PA)**
biphasic calcium phosphates, BCP (HA + β-TCP)	**MBCP (Biomatlante, France),**
	Triosite (Zimmer, IN)
	Osteosynt (Einco Ltd., Brazil)
	Tribone (Stryker, Europe)
BCP/collagen	**Allograft (Zimmer, IN)**
BCP/fibrin Tricos	**(Baxter BioScience,France)**
BCP/silicon	**FlexHA (Xomed, FL)**
CHA/collagen	**Healos (Orquest Inc., CA)**

Although both collagen and HA were found to enhance osteoblast differentiation, they were shown to accelerate osteogenesis when combined together [2, 23, 32, 38, 39]. Calcium phosphate ceramics with collagen composites have been extensively investigated as they produce similar physico-chemical characteristics that are found in human bone or tooth [2, 7-10, 12-14, 24, 25, 31, 32, 40]. A composite matrix when embedded with human osteoblast like cells, showed better osteocondcuctive properties compared to monolithic HA and produced calcification of identical bone matrix [23].

Preparation of Bone-like Composites

Synthetic bone-like biocomposites structured by collagen matrix impregnated with oriented calcium phosphate biominerals have been significantly progressed by mimicking biological processes. HA/collagen composites are generally formulated by freeze-drying HA/collagen mixture [9, 10, 29]. The mechanical properties of these porous scaffolds are poor, thus highly cross-linking of the composite is critical for the better biological stability.

Glutaraldehyde (OHC CH_2 CH_2 CH_2 CHO) has two functional groups enable to link with free amino groups of lysine or hydroxylysine amino acids residues of the polypeptide chain in

collagen. It is known that all available free amine groups of collagen react with the aldehyde groups of glutaraldehyde from Schiff bases [9, 10].

This can also decrease biocompatibility while the cross-linking agents such as glutaraldehyde gives a cytotoxic reaction [12, 13]. Therefore, the development of bone-like composites that are naturally synthesized and need not additional treatments is required.

The size distribution, composition, and crystallization of the calcium phosphate particles may have an important role in the self organization of the composites. Reducing the diameter can increase the specific surface area of calcium phosphate particles that can increase the specific surface area of the particles that can conjugate organic molecules [41, 42]. Several kind of calcium phosphate compounds have been investigated in synthesis of bone-like composites [7, 11-13, 20, 35, 36]. In particular, β tricalcium phosphate (β-TCP) or Octa calcium phosphate (OCP) have been candidates in development of self organized bone-like composites as they yield complex macromolecular assemblies in comparison with HA, although the mechanism has not been fully elucidated [7, 13].

Shibata et al prepared a colloidal nano-scale β-TCP/collagen composite that does not need any extra treatments [12]. Systematic studies on the β-TCP/collagen composite using combined analytical methods namely, Fourier transformed infrared (FTIR) and x-ray photoelectron spectroscopy (XPS) led to the conclusion that HA is not a sufficient starting material in synthesis of self organized bone-like composites [12, 13].

FTIR is a useful tool for structural investigations because we know the origins of amide bond vibrations, the sensitivity of some of these position to conformation, and the possibility of predicting band positions for a given helical or extended collagen conformation [9] while the cross-linking between calcium phosphate and collagen fibers is detectable by XPS [10].

It was reported that collagen molecules were polymerized together to form stable collagen fibers in the β-TCP/collagen composite. The phosphate vibration mode of β-TCP/collagen composite indicated that a –P-O-P- polymerization chain was produced in this composite after mixing. Since polyphosphate has been used as a catalyst in organic molecules [38, 39, 43, 44], the generation of a –P-O-P- polymerization chain might play an important role in the polymerization of collagen fibers in the composite.

In the XPS, the energy position of Ca peak increases with cross-linking between calcium phosphate and collagen molecules, because the bonding strength of Ca^{++}-COO^{-} is higher than that of inorganic Ca^{++}-PO_4^{-} [10]. The XPS spectra revealed that electron-binding states of Ca in the β-TCP/collagen composite, which was coordinated with both inorganic PO_4^{-} and RCOO- groups, was much higher than those in the HA/collagen composite. The cross-linking between the minerals and collagen molecules was greatly increased in the former. This conformational change was also obtainable in the OCP/collagen composites [7, 8]. The highly biological stability could be expected in the β-TCP or OCP/collagen composite without cross-linking agents such as glutaraldehyde. The reasons why HA does not well coordinated with collagen molecules in comparison with β-TCP or OCP is not fully elucidated. However, since both β-TCP and OCP generate polyphosphate chains while they coordinated with collagen molecules, catalytic action of the polyphosphate might be responsible for the superior structural manipulation in the self organized bone-like composites.

CONCLUSION

Highly ordered nanostuructures inspired by natural biocomposites, such as those found in bone and teeth, are required for the production of advanced biomaterials.

Synthesis of bone-like composite is recognized as being difficult, because it involves two dissimilar organic and inorganic nanophases that have a specific spatial relationship with one another. Though the HA is a main inorganic components found in bone or tooth, HA itself is not an adequate starting material in development of the self-organized bone-like composites without cross-liking agents. The other calcium phosphate compounds such as β-TCP or OCP are superior candidate for use in self-organized bone-like composites because the catalytic action of the generated polyphosphate chain during processing is responsible for the cross-linking between the biominerals and collagen fibers which yield biological stability of the composites.

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Chapter 11

THE ANNUAL EFFECTIVE DOSE ASSESSMENT DURING PANORAMIC / ORAL RADIOGRAPHY INCLUDING THE DENTAL ERRORS EXAMINATIONS TO THE PATIENTS IN ALBANIA

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ABSTRACT

Diagnostic medical and oral dental radiography comprises 82% of all man-made radiation exposure of the population in Albania. Although dental radiography does not make a major contribution to radiation dose, we performed a detailed assessment of radiation risk from panoramic and oral dental radiography, evaluating the annual effective dose in dentistry clinics of Tirana.

Since 1996, a computerized registration system of occupational exposure was established and the central national dosimetric register allows authorized users with basic knowledge of programming to make various inquiries on persons or statistical analyses in connection with doses, has also been improved.

The measures of effective doses were carried out using TLD-100 cards for different parameters of X-ray tubes. After exposure of patients, we carried out measurements of crystals by TLD Harshaw Reader 4500 apparatus, furnished by IAEA. The assessment of TLD-100 cards (crystals) was performed through the TLD-REMS program, and processing of the results was carried out by RAIS program. A National Dose Registry is established for this purpose, which contains radiation doses for occupational exposures and patients of our study etc. The doses were evaluated for mandibular and maxillary incisors, canines, premolars and molars, given to more as 1000 patients aged 5-60 years. The average dose was 4,1 mGy (miligrey) and dose limits were from 0,7 – 144 mGy. The reference level recommended by IAEA and ICRP for dental radiography examinations is 7 mGy (surface dose), while the Albanian Commission of Radiation Protection (ACRP) has evaluated and recommended the value of 5 mGy. The dose from 5 mGy in oral dental radiography examinations is equivalent to effective dose of 5 μSv (microsivert).

In practically all techniques the films for dental procedures of the radiography are basically same. The examinations of dental radiography performed by panoramic apparatus are a unique film technique that allows the dentist to view the entire dentition and related structures, from condyle to condyle, on one film. Oral dental radiographies in our study are carried out using ISO 2 Agfa film plaque; ISO E- Safety, ISO 3665/5799 maximum 23 0C; λ= 500 nm. Dental radiography is one of the largest single group examination performed, although the effective dose per radiograph is small. The individual risks from dental radiography are low, but it has identified a significant potential for reduction in the collective dose and for improvements in the diagnostic quality of dental radiography.

The economic impact of our recommendations suggestion to cover all aspects of dental radiography: training and examination regimes for dentist and staff, patients' selection and clinical justification for radiography, diagnostic interpretation, equipment and procedural aspects, and finally the question of quality assurance in dental radiography.

The measurements obtained in our study showed that the system of radiation protection of X-ray machines in operation phase in all dental practices, where we performed activities was satisfactory levels, ensuring protection of staff patients and population.

Key words: dental and panoramic radiography, X- ray examination, mandible, maxilla, pre-molar tooth, molar, radiation protection, effective dose, annual patient dose, ALARA principle.

INTRODUCTION

A) Panoramic Radiography

The procedures and equipment for obtaining a panoramic radiography have steadily improved since the first one taken in 1934. A panoramic radiograph can be made with patient sitting, standing or lying down. In all situations, the manufacturer's instructions must be carefully followed and the patients must remain perfectly still while the X-ray beam and image receptor (film screen combination) rotate together around the patient's head.

Diagnostic medical and dental radiography comprise about 82% of all man-made radiation exposure of the population in the world [1]. The need to reduce patient doses depends on the level of risk, to both populations and individuals, associated with X-ray examinations. Patient dose reduction is achieved by adherence to the general principles[2].

All diagnostic practices should be justified at a broad level and the expected clinical benefit demonstrated to be sufficient to offset the radiation detriment, there should be a valid clinical indication for all medical exposures at the level of individual procedures, there should be a commitment to optimization of radiological protection at the all levels.

In this context, optimization of radiological protection means that patient's doses should be kept as low as reasonably practicable (ALARP), consistent with achieving diagnostic objectives and in Albania this is a legal requirement[1].

Although dental radiography does not make a major contribution to radiation dose, it is one of the largest single groups of radiographic examination performed, as illustrated in figure No. 1

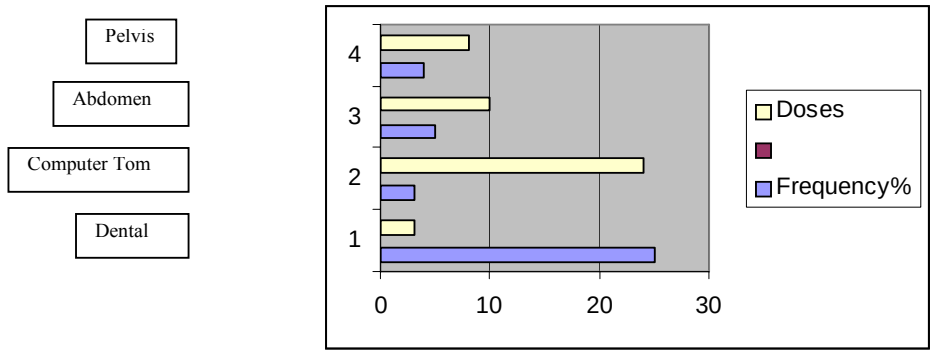

Figure 1. Frequency of and collective dose from different radiological examinations in Albania.

Based at the recommendation of ICRP and Albanian Radiation Protection Commission (ARPC), related to the annual effective dose obtained from staff and patients during radiological examination (ALARA-as low as reasonably achieving principle) it should be within the limit dose approved by above-mentioned authorities[2,3].

The cast of factors for radiation protection of staff and patients such: technical parameters of exposure tube setting, distance from ionizing radiation source, the exposure time with ionizing radiation source to perform the examination procedure, are much more significant parameters to decrease the annual effective dose.

The average kVp and / or mA setting are recommended by the unit's manufacturer, but can vary from patient-to-patient due to size, dentition, etc. In panoramic radiography, the exposure time is fixed by the time required to complete one full excursion of the assembly[4]. There are other factors that can affect the average exposure setting recommended by the equipment manufacturer, which are shown in table No. 1

Table 1. The list of common factors that affect exposure

Factor to consider	Exposure setting
Obese patient	Use the next highest kVp or mA setting
Patient with large bone structure	Use the next highest kVp or mA setting
Patient with small bone structure	Use the next lower kVp or mA setting
Patient that is edentulous (toothless)	Use the next lower kVp or mA setting

The patient should be positioned to the manufacturer's recommendations. Ask the patient to remove glasses, all jewelry or other metallic ornaments, or devices on and around the head and neck areas. Full or partial dentures should also be removed. Be sure to instruct the patient how to bite on the bite block, close his/her lips and place tongue against the roof of the mouth [3,5]. Panoramic-leaded aprons should be used.

Some probable errors are illustrated in our study during practices of panoramic examinations to the patients and some recommendations for their corrections are shown to study cases as following:

First Case

Anterior teeth in both arches are out-of-focus, they are blurred and narrow in appearance; spine is superimposed on ramus areas, pre-molars are severely overlapped. This fact was explained; the patient was positioned too far forward in relation to the image layer.

How To Correct?

Check to be sure the patient's teeth are correctly biting the bite block. The anterior incisors must be in the groove indicated on the block. Reposition the patient in chin rest according to manufacturers' recommendation. This case usually was met, when the anterior teeth are missing. In order to maintain the slight distance that the anterior teeth would have had in relation to the ridge, the patient's ridge should be placed slightly behind the groove (toward the X-ray source) in the bite block (Photo 1).

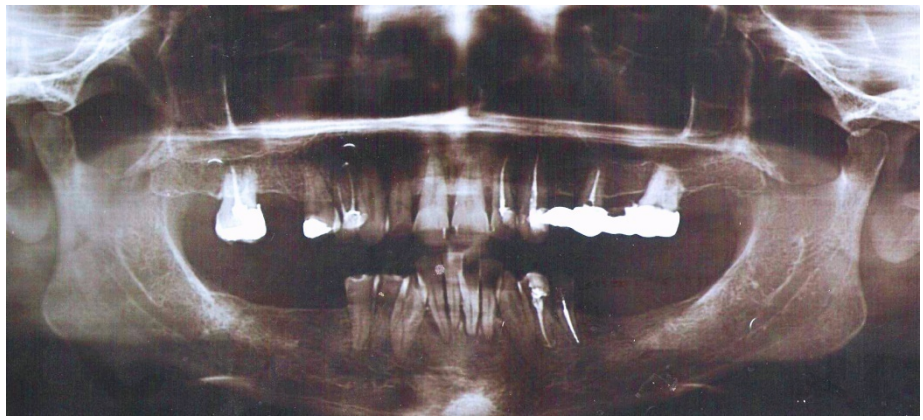

Photo 1. Correction of the bad position through the reposition of the patient in bite block.

Second Case

Apices of lower incisors are out-of-focus and blurred; shadow of hyoid bone is superimposed on anterior mandible; condyles may be cut off at the top radiograph; pre-molars are severely overlapped. This fact was explained; the patient's head is tilted downward; chin is positioned back while forehead is positioned forward.

How to Correct this Incorrect Image of the Patient Examination, Decreasing the Dose

Rate for Such Errors?

Follow the directions by the equipment manufacturer on how to position anatomical points on the face with reference line on the unit. Each panoramic equipment manufacturer has different instructions on how to align anatomical structures with specific lines on the machines. If the reference lines missing than the alignment would be closer to being correct if the occlusal plane was positioned approximately minus 5 degrees from parallel to the floor (Photo 2).

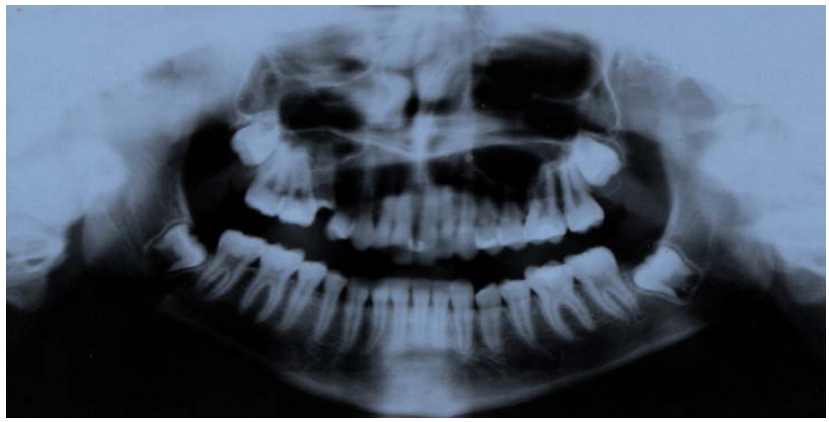

Photo 2. Correction of the bad position through the reposition -5^0 parallel to the floor.

Third Case

One condyle is definitely larger than the opposing one; the neck also is longer on the large side; image appears to be tilted; one angle of the mandible is higher than the other. The above-mentioned was caused, because the patient's head is tilted to one side, anatomical variations; film is crooked in cassette.

The Correction of this Defect Image was Made.

First determine if the problem is anatomical / pathological rather than an error. If it is an error, and the panoramic unit is equipped with a positioning light, adjust the patient's head until the vertical positioning light aligns with the mid-sagittal line of the patient. If there is no positioning light, align the mid-sagittal plane visually so that it is perpendicular to the floor. There may be an anatomical/pathological reason for a difference in condyle size between right and left. If the occlusal plane is parallel to the bottom edge of the film, the difference is probably anatomical / pathological [5,6]. On machines with mirrors, we can tape a vertical line on the mirror that the patient can use as a reference point (Photo 3).

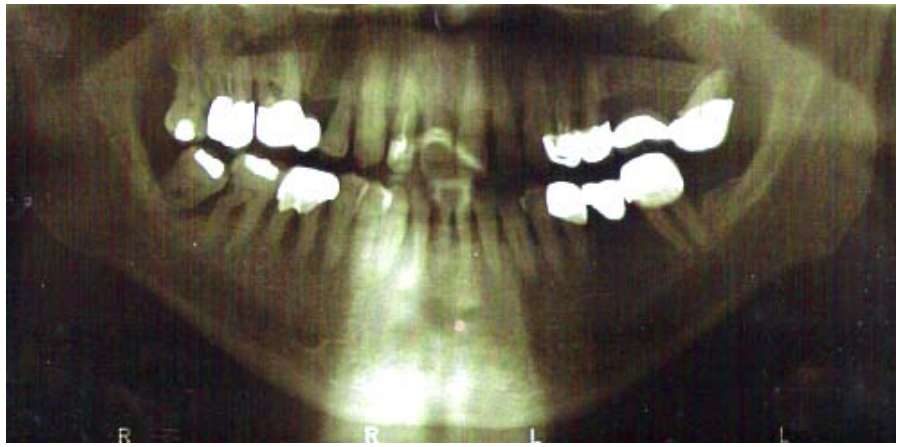

Photo 3. Correction of the position through the vertical line on the mirror of equipment.

Fourth Case

Patient's tongue was not held closely to the roof of the mouth during the exposure. For that reason, at the panoramic image the dark shadow in the maxilla below the palate will show and maxillary apices are obscured.

How to Correct it?

The correction of this incorrect image was made performing: asking the patient to place tongue fully against the roof of the mouth and to hold it there during the exposure. If only a portion on the film shows a dark area, patient may have lowered the tongue during the exposure. Usually the patient was helped from radiologist technician explaining to understand about placing the tongue, asks the patient to swallow and note how the tongue feels against the roof of the mouth. Then, ask the patient to hold that position for the duration of the exposure (Photo 4).

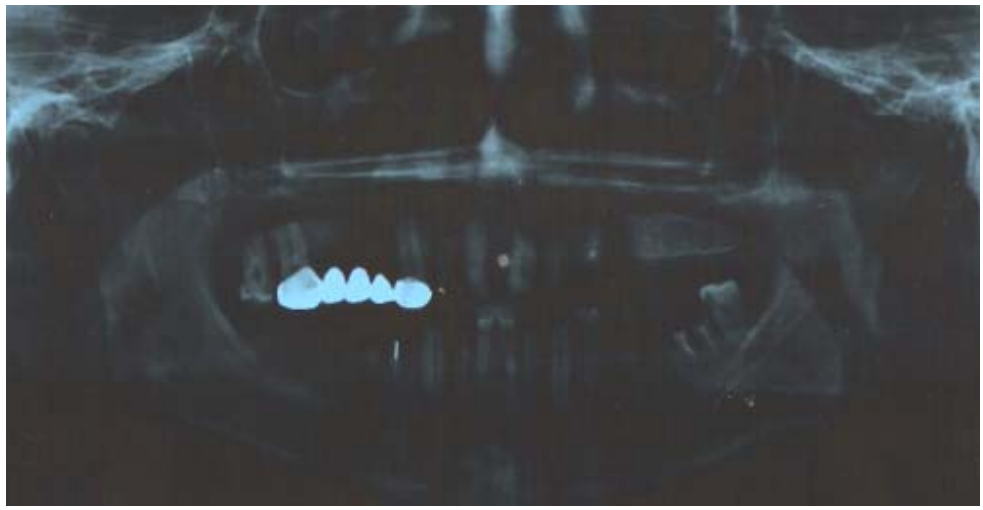

Photo 4. Correction of position through the patient tongue fully against the roof of the mouth.

In 2008, 2009, measurements of molar X-ray doses were taken in Tirana dental X-ray units. The doses correspond to the entrance dose on the surface of the cheek when a molar tooth is X-rayed. The distribution of the measured dose shown that the mean dose was 5 mGy and the range of doses 0,5-15,5 mGy. The reference value recommended by IAEA for the limit of a dental X-ray dose is 7 mGy, which correspond the value of the entrance surface dose. The corresponding reference level given by ARPC is 5 mGy, which dose in dental X-rays is equivalent to an effective dose of about 7 μSv [7].

B) Oral Radiography

Oral radiography methods give images of the tooth changes obtained in film plaques, in order to verify and determine exact depth of carries, changes in other tissues, etc. By observing radiography only, we cannot determine the exact depth of a lesion, or its

topography; if it is needed, it is necessary to perform at least 2 radiographies in 2 projections, with a rotation of 900 for each.

For teeth of the upper jaw it is impossible, as gingival and palate impede the film plaque to be parallel with tooth. In this case the film plaque should be put into the mouth touching the tooth's crown, gingiva and palate, establishing an angle with axe of tooth having pick at the point where it touches the tooth. For teeth's of the lower jaw, the gingival, the arc of mandible and soft tissues of the floor of the mouth impede the film plaque to be put parallel with axe of tooth. We have used the "isometric rule" in order to have real tooth dimensions, which permit the central beam to be perpendicular on bisecting line of the angle established by the axe of the tooth's root. In oral radiography with contact, the film plaque needs to be put into the mouth to be jointed with the tooth's crown, gingival, hard palate (in the maxilla) and floor of the mouth (in the mandible) [7,8].

The effective dose of patients during oral radiographic examinations with contact of the film plaque to the incisors, canines, premolars and molars of the maxilla and mandible, are performed by X-ray irradiation machines in our study and Table 2 shows the effective dose measured to the molars.

Table 2. The effective dose measured to the molars

Parameters	Maxillary vertical angle (+200)	Mandible vertical angle (-50)
Tube 60 kV-7mA Exposure time 8"-12" Effective dose (μSv)	16/26 17/27 18/28 0.40 - 0.80 5,21 5,11 5,07	46/36 47/37 48/38 0.32 0.64 6,42 6,31 6,18
Tube 70 kV-7mA Exposure time 8"-12" Effective dose (μSv)	16/26 17/27 18/28 0.20 - 0.40 5,32 5,23 5,17	46/36 47/37 48/38 0.16 0.32 6,60 6,53 6,39
Tube 65 kV-10mA Exposure time 8"-12" Effective dose (μSv)	16/26 17/27 18/28 0.25 - 0.50 5,29 5,20 5,22	46/36 47/37 48/38 0.20 0.40 6,51 6,34 6,37
Tube 70 kV-10mA Exposure time 8"-12" Effective dose (μSv)	16/26 17/27 18/28 0.16 - 0.32 5,25 5,16 5,18	46/36 47/37 48/38 0.12 0.25 6,37 6,36 6,29

Being based at the results presented in table 2, we found that limits of the average effective doses to the maxillary molars (16/26; 17/27; 18/28) were between 5.07 – 5.32 μSv for parameters of 60 kV, 70 kV – 7 mA and for the exposure time 8 – 12" within the interval of 0.20 – 0.80, while limits of the average effective doses to the mandibles molars (46/36; 47/37; 48/38) were 6.18 – 6.60 μSv for parameters of 60 kV, 70 kV – 7 mA and for exposure time 8 – 12" within the interval of 0,16 – 0,64.

In our study, the average dose was 4,1 mGy (miligrey) and dose limits were from 0,7 – 144 mGy. The reference level recommended by IAEA and ICRP for dental radiography examinations is 7 mGy (surface dose), while the Albanian Commission of Radiation Protection (ACRP) has evaluated and recommended the value of 5 mGy. The dose from 5 mGy in oral dental radiography examinations is equivalent to effective dose of 5 μSv (microsivert) [7,8,9].

Study of Maxillary and Mandibular Anatomy by Intra-Oral Radiography (Studied Cases)

For patients who undergone intra-oral radiography in the mandible, we studied the important radiographs landmarks as following:

a) The mandible pot, was observed at the 22 patients (2,7% of the total number of patients), we have found small holes on the lingual side of the mandible, approximately halfway between the upper and lower border of the mandible of the patients.
b) The genial tubercle was seen in several variants in the 45 patients (6.25%), as a small prominence on the lingual side.
c) In most radiographs of the bicuspid region, carried out in 120 patients (16.6%), the borders of the mental foramen were observed in all cases, and the existing canal was visible. In some cases it was superimposed the apex of one bicuspid [10,11].
d) The inferior dental canal was usually visible in radiographs covering the posterior mandible and the average effective dose rate in all cases of our study was approximately 5 μSv.
e) The angle of the mandible was seen in 72 patients (10%), and the measurement carried out by TLD 100 cards (crystals) gave the average effective dose of 6,82 μSv.
f) Common to the mandible and maxilla is the lamina dura. The appearance of lamina dura brought about by the shape of the root and the relative angulations of the X-ray beam. Variations in the appearance of lamina dura were caused by the different in exposure of X-ray parameters and the effective dose was evaluated to be between 5.45 and 6.12 μSv.

For patients who undergone intra-oral radiography in the maxilla, we studied the important radiographs landmarks as following:

1) Inter-maxillary suture structure which causes a thin linear radiolucency in the midline of the palate, occasionally extending to the crest of the alveolus. This fact was most visible in 25 children patients (8.9% of the 280 total number of the children aged 5-12 years), and rarely in adults. The average effective dose was evaluated to be 6.32 μSv.
2) Incisive canal of the maxilla was observed in 185 patients (25.6%). In some cases we found that parallax has caused an apparent change in the position of the canal relative to the teeth.
3) The incisive fossa was observed on the bucal aspect of the maxilla, anterior to the canine. Radiographically, it caused a radiolucency in some cases, which was seen clearly between the lateral incisor and the canine [10,11].
4) Study of zygomatic buttress of the maxilla was observed in 64 patients (8.8%) in region of first and second molars. The effective dose limits were 5.07 – 5.32 μSv for the different exposure parameters.

QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA) TO THE DENTAL EXAMINATION PROCEDURES

All persons involved in the internal-external exposure assessment programme are responsible for its quality and therefore for implementing its Quality Assurance (QA) and Quality Control (QC) procedures.

QA – all those planned and systematic actions necessary to provide adequate confidence that a structure, system, component or procedure will perform satisfactory complying with agreed standards.

QC – is a part of QA – the set of operations (programming, coordinating, implementing) intended to maintain or to improve quality. It covers monitoring, evaluation and maintenance at required levels of all characteristics of performance of equipment than can be defined, measurement and controlled [12].

Responsibility for the quality of a particular operation should be delegated to the persons actually performing the operation. Such persons should be actively involved in the development of QC procedures, and trained methods of detection non-compliance. Management should motivate staff to detect report and correct non-compliance. QA built into a programme from bottom up is more effective than QA imposed from the top down. A QA program should be established to ensure high-quality radiographic images. The quality control was carried out to the actions providing means to control and measure the characteristics of parameters, methods carried out for the panoramic / oral radiographies in accordance with quality assurance.

The purpose of QA in dental radiography is to ensure consistence adequate diagnostic information, whiles radiation doses to patients and staffs are controlled to be as low as reasonably practice (ALARP). The QA programme will need to take account of relevant statutory requirements and this will determine many of the operational objectives.

Computerized Registration System of Occupational Radiation Exposure

At the beginning of 1996, the CANP started to develop a computerized registration system of occupational radiation exposures for workers that work in ionizing radiation fields in Albania. Introduction of the national fundamental control register at the CANP makes it possible to supervise all data on individual and collective exposures, and to make statistical analyses and reports on occupational radiation exposures. At present, the Register includes data on about 500 persons, among them approximately 150 workers who are or involved in X-ray dental practices in Albania [7,9,11].

The legal basis for the introduction of the Register of received doses is the valid legislation of the Republic of Albania: the Act on Radiation Protection approved on November 1996 and amended on January 2008. The CANP established the Register in accordance with the requirements of the EU directives – number 96/29/EUROATOM from 13 May 1996 and number 90/64/EUROATOM from 4 December 1990.

The TLD-REMS program is used as the basic computer software. Its advantage is the possibility to receive data on occupational exposure in the original electronic form the

dosimetric service in Albania. An application called: "AFC Register – Albanian Fundamental Control Register, allows data input into the register by hand or automatically independently from the software and hardware used by Dosimetric service of CANP.

The register is the fundamental data collection of received doses, personal data of workers and facilities, equipment, apparatus etc. The data include:

1) Data on received doses include the monthly dose, the quarterly dose, the dose received in the current year, the dose received in the last twelve months, the dose received in the last five years and the lifetime dose.

2) The personal data of workers include the identification number, name and surname, sex, date of birth, education, date of beginning of work with ionizing radiation, specification on whether the worker is an employee or a contractor, the facility/ equipment where the dose is/was received, job classification, the employer, and data on medical examination and on the certificate on the radiation protection exam.

3) Data on facilities/equipment/apparatus include the name and sign, address, town, country, phone /fax number, e-mail address etc.

4) The fundamental register makes it possible to examine the whole history of occupational exposure of an individual worker-when and where he/she was exposed in the workplace or to the time by years of the work life (by years).

5) The register allows an immediate review of the cumulative collective effective dose, as well as the annual collective effective dose by type of work or activity, as well as a year-after-year review by employers and by employees and workplaces.

DISCUSSION

The present study has indicated, on the basis of annual effective dose values, that patient's radiation exposures were compatible with basic safety standards for radiation protection of the staff (20 mSv/years), patients-population (1 mSv/year) and ALARA principle [1,2,7].

In our study we have used the "isometric rule" in order to have the real tooth dimensions, which permits the central beam to be perpendicular on bisecting line of the angle established by the axe of the tooth and film plaque, passing through pick of the tooth's root.

While, in oral radiography with contact, the film plaque needs to be put into the mouth to be jointed with the tooth's crown, gingival, hard palate (in the maxilla) and floor of the mouth (in the mandible).

CONCLUSION

The applying of guides of Code of Practice and recommendation of ACRP and ICRP for dental problems is able the application of basic standards in health dental care, which are shown in follow conclusions:

- Code of Practice shown and contain the technical parameters ensuring the staff, public and population, protecting during the procedures of dental examinations;
- The individual irradiation doses and individual risk from radiological dental examinations are lower as in all other cases of radiological examinations;
- Is more important the reduction and decrease of collective dose for patients and consequently the benefits from society by dental examinations;
- The annual mean dose is 5 mGy, which correspond this value correspond with the entrance surface dose and which dose in dental X-rays is equivalent to an effective dose of about 7 μSv.
- In scope of social - financial - treatment of procedures by dental radiological examinations, was needed to be upgraded the system of procedures, aiming the radiation protection by ionizing radiation [1,2,13].

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Chapter 12

EFFECTS OF USING RECYCLED NICKEL-CHROME BASED ALLOYS IN THE PREPARATION OF METALLIC RESTORATIONS

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ABSTRACT

The objective of this study was to investigate, the vaporization of alloying elements, variation in tensile strength and changes in the microstructure of the recycled Ni-Cr based dental alloys used in the preparation of metallic restorations like crowns and bridges. The commercial dental laboratories have the practice of using material left out in the form of sprue and buttons after every casting of a metallic restoration. The use of recycled alloys showed weaker tensile strength values and also inclusion of burnt oxides, silica and investment material after every repeated melting. The grain boundaries formed were found non uniform and scattered when observed under SEM (scanning electron microscope). The study established the quality of these restorations depends on the composition of the new alloy. During the conduct of experiments ten tensile test specimens having 30 mm gauge length and 5 mm diameter were casted for each group of repeated melting. The results were recorded and it was observed the considerable quantity of alloying elements vaporized. The loss of alloying elements resulted in decrease of tensile strength from a maximum of approximately 695 Mpa to a minimum 470 Mpa. Further, the fractured surfaces showed the presence of foreign particles under metallurgical microscope. Theoretical validation was also carried out using Hall-petch equation. Finally, it was recommended to use new (fresh) alloy to have a good quality of metallic restorations with a considerable reduction in failure rates.

Key words: Tensile strength, crowns, bridges, Hall-Petch, gauge length and SEM

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1. INTRODUCTION

The use of Nickel-Chrome based alloys for making metallic restorations like crowns and bridges in dentistry is ever increasing due to its excellent mechanical properties. The quality of these restorations depends on the composition of the alloy [1]. It is the practice for the commercial dentistry laboratories to use the recycled alloys extensively, due to the very high cost of the imported new (fresh) base metal alloys. This reason definitely spoils the quality of the restorations. Metallic crown is a single unit to be mounted on a partially trimmed tooth due to decay and a bridge is again a single unit to be mounted on two supporting teeth with a tooth lost in between. The use of metal alloys is universal and ever increasing due their excellent mechanical properties. Controlled clinical trials have shown that the failures of these restorations are about 10 to 15% over a length of service of 8 years and are likely to be much higher in general dental practice [2,3]. A large percentage of these failures are established to be as result of improper flow of molten metal to all the corners of the mould cavity and uneven thickness of the cast metal. The other reasons for the failures include intricacy of the mould cavity, laboratory procedure, pattern allowances and inclusion of foreign particles. etc. [4-6]

2. OBJECTIVE OF THE STUDY

The main objective of this study was to investigate the following.

1. Vaporization of alloying elements during repeated melting.
2. Tensile strength of the metal alloys after every repeated melting.
3. Changes in the microstructure of the alloys.

3. MATERIALS AND METHODS

The materials used in this study are presented in the table. 1.0. Wirolloy (BEGO,Germany) the Ni-Cr based alloy was used for preparing specimens. New and recycled alloys were standardized and divided into four groups of 10 specimens each. Each specimen has a gauge length of 30mm and diameter 5mm (Fig 1.0).

Table 1.1. Materials used in the Study

Brand name	Description	Manufacturer
Wirolloy	Pallets	BEGO, Germany
Bellvest-T	Phosphate bond graphite free precision casting investment.	BEGO, 12347.k 1768
Bego sol	Mixing liquid	BEGO,80204
Silicast	Ceramic crucibles	15300

Figure 1.0. Ready specimen.

Wax patterns were prepared from a mould of standard dimensions and ten specimens were cast from each of the four groups of metal alloys. A phosphate bond graphite free casting investment (Whip mix) was used and a two stage burn out procedure was used with a peak temperature of 850^{o} C.(using KOVA type 5635). The alloy each group was heated to its melting temperature i.e. 1350^{o} C in individual ceramic crucibles (make silicast) and cast using high frequency centrifugal induction casting machine (Degussa, Degutron model 1088 Germany make). After devesting, the specimens were sand blasted 60μm Al_2O_3 and recovered after ultrasonic cleaning in distilled water for 10minutes and dried.

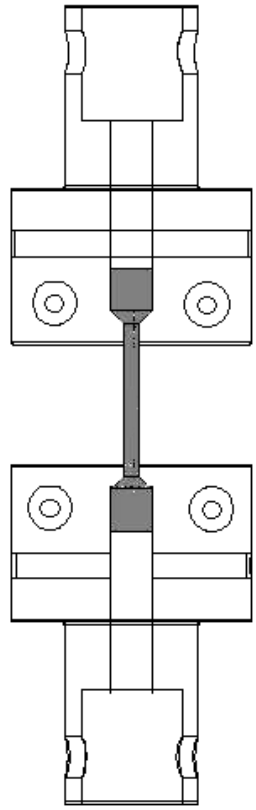


Figure 1.1. Tensile test arrangement.

Ready test specimens were fastened individually on the universal-testing machine using a special fixture to hold precisely the specimen in the axial direction. Figure 1.1 shows the arrangement of holding the test specimens. The load was applied axially to the gauge length by moving the upper jaw in the vertical direction, which is connected to the testing machine by the upper hole where the upward forces is applied. Lower jaw is fixed to testing machine by the lower hole. The cross head speed selected was 0.5mm/min. The load was applied until the test specimens were fractured. Tensile strength data were analyzed statistically using one-way analysis of variance. The load at which the specimen fractured was noted and the mean was calculated for each group of alloy.

All the four groups of base metal alloys were subjected to polishing using 240-through 1200-grit Sic metallographic paper and finished with fine polishing alumina. They were ultrasonically cleaned in distilled water for five minutes and dried. The final polishing of specimens was carried out on chamois leather. After etching with a suitable standard etchant the surfaces were examined under SEM to observe the microstructures. Composition analysis was carried out using SEM with EDS (energy dispersive x-ray spectrometer). Figure 1.4 a, b, c and d shows the variations in the microstructure after each melting.

4. RESULTS

The tensile strength values for each group of alloys are presented in table 1.2. The tensile strength results of the study showed that group 1 alloy consisting of new alloy (Wirolloy, BEGO, Germany) showed the highest values compared to the recycled alloys. Figures 1.2 shows the nature of tensile curves obtained during the tests. Table 1.3 shows the SEM with EDS results indicating the evaporation of alloying elements in the process of repeated melting of the metal alloys. The figures 1.4 a b c & d show the SEM photographs, which show the formation of grain boundaries and inclusion of foreign particles in the case of each group of alloys.

Theoretical validation was carried out using Hall-petch equation $\sigma_t = \sigma_0 + k\ 1/\sqrt{d}$, where σ_t is tensile strength, σ_0 is material constant, k is Hall-petch constant and d is average grain size diameter. Table 1.4 shows clearly that as the grain size diameter increases, it is observed the decrease in the tensile strength values.

Table 1.2. Tensile strength values

Sl.No. (alloy)	Melting	Tensile strength values in, Mpa	Mean tensile strength in,Mpa	SD*	CV*
1.Group I (New)	First	710 690 675 685 715 698 705 714 680 682	695.4	4.95	2.15
2.Group II (recycled)	Second	625 605 589 595 602 622 640 574 599 615	606.6	19.29	3.18
3.Group III (recycled)	Third	545 506 524 565 574 520 510 515 540 538	533.7	22.97	4.3
4.Group IV (recycled)	Fourth	456 500 540 465 425 480 504 465 439 428	470.2	36.59	7.78

SD – Standard deviation, CV – Coefficient of variation

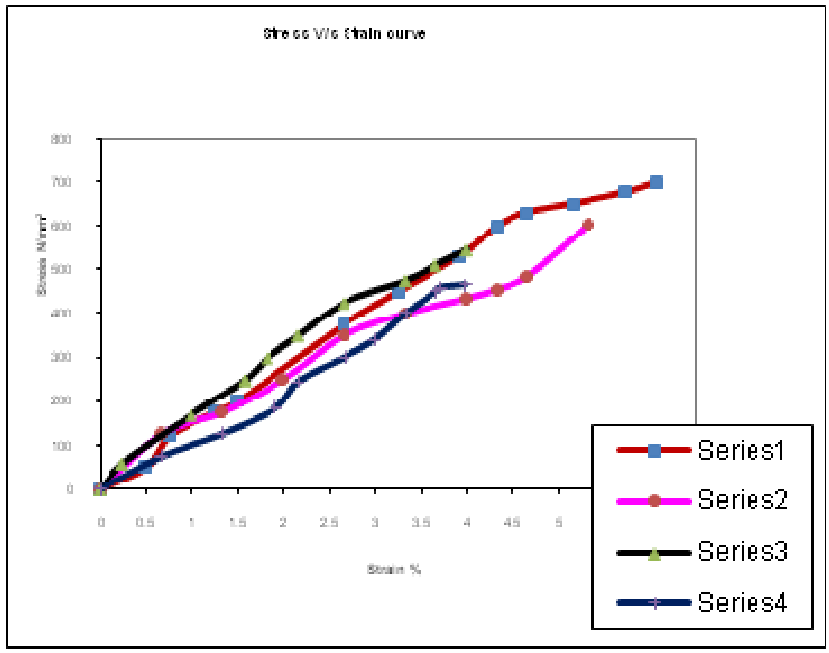


Figure 1.2. Stress- strain curves.

Table 1.3. Percent composition of test alloys (wt %)

Metal alloys	Alloying elements (%)					
	Ni	Cr	Fe	Mo	Si	Mn
Group I	63.5	23.0	9.0	3.0	1.0	0.5
Group II	62.6	23.5	8.7	3.75	0.8	0.6
Group III	62.03	25.8	6.9	4.37	0.65	0.25
Group IV	59.5	27.0	6.9	6.0	0.5	0.10

Table 1.4. Tensile strength values as per Hall-petch validation

Sl.No	Average grain size in, mm	Tensile strength values, in Mpa
Group I	$2.4*10^{-6}$	695.4
Group II	$3.3*10^{-6}$	596.51
Group III	$4.6*10^{-6}$	520.06
Group IV	$5.9*10^{-6}$	470.39

4.1. Discussions and Conclusions

The results presented in table 1.2 clearly explain that the reuse of metal alloys should be avoided totally in the process of obtaining good quality metallic restorations. The use of recycled alloys was found to show weaker tensile strength values. In view of high cost of the metal alloys, the commercial laboratories and the manufactures of the alloys suggest reusing the excess metal collected in the form of sprues and cones with addition of some percentage

of new base metal alloys. Repeated casting procedure may cause excessive quantities of these constituents of base metal to be lost thus potentially affecting the tensile strength values. The results of this study showed that considerable decrease in tensile strength was observed when recycled alloys were used. Figure1.3 bar chart makes it clear that the increase in the Standard deviation increases the values of Coefficient of variation. When the new base metal alloys were used for the group 1 specimen the tensile strength values were found to varying in the range 675-715. So the value of coefficient of variation found at a lower level. But with the other groups the range of tensile strength values are found widening gradually together with the increase in the values of the coefficient of variation. It is a very important factor to be considered because as the value of coefficient of variation increases the quality of the metal alloy will be under suspect.

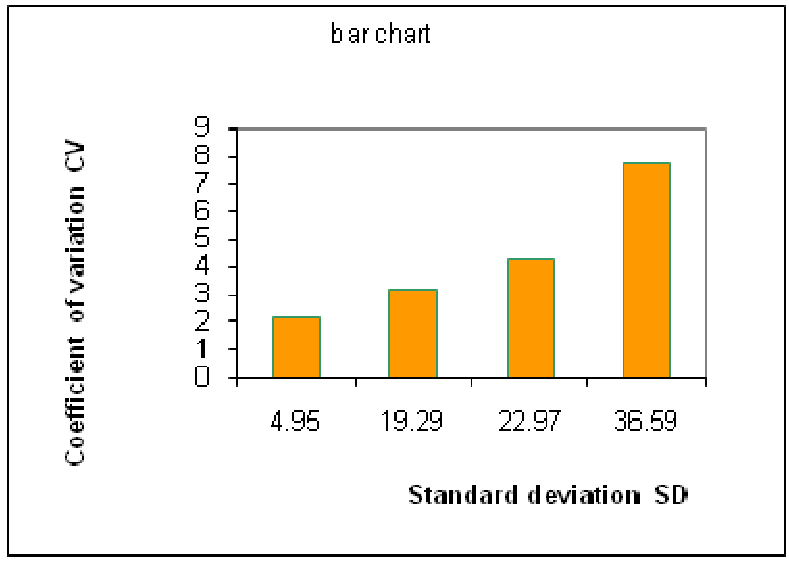
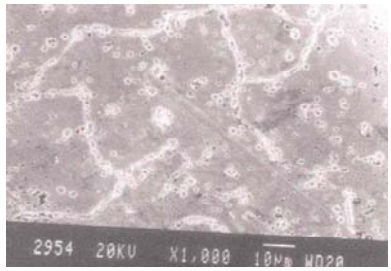


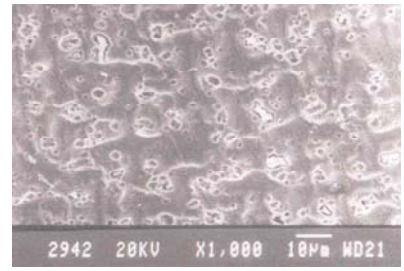
Figure 1.3. Bar-Charts.

Figures 1.2 is the tensile stress- strain curves for the alloy groups I to IV respectively and their ultimate tensile strengths of them. This explains the greater elongation of the group I specimens compared to other groups of alloys. This means new base metal alloy posses more toughness.

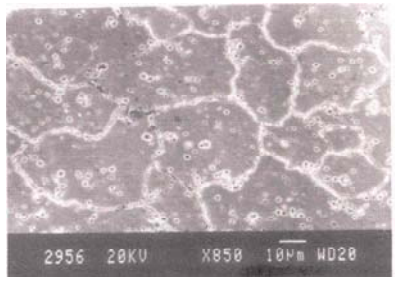
The analysis of fracture surfaces under metallurgical microscope showed the presence of foreign particles when recycled alloys were used. Based on the results it was recommended to use only the fresh alloy and not the recycled alloys while preparing any metallic restorations. The SEM photographs shown in Fig 1.4 a-d represent the variation in the microstructure of all the groups of alloys. The inclusion of foreign particles such as burnt oxides, silica and investment material are found in excess especially in the SEM microstructure of the group IV alloy. This is very clear from the black spots at the grain boundary region. So, also the grain boundaries are more non-uniform and scattered. Finally, the laboratory technicians were suggested to only the new base metal alloys to have good life span. There is scope for adding some percentage of new alloy to the previously melted alloy and carry out further work.



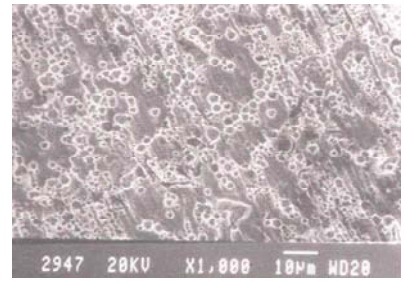
(a) Micro structure group I



(c) Micro structure group III



(b) Micro structure group II



(d) Micro structure group IV

Figure 1.4. a, b, c, & d shows the microstructures.

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Chapter 13

DEVELOPMENT OF LOW-SHRINKAGE DENTAL COMPOSITES

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ABSTRACT

Dental composite resins have revolutionized modern clinical dentistry. They are widely used for restoring teeth and cosmetic dentistry due to their esthetic and handling properties. Despite their wide applications, present day composite resins shrink when cured. This polymerization shrinkage generate stresses which affect the marginal seal between the tooth/restoration interfaces leading to secondary caries, post-operative sensitivity, tooth fracture, bond failure and marginal leakage. Other problems associated with current dental composites include inadequate wear resistance and the leaching of uncured organic monomers. The development of low shrinkage resins is therefore an important research focus in dentistry and remains a challenge. In this review, different polymerization techniques such as soft-start, pulse cure and pulse delay used to minimize shrinkage clinically will be discussed. The effect of the different light-curing techniques on the crosslink density of composites will also be reported. Recent developments of low shrinkage composites including some of our work on silsesquioxane in the laboratory will also be highlighted.

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INTRODUCTION

The modernization of clinical dentistry evolved from the development of light-activated dental composite resins. They are viewed as an attractive alternative to amalgam fillings and have superseded chemically cured counterparts. What are light-activated dental composites? What are their superior advantages over chemically cured composite resins and dental amalgam?

Composite materials refer to a mixture of two or more distinctly different materials with properties that are superior or intermediate to those of the individual constituents. Light-activated dental composites are tooth-coloured filling materials made up of synthetic polymers such as 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane (Bis-GMA) / triethyleneglycol dimethacrylate (TEGDMA) (most commonly used resins) (Figure 1), inorganic particulate fillers, initiators and activators, silane coupling agents (for bonding of the fillers to the matrix), pigments and stabilizers [1]. They undergo polymerization upon blue light-irradiation in the region of 410-500 nm to form a cross-linked polymer network. Light in this region is absorbed efficiently by photoinitiators usually camphorquione (CQ), which creates an excited state that reacts with an amine reducing agent (activators) such as *N,N*-dimethylaminoethyl methacrylate (DMAEMA) or ethyl *p*-dimethylaminobenzoate (DMAB) to produce free radicals for the polymerization of the organic matrix (Figure 2) [2, 3].

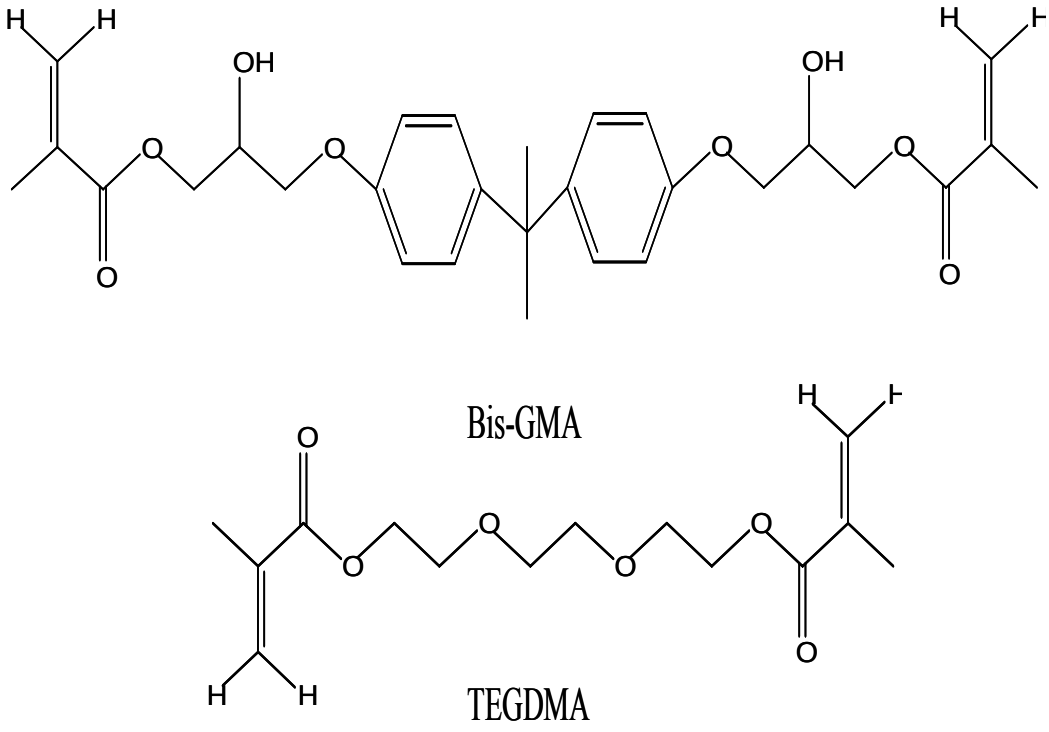

Figure 1. Chemical structure of conventional dental monomers, bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane (Bis-GMA) / triethyleneglycol dimethacrylate (TEGDMA).

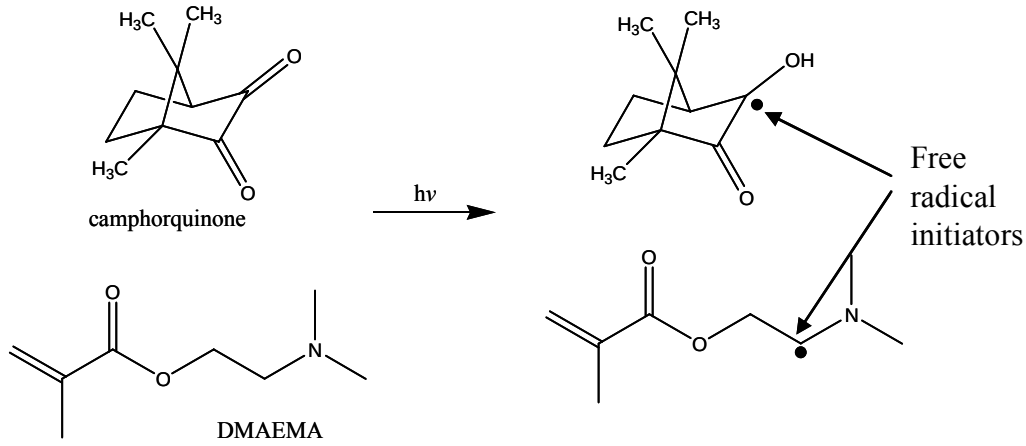

Figure 2. Light activation mechanism of camphorquinone with an amine reducing agent, *N,N*-dimethylaminoethyl methacrylate (DMAEMA).

When compared to chemically cured composites, light-activated composite resins offer the advantages of higher wear resistance, lower thermal expansion, lower polymerization shrinkage and controlled working time where clinicians have the freedom to time their polymerization thus getting rid of the time consuming blending procedures that often introduce unnecessary porosities such as oxygen to the restorations as observed in chemically cured composites. Chemically cured composites also known as self or auto cured composites, involved the blending of two pastes that brought the monomers matrix and the initiator (dibenzoyl peroxide) / activator (*N,N*-di-(2-hydroxyethyl)-*p*-toluidine (DHEPT) or *N,N*-dimethy-*p*-toluidine (DMPT)) system for the initiation of the cross-linking reactions. The lack of wear resistance and strength prevented them from preserving restoration contour in areas subject to abrasion or attrition and also affected their use in high-stress areas due to flowability of the materials under load. Besides that, self-cured composites were also found to be associated with high polymerization shrinkage and coefficient of thermal expansion which led to problems such as microleakage and discoloration at the margins due to percolation [4]. Furthermore, clinicians were also restricted by the polymerization setting time when placing and shaping the restorations. Clinical studies conducted have also showed that over a period of time, restorations that were restored with chemically-cured composites tend to undergo more darkening when compared to light-activated composites [5]. Thus, the aforementioned limitations of self-cured composites have led to the development of light-activated ones.

Besides the superior properties displayed by light-activated resins when compared to chemically cured composites, tooth-coloured composite resins were also viewed as an attractive amalgam substitution due to concerns over mercury toxicity and increasing aesthetic demands by patients and clinicians [6, 7]. Thus, modern day composites are widely used for restorations in the anterior and posterior regions, indirect inlays and onlays, pit and fissure sealants as well as crowns and implants.

The development of light-activated composites to date has progress rapidly with major improvements made in the area of increased filler loading along with variation in distribution, size, shape and composition. Modifications to the filler components bring improvements in wear resistance, color stability, strength, radiopacity and degree of conversion of dental composites and thus the overall improvement in clinical performance of these materials.

However, despite vast improvements in composite materials and their mechanical properties, present day composite resins still have shortcomings limiting their application. Inadequate resistance to wear (loss of anatomic form) under masticatory attrition, fracture of restorations, incomplete conversion and cross-linking of the organic matrix, discoloration, marginal adaptation, secondary caries and marginal leakage due to polymerization shrinkage are some of the factors limiting the longevity of composite resins [8, 9].

LIMITATIONS OF CURRENT DENTAL COMPOSITES

Commercial dental composites exhibit 2–14% volumetric shrinkage during the polymerization process [10-12] and are affected by factors such as constituents of the resin-based composite material, configuration of the cavity preparation, spectral and power distribution of the visible light-curing unit, and finally clinical technique [13]. When composites shrink, stresses are generated at the composite/tooth interface. These shrinkage stresses can cause marginal openings if the bonding system is unable to withstand the polymerization forces and thus lead to leakage and ultimately caries. Despite the dramatic improvements in the formulation of newer generation bonding agents with enhanced marginal adaptation and bond strengths, the goal of a perfect marginal seal is still not achievable. Clinical studies carried out for resin-based composite restorations for Class I and II cavities for a period of 3 to 6 years have also shown that secondary and/or recurrent caries were the main reasons for restoration failure [8, 14-16] and polymerization shrinkage has been cited as one of the most significant factors influencing the seal between tooth structure and polymer-based restorative materials.

All composites shrink upon light-activation and the total shrinkage of composite materials can be divided into pre-gel and post-gel phases. During the pre-gel polymerization, the composite is able to flow and stresses within the structure are relieved [17]. After gelation, viscosity increases significantly and stresses due to shrinkage cannot be compensated. Post-gel polymerization thus results in significant stresses in the surrounding tooth structure and composite tooth bond [18] that may lead to bond failure, microleakage, post-operative sensitivity and recurrent caries. These stresses could also result in deformation of the surrounding tooth structure if the composite-tooth bond is strong, causing the tooth to fracture [19].

As aforementioned, the stress associated with polymerization shrinkage is one of the most significant problems associated with current composite materials as it adversely affects the seal at the composite/tooth interface which led to the occurrence of secondary caries [20]. When bonding of the adhesive to the tooth structure is inadequate, composite shrinks and pulls away from the cavity walls, forming an opening. This opening at the restoration interface leads to clinical problems such as microleakage, straining, sensitivity, and/or recurrent caries. However, when the bonding to tooth structure is strong enough, polymerization stress is applied to the tooth as composites shrink which leads to problems such as fractured cusps, movement of cusps, and/or postoperative sensitivity [13].

Besides, differences in monomer chemistry, various degrees of final polymerization, filler types and filler concentrations, the amount of stress generated due to polymerization shrinkage is also dependent on the configuration of the cavity preparation by clinicians. Configuration factor, commonly known as the C-factor, is defined as the ratio of the bonded

area of the restoration to the unbonded area [18]. High C-factor are often associated with a higher stress on the bonded surfaces [21]. Since composite flow is more likely to occur from the free surfaces of the specimen, a higher proportion of free composite surface would correspond to a smaller restriction to shrinkage, thereby reducing stress. When the free surface is reduced, the ability to flow and compensate for shrinkage is restricted by the bonded surfaces thus, increasing stress. As cavity preparations present a much more complex geometry with heterogeneous stress distribution [22], the application of the C-factor concept to clinical practice must be performed carefully by the clinicians in order to minimize shrinkage and its accompanying stress.

Besides C-factor, other clinical techniques used for minimizing polymerization shrinkage and its accompanying stresses includes incremental layering of composite during placement [23] and application of a low elastic modulus liner as a stress absorber between the tooth and shrinking composite restorative [24]. One recent method which has gained popularity over the years among researchers and clinicians for the reduction of polymerization shrinkage without affecting the degree of conversion in light-activated composites is to reduce the viscosity during setting by means of controlled polymerization.

CONTROLLED POLYMERIZATION

Controlled polymerization refers to the various curing techniques employed by clinicians and researchers for the reduction of polymerization shrinkage and its accompanying stresses. In contrast to the conventional continuous curing method, controlled polymerization set to reduce shrinkage by slowing down the rate of polymerization. Various controlled polymerization techniques include curing with an intensity that increases exponentially from low to high (ramp/ exponential), application of short pulses of energy (pulse activation), delays between exposures (pulse delay) or pre-polymerization at low-intensity light followed by a final cure at high intensity (soft-start techniques) (Figure 3). It was postulated that with controlled polymerization, clinicians can reduce polymerization shrinkage and stresses of various materials by timing and controlling their polymerization more efficiently. The delay, dark period and low-intensity curing associated with the various curing modes allowed for composite flow to take place by delaying the gel-point. Gel-point refers to the transformation of the composites from a viscous state to a rigid solid while composite flow is defined as the capability of the molecules to go into a new position before cross-linking. Thus the low modulus developed by the composites in the early polymerization phase of the controlled curing techniques helps in relieving stress through composite flow and finally the reduction of polymerization shrinkage. In recent years, there have been tremendous studies and research carried out to determine the effectiveness of reducing polymerization shrinkage and stresses through controlled polymerization light curing techniques.

Uno & Asmussen [25], Goracci et al. [26] and Koran & Kürschner [27] have demonstrated through studies in the 90s that marginal gap reduction, better marginal adhesion were observed in composite cured through a sequential irradiation approach when compared to the continuous irradiation mode. In addition, Koran & Kürschner [27] have also demonstrated that even though the degree of conversion and final shrinkage did not differ significantly with either approach when the total light energy density applied was kept constant, the dynamic of shrinkage were found to be superior with the two step approach. In

2002, Lim et al. [28] demonstrated that curing composites using the pulse delay mode helps to reduce polymerization contraction stresses. No significant reduction in polymerization stresses was observed when composites were cured using the soft-start approach. Results obtained correlate well to that of Friedl et al. [29] and Bouschlicher et al. [30] where marginal adaption and shrinkage forces of composites were not influenced by either the conventional or two step curing modes. Thus, the introduction of a gap period between pulses is important for enhancement of composite flow which helps in the reduction of composite shrinkage and stresses [31, 32].

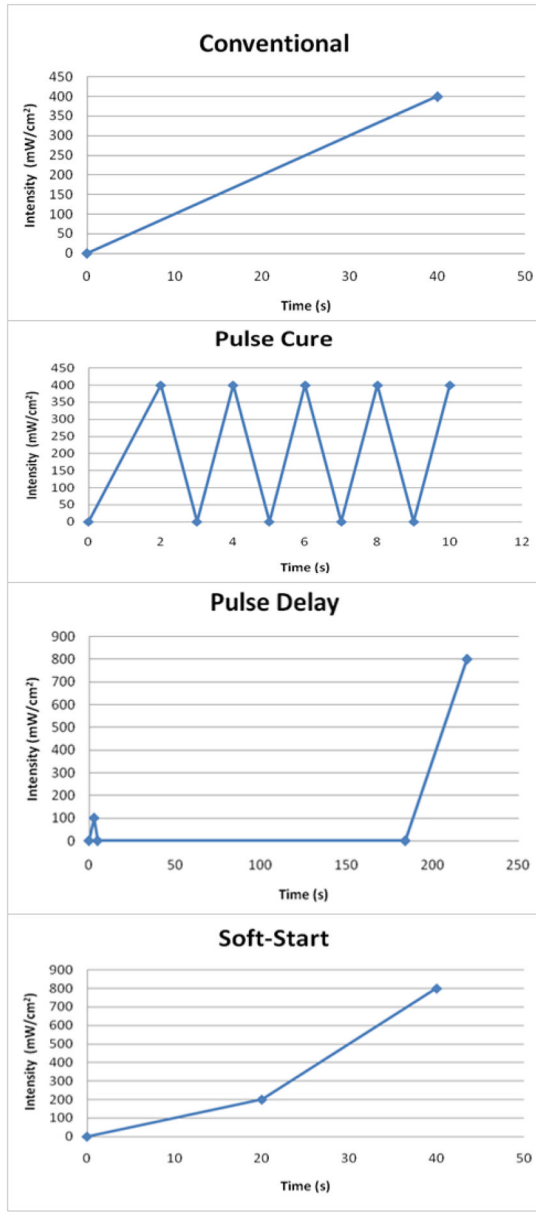

Figure 3. Examples of the different light-curing profiles (a) conventional, (b) pulse cure, (c) pulse delay, (d) soft-start used for polymerization of dental composites.

While studies [27, 28, 33, 34] have shown that curing of composites using soft-start technique did not result in significant reduction in shrinkage and stresses when compared to continuous mode, others have found that controlled polymerization helps to reduce gap formation [35-37], microleakage [38, 39], polymerization shrinkage and stresses [32, 40-42] without affecting their degree of conversion and mechanical properties. Thus, their use in clinical practice has been encouraged by Alonso et al. [35].

In order to evaluate the usefulness of controlled polymerization in clinical practice, we have also carried out studies to determine the effectiveness of different light-curing modes on composite cure and shrinkage [43, 44]. In the studies, it was found that the use of pulse delay mode helps to reduce shrinkage when compared to the continuous mode. However, the technique also results in lower bottom hardness value when cured on a 2 mm thick specimens. In order to have a fair comparison, we conducted another study to evaluate the different light curing regimens at a fixed light energy density [45]. It was found that pulse delay and soft-start curing techniques does help to decrease post-gel polymerization shrinkage when compared to the conventional light-curing mode. The effectiveness of cure may also be enhanced by the use of soft-start curing technique [46]. It was also observed that the use of soft-start and pulse activation modes of some light curing units (Halogen vs. LED (light-emitting diodes)) may help in the reduction of post-gel shrinkage [10]. Thus, the reduction of polymerization shrinkage of composites was found to be not only materials dependent, mode dependent but also light curing unit dependent.

While studies on the usefulness of controlled polymerization for the reduction of polymerization shrinkage and stresses remains to be debatable, Asmussen & Peutzfeldt [47, 48] discovered that the use of pulse delay and soft-start modes resulted in a different polymer structure compared to composite cured using the conventional continuous mode despite similar degree of cure. In the study, it was postulated that the use of a continuous mode resulted in a more crosslink structure due to its ability to initiate more camphoroquinone and thus the formation of more growth centers compared to the pulse delay and soft-start modes where fewer growth centers were formed as a result of lower and shorter initial curing. Thus, polymerization using the controlled curing technique (pulse delay and soft-start) resulted in a more linear polymer structure. The linear polymer structure obtained as a result of pulse delay and soft-start curing causes an increased susceptibility to softening in ethanol. The results obtained suggest that composites cured by controlled polymerization techniques may be more susceptible to softening by food and beverages and also an increased in free monomer leaching. While pulse delay and soft-start techniques have been found to be useful for the reduction of marginal gap and polymerization shrinkage and its accompanying stress, the use of these techniques may become less attractive in the dental community. However, studies in this aspect are still limited.

In order to evaluate the aforementioned results further, we conducted a study to evaluate the crosslinking density of composite cured with both the conventional and controlled polymerization techniques under the condition whereby constant light energy density were applied for all curing modes [49]. In this study, the degree of crosslinking density was measured both directly and indirectly through measurements of the glass transition temperature and hardness after ethanol storage, respectively. The glass transition temperature (T_g) associated with the controlled polymerization curing modes in this study was found to be lower than the conventional cure mode. As T_g values of a polymer system are more dependent upon crosslinking, the lower T_g values obtained for the controlled polymerization techniques

suggest less crosslinking. Results obtained in this study also suggest that polymerization with pulse delay mode resulted in a lower crosslink density and gave rise to polymers with an increased susceptibility to softening in ethanol. However, when we investigated the amount of uncured monomers that leached out of the composite when cured by the different light-curing techniques, we found that leaching of the monomers are light-curing units dependent rather than polymer structures or light-curing modes dependent [50]. While the usefulness of controlled polymerization for the reduction of polymerization shrinkage remains an elusive target, one of the greatest challenges and the ultimate solution to polymerization shrinkage is to develop expanding, low-shrinking or non-shrinking resins.

DEVELOPMENT OF LOW SHRINKING COMPOSITES

While shrinkage stresses can be reduced by increasing filler loading, the ultimate solution to polymerization shrinkage is to develop "non-shrinking" resins. Although earlier efforts to synthesize such resins were not successful, several developments in the last decade are more encouraging.

RING-OPENING MONOMERS

In order to reduce shrinkage, expanding monomers such as spiro-orthocarbonates (SOCs) derivatives have been synthesized by Stansbury [51,52] in 1992. These SOCs monomers expand during polymerization through "a double-ring opening process". The SOCs derivatives were attached with polymerizable group such as the methylene (Figure 4) and the methacrylate (Figure 5) group to enable cross-linking to take place upon curing. The first series of SOCs synthesized base on the methylene side chain was found to have slow reactivity when compared to the SOCs compounds with methacrylate as side chain. The methacrylate substituted SOCs were found to be very reactive when polymerized in dilute solutions with almost complete ring opening of the SOCs compounds. However, reactivity decreases with bulk curing and hence resulted in 1 % shrinkage.

Besides the above mentioned compounds, a six-membered SOCs (trans/trans-2,3,8,9-di(tetramethylene)-1,5,7,11-tetraoxaspiro[5.5] undecane) (Figure 6) which exhibited a 3.5 % volume expansion when polymerized with cationic initiators such as (4-octyloxyphenyl)-phenyliodonium hexafluoroantimonate, has also been synthesized [53, 54]. When used as copolymers, results showed that the addition of as little as 5 % of the SOC monomers in an epoxy base produced a compound with substantial tensile strength and modulus, acceptable water sorption and solubility, and a slight expansion. While results of these studies are promising, no commercial materials are available to date. This may be due to the high cost of the monomer.

Further to SOCs, synthesis of SOEs (spiro ortho esthers) derivatives has also been reported by Miyazaki *et al.* [55]. These SOEs which contained polymerizable group such as the acrylate and methacrylate can undergo heat, ionic and free radical polymerization. However, the composites after polymerization were found to be low in strength without much reduction in shrinkage.

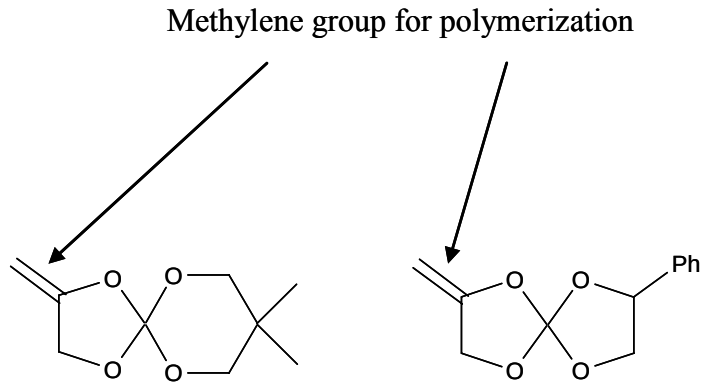

Figure 4. Examples of the different spiro-orthocarbonates (SOCs) containing methylene groups synthesized as ring-opening monomers.

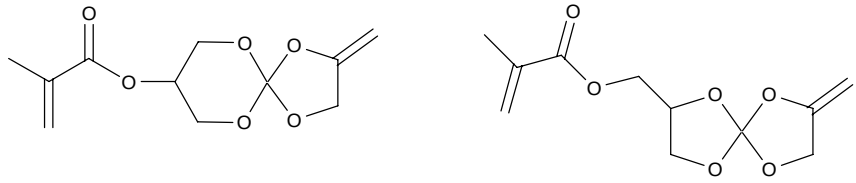

Figure 5. Chemical structures of spiro-orthocarbonate substituted methacrylate.

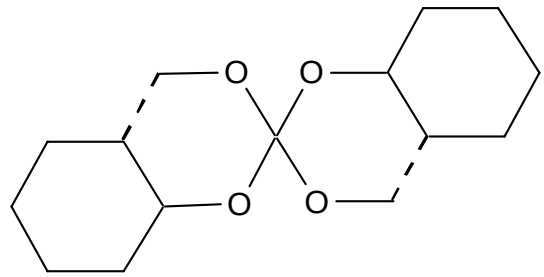

Figure 6. A cationic polymerizable spiro-orthocarbonate, trans/trans-2,3,8,9-di(tetramethylene)-1,5,7,11-tetraoxaspiro[5.5] undecane.

Besides SOCs, cationic photopolymerizable epoxy monomer such as cycloaliphatic epoxy is another class of compounds developed. These epoxy monomers can be polymerized using blue light through a three component initiating system which is made up of cationic initiators, camphorquinone and an amine activator. When compared to dental methacrylate, the cycloaliphatic epoxy resulted in a lower shrinkage. One example in the application of the epoxy resins is that of epoxy-polyol mixtures. Tilbrook *et al.* [56] has demonstrated through their study that with the correct choice of polyol and ratio of epoxy to polyol groups, a low shrinkage composite with comparable strength and stiffness can be obtained. However, the use of epoxy-polyol was not encouraged due to its high water sorption (polyol is hydrophilic in nature) which leads to cracking. One other area of development for the cycloaliphatic epoxy monomers is the development of Siloranes™ which will be discussed further in the later part of this review.

In addition to epoxy resins, ring opening monomers such as oxetanes [57, 58], cyclic acetals [59, 60], cyclic allyl sulphides [61] and vinylcyclopropanes [62, 63] have also been evaluated for dental applications. However, none of them were found to be promising. While oxetanes has a higher basicity with the reactivity of polymerization being atmosphere dependent (more reactive in nitrogen) [58], cyclic acetals, cyclic allyl sulfides and vinylcyclopropanes compounds were found to be either unstable, have low reactivity, exhibited glass transition temperature (T_g) that were unacceptable for dental applications or resulted in polymers that have high flexibility.

LIQUID CRYSTALLINE MONOMERS

As the search for low-shrinking polymers continue, liquid crystalline or highly branched molecules have also been synthesized and evaluated for their usefulness as low-shrinking monomers. While liquid crystalline monomers have the advantages of low viscosity, high degree of conversion and low shrinkage properties, they were found to have melting temperatures greater than 80 ^{o}C making polymerization difficult. In an effort to overcome this problem, several new liquid crystalline dimethacrylates [64, 65] (Figure 7) and/or branched liquid crystalline bismethacrylates [66, 67] (Figure 8) monomers with polymerization shrinkage ranging from 1.3 – 2.5 vol% have been synthesized through chain modifications. While these monomers resulted in low polymerization shrinkage, their mechanical properties were not promising due to the more flexible network.

ORMOCERS

Ormocers (organically modified ceramics), which refers to an inorganic-organic hybrid dental materials is another type of nanostructured hybrid dental materials developed with the purpose of reducing polymerization shrinkage, improving marginal adaption, abrasion resistance and biocompatibility. The ormocers which have an inorganic backbone based on SiO_2 are functionalized with polymerizable organic units such as dimethacrylate and are separated from the trialkoxysilane moiety by different spacer groups (Figure 9). The more rigid the spacer groups, the higher the modulus of elasticity. The nanostructured ormocers can be synthesized from “an alkoxy silane functionalized with a polymerizable group, followed by hydrolysis and condensation which led to an oligomeric Si-O-Si nanostructure” (Figure 10) [68]. However, due to its high viscosity, a diluent, TEGDMA, is often needed. Despite comparable marginal adaptation, a recent study conducted on low shrinkage composites showed that a considerable amount of polymerization shrinkage is still present with this class of materials [11].

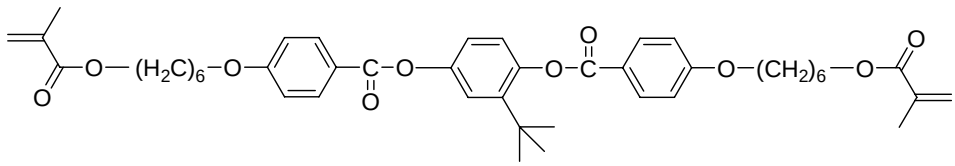

Figure 7. Example of near room temperature polymerizable liquid crystalline dimethacrylate.

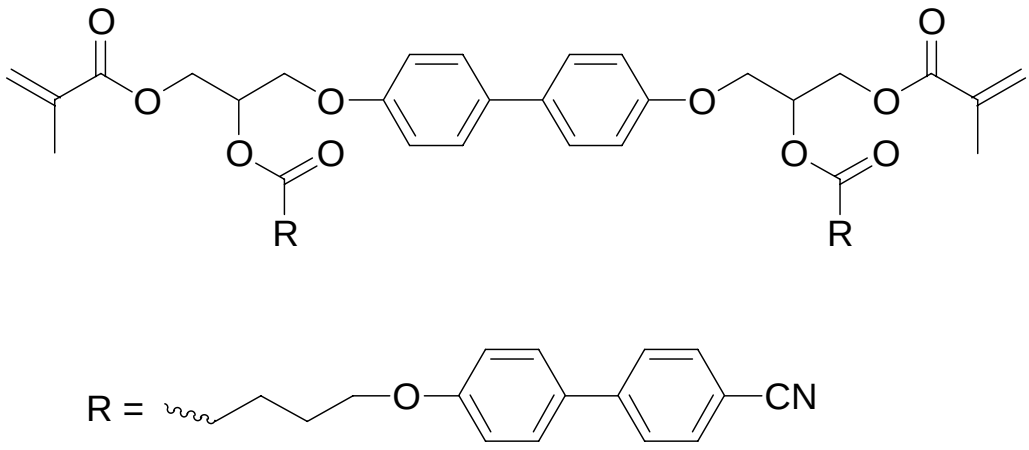

Figure 8. Branched liquid crystalline bismethacrylates.

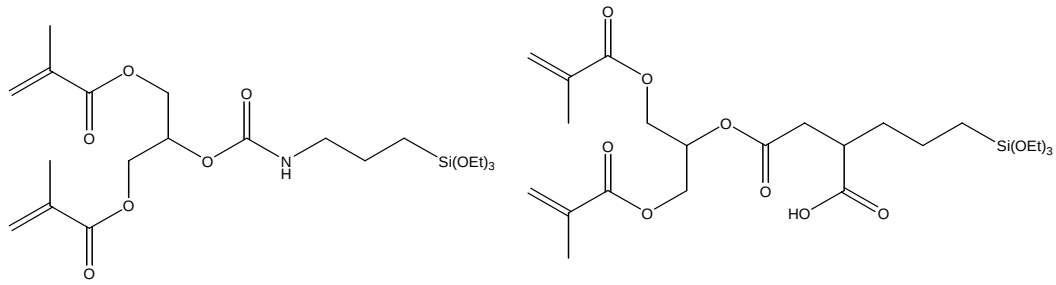

Figure 9. Example of commercially available methacrylate silanes with different spacer group.

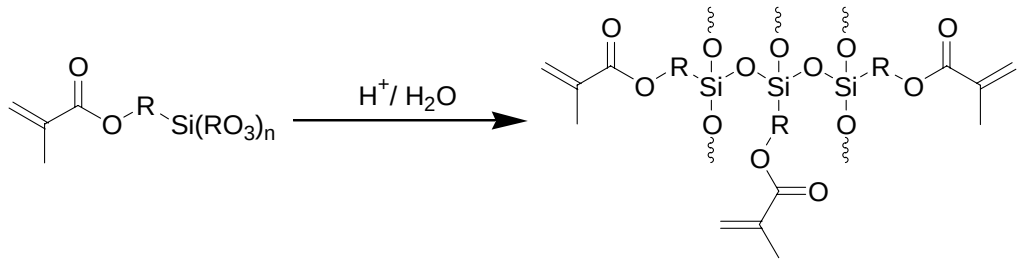

Figure 10. The synthetic route of SiO_2 nanostructures.

SILOXANE

Siloxane dendrimers (Figure 11), which were based on cyclic siloxane cores with photo-polymerizable side groups, were another class of cross-linking monomers developed [69]. This group of material demonstrated increased hardness and reduced viscosity that allowed for high amount of filler loading. As the monomers are designed to link with the siloxane core, uncured monomers linked to the core cannot be easily leached out into surrounding gum tissue. Siloxane dendrimers were found to have useful applications in area of restorative fillings, crowns, bridges or cast restorations.

In recent years, 3M ESPE has successfully developed and commercialized the Siloranes™. Siloranes™ (Figure 12), was developed based on the siloxane core and oxiranes. It is a class of cationic ring opening monomers developed to reduce shrinkage. Though initial study based on the Siloranes™ has shown low polymerization shrinkage with comparable elastic modulus and flexural strength, more studies are still warranted [70].

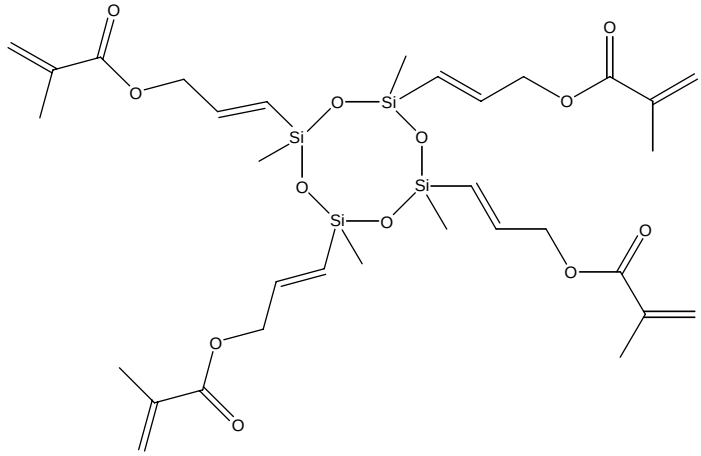

Figure 11. Siloxane dendrimers.

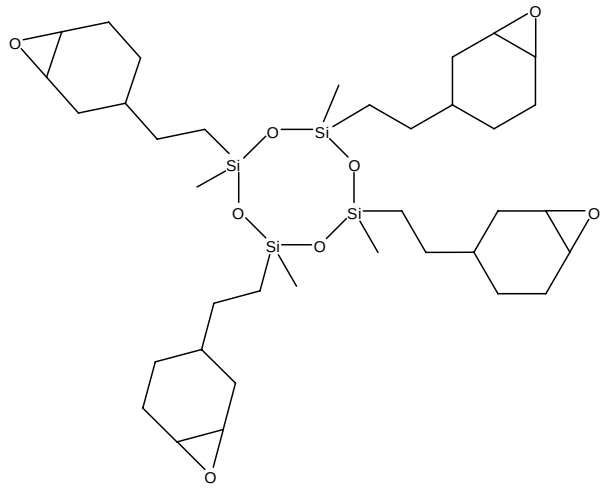

Figure 12. Siloranes™ dendrimers.

POLYHEDRAL OLIGOMERIC SILSESQUIOXANE

Polyhedral Oligomeric Silsesquioxane (POSS™) $(RSiO_{1.5})_x$, with a diameter of 0.54 nm, is one other hybrid organic-inorganic nanocomposite material evaluated for dental applications. POSS™, which can be regarded as the smallest particle of silica, is generally obtained by hydrolysis and condensation of trialkoxy or trichlorosilanes. With a unique well-defined structure, POSS™ is often used for the preparation of hybrid materials with well-

defined structures [71]. It can also be chemically functionalized and behave as a platform from which to synthesize organic/inorganic nanocomposites for use in a variety of applications such as performance materials and abrasion resistant coatings.

Incorporation of POSS™ derivatives into polymeric materials can help to improve mechanical properties, increase thermal stability, oxidation resistance and surface hardening as well as to reduce flammability and viscosity during processing. POSS™ monomers, which do not require significant changes in processing, are simply mixed and copolymerized by traditional methods. They form true molecular dispersions when mixed into polymer formulations with no phase separation and hence represent a significant advantage over current filler technologies [72].

In recent years, several POSS™ molecules have been synthesized and investigated for dental applications. Mono-methacrylate functionalized POSS™ (Figure 13) synthesized by Gao *et al.* [73] have been evaluated and used for copolymerization with methacrylate monomers. It was found that incorporation of small amounts (5% *w/w*) of POSS™ molecules resulted in improved mechanical properties and reduced shrinkage. The potential of using POSS™-MA (methacryl-POSS™ cage mixture) as a replacement for Bis-GMA has also been investigated [74]. It was also found that a small percentage substitution (mass fraction of 10% or less) of Bis-GMA with POSS™-MA improved flexural strength and Young's modulus of composites but large percentage substitution (mass fraction of 25% or more) resulted in undesirable mechanical properties, lower degree of conversion and lower reactivity. Liquid epoxy-functionalized cubes (Figure 14) were other POSS™ structures designed for single phase composites with potential application for dental restoratives. This epoxy POSS™ containing up to 65% masked silica was capable of producing hard, scratch- and solvent-resistant materials when photochemically cured [75]. The functionalized POSS™ materials developed thus far for dental applications are all monofunctionalized and work on fully functionalized materials have not been fully explored yet.

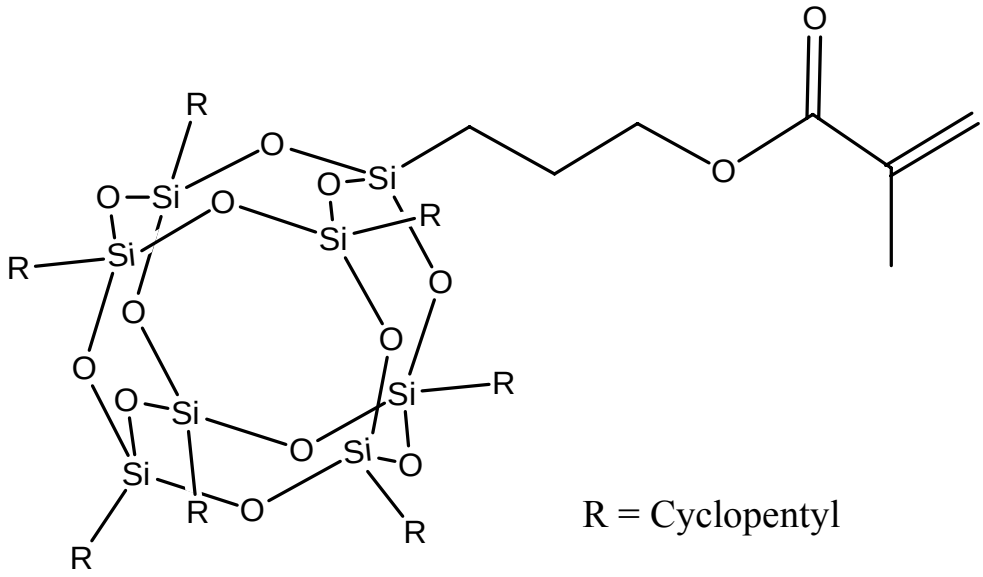

Figure 13. Mono-methacrylate functionalized POSS™.

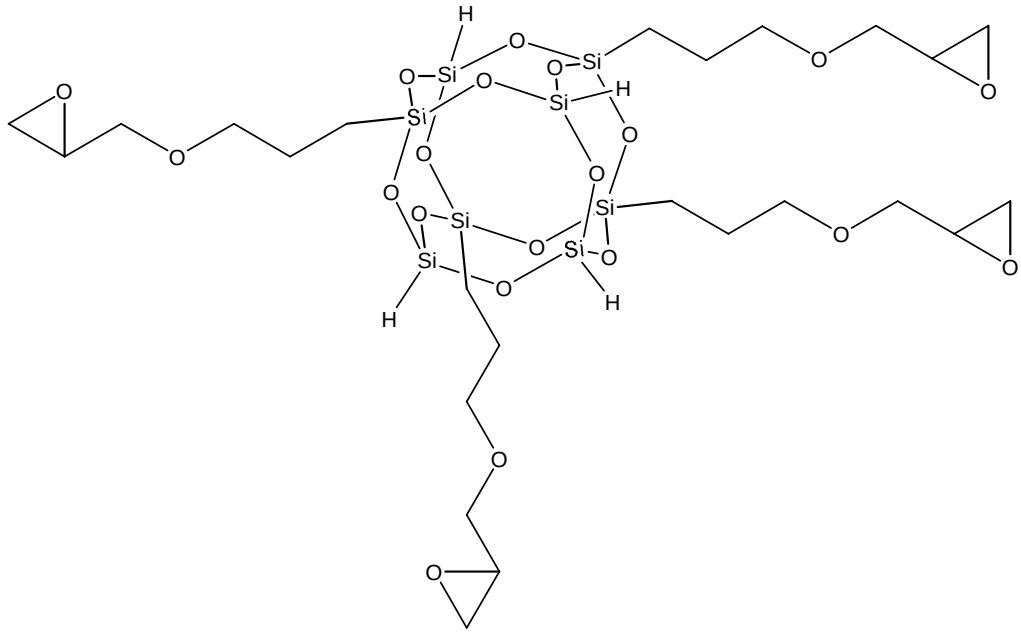

Figure 14. Epoxy functionalized POSS™ structure.

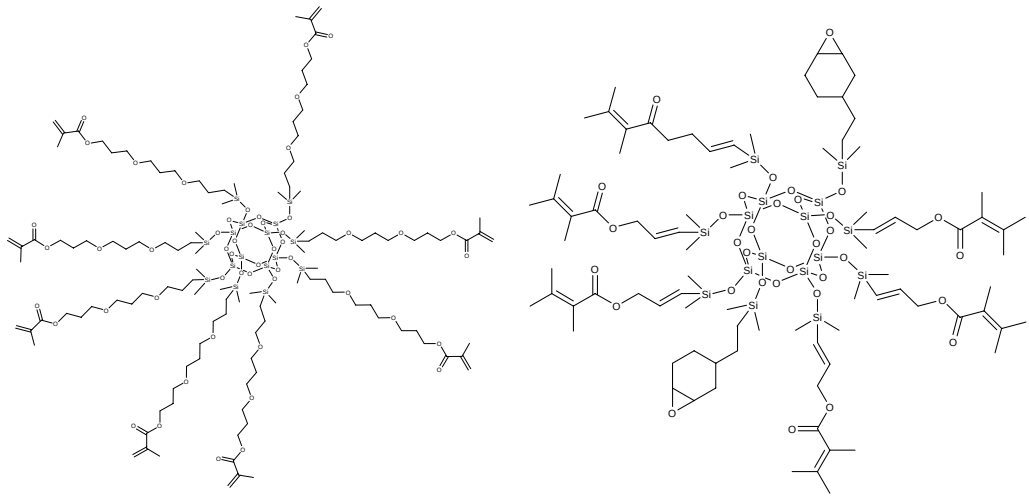

Figure 15. Example of fully functionalized silsesquioxane (SSQ) monomers.

To further evaluate the potential application of POSS™ modified resins as dental monomers, we have designed and developed novel low shrinkage silsesquioxane (SSQ) materials fully functionalized with various methacrylates and epoxy for dental applications (Figure 15) [76-78]. These novel multifunctional SSQ materials were found to have the advantages of being able to be isolated in high yields (>80%) and are viscous liquids that can be polymerized both thermally and photochemically. The SSQ resins were synthesized based on inexpensive starting materials and contained up to 48% masked silica making them ideal for dental materials. The post-gel shrinkage associated with these SSQ compounds were found be significantly lower than the conventional Bis-GMA/TEGDMA mixture. Shrinkage

was found to decrease with decrease in the number of methacrylate group attached. In our studies, we have also demonstrated that besides shrinkage, various physico-mechanical properties such as modulus, hardness, stability and cross-link density can also be improved through chain modifications and strategic selection of the polymerizable groups attached to the SSQ core. The addition of as little as 5 wt% of the SSQ resins into dental monomers helps to reduce shrinkage by as much as 20% [78]. Furthermore, it is anticipated in our study that SSQ compounds will have the advantage of potentially reducing cytotoxic properties as a result of their relatively large size and multi-functionality. For example in current dental composites, because of their small size and limited number of functional groups, uncured monomers can slowly leach out of the composite matrix into surrounding gum/ pulp tissue and cause cytotoxic responses. In the case of SSQs described here, the combined properties of large molecular size (prevents migration) and multiple methacrylate groups (just one reactive site to anchor) makes the probability of leaching out from the matrix extremely low.

KALORE

Kalore is the newest class of low shrinking composite successfully developed and commercialized by GC Corporation. Development of the low shrinking Kalore is based on new monomer technology from DuPont (Figure 16). It is a class of methacrylate based monomer with long and rigid center core developed to control polymerization shrinkage. While manufacturer has claimed that Kalore exhibited a lower shrinkage stress when compared to the Silorane™, more studies on polymerization shrinkage as well as the mechanical properties of the new material are warranted to evaluate the overall performance of the composite.

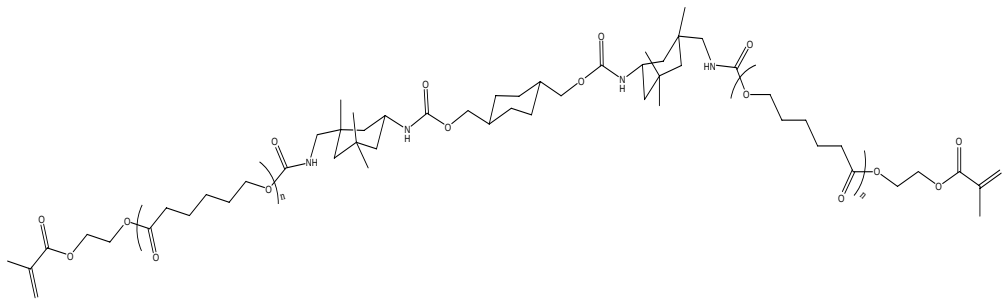

Figure 16. DuPont monomer developed with long and rigid center core and flexible methacrylate arms.

CONCLUSION

Research has well documented over the past years that all dental composite shrink during polymerization. While efforts have been made clinically to reduce shrinkage through development of various controlled polymerization techniques, none has been successfully implemented across the whole dental community due to controversy in research findings. While some studies have shown that controlled polymerization techniques help to reduce polymerization shrinkage, stresses and marginal adaption, others have shown otherwise. The use of light-curing techniques such as pulse delay mode was found to result in a more linear

polymer structures that is more susceptible to ethanol softening, thus making the technique less attractive for use by clinicians. In order to overcome the problem of polymerization shrinkage, efforts have also been made to develop low/non shrinking materials in the laboratory. While various monomers such as epoxy, spiro-orthocarbonates, ormocers have been developed, none has been successfully implemented commercially. The biggest breakthrough in dental materials research came with the commercialization of the Silorane™ by 3M ESPE. Although shrinkage results are encouraging, more studies are warranted. The development of low shrinking silsesquioxane (SSQ) mutifunctionalized materials developed by our group was also found to be rather encouraging. Thus, the search for dental materials with zero net shrinkage continues to be one of the greatest challenges in composite resins technology.

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Chapter 14

DESIGN, CHARACTERIZATION AND EVALUATION OF BIOMIMETIC POLYMERIC DENTAL COMPOSITES WITH REMINERALIZATION POTENTIAL

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1. INTRODUCTION

During the last century, much has been learned about the process of dental caries, a localized destruction of tooth tissue by plaque microorganisms that ferment dietary carbohydrates into organic acids which then cause dissolution of tooth mineral. Teeth are constantly going through cycles of mineral loss (when oral pH is below the point at which tooth mineral begins to dissolve) and repair (neutral and/or basic pH conditions that favor the redeposition of mineral). The net loss or gain in mineral over time ultimately determines whether tooth decay (demineralization) will advance, stabilize and/or regress. The major goal of clinical intervention is the preservation of tooth structure and the prevention of lesion progression to the point where restoration is needed. Caries prevention strategies have focused on reducing bacterial growth, neutralizing oral acids and using various remineralizing agents. Traditionally, remineralizing caries-arresting approaches are based on calcium and phosphate ion delivery through the use of dentifrices, chewing gums and mouthwashes, and systemic and/or topical fluoridation. However, restoring the lost mineral by using remineralization solutions containing calcium and phosphate ions often fails clinically because of the low solubility of calcium phosphates, particularly in the presence of fluoride ions and their inability to incorporate into plaque and localize at the tooth surface [1]. Incorporation of fluoride into dental materials is viewed by many as the scientific cornerstone for caries prevention [2-14]. It is generally recognized that fluoride regenerates damaged tooth enamel via incorporation in tooth mineral as fluoroapatite or fluoride-enriched hydroxyapatite, therefore decreasing the solubility of tooth enamel. Fluoride has been less effective as dentin remineralizing vehicle [2, 11, 13, 14]. In addition to the various fluoride treatments, remineralization of enamel has been successfully achieved by two

distinct new technologies: a) casein-phosphopeptide stabilized amorphous calcium phosphate (ACP) in the form of mouthrinses and sugar-free chewing gums [15-17] and b) ACP based polymeric composites [18-20]. Dental applications of ACP also include varnishes, dentifrices and desensitizing agents [21-24]. The development of the ACP based remineralizing composites is discussed in this Chapter with emphasis on the structure-composition-property relationships and comprehensive physicochemical evaluation of composites formulated for various dental applications.

1.1. Amorphous Calcium Phosphate (ACP): Chemistry and Dental Importance

Calcium phosphates (CaPs) are of significant interest to dentistry due to their involvement in both normal dentition as well as pathological mineralization (dental calculus) and demineralization (dental caries). ACP is unique among the CaPs as the only non-crystalline compound in the group (Table 1). The crystalline CaPs differ significantly in their calcium/phosphate ratio, crystal morphology and solubility. Studies of the synthetic CaP systems indicate that different CaPs form and undergo transformation under specific solution composition, temperature and pH conditions [25]. ACP forms instantaneously during the spontaneous precipitation from supersaturated basic calcium and phosphate solutions. ACP is generally viewed [26, 27] as a precursor in the formation of hydroxyapatite (HAP), a thermodynamically stable form of CaP in neutral and basic environments. The rate of ACP conversion to HAP depends primarily on the chemistry of the microenvironment. The process can be affected by the presence of inorganic anions, cations, or organic molecules which can adsorb on the ACP surface, incorporate into the ACP structure and/or co-precipitate with ACP.

Table 1. Calcium phosphate (CaP) variants

CaP	Compositional formula	Acronym*
Amorphous calcium phosphate	$Ca_3(PO_4)_2 \cdot 3H_2O$**	ACP
Monocalacium phosphate anhydrous	$Ca(H_2PO_4)_2$	MCPA
Dicalcium phosphate anhydrous	$CaHPO_4$	DCPA
Dicalcium phosphate dihydrate	$CaHPO_4.2H_2O$	DCPD
Tricalcium phosphate (α- or β- form)	α- or β-$Ca_3(PO_4)_2$	α-TCP; β-TCP
Tetracalcium phosphate	$Ca_4O(PO_4)_2$	TTCP
Octacalcium phosphate pentahydrate	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	OCP
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	HAP
Fluorapatite	$Ca_{10}(PO_4)_6F_2$	FAP

* Indicated acronyms will be used throughout this Chapter. The list of all acronyms is provided in Appendix 1.

** Approximate formula.

CaP-based materials intended for hard tissue regeneration are generally appealing due to their biocompatibility. ACP-based materials, by providing an extended supply of calcium and phosphate ions needed to reform damaged mineral structures, successfully counteract recurrent decay, known to develop near the surfaces of teeth in contact with conventional fillings. It is well documented that almost 50 % of all dental fillings require replacement because of recurrent caries (a destructive mineral loss in tooth enamel, dentine and/or cementum). Depending on the extent of tooth destruction, various treatments are used to restore teeth to proper form, function, and aesthetics, but there is no known method to regenerate large amounts of tooth structure. Anti-cariogenic/remineralizing ACP composites are seen as promising preventive and, possibly, restorative dental materials. The potential dental benefits of bioactive ACP-based composites are listed in Table 2. Functional differences between the biostable and bioactive dental materials are presented in Table 3.

Table 2. Potential dental benefits of bioactive, remineralizing ACP composites.

Dental field (specialty)	Foreseen benefit
Preventive dentistry	Counteracting recurrent decay, known to develop at the restoration/tooth intephase Particularly useful for patients that are especially susceptible to caries as a result of radiation therapy and/or diseases/medications that cause dry mouth Ameliorate the development and promote healing of root caries Desensitizing agent for patients with tooth sensitivity
Orthodontics	Adhesive cement capable of minimizing demineralization that frequently occurs under orthodontic brackets
Endodontics	Biocompatible, easy-to-manipulate root canal sealers and/or filling materials

Table 3. Functional differences between biostable and bioactive dental materials.

Type of material	Constituent(s): function(s)
Biostable	Methacrylate resin + initiator system: provides polymeric matrix Silanized glass or ceramic filler: reinforces matrix phase
Bioactive	*Glass ionomer/resin-modified ionomer* Polyalkenoate/resin-modified polyalkenoate: forms polyelectrolyte resin matrix Ion-leachable glass filler: releases fluoride ions *ACP composite* Methacrylate monomer + initiator system: provides resin-derived matrix ACP filler: releases calcium and phosphate ions to form apatitic mineral

1.2. Dental Composites: Structure/Property Relationships

As shown in Table 3, dental composites are made up of a polymer matrix (usually methacrylate based) and filler particles (commonly glass, quartz or ceramic oxides; in our case ACP). A composite material is a result of inter-atomic or molecular interactions between these two components. Coupling agents such as silanes are commonly utilized to improve the bonding at the glass filler/polymer matrix interface. It is important to review the chemistry of composites for an appreciation of the potential for interactions of monomer components of these materials and the oral environment. It is also important to consider the biodegradation of composite/adhesive chemistries at the interface with tooth structures. The susceptibility to degradation (inherent in the choice of chemistries selected for the formulation of dental composites) is promoted by salivary enzymes and related co-factors, generating defined chemical products [28]. The latter products may modulate the biological activity of cells and oral bacteria which interface with restorative material.

Typically dental resins contain a relatively viscous base monomer and one or more diluent comonomers. The base monomer in the resin serves to minimize the polymerization shrinkage by virtue of its relatively large molecular volume and enhance the modulus of the cured polymer, while diluent monomers provide good handling properties and improves copolymer conversion due to their greater flexibility and smaller molecular volume [29]. The most commonly utilized copolymers are based on the base monomer 2,2-bis(p-2'-hydroxy-3'-methcryloxypropoxy)phenyl-propane (Bis-GMA) and the diluent monomer triethylene glycol dimethacrylate (TEGDMA). The hydroxyl groups of Bis-GMA and the ethylene oxide segments of TEGDMA contribute to the relatively high water sorption (WS) of Bis-GMA/TEGDMA copolymers [30]. High concentrations of the more rigid structure of Bis-GMA typically result in monomer systems with relatively low degrees of cure or vinyl conversion (DC). Polymerization shrinkage (PS), relatively low cure efficiency at ambient temperatures and plasticization of Bis-GMA/TEGDMA copolymers by oral fluids affect the service life of these composites. Alternative base monomers and/or diluent monomers have been explored to overcome some of the known shortcomings of the Bis-GMA/TEGDMA copolymers. Dental polymers based on ethoxylated bisphenol A dimethacrylate (EBPADMA), a relatively hydrophobic analog of Bis-GMA with a more flexible structure and lower viscosity, reportedly show higher DC and lower PS than Bis-GMA/TEGDMA resins [31]. For the list of monomers and components of the initiator systems with their chemical names and acronyms that are used throughout this Chapter consult Table 4.

The mechanical properties of polymeric composites, as expected, depend upon the condition of the interface between surfaces of the inorganic filler particles and the polymerized organic resin in which the filler particles are embedded [32]. In order to better resist the destructive environment that exists inside the human oral cavity it is essential to achieve a fairly uniform distribution of the filler particles within the polymer matrix, i.e. minimize the uneven formation of filler-rich and filler-poor areas (voids, non-bonding spaces). The homogeneity of the filler/matrix interface and the entire composite may directly control the sorption of water and the subsequent release of potentially irritative organic moieties.

Table 4. Methacrylate monomers and the components of the polymerization-initiating systems

Component	Chemical name	Acronym
Base monomers	2,2-Bis(p-2'-hydroxy-3'-methcryloxypropoxy)phenyl-propane Ethoxylated bisphenol A dimethacrylate Urethane dimethacrylate	Bis-GMA EBPADMA UDMA
Diluent monomers	2-hydroxyethyl methacrylate Triethylene glycol dimethacrylate	HEMA TEGDMA
Adhesive monomer	Methacryloyloxyethyl phthalate Pyromellitic glycerol tetramethacrylate	MEP PMGTMA
Polymerization-initiating systems	Benzoyl peroxide Camphorquinone 2,2'-Dihydroxyethyl-p-toluidine Ethyl-4-N,N-dimethylamino benzoate Bis(2,6-dimethoxybenzoyl)-2,4,4-trimethylpentyl phosphine oxide & 1-hydroxycyclohexyl phenyl ketone	BPO CQ DHEPT EDMAB IRGACURE 1850

Usually, the improved mechanical performance of composite is achieved with high vinyl conversion and cross-linking. However, the excessive cross-linking that enhances the DC can also lead to the clinically unfavorable condition, i.e., the enhanced PS which leads to a build-up of internal stresses, and consequently to adhesive or cohesive failures. PS can also cause micro-leakage (passage of fluids, bacteria, ions, molecules and air between a restorative material and a prepared tooth surface). This micro-environment can yield to secondary caries that can further affect the bio-stability of the material [33].

When embedded in polymerized methacrylate matrices [34, 35] and exposed to an aqueous environment, ACP releases sufficient levels of remineralizing calcium and phosphate ions in a sustained manner to promote redeposition of thermodynamically stable, apatitic tooth mineral [19, 36]. A problem with dental composites of all types is their inability to resist cracking under masticatory stress due to their low strength and toughness. In the case of ACP composites the uncontrolled aggregation of ACP particles was identified as one of the main reasons for a poor interfacial interaction with dental resins that leads to the mechanical instability for these materials [37] and their inferiority when compared to glass-reinforced composites. To overcome this shortcoming we have focused on developing strategies for improving the ACP filler/polymer matrix interfacial properties (and, in turn, composite properties) by better controlling the particle size distribution and surface properties of ACP fillers and/or by fine-tuning of the resin [38-41]. The working hypothesis was that the physicochemical properties of ACP composites can be tailored through the choice of resin system and ACP's dispersion throughout the composite could be controlled by either its surface modification or by milling (in both cases the goal was to reduce the uncontrolled ACP's agglomeration). Remineralizing composites formulated by embedding better dispersed ACP filler into resin systems with improved DC are expected to have minimal biocompatibility concerns. In addition to their dental value, our studies also contribute to the fundamental understanding of the relationship(s) between the chemical structure, degree of

cure and relative cross-link density of polymeric matrices and the thermodynamic stability and mechanical behavior of their ACP composites.

1.3. ACP Composites: Cytotoxicity Considerations

The main advantages of CaP-based biomaterials designed for dental and/or orthopedic bone tissue regeneration are their osteoconductivity and biocompatibility [42-44]. Both vary with the type of CaP utilized [45-49]. Generally, CaP's biocompatibility arises from their chemical composition which resembles that of the inorganic phase of natural bone tissues. CaPs with solubility above that of hydroxyapatite (HAP) are reactive and would be expected to contribute to bone formation by osteoblasts since both calcium and phosphate ions can be used in metabolism [44, 50]. Reportedly, the biocompatibility of various CaP cements [51-55] is attributed to either the biocompatibility of their individual constituents (α- and β-TCP, DCPD, TTCP and HAP) or the biocompatibility of the cement setting product(s) (predominantly Ca-deficient HAP, traces of DCPD). Despite considerable research efforts, the mechanism by which the more soluble CaPs promote osteogenesis still remains unclear [53, 56-58]. We have found that copolymers derived from highly converted resins (high DC) also yield polymeric ACP composites with low leachability of unreacted monomeric species (which is taken as an indirect measure of high biocompatibility) and favorable calcium and phosphate ion release profiles [37, 41]. In our evaluations thus far we have used the DC as an indirect predictor of the material's biocompatibility.

The majority of ACP used in our experimental composites was synthesized in the presence of zirconyl chloride for the purpose of improving ACP's stability upon exposure to aqueous milieu [59]. Such ACP usually contains a mass fraction of (8.6 ± 1.4) % zirconia [19]. In contrast, the fast-setting CaP cements described in the literature are formulated from the solutions containing no ions other than calcium, phosphate, sodium and/or potassium. The significance of calcium ions in bone mineralization is well established but the ability of extracellular calcium to regulate specific cell responses has been demonstrated only recently [60, 61]. The ability of osteoblasts to transport phosphate was also recognized as a prerequisite for bone mineralization [62]. Cellular receptors for both calcium and phosphate have been identified [60, 63]. There is also evidence that silica plays an important role in bone metabolism [61, 62] but a cellular receptor for silica has not been identified. At this point, no evidence on the potential role of zirconia in hard tissue mineralization and its interaction(s) is available. It is, however, possible that the co-precipitation of zirconia salts into the ACP solid could have some effect on mitochondrial dehydrogenase activity of cells cultured in the extract of zirconia-ACP-filled resin matrices. A series of cell viability experiments may be necessary to test this possibility.

Polymerization of dental resin composites is usually less complete than that of the unfilled resin, and almost every component can be detected in the extracts of polymerized materials [64, 65]. Some of the released, unpolymerized resin monomers may elicit various biological effects such as genetic mutations *in vitro*. Among commonly used methacrylate monomers TEGDMA has been reported as directly mutagenic in a mammalian cell gene mutation assay while no mutagenic effects were detected with UDMA and HEMA [66]. No information was available for EBPADMA, a base monomer utilized in our experimental

copolymers and composites. On the other hand, cytotoxicity of the resin components of composites and adhesives (expressed as a concentration that suppresses the mitochondrial activity by 50 %, i.e., TC_{50} concentration) was ranked as follows UDMA > TEGDMA > HEMA after 72 h exposure to Balb/c 3T3 mouse fibroblasts [67].

The chemical structure/property relations of the constituent monomers, compositional differences involving polymers and initiator systems, and the attainable DC, especially as it relates to the leachable monomers, are important contributing factors that control the cellular response. The total residual vinyl unsaturation upon polymerization that is measured by infrared spectroscopy consists of the pendant vinyl groups in the matrix phase plus residual monomers and other unsaturated species that arise from the polymerization process. Cytotoxicity is more likely to depend on leachable residual monomers and other leachable organic species in the composite. Therefore, to better understand the correlation between the cytotoxicity and the extent of vinyl conversion it may be necessary to assess leachable organic moieties as well as the total vinyl unsaturation in the composite.

Table 5. Methods/techniques (listed in alphabetical order) utilized to evaluate ACP fillers, monomers, copolymers and composites

Method	Property
Colorimetry	Cell viability of the extracts from copolymers and/or ACP composites (Wst-1 assay)
Dilatometry	Volumetric shrinkage of composites upon polymerization (PS)
Fourier-transform infrared (FTIR) spectroscopy	Structure/composition of the monomers, ACP fillers and composites Degree of vinyl conversion (DC) of copolymers and composites
Gravimetry	Water sorption (WS) of copolymers and composites
Mechanical milling	Reducing the particle size of ACP fillers by wet milling
Mechanical strength tests	Biaxial flexure strength (BFS) of copolymers and composites Shear bond strength (SBS) of composites
Microradiography	Quantitative assessment of the changes in tooth mineral density – measure of the remineralization efficacy of ACP composites
Particle size analysis (PSA)	Particle size distribution of ACP fillers
Phase contrast microscopy	Morphology of cells cultured in copolymer and/or ACP composite extracts
Scanning electron microscopy (SEM)	Morphology/topology of ACP fillers
Thermogravimetry (TGA)	Water content and thermal stability of the ACP fillers
Tensometry	Kinetics of the shrinkage stress (PSS) development in composites
Ultraviolet/visible (UV/VIS) spectrophotometry	Compositional analysis of ACP fillers Kinetics of calcium and phosphate ion release from composites
X-ray diffraction (XRD)	Long-range crystalline order of the fillers

2. EXPERIMENTAL DESIGN AND METHODS

The methodologies and techniques utilized to validate ACP fillers and evaluate the unfilled resins (copolymers) and their ACP composites are summarized in Table 5. Indicated acronyms are used throughout this Chapter. A typical sequence of the experimental steps is presented in Figure 1.

2.1. ACP Synthesis

ACP fillers were synthesized as detailed in our earlier papers [18-20]. Generally, ACP precipitated instantaneously in a closed system at 23 ^{o}C upon rapidly mixing equal volumes of a 80 mmol/L calcium nitrate solution, a 54 mmol/L sodium hydrogen phosphate solution that contained a molar fraction of 2 % sodium pyrophosphate, a component that inhibits HAP formation. Various additives were introduced during the synthesis (see subsection 3.1.). The reaction pH was maintained between 8.5 and 9.0. The suspension was filtered, the solid phase washed subsequently with ice-cold ammoniated water and acetone, freeze-dried and then lyophilized. Dry solid was used as-synthesized ACP (as-made or am-ACP) or was subjected to milling (milled or m-ACP [68, 69]). Both am- and m-ACP fillers were stored under vacuum to avoid exposure to humidity and possible conversion to HAP prior to its utilization in the composite preparation and the subsequent physicochemical and cytotoxicity evaluation of composites.

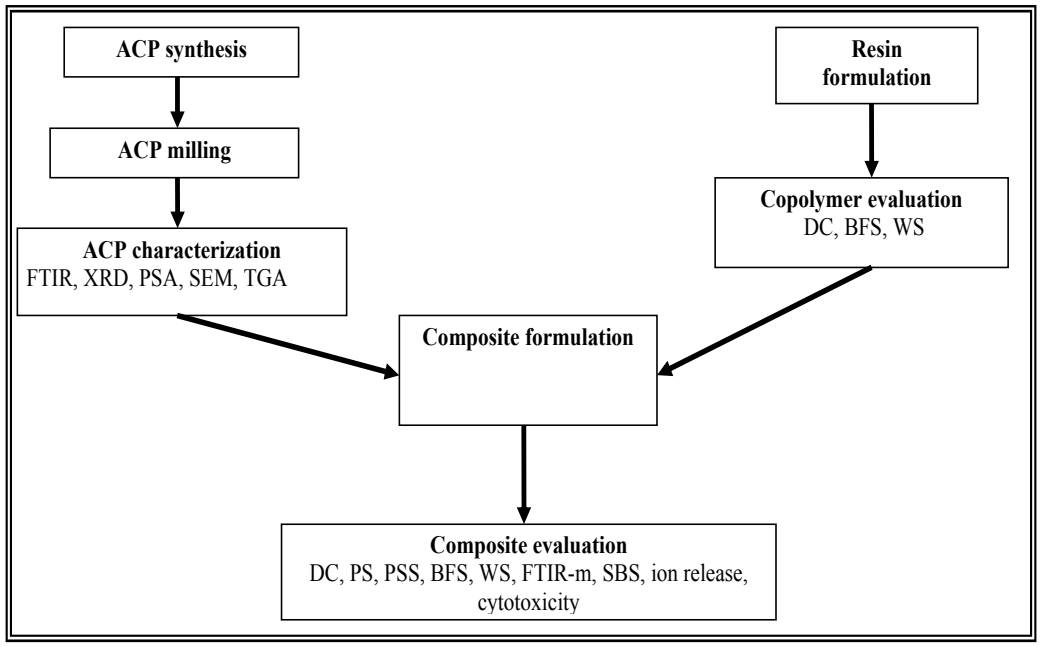

Figure 1. The sequence of the experimental steps involved in ACP composite design.

2.2. Mechanical Milling of ACP Fillers

To reduce the average particle size of ACP filler, mechanical milling was performed as follows: ACP solid and high-density zirconia oxide balls (3 mm in diameter; Glen Mills Inc., Clifton, NJ, USA) at an approx. mass ratio 1:25 were combined with 150 mL isopropanol of analytical grade and sealed in a grinding jar. The jar was clamped into the latching brackets, counterbalanced and milling with rotation reversed every 15 min was performed for 2 h at 42 rad/s (planetary ball mill PM 100, Retch Inc., Newton, PA, USA). Milled ACP was removed from the zirconia oxide balls by sieving and isopropanol was then evaporated in a vacuum oven (Squaroid Labline, Melrose Park, Il, USA) at 70 ^{o}C for 24 h. Approximately 80 % by mass of the initial ACP was retrieved on average after the ball milling and recovery processing.

2.3. Formulation of Resins

The experimental resins were formulated from commercially available dental monomers. In light-activated formulations, a visible light initiator system consisting of CQ plus EDMAB or IRGACURE 1850 was combined with appropriate monomers and their mixture was magnetically stirred (38 rad/s; safe lighting) at room temperature until a uniform mixture was achieved. Chemically-cured formulations were prepared by first combining the monomers and homogenizing the mixture via magnetic stirring. The homogeneous monomer mixture was than split into two equal parts by mass. The components of the chemical-cure system (BPO and DHEPT) were then added separately to each part of the monomer mixture, and each was stirred magnetically until fully blended.

2.4. Fabrication of Composite and Copolymer Specimens

Composite pastes were made by mixing the resin (mass fraction 60 %) and ACP filler (mass fraction 40 %) by hand spatulation. Once homogenized, pastes were kept under a moderate vacuum (2.7 kPa) overnight to eliminate the air entrained during mixing. Light-curable composite pastes were then packed into Teflon molds (the molds for BFS testing were (15.0 ± 0.5) mm in diameter and (1.5 ± 0.2) mm in thickness and the molds for cytotoxicity tests were (5.3 ± 0.1) mm in diameter and (3.1 ± 0.1) mm in thickness), each opening of the mold was covered with a thin Mylar film and a glass slide, and the assembly clamped in place by spring clips. The clamped specimens were photo-polymerized by irradiating sequentially each side of the mold assembly for 120 s with visible light (Triad 2000, Dentsply International, York, PA, USA). The BPO-containing paste and the DHEPT-containing paste were combined in 1:1 mass ratio before packing the mixture into the molds in the same manner as for the light-cured specimens. In the cases where dual cure (chemical + light) initiation was used, chemically polymerized specimens were additionally irradiated and then stored for 24 h in air at 23 ^{o}C before being randomly selected for either dry BFS, wet BFS, ion release or cytotoxicity testing. Water sorption tests were performed with the broken disk specimens collected after dry BFS determinations. Light-, chemically- and dual-cured

copolymer (unfilled resin) specimens were prepared following the same procedures utilized for fabrication of the composite specimens. When the composite samples were made from commercial materials (commercial controls), manufacturer-recommended curing procedures were followed.

2.5. X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) Spectroscopy and FTIR-Microspectroscopy (FTIR-m)

The amorphous state of ACP filler was verified by powder X-ray diffraction (XRD; DMAX 2000 diffractometer, Rigaku/USA Inc., Danvers, MA, USA) and Fourier-transform spectroscopy (FTIR: Nicolet Magna-IR FTIR 550 spectrophotometer, Nicolet Instrumentation Inc., Madison, WI, USA [18, 19, 35, 40]). XRD patterns were recorded from 4^o to 60^o 2θ with CuKα radiation (λ = 0.154 nm) at 40 kV and 40 mA. The samples were step-scanned in intervals of 0.010^o 2θ at a scanning speed of 1.000 deg/min. The FTIR spectra (4000 cm^{-1} to 400 cm^{-1}) were recorded using a KBr pellet technique (0.8 mg to 1.0 mg solid/400 mg KBr). The FTIR spectra of the resins were obtained in transmission mode from thin films of the neat resins between KBr plates.

Mid-FTIR or near-IR (NIR) screening was utilized to determine DC of the unfilled resins (copolymers) and their ACP-filled composites. Mid-FTIR measurements included monitoring the reduction in the 1637 cm-1 absorption band for the vinyl group against that of an unchanged aromatic peak (1538 cm-1; internal standard) [39]. Spectra were acquired before cure and at predetermined time intervals after cure (usually immediately after cure and 24 h post-cure) by collecting 64 scans at 2 wave-number resolution. DC values determined by NIR method [29] were calculated from the % change in the integrated peak area of the 6165 cm-1 methacrylate=CH_2 absorption band between the cured specimen (polymer) and the uncured specimen (monomer). Use of an internal reference was not required for the NIR measurements, provided that the thickness of monomer and polymer sample was measured.

The FTIR-m (a Nicolet Magna-IR 550 FTIR spectrophotometer equipped with a video camera, a liquid nitrogen cooled-mercury cadmium telluride detector, a computerized, motorized mapping stage and the Omnic® Atlus™ software, Spectra-Tech Inc., Shelton, CT, USA) was utilized to analyze the intact copolymer and composite surfaces as well as cross-sections of copolymer and composite specimens in dry and wet states (after exposure to aqueous environment). The usefulness of FTIR-m in producing functional group maps representative of the organic matrix and the inorganic filler distributions was previously discussed [37].

2.6. Particle Size Analysis (PSA)

The particle size distribution of the ACP fillers was measured using a laser light scattering particle size analyzer (CIS-100, Ankersmid, Metropolitan Computing Corporation, E. Hanover, NJ, USA). ACP powders were measured dry and/or dispersed in isopropanol and utrasonicated for 10 min at room temperature prior to the analysis. From the PSA data, the median particle size diameter (d_m) of the sample was obtained. Changes in d_m were taken as a

primary indicator of alterations in the agglomeration of the ACP particulates (the higher the d_m value, the more aggregated the ACP [68-70].

2.7. Scanning Electron Microscopy (SEM)

The morphology/topology of ACP powders, after the specimens were sputter-coated with gold, was determined by SEM using a JEOL 35C instrument, JEOL, Inc., Peabody, MA, USA.

2.8. Thermogravimetric Analysis (TGA)

Thermal decomposition profiles of ACP fillers were determined by TGA (7 Series Thermal Analysis System, Perkin Elmer, Norwalk, CT, USA) by heating powdered ACP samples (initial weight (5 to 10) mg) at the rate of 20 ^{o}/min (temperature range: (30 to 600) ^{o}C) in air. The overall water content and the relative ratio of surface-bound vs. structurally incorporated water was then determined from mass loss curves.

2.9. Biaxial Flexure Strength (BFS)

BFS values of dry (stored for 24 h in the air at 23 ^{o}C) and wet (after immersion in HEPES-buffered, pH=7.4, saline solutions at 23 ^{o}C for a minimum of one month) copolymer and composite disk specimens (prepared as described in 2.4.) were determined using a piston-on-three-ball loading cell and a computer-controlled Universal Testing Machine (Instron 5500R, Instron Corp., Canton, MA, USA) operated by Testworks 4 software. The BFS values were calculated according to the mathematical expressions defined in ASTM F394-78 specification [71]:

$$BFS = A \cdot L/t^2 \quad (1)$$

where $A = -[3/4\pi(X-Y)]$, $X = (1+\nu)\cdot\ln(r_1/r_s)^2 + [(1-\nu)/2]\cdot(r_1/r_s)^2$, $Y = (1+\nu)\cdot[1 + \ln(r_{sc}/r_s)^2]$, ν is the Poisson's ratio (value of 0.24 was used in accordance with the published data on elastic properties of resin-based composites [72]), r_1 is the radius of the piston applying the load at the surface of contact, r_{sc} is the radius of the support circle, r_s is the radius of the disk specimen, L is the applied load at failure, and t is the thickness of the disk specimen.

2.10. Water Sorption (WS)

WS of copolymer and composite specimens was determined as follows. After initially drying the specimens over anhydrous $CaSO_4$ until a constant mass was achieved (± 0.1 mg), specimens were then immersed in saline solutions as described in the BFS measurements. Gravimetric mass changes of dry-tissue padded specimens were recorded at predetermined

time intervals. The degree of WS of any individual specimen at a given time interval (t), expressed as a % mass fraction, was calculated using the equation:

$$WS = [(W_t - W_o)/W_o] \times 100 \quad (2)$$

where W_t represents the sample mass at the time t, and W_o is the initial mass of dry sample.

WS data were used to calculate the diffusion coefficient (D) according to diffusion theory [73], which is conveniently used to assess the water uptake of dental materials [74-76]. D was calculated by applying the simplified Fick's model (valid for the sorption stages when $W_t/W_{eq} \leq 0.6$):

$$W_t/W_{eq} = (2/L)\cdot(Dt/\pi)^{1/2} \quad (3)$$

where W_t is the mass gain at time t, W_{eq} is the mass gain at equilibrium, and L is the thickness of the specimen. D is calculated from the slope of the plot W_t/W_{eq} vs. $t^{1/2}$.

In some systems WS was determined by exposing dry specimens to an air atmosphere of 75 % relative humidity (RH) at 23 ^{o}C (specimens were suspended over saturated aqueous NaCl slurry in closed systems). Gravimetric mass changes were recorded in the same manner as with the saline-immersed specimens and calculated according to Eq. (2). Data collected at 75 % RH provided information on the water uptake that was not affected by the ACP filler dissolution and leaching out of any water-soluble monomeric and/or polymer degradation species (processes that may take place parallel to water sorption of the specimens immersed in saline).

2.11. Polymerization Shrinkage (PS)

The PS of composite resin samples was measured by a computer-controlled mercury dilatometer (Paffenbarger Research Center (PRC); American Dental Association Foundation (ADAF), Gaithersburg, MD, USA). Composite pastes were cured using a standard 60s plus 30s exposure and data acquisition of 60 min + 30 min. PS of a specimen corrected for temperature fluctuations during the measurement was plotted as a function of time. The overall shrinkage (volume fraction, %) was calculated based on the known mass of the sample (50–100 mg) and its density. The latter was determined by means of the Archimedean displacement principle using an attachment to a microbalance (Sartorius YDK01 Density Determination Kit; Sartorius, Goettingen, Germany).

2.12. Polymerization Shrinkage Stress (PSS)

A shrinkage stress measurement device (tensometer; designed and fabricated at the PRC-ADAF, Gaithersburg, MD, USA) was utilized to assess the PSS of the experimental composites. The corresponding software program has also been developed at PRC/ADAF. The tensometer, shown as highly effective tool for investigating the PSS kinetics as well as for probing various aspects that dictate PSS developments, is based on the cantilever beam

deflection theory that a tensile force generated by the bonded polymerizing sample causes a cantilever beam to deflect. The design of the sample assembly facilitates convenient sample insertion, experimental reproducibility and a short preparation time between the consecutive measurements. For a rectangular prismatic cantilever beam of a linearly elastic material with a small deflection which is under a concentrated normal load F, the displacement at the end of the cantilever beam is defined by the following expression [77]:

$$\varepsilon/F = 2a^2(3L-a)/Ebd^3 \quad (4)$$

In Eq. (4), ε is the displacement at the beam end (μm); E is the Young's modulus of the cantilever beam (MPa); F is the load (N) needed to generate the displacement ε; L is the total beam length (cm); a is the distance from the load applied position to the end of the beam (cm); b is the width of the beam (cm) and d is the height of the beam (cm). The deflection of the cantilever beam was measured with a linear variable differential transformer. The force was calculated from a beam length (12.5 cm) and a calibration constant (3.9 N/μm). PSS was obtained by dividing the measured force by the cross sectional area of the sample (diameter = 6 mm). Detailed description of the technique and its use as an integral part of the novel methodology for the simultaneous evaluation of polymerization PSS and DC is provided in refs. [77, 78].

2.13. Shear Bond Strength (SBS)

Shear bond strength (SBS) to dentin was tested on extracted human molars and pre-molars embedded with cold-cured resin in polycarbonate cups. Exposed dentin surfaces of the tooth samples were ground flat at a 90^{o} angle to the longitudinal axis of the polycarbonate holder. Photo-activated pyromellitic glycerol tetramethacrylate (PMGTMA) in acetone solution was used to establish an adhesive layer between the dentin and composite. A brass ring (4 mm in diameter and 1.5 mm in thickness) was used as a mold for the composite. Teflon tape (0.3 mm thick) with an aperture coinciding with the hole in the ring was placed under the brass ring to prevent the ring from adhering to the dentin. Both the ring and the tape were placed in the center of the dentin surface and held down with a lead weight (450 g). The cavity in the brass ring was filled with the composite, irradiated for 1 min with a commercial visible-light source, and stored (at 37 ^{o}C) immersed in distilled water for various time intervals prior to de-bonding. The assembly was placed against the vertical surface of a nylon block and the ring-enclosed composite was sheared off at a cross-head speed of 0.5 mm/min with a flat chisel pressing against the edge of the brass ring and connected to the load cell of the testing machine. The analysis of fractured specimens was performed by digitally photographing (Leica MZ16 Optical Stereomicroscope, Wetzlar, Germany) tooth and iris, and measuring the surface areas of different failure modes (ImageJ, National Institutes of Health, Bethesda, MD, USA) [79].

2.14. Ion Release from Composites

Mineral ion release from the individual composite disk specimens was examined at 23 ^{0}C, in continuously stirred HEPES-buffered (pH=7.4) saline solutions (100 mL). Kinetic changes in the calcium and phosphate levels were determined by utilizing spectroscopic methods (UV/VIS spectrophotometer Carey Model 219; Varian Analytical Instrumentats, Palo Alto, CA, USA) described in refs. [19, 20, 41, 59]. Ion release data were corrected for variations in the total area of the composite disk specimen exposed to the immersion solution using the simple relation for a given surface area, A: normalized value = measured value X (500/A).

2.15. Microradiography: Quantitative Assessment of the Remineralization Efficacy

To assess the efficacy of ACP polymeric materials in restoring the mineral lost due to acid attack, these materials were tested *in vitro* by quantifying changes in mineral content of the lesions by digital image analysis of their contact microradiographs before and after exposure to the pH-cycling regimens that simulate environmental changes in the oral milieu.

2.15.1. Tooth specimens

Two separate studies were carried out: one using bovine enamel and the other using human enamel as a testing substrate. Bovine incisors free of macroscopic cracks were extracted from (360 to 600) d old cows. Tooth surfaces were mechanically scraped and mildly cleaned with pumice, then stored in in an antiseptic solution containing a mass fraction of 0.1 % thymol until use. The human tooth project received an Institutional Review Board exemption, since the teeth were collected from local dentists and have no associated patient identification. Teeth were initially soaked for 4 h in a 0.005 % promodyne disinfectant solution, after which they were mechanically scraped to remove any soft tissue and stored in refrigerated distilled water until use.

2.15.2. Preparation of tooth sections

To create subsurface enamel lesions microradiographically similar to those seen in early caries, wax-coated bovine tooth specimens with only the labial surfaces exposed, were immersed in fluoride-free, lactic acid-containing demineralizing solution (DS; pH = 4.0) [80] for 3 d at 37 ^{o}C (5 mL DS/tooth). The DS solution used to create subsurface lesion in human teeth contained 0.001 mmol/L sodium fluoride [81] and each individual tooth was immersed in 40 mL DS solution for 3 d at 37 °C with no stirring. After demineralization, sagittal sections were cut from each tooth with a circular diamond saw blade (Isomet, Buehler Ltd., Lake Bluff, Il, USA) and ground by hand on wet sandpaper to a thickness ranging from 120 μm to 180 μm. In order to aid in the alignment of ‘before’ and ‘after’ microradiographic images, copper grids were adhered to the sound enamel portion of each tooth slice.

2.15.3. Microradiography

Contact microradiographs of bovine and human tooth sections, respectively, were produced on Kodak SO434 (Eastman Kodak Co., Rochester, NY, USA) or the holographic film (Integraf LLC, Kirkland, WA, USA) exposed for 13 min or 30 min to CuKα radiation (40 kV, 3 mA; Faxitron Model #43855A, Hewlett Packard, McMinnville, OR, USA) and developed according to manufacturer's recommendations. An Al step wedge exposed along with the tooth sections was used to normalize film exposure/development variations. Enamel mineral density was established relative to sound enamel for each sample [81, 82].

2.15.4. Application of composites to tooth section specimens

After the initial contact microradiographs were taken, the sections were randomly grouped and then sandwiched between parafilm and glass cover slips in the following order: cover slip, parafilm, tooth slice, parafilm, cover slip (keeping the demineralized edge of the slice even with one side of the sandwich). This assembly was wrapped and embedded in strips of parafilm to seal around the edges, leaving only the demineralized edge exposed. It was then sandwiched between two glass slides, with the exposed edge of the tooth section positioned approximately 1 mm below the slide edges. The demineralized surface was coated with a (1.0 ± 0.1) mm thick layer of the experimental composites, commercial control or left untreated. Composites were then irradiated with visible light for 60 s (Triad 2000, Dentsply International, York, PA, USA). After photo-curing, the individual assemblies of composite specimens and uncoated control sections that required no curing were subjected to a cycling demineralization/remineralization treatment.

2.15.5. Demineralization/remineralization cycling regimen

The demineralizing solution (DS; [83]) used to mimic demineralizing oral fluid conditions had the following composition: 3.0mmol/L $CaCl_2$, 1.8 mmol/L K_2HPO_4, 0.1 mol/L lactic acid, mass fraction 1 % carboxymethylcellulose, pH = 4.0. The remineralizing solution (RS; [84]) used to simulate remineralizing oral fluid conditions contained 1.2 mmol/L $CaCl_2$, 0.72 mmol/L K_2HPO_4, 2.6 µmol/L F, 50 mmol/L HEPES buffer, pH = 7.0. The assemblies were alternately immersed at 37 ^oC, under continuous magnetic stirring (38 rad/s), in DS (0.5 h) and RS (11.5 h) for 14 d (bovine teeth series; 15 mL DS or RS/specimen), or in DS (1 h) and RS (23 h) for 30 d (human teeth series; 20 mL DS or RS/specimen) excluding weekends, when specimens were stored in distilled water. At every solution exchange, the assemblies were rinsed with distilled water.

2.15.6. Mineral content of the lesions

The mineral profiles of each specimen before and after the demineralization/ remineralization regimen were determined by quantitative analysis of contact micro radiographs taken before and after treatment using the commercial digital-image-analysis system Bioquant IV (R&M Biometrics Inc., Nashville, TN, USA) interfaced with an optical microscope (Leitz, Ortholux, Germany) in the bovine teeth series and Scion Image – release Alpha 4.0.3.2 (National Institute of Health, Bethesda, MD, USA) interfaced with an optical microscope (Olympus BX50F; Olympus Optical Co., Ltd., Japan) and digital camera (RGB/YC/NTSC; Microimage Video Systems, Boyerstown, PA, USA) in the human teeth series. Principles of operation, data collection and the corresponding calculations are

described in detail in refs. [81-84]. Changes in lesion depth and mineral loss (ΔZ) were compared for each imaged area before and after treatment.The difference in summed ΔZ values across the depth of each lesion before and after the pH-cycling regimen, i.e., the relative change in mineral content, Δ(ΔZ) in %, was calculated according to the following equation:

$$\Delta(\Delta Z) = \{(\Delta Z_{before} - \Delta Z_{after})/\Delta Z_{before}\} \cdot 100 \quad (5)$$

The mean Δ(ΔZ) values obtained for all image areas of each group of specimens were used to indicate remineralization (positive (+) Δ(ΔZ) values) or further demineralization of the lesions (negative (-) Δ(ΔZ) values) as a result of pH-cycling treatment.

2.16. In Vitro Cytotoxicity Assessments

To assess the cellular response to experimental copolymers and their corresponding ACP composites, specimens were extracted in media overnight and then the osteoblast-like cells were cultured in these extracts [85]. Cytotoxicity was evaluated by phase contrast microscopy and an enzymatic assay for mitochondrial dehydrogenase activity (Wst-1).

2.16.1. Cell culture maintenance

Osteoblast-like MC3T3-E1 cells (Riken Cell Bank, Hirosaka, Japan) were maintained in α-modification of Eagle's minimum essential medium (Biowhittaker, Walkerville, MD, USA) with a volume fraction of 10 % fetal bovine serum (Gibco-BRL-Life Technologies, Rockville, MD, USA) and 60 mg/L kanamycin sulfate (Sigma, St Louis, MO, USA) in a fully humidified atmosphere with a volume fraction of 5 % CO_2 at 37 ^{o}C. The medium was changed twice a week. Cultures were passaged with EDTA-containing (1 mmol/L) trypsin solution (mass fraction of 0.25 %; Gibco, Rockville, MD, USA) once a week.

2.16.2. Extraction experiments and cell morphology examination

All disk specimens were sterilized with 70 % ethanol prior to extraction experiments. Each disk was then washed with 2 mL of media for 1 h and then fresh media was placed on each disk for an overnight extraction in the cell incubator. Positive control (containing media with Triton X-100 detergent (Res. Prod. International, Elk Grove Village, Il, USA; 0.1% mass fraction)) and negative control (containing only media) were evaluated in addition to ACP composite and copolymer specimens. In parallel, a flask of 80 % confluent MC3T3-E1 cells were passaged, cells seeded into well plates with 10,000 cells per well in 2 mL of media, and then placed in the incubator overnight. On the second day of the experiment, the medium from each "cell well" was removed and replaced with the 2 mL of extraction medium from one of the disk specimens (or with the positive or negative control media). The cells were incubated in the extracts for 3 d, photographed (digital photography using an inverted phase contrast microscope, Nikon TE300, Melville, NY, USA) and then prepared for the cytotoxicity assays.

2.16.3. Wst-1 viability assay

To measure cellular dehydrogenase activity, the Wst-1 colorimetric assays [86] were performed according to the following procedure: Extract-cultured cells and the controls without cells were combined with a Wst-1 (2-(4-iodophenyl)-3-(4-nitophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt; Dojindo, Gaithersburg, MD, USA) solution in HEPES buffer, individually added to wells and incubated for 2 h at 37 ^{o}C. Aliquots from each well were transferred to a well-plate and absorbance was read at 450 n with a plate-reader (Wallac 1420 Victor^{2}, Perkin Elmer Life Sciences, Gaithersburg, MD, USA).

2.17. Statistical Methodology

The number of test specimen for each evaluation step was chosen so that there is a reasonable chance (power) to detect the minimum desired difference between the groups [87]. The variance estimates to be used in the calculation was obtained from previous work. Where no historical information is available, pilot experiments were performed to obtain the variability information. The use of statistically designed experiments ensured that the relevant comparisons of treatments and controls was made unambiguously even when multiple factors are studied simultaneously.

Graphical data analysis, analysis of variance (ANOVA), and other related tests [88, 89] were performed to evaluate the experimental data as a function of composite makeup, storage times or any other relevant factor involved in the experimental design. For the cases where the overall statistically significant effects are found with ANOVA, further tests were performed to determine the significant differences between the specific groups using a multiple comparison procedure. All tests were 2-sided at $\alpha = 0.05$. Statistical analyses of the data were done by means of Microsoft Office Excel 2007, NISTDataplot (NIST, Gaithersburg, MD, USA), SYSTAT9 or SigmaStat version 2.03 (SPSS Inc., Chicago, IL).

One standard deviation (SD) is identified in this paper for comparative purposes as the estimated uncertainty of the measurements.

3. RESULTS AND DISCUSSION

3.1. Surface Modification of ACP Fillers

Random clustering of highly aggregated Zr-ACP particles in polymerized methacrylate matrices leads to inferior mechanical behavior and enhanced water sorption of ACP composites upon exposure to aqueous milieus [37]. To test if certain additives introduced during ACP synthesis could reduce spontaneous ACP agglomeration and, in turn, improve the hydrolytic stability of the fillers, the following additives were tested: (a) cations - silver, iron (II and III), zinc, aluminum, silica and zirconia; (b) surfactants - non-ionic [alkyl aryl polyether alcohol (TRITON-100), poly(oxyethylene) sorbitan monolaureate (TWEEN-80), a fluoro-surfactant (ZONYL FSN)] and an anionic surfactant (ZONYL FSP); and (c) polymers – [poly(ethylene oxide; PEO] and [poly(acrylic acid); PAA]. Relevant tasks were to validate the amorphous character of the solids precipitated in the presence of additives, evaluate their

stability upon aqueous exposure and assess the effect of additives on DC and mechanical strength of composites formulated with photo-activated methacrylate resins. The results of particle size and thermogravimetric analysis of the fillers synthesized in the presence of additives and the DC and BFS attained in composites based on such ACPs are summarized in Table 6 [19, 20, 90, 91].

The PSA data revealed the following order of decreasing d_m in cation-ACP series: (Si-ACP, Zr-ACP) > (Ag-, Fe(II)-, Al-, Fe(III)-ACP) > Zn-ACP. However, both Fe(II)- and Fe(III)-ACP showed the signs of an early conversion to apatite (FTIR and XRD data) and the unwanted color change due to the co-precipitation of Fe-phosphates. Ag-ACP also showed color instability probably due to the formation of light-sensitive Ag-phosphate. The mechanical strength of cation composites decreased in the following order: (Zr-, Zn-ACP) > Si-ACP > Al-ACP > (Ag-, Fe(II)-, Fe(III)-ACP; BFS = 0, i.e., these composites disintegrated upon immersion). In addition to the highest BFS values, Zr-ACP also showed the highest DC of all cation-modified ACPs. Introduction of surfactants and/or polymers with the exception of the anionic Zonyl surfactant did not reduce the ACPs particle size and yielded weaker composites than those formulated with Zr-ACP. Their DCs were not measured. Interestingly, water content (TGA results) of all modified ACPs (on average (15.8 ± 1.7) mass %) appeared unaffected by the type of additive used during the synthesis. Due to the superior DC values and the highest BFS attained in Zr-ACP composites, Zr-ACP filler was chosen as "gold standard" filler and was used in all subsequent studies described in this Chapter. This does not exclude the possibility that other types of surfactants and/or polymers as well as the alterations in surface modification protocol would yield ACP fillers that may better disperse within the polymer matrix and have improved mechanical stability.

Table 6. Effect of additives on the particle size and water content of ACP fillers and degree of vinyl conversion (24h post-cure) and the mechanical strength (BFS of specimens immersed for one month in saline) of their respective composites. Indicated are mean values with one standard deviation in parenthesis.
Resin matrix: [a]EBPADMA/PMGTMA, [b]Bis-GMA/TEGDMA and [c]Bis-GMA/TEGDMA/HEMA/ZrDMA. nd – not determined

Additive	Median diameter d_m (μm)	Water (mass %)	DC (%)	BFS (MPa)
Cations				
Silver[a]	3.5 (1.9)	14.0 (2.2)	63.3 (1.9)	disintegrated
Iron (II)[a]	3.8 (1.8)	15.4 (1.2)	65.7 (1.8)	disintegrated
Zinc[a]	1.4 (0.5)	16.6 (2.5)	63.7 (2.6)	48.4 (5.3)
Aluminum[a]	2.2 (1.3)	14.1 (2.3)	56.0 (3.3)	19.8 (4.7)
Iron (III)[a]	2.1 (0.6)	16.8 (2.8)	56.7 (2.6)	disintegrated
Silica [a,b,c]	5.8 (1.6)	14.1 (1.2)	72.5 (2.5)	40.0 (9.0)
Zirconia[a,b,c]	6.7 (1.9)	16.1 (2.0)	80.1 (3.3)	53.4 (12.0)
Surfactants				
TRITON-100[b]	8.3 (1.4)	16.3 (1.2)	nd	27.7 (3.3)
TWEEN-80[b]	8.9 (2.1)	16.9 (0.9)	nd	32.4 (10.8)
ZONYL FSN[b]	6.5 (1.2)	17.4 (1.1)	nd	28.1 (2.5)
ZONYL FSP[b]	4.1 (0.4)	17.6 (2.1)	nd	31.9 (10.4)
Polymers				
PEO[b]	14.1 (4.7)	14.7 (1.2)	nd	23.4 (4.3)
PAA[b]	9.2 (1.9)	15.8 (1.0)	nd	34.1 (9.9)

3.2. Silanization of Zr-ACP Filler

To test the hypothesis that silanization of the ACP filler might reduce the interfacial penetration of water between the ACP particles and polymer matrix, 3-aminopropyltrimethoxysilane (APTMS) and methacryloxypropyltrimethoxysilane (MPTMS) were applied individually at 2 mass % relative to Zr-ACP. To catalyze the hydrolysis of the methoxy groups, MPTMS was deposited from an aqueous/alcohol (5/95 vol %) solution adjusted to pH 5.5 by addition of acetic acid. For APTMS acidification was not necessary, since its amino group auto-catalyzed the hydrolysis/condensation reaction. Five minutes was allowed for completion of hydrolysis and silanol formation following initial mixing-in of the ACP powder and stirring for 30 min. The pH of the MPTMS/ACP slurry was then adjusted to 10 by the addition of 100 mmol/L KOH solution to facilitate the condensation and formation of siloxanols. After filtration and drying at room temperature, the silanized ACP was heated at 100^{o}C for 30 min to strengthen the coating by secondary formation of polysiloxane network structures. Unbonded silane molecules were washed-out with ethanol and the silane-coated ACPs (their amorphous character was confirmed by FTIR and XRD) were kept dry in a desiccator until used for composite preparation. The BFS of EBPADMA/TEGDMA/ HEMA/MEP (ETHM resin) dry and wet composite specimens utilizing silanized ACPs is presented in Figure 2. The BFS results obtained with the unsilanized Zr-ACP/ETHM composites are shown for comparison. In dry state, MPTMS-ACP composites attained high strength [(73.3 ± 7.5) MPa] compared to APTMS-ACP [(46.4 ± 9.8) MPa] and Zr-ACP control composites [(46.7 ± 7.5) MPa]. Moreover, upon soaking MPTMS-ACP composites were approximately 60 % stronger than Zr-ACP based composites, suggesting that the silanization of the ACP filler can ameliorate the overall plasticization of composites and may be potentially useful in designing remineralizing composites with moderately improved mechanical properties.

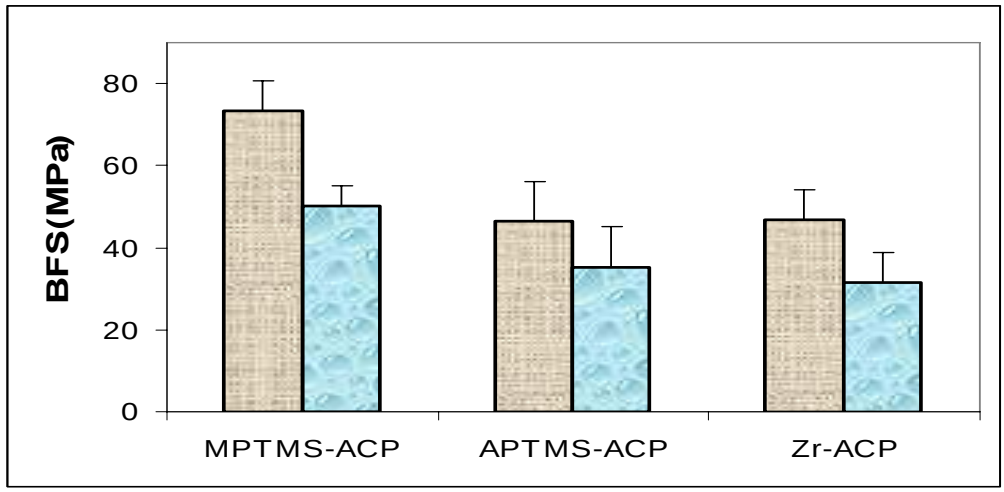

Figure 2. Effect of silanization on the BFS of composites. In each experimental group, left column indicates mean dry BFS values and right column indicates mean wet BFS values. Standard deviation of the means is shown by bars.

Table 7. Particle size analysis of the am- and m-ACP fillers and the mechanical strength (after 1 mo aqueous immersion), water sorption at 75 % relative humidity and the anti-demineralizing/remineralizing capacity of composites. Values represent a group mean + one standard deviation (SD). Number of specimens in each group: n ≥ 7 (d_m, SSA, BFS and WS) and n =3 (E). Resin matrix: [a]ETH; [b]ETHM

Parameter	am-ACP	m-ACP
Particle size range (μm)	0.3 to 80.0	0.2 to 3.0
Median particle diameter, d_m (μm)	6.4 (0.7)	0.9 (0.2)
Specific surface area, SSA (m^2/g)	0.5 (0.1)	3.8 (1.0)
Biaxial flexure strength, BFS (MPa)	45.3 (5.4)[a] 47.0 (11.9)[b]	59.3 (7.6)[a] 73.0 (11.7)[b]
Ion activity product, IAP	99.3 (0.7)[a]	101.2 (1.0)[a]
Thermodynamic stability, ΔG^0 (kJ/mol)	-5.7 (0.2)[a]	-5.1 (0.3)[a]
Water sorption, WS (mass %)	2.2 (0.2)[b]	1.7 (0.1)[b]

3.3. Effect of Mechanical Milling on the Properties of Zr-ACP Filler and Its Composites

In addition to the surface-modification via *ab initio* additive introduction or silanization of the filler, mechanical ball milling (experimental details are provided in section 2.2.) of Zr-ACP prior to its utilization as filler in composites was evaluated as an alternative way to reduce the average size of ACP by breaking up large aggregates into smaller agglomerates that will more intimately interact with the resin and disperse more evenly in the composite. Relevant tasks were to formulate the composites with un-milled (as made or am-ACP) and milled (m-ACP) filler and compare their mechanical properties, water sorption and the kinetics of mineral ion release. Such an assessment is deemed necessary in order to ensure that improvement in mechanical strength of these composites is achieved without compromising their anti-demineralizing/remineralizing ability. Composite specimens were made from a photo-activated EBPADMA/TEGDMA/HEMA (ETH) resin or EBPADMA/ TEGDMA/HEMA/MEP (ETHM) resin admixed with a mass fraction of 40 % of un-milled (as-made or am-ACP) or milled Zr-ACP (m-ACP). The results of the physicochemical evaluation are shown in Table 7.

While having no apparent effect on the structure and/or composition of the fillers (no changes were observed in their FTIR and XRD spectra) milling significantly reduced the average size of Zr-ACP particulates (d_m decreased from (6.4 ± 0.7) μm to (0.9 ± 0.2) μm, i.e. approximately 85 % in going from am-ACP to m-ACP) and the spread of their particle dimensions. Changes in size distribution following the milling were also confirmed by SEM. Better dispersion of m-ACP in the resins resulted in the improved BFS of their composites, especially after aqueous immersion. The m-ACP/ETH composites also maintained a satisfactory ion release. They steadily released calcium and phosphate ions into buffered saline solution (Figure 3). The attained levels of the mineralizing ions were adequate to achieve the super-saturation required for the precipitation of HAP. The thermodynamic stability of the immersion solutions containing the maximum concentrations of calcium and

phosphate ions released from the composite disk specimens was calculated with respect to stoichiometric HAP using the Gibbs free-energy expression [92]:

$$\Delta G^0 = - 2.303(RT/n)\ln(IAP/K_{sp}) \quad (6)$$

where IAP is the ion activity product for HAP, K_{sp} is the corresponding thermodynamic solubility product, R is the ideal gas constant, T is the absolute temperature, and n is the number of ions in the IAP (n=18).

The reduced WS of the milled composites compared to am-ACP composites was linked to a lesser number of voids (defects) existing throughout the body of composites disk specimens (micro-FTIR analysis [37]).

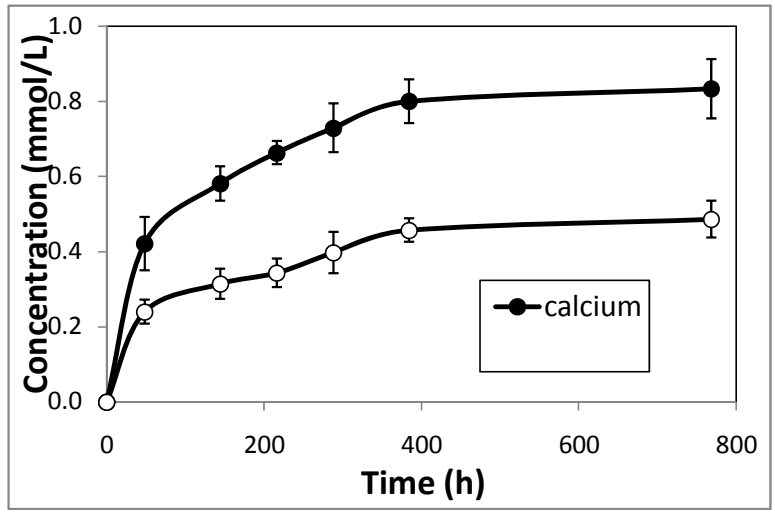

Figure 3. Mineral ion release from m-ACP ETHM composites. Shown are mean values ± standard deviation; number of runs 3/group.

Table 8. Compositions of the experimental EBPADMA/TEGDMA/HEMA/MEP (ETHM) resins (mass %)

Monomer/ resin	ETHM 0.50	ETHM 0.33	ETHM 0.25	ETHM 0.13	*ETHM 0.50**	*ETHM 0.85*	*ETHM 1.35*
EBPADMA	42.00	33.60	28.00	16.80	43.17	54.45	62.85
TEGDMA	42.00	50.40	56.00	67.20	43.24	31.94	23.22
HEMA	10.00	10.00	10.00	10.00	10.08	10.00	10.36
MEP	5.00	5.00	5.00	5.00	2.51	2.56	2.57
IRGACURE 1850	1.00	1.00	1.00	1.00	-	-	-
CQ	-	-	-	-	0.20	0.20	0.20
4EDMAB	-	-	-	-	0.80	0.80	0.80
EBPADMA/ TEGDMA molar ratio	0.50	0.33	0.25	0.13	0.50	0.85	1.35

* EBPADMA/TEGDMA molar ratio of 0.50 with MEP concentration reduced 50 % compared to ETHM0.50 formulation.

3.4. Fine Tuning of the Resins: Effect on Physicochemical Properties

The effect(s) of the fine-tuning of the resin matrix on DC, WS, BFS, PS and the anti-demineralizing/remineralizing ability of composites were assessed in two separate studies. The am-ACP and/or m-ACP fillers and EBPADMA-based resins were formulated into a composites with two series of matrices: one with the decreasing EBPADMA/TEGDMA molar ratio in the range 0.50 to 0.125 and a constant HEMA/MEP molar ratio of 4.28 (ETHM0.50, ETHM0.33, ETHM025 and ETHM0.13; photoinitiator IRGACURE 1850), and the other with the increasing EBPADMA/TEGDMA molar ratios in the range 0.50 to 1.35 and the HEMA/MEP molar ratio of (8.26 ± 0.33) (*ETHM0.50**, *ETHM0.85* and *ETHM1.35*: photoinitiator system CQ and 4EDMAB). The composition of the resins are provided in Table 8. The results of the physicochemical and mechanical testing are summarized in Table 9.

Fine-tuning of EBPADMA-based resin matrix did not significantly affect the DC, WS or the BFS of their ACP composites. The reduction in Ca release observed in formulations with the higher MEP levels only marginally affected the overall supersaturation levels (ΔG^0 values). The attained superstaurations in all formulations were significantly above the theoretical minimum necessary for remineralization ($\Delta G^0 < 0$). However, the PS of composites increased with lowering the EBPADMA/TEGDMA molar ratio in the resin. The factor that controls the PS of ETHM formulations appears to be the relative content of the high molecular mass EBPADMA. Higher DC and BFS values, accompanied with reduced WS, were achieved by replacing the as-synthesized ACP filler with milled ACP in *ETHM0.50** - *ETHM0.85* – *ETHM1.35* series. The improved strength and lower WS of m-ACP based composites is related to the improved dispersion of milled ACP filler throughout the composites compared to the am-ACP based composites. Ultimately, m-ACP composites have the lesser number of water sorption-prone voids/defects which have been identified [37] as the main reason for the poor mechanical stability of the am- ACP composites.

Table 9. DC, PS, WS, BFS (wet specimens) and the supersaturation (ΔG^0) corresponding to the maximum relase of calcium and phosphate ions from the am- and m-ACP/ETHM composites. Indicated are mean values with standard deviation in the parenthesis. Number of runs: n = 3 (PS), n = 4 (ΔG^0), n = 5 (WS, BFS) and n ≥ 6 (DC)

Monomer/ Resin	ETHM 0.50	ETHM 0.33	ETHM 0.25	ETHM 0.13	*ETHM 0.50*	*ETHM 0.85*	*ETHM 1.35*
DC (%) am-P m-ACP	85.8(5.3) -	84.4 (5.0) -	82.2 (6.5) -	86.6 (5.0) -	76.9 (3.5) 80.3 (1.4)	70.5 (3.8) 74.8(3.9)	69.1 (4.2) 75.1 (1.2)
PS (vol %) am-P	6.1 (0.5)	6.5 (0.7)	7.2 (0.4)	7.8 (0.6)	-	-	-
WS (mass %) am-P m-ACP	3.3 (0.5) -	3.6 (0.5) -	3.2 (0.5) -	3.3 (0.5) -	2.5 (0.2) 2.1 (0.2)	2.3 (0.2) 1.8 (0.2)	2.5 (0.3) 1.6 (0.1)
BFS_{wet} (MPa) am-P m-ACP	43.6(7.8) -	48.5 (6.9) -	45.9 (4.2) -	44.4 (8.2) -	36.3 (4.9) 60.9 (9.4)	35.0 (4.2) 52.9 (8.9)	36.4 (6.7) 55.8 (17.2)
ΔG^0 (kJ/mol) am-P m-ACP	-4.5 (0.2) -	-4.6 (0.1) -	-4.4 (0.3) -	-5.1 (0.3) -	-4.4 (0.2) -3.4 (0.4)	-4.7 (0.2) -4.3 (0.4)	-4.7 (0.2) -4.8 (0. 0)

We have used the DC attained upon polymerization to indirectly assess the potential leachability of unreacted monomeric species from bioactive ACP composites [19, 20, 34-36, 38, 39, 93, 94]. In these studies, Bis-GMA, EBPADMA and/or UDMA were utilized as base monomers. Regardless of the type of base monomer, all copolymers (unfilled resins), and to somewhat lesser extent their ACP composites, achieved high DCs (70 % or higher). Typical DC values reported for the most commonly used Bis-GMA/TEGDMA resins range between as low as 55 % and only seldom reach 75 % conversion [95]. In ternary and quarternary HEMA-containing formulations [19, 20, 92, 94, 96], the high DC values were generally attributed to the high diffusivity of this monomer (the effect increases with the increasing HEMA level in the formulation). We have found no enhancement in DC of UDMA-based resins compared to Bis-GMA ones [19, 20, 36] although an increase has been expected since UDMA is a less viscous and more flexible methacrylate with lower hydroxyl type hydrogen bonding interactions than Bis-GMA. UDMA based formulations, however, showed lesser reduction in DC (up to 5 %) in going from copolymer to ACP composite compared to both Bis-GMA and EBPADMA formulations (reduction up to 14 %).

Since PS is directly proportional to DC, it is of no surprise that the ACP composites formulated with resins that achieved up to 86 % double bond conversion also exhibited high volumetric shrinkage upon polymerization ((6.9 ± 1.3) % on average). Undesirable consequence of high PS would be higher probability of development of intra-composite micro-structural strains and gaps at the composite/tooth interphase, potentially leading to micro-leakage. The average PS values of the experimental ACP composites were close to the upper end of PS values reported for the commercial flowable composites and the lower end of PS values for adhesive resins (6.0 % and 6.7%, respectively [97]). It certainly appears that from the PS standpoint that our experimental resins would require additional compositional adjustments in order to reduce PS. One option would be exploring beyond methacrylates [98] and/or employing ring-opening monomers [99] in resin formulations. Alternatively, utilizing organic additives capable of inducing physical gelation in a variety of organic monomers and polymers by forming self-assembled networks may be a practical way of reducing PS without compromising the biocompatibility of the resins. The second option was experimentally tested by introducing an organogelator, dibenzylidene sorbitol (DBS) into EBPADMA resins and evaluating the unfilled resins and am-ACP composites for DC, PS and PSS [100]. Results of this study showed that via incorporation of DBS a significant reduction in the PS and associated shrinkage stress in composites can be achieved without adversely affecting the degree of double bond conversion.

A concern intrinsic to all resin formulations containing high levels of HEMA is the leachability of residual HEMA and possible adverse effect of this low mass monomer on PS and PSS. Replacing mono-functional HEMA with a high molecular mass oligomeric urethane dimethacrylate co-monomer (poly(ethylene glycol) extended UDMA; PEG-U) in UDMA-based resins or substituting ethyl α-hydroxymethylacrylate (EHMA), an unique isomer of HEMA, for HEMA in Bis-GMA based resins were investigated as possible ways to reduce the above HEMA-related concerns. Inclusion of PEG-U into UDMA based resins improved DC and had no undesirable effect on PS, PSS and BFS of the composites, therefore, resulting in dental materials with higher expected biocompatibility. EHMA showed decreased WS compared to HEMA, EHMA homo-polymers and co-polymers attained DCs and ion release levels equivalent to those of HEMA counterparts. In the light of these results, both PEG-U

and EHMA can be considered as the suitable substitutes for HEMA in dental and, possibly, biomedical applications [101, 102].

Recently we have focused our attention on the processing factors that, in addition to material factors (filler type and content, resin type and composition, polymerization mode), determine the PS, elastic modulus and PSS of composite materials. Although the PSS in photo-polymerized dimethacrylate monomer systems has been studied quite extensively [97, 103-109], a fuller understanding of the kinetics of both PS and PSS is still lacking. Similarly, how cavity configuration (C-factor; 110-112] affects the performance of bonded dental restorative materials also needs to be better understood. To investigate whether larger surface area (lower C-factor) yields lower PSS values by allowing greater plastic deformation to occur during polymerization before the gel point is reached (as proposed by Feilzer [112)], we have assessed by tensometry [77] the effect of variations in C-factor on PSS in a typical ACP remineralizing composite and a typical commercial glass-filled composite (TPH). The heights (h) of unpolymerized composite cylindrical specimens were systematically varied between 0.5 mm and 3.75 mm to give C-factors ranging from 6.0 to 0.8. For a circular quartz rod of diameter 2r and a specimen of height h, C-factor was calculated as the ratio of bonded composite area (the silinated ends of the silica rods) to the unbonded area (the compliant plastic enclosure encasing the composite cylinder) according to the expression:

$$\text{C-factor} = 2\pi r^2/2\pi rh = r/h \quad (7)$$

The composites were irradiated through the lower quartz rod with a visible light (curing unit, Dentsply, York, PA, USA) for 60 s to initiate polymerization, and the PSS was then measured after 60 min. Because all specimens had the same diameter, h becomes the determinant of the composite specimen mass as well as its C-factor. The measured PSS values (PSS_{meas}) for specimens with variable heights were normalized for mass to a control specimen with h = 2.25 mm (C-factor = 1.33) to give calculated PSS values (PSS_{calc}) using the following expression:

$$PSS_{calc} = PSS_{meas} \cdot (h_{control}/h_{variable}) \quad (8)$$

Table 10. The PSS_{meas} values (mean value (in MPa) ± SD of three repetitive measurements) and the corresponding PSS_{calc} for BT/ACP and TPH composites as a function of C-factor

C-factor	Composite height (mm)	BT/ACP composite PSS_{meas} PSS_{calc}	TPH composite PSS_{meas} PSS_{calc}
0.80	3.75	nd	2.78 ± 0.07 1.67
0.86	3.50	5.80 ± 0.55 4.39	2.91 ± 0.05 1.87
1.00	3.00	6.21 ± 0.27 4.66	2.70 ± 0.07 2.03
1.33	2.25	6.55 ± 0.19 6.55	3.16 ± 0.19 3.16
2.50	1.20	6.79 ± 0.34 12.73	3.37 ± 0.08 6.32
3.00	1.00	6.96 ± 0.06 14.73	nd
6.00	0.50	6.83 ± 0.68 26.09	2.82 ± 0.17 12.69

PSS_{meas} and PSS_{calc} data obtained for the Bis-GMA/TEGDMA (BT)/ACP and TPH composite specimens for a range of cavity configuration factors are given in Table 10.

The PSD_{meas} varied between 5.80 MPa and 6.96 MPa in the BT/ACP composite series and between 2.78 MPa and 3.37 MPa in the TPH composites. For any given C-factor, the value of PSD_{meas} in BT/ACP composites more than doubled the value obtained in the corresponding TPH control. Also, data scattering was significantly lower for the TPH compared to BT/ACP composite specimens (SD values ranging from 0.05 MPa to 0.19 MPa vs. 0.06 MPa to 0.68 MPa). Plotting PSD_{meas} values obtained either for BT/ACP or for the TPH composite specimens as a function of composite specimen height showed practically no correlation between the two. However, the corresponding PSD_{calc} values decreased with the increasing specimen thickness for both the experimental and control groups according to the following exponential functions (R^2 is the correlation coefficient):

$$PSD(ACP)_{calc} = 28.3 \cdot e^{-0.58h} \ (R^2 = 0.9542) \quad (9)$$

$$PSD(TPH)_{calc} = 14.5 \cdot e^{-0.61h} \ (R^2 = 0.9680) \quad (10)$$

Similarly, no correlation existed between the PSD_{meas} and C-factor for both types of composites. On the other hand, PSD_{calc} and the C-factor for both the BT/ACP and TPH composites showed linear correlations that can be described by the following equations, respectively:

$$PSD(ACP)_{calc} = 4.28 \cdot \text{C-factor} + 1.05 \ (R^2 = 0.9923) \quad (11)$$

$$PSD(TPH)_{calc} = 2.13 \cdot \text{C-factor} + 0.20 \ (R^2 = 0.9903) \quad (12)$$

The results of this study clearly show that configuration factor needs to be considered in minimizing the polymerization stress development and, in turn, improving the quality of the interface between the composite and tooth structures. Additionally, tensometry has the potential for aiding in optimizing the material and processing factors that can lead to the development of polymeric materials with favorable PSS values.

3.5. Bonding Properties of ACP Composites

Effect of water aging on dentin bonding of the experimental Bis-GMA/TEGDMA /HEMA/ZrDMA ACP remineralizing basing composites formulated with the am- and m-ACP filler and Sr-glass filled composite as a control, was evaluated at 24 h, 14 d and one month of aqueous exposure. Shear bond strengths (SBS) values of both experimental ACP systems showed no adverse effect on the dentin bonding compared with the inert glass control indicating the suitability of ACP fillers for the intended application as basing materials (Figure 4; [113]).

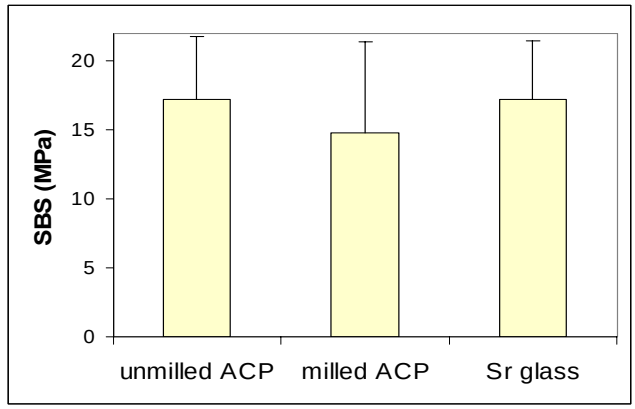

Figure 4. Shear bond strength (after 30 d immersion) of the am- and m-ACP/Bis-GMA/TEGDMA/HEMA/ZrDMA composites compared to Sr-glass filled composite.

In a separate study, SBS testing was used to compare the adhesiveness to tooth surfaces of an experimental ETHM orthodontic resin (*ETHM1.35*; Table 8) utilizing again am- and m-ACP. The SBS tests were performed on extracted human molars and pre-molars embedded with cold-cured resin in polycarbonate cups. The specimens were immersed in an artificial saliva solution [114] at 37 ^{o}C for 24 h, 1 month, 3 months and 6 months prior to debonding. The SBS values of the experimental ACP orthodontic adhesives remained comparable to the SBS value of the commercial light cured adhesive after prolonged aqueous exposure (data not shown).

Based on both adhesion studies it was concluded that the experimental ACP composites are good candidates for future clinical testing as remineralizing adhesives, protective liners and bases, orthodontic cements and/or pit-and-fissure sealants. To improve their clinical appeal further work may be needed on formulating resins that will have enhanced long-term dentin-bonding performance.

3.6. *In Vitro* Remineralization with ACP Composites

Remineralization efficacy of the experimental, photo-cured, basing am-ACP/Bis-GMA/TEGDMA/HEMA/ZrDMA composite and orthodontic am-ACP/EBPADMA/ TEGDMA/HEMA/MEP composite was tested microradiographically using bovine and human tooth substrates, respectively. Results of both studies are summarized in Table 11. Quantitative digital image analysis of the matched areas from the contact microradiographs taken of the sections before and after treatment (experimental details are provided in Sections 2.15.1.- 2.15.6.) indicated superior remineralization activity of both types of ACP composites compared to the various controls. Mineral recovery attained with the basing ACP composites was significantly higher that of the corresponding HAP- and silica- composites or the uncoated (negative control) specimens. Similarly, ACP composite formulated for orthodontic utility recovered, on average, significantly more mineral lost to acid attack than a fluoride-releasing commercial control while the uncoated control specimens lost on average an additional 55 % of the mineral.

Table 11. Mineral recovery (remineralization, positive values; %) or mineral loss (demineralization; negative values, %) observed in bovine or human tooth specimens coated with various composites or left uncoted (negative controls) and exposed to pH-cycling mimicking changes in oral environment. Indicated Δ(ΔZ) values represent mean with standard deviation given in parenthesis

Substrate	Resin matrix	Treatment	Δ(ΔZ) (%)
Bovine teeth	Bis-MA/TEGDMA/HEMA/ZrDMA (BTHZ resin)	ACP composite	37.9 (6.4)
		HAP-composite	- 5.7 (4.1)
		Glass-composite	- 9.4 (3.6)
		Uncoated control	- 7.3 (6.4)
Human teeth	EBPADMA/TEGDMA/HEMA/MEP (ETHM resin)	ACP composite	14.4 (16.7)
		Fluoride-releasing composite	4.4 (14.2)
		Uncoated control	- 55.4 (24.4)

Importantly, in both bovine and human series, remineralization with ACP composites took place throughout the depth of the lesions rather than being confined to the regions near the surface. For these reasons, both types of composites may be useful adjuvants in the control of dental caries, especially when used in conjunction with fluoride-based dentifrices and/or mouthwashes where the remineralization efficacy of fluoride can be augmented by the controlled release of calcium and phosphate ions from the ACP filler phase of composites.

3.7. Cytotoxicity of the Experimental ACP Orthodontic Adhesive

In order to shed light on the interactions between the ACP filler and/or ACP composites and osteoblast-like cells we have performed an *in vitro* study of their cytotoxicity [85]. EBPADMA/UDMA/TEGDMA/HEMA copolymer, m-ACP powder and the corresponding composite were extracted in media overnight and the osteoblast-like cells were then cultured in extracts for 3 d. Phase contrast images of MC3T3-E1 cells cultured in extracts from ACP powder and different resin composites for 3 d (not shown) indicated a normal, spread, polygonal morphology. Only cell remnants were seen in a positive control, detergent-containing samples, indicating that 0.1 mass % detergent in the medium was cytotoxic. Qualitatively, an approximately equivalent amount of cells was found in each experimental system suggesting no adverse cellular response to ACP powder, composite, copolymer or COA. Based on a colorimetric assay of cellular dehydrogenase activity Wst-1 (Figure 5), the extracts from the resins caused a mild drop in the viability of cells compared to the negative control. It is likely that the mild effects of the resins on cell viability observed in our study are a result of the effects of leachable monomers or initiator components or byproducts on cells. To properly correlate the cytotoxicity findings with the DC data, an assessment of leachable organic moieties as well as the total vinyl unsaturation in the composite would be necessary.

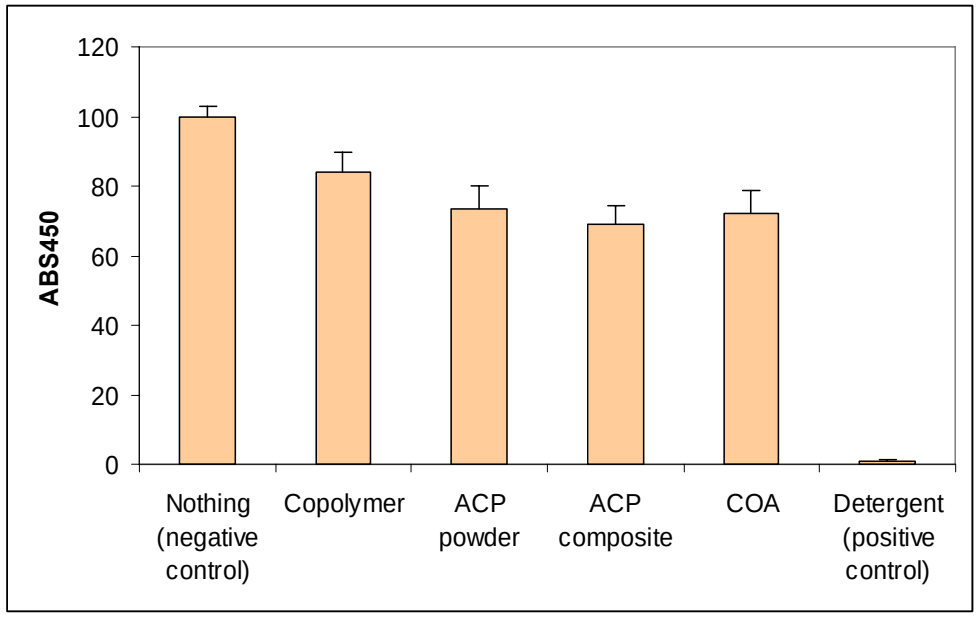

Figure 5. Cell viability for EBPADMA/UDMA/TEGDMA/HEMA copolymer, m-ACP powder and their composite compared to commercial (COA). Positive control: 0.1 mass % detergent.

CONCLUSIONS/FUTURE DIRECTIONS

The comprehensive physicochemical evaluation of ACP composites improved our understanding of the structure/composition/property relationships of ACP fillers and the complex mechanisms governing intra-composite ACP filler/multi-component resin matrix interactions. The intention of our research is to stimulate studies that involve biocompatibility issues related to composites and promote the development of alternate polymeric chemistries and composite formulations that would yield materials based not only on suitable mechanical properties but also on improved biological and chemical performance. Our current efforts are aimed towards formulating ACP composites for endodontic utility. In broad terms, the biological risks of resin-based materials to the dentin-pulp complex originate from the toxicological properties of the materials themselves (direct bio-risks) and those steaming from microbiological leakage (indirect bio-risks). Therefore, the extensive cytotoxicity and biocompatibility studies will be performed before testing the anti-demineralizing/ remineralizing endodontic composites in clinical trial with human subjects. It is expected that the findings of our continuing research will be useful as guideline(s) in future design of ACP/biodegradable polymeric materials for the generalized bone repair applications.

DISCLAIMER

Certain commercial materials and equipment are identified in this article for adequate definition of the experimental procedures. In no instance does such identification imply recommendation or endorsement by the American Dental Association Foundation or the

National Institute of Standards and Technology or that the material and the equipment identified are necessarily the best available for the purpose.

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APPENDIX 1. LIST OF ACRONYMS USED THROUGHOUT THE CHAPTER

ACP	amorphous calcium phosphate
ADAF	American Dental Association Foundation
am-ACP	as made ACP
ANOVA	analysis of variance
APTMS	3-aminopropyltrimethoxysilane
ASTM	American Society for Testing and Materials
BFS	biaxial flexural strength
Bis	GMA–2,2-bis[p-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane
BPO	benzyl peroxide
BT	Bis-GMA/TEGDMA resin
BTHZ	Bis-GMA/TEGDMA/HEMA/ZrDMA resin
C factor	cavity configuration factor
CaP	calcium phosphate
COA	commercial orthodontic adhesive
CQ	camphorquinone
D	diffusion coefficient
DBS	dibenzylidene sorbitol
DC	degree of vinyl conversion
DCPA	dicalcium phosphate anhydrous
DCPD	dicalcium phosphate dehydrate
DHEPT	2,2'-dihydroxyethyl-p-toluidine
d_m	median particle diameter
DS	demineralizing solution
EBPADMA	ethoxylated bisphenol A dimethacrylate
EDMAB	ethyl-4-N,N-dimethylamino benzoate
EDTA	ethylenediamine tetraacetic acid
ETH	EBPADMA/TEGDMA/HEMA resin
ETHM	EBPADMA/TEGDMA/HEMA/MEP resin
FAP	fluorapatite
FTIR	Fourier transform infrared spectroscopy
FTIR-m	FTIR micro-spectroscopy
ΔG^o	Gibbs free energy

HAP	hydroxyapatite
HEMA	2-hydroxyethyl methacrylate
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
IAP	ion activity product
IRGACURE 1850	commercial photo-initiator system
K_{sp}	thermodynamic solubility product
m-ACP	milled ACP
MCPA	monocalcium phosphate anhydrous
MEP	methcryloyloxyethyl phthalate
MPTMS	methacryloxypropyltrimethoxysilane
NIR	near infrared spectroscopy
OCP	octacalcium phosphate pentahydrate
PAA	poly(acrylic acid)
PEG-U	poly(ethylene glycol) extended urethane dimethacrylate
PEO	poly(ethylene oxide)
PMGTMA	pyromellitic glycerol tetramethacrylate
PRC	Paffenbarger Research Center
PS	polymerization shrinkage
PSA	particle size analysis
PSS	polymerization shrinkage stress
R	ideal gas constant
RH	relative humidity
RS	remineralizing solution
SBS	shear bond strength
SEM	scanning electron microscopy
SD	standard deviation
SSA	specific surface area
T	absolute temperature
TCP	tricalcium phosphate
TEGDMA	triethylene glycol dimethacrylate
TGA	thermogravimetric analysis
TPH	commercial glass-filled composite
TRITON	alkyl aryl polyether alcohol
TTCP	tetracalcium phosphate
TWEEN	poly(oxyethylene) sorbitan monolaureate
UDMA	urethane dimethacrylate
UV/VIS	ultraviolet/visible spectroscopy
WS	water sorption
Wst1	mitochondrial dehydrogenase activity assay
XRD	X-ray diffraction
ΔZ	relative mineral content of the lesion
ZONYL FSN	non-ionic fluoro-surfactant
ZONYL FSP	anionic fluoro-surfactant
ZrDMA	zirconyl dimethacrylate

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Chapter 15

INTERIM RESTORATIONS IN IMPLANT DENTISTRY: BASIC CONSIDERATIONS AND TECHNIQUES

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ABSTRACT

Implant dentistry provides unique and predictable methods for the replacement of missing teeth. The need for provisionalization of implant-supported prostheses is increasing. Interim denture is a prosthesis designed to enhance esthetic, provide stability and function for a limited of time and should be replaced by definitive prosthesis after a period of time.

A challenge exists for prosthodontist in treatment planning and providing treatment for the patient before surgical phases. The interim restoration is an important diagnostic tool and a key factor for patient, dentist, technician and surgeon communication.

Immediate provisionalization of implants at the time of placement can provide a tooth-like restoration for the patient. In addition, interim restorations are frequently used for immediate or early occlusal loading of implants, for soft tissue management to obtain a natural emergence profile and during the fabrication period of the definitive prostheses.

Achieving esthetics in the anterior maxilla depends on natural looking teeth enveloped by normal soft tissue. Soft tissue will recede if not supported by alveolar bone, tooth structures or restorations, resulting in an esthetic failure.

Interim restorations can be either fixed or removable but certain key principles should be followed to prevent deleterious effects on the tissue in the edentulous space.

Although implant-supported provisional restorations are usually fabricated in the laboratory, there are some clinical situations that demand the direct fabrication of provisional restorations, especially when there is a high esthetic demand.

Various techniques to make implant-supported interim restorations have been described in the literature. This chapter includes indications and the types of interim restorations in implant dentistry. The interim restorative materials and different techniques for making interim restorations will also be discussed.

INTRODUCTION

Fabrication of interim restorations is an important step toward achieving a successful prosthetic treatment.[1-3] Interim denture is a prosthesis designed to enhance esthetic, provide stability and function for a limited of time and should be replaced by definitive prosthesis after a period of time.[4]

Interim restoration in conventional prosthodontics is needed to maintain gingival health and protect the pulp of prepared teeth.[3, 5-8] They are also used to provide esthetics, prevent migration of abutment teeth, establish anterior guidance, and assist in the development of occlusal schemes and occlusal vertical dimension.[8, 9]

Even though a definitive restoration may be placed quickly after tooth preparation, the interim fixed restoration must satisfy important needs of the patient and dentist. An interim restoration should not fabricate on the basis of short term use. An interim fixed restoration may have to function for an extended period because of laboratory delays.[10] In addition these restorations are worn over a long period of time to assess the result of periodontal, endodontic and also temporomandibular joint dysfunction therapies and during restorative phase of implant reconstructive procedures.

Interim Restorations in Implant Dentistry

Implant-supported restorations for partially and fully edentulous patients are a well-documented and predictable treatment. Success rate of implant-retained prostheses for complete and partial edentulous patients has been shown to be over 90 percent.[11-13]

According to the conventional loading protocols, the implants are left unloaded for 3 to 6 months to allow the osseointegration process to take place.[11] In addition, the treatment of edentulous span in the esthetic zone with pre-existing soft and/or hard tissue deficiencies will require surgical augmentation procedures before implant placement that can effectively extend the treatment time.[14] During this healing period, patients need to wear an interim prosthesis prior to delivery of the final prosthesis, especially in the aesthetic zone. In the nonesthetic zone, clinician may decide not to construct interim restoration.

Interim restoration must: [14]

1. satisfy the patient's esthetic expectations.
2. be easy to make and maintain.
3. eliminate intermittent pressure.
4. be durable.
5. provide diagnostic value.

Functions of Interim Restorations in Implant Dentistry

Interim restorations in implant dentistry not only can provide needs of the conventional dentistry but also can be helpful in diagnosis, improving peri-implant tissues, conditioning of implant site and communication between treatment team.

1. Diagnosis

In restoration-driven implant placement,[15,16] implants are positioned in relation to anticipated needs of the final restoration rather than the availability of bone. Interim

restorations can be used as a diagnostic tool to evaluate the position and contours of the planned definitive restoration prior to surgical implant placement and during the healing phase. A pretreatment prosthesis can provide the information required to determine whether a fixed partial denture (FPD) will compromise esthetics, support or hygiene. Provisional restorations should be used to evaluate esthetic, phonetic and occlusal function prior to delivery of the definitive implant-supported restorations. (Figure 1)

2. Preserving and enhancing the peri-implant and gingival tissue health and contour

An interim restoration immediately placed with ovate pontics extending into the extraction sockets can be used to preserve the pre-extraction soft tissue morphology. After implant insertion, prosthetic-guided healing of the peri-implant tissue enhances esthetic outcomes and allows the clinician to determine any necessary phonetic or esthetic adjustments.[17] Achieving esthetics depends on natural-looking teeth enveloped by normal soft tissue. Proper emergence profile of an implant-supported restoration is important for hygiene, gingival health, and appearance. Most healing abutments and transfer copings are round and do not simulate the normal cross section of teeth, resulting in an unnatural sulcular form around implant abutments. Therefore, to develop natural-looking replacement, interim restorations must change peri-implant tissue contour from a round shape into a crown shape. (Figure 2) [18]

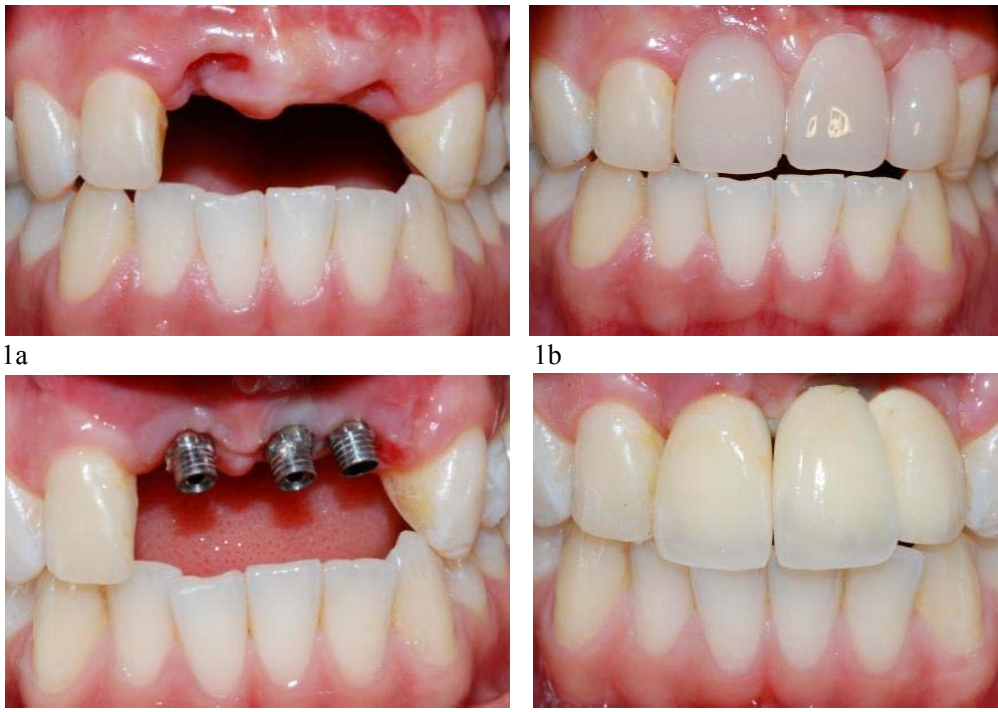

Figure 1. An interim restoration can be used as diagnostic tool to evaluate the position and contours of the planned definitive restoration prior to surgical implant placement, a. Preoperative facial view, b. Pretreatment prosthesis, c. Definitive abutments, d. Definitive restoration.

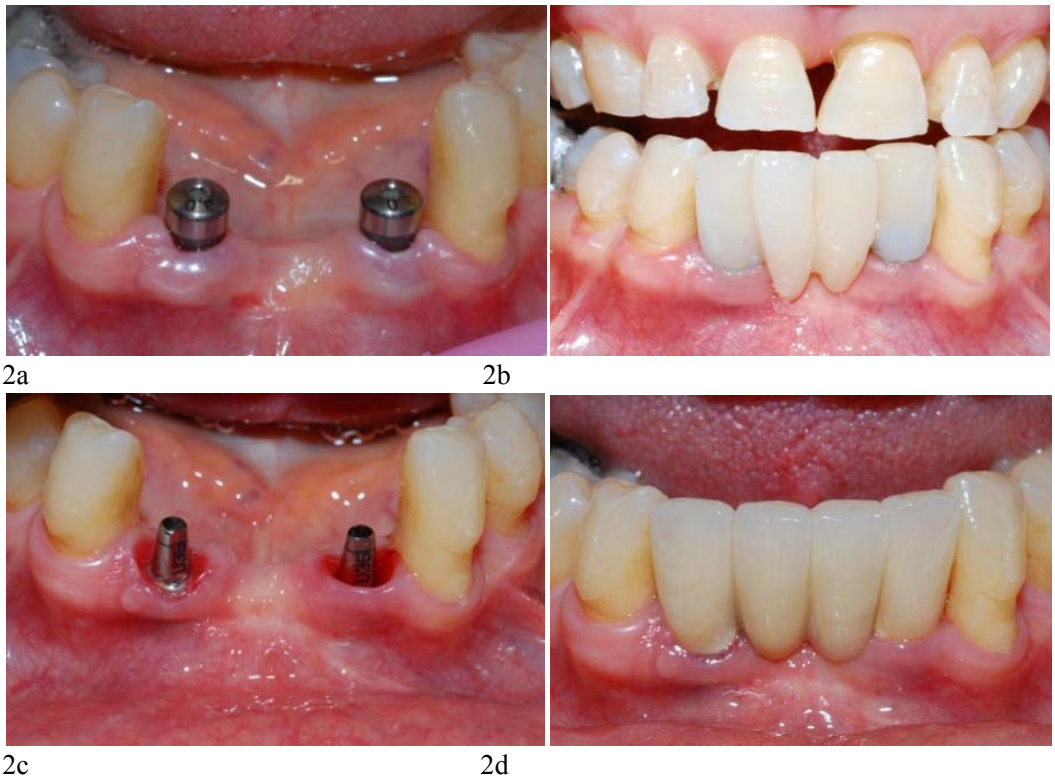

Figure 2. The interim restoration can change the peri-implant tissue contour to develop a natural-looking replacement, a. Implants with cover screws, b. Provisional FPD, c. Moulded soft tissue resulting in the provisional FPD, d. Definitive restoration.

If soft tissue is not supported by alveolar bone, tooth structures, or restorations, it will recede, resulting in an esthetic failure.[19] The morphology of interdental papillae between natural teeth can be consistently improved by manipulating restorative contours of the adjacent teeth, if there is sufficient interproximal bone support underlying the papilla. The papilla can be supported by the interproximal bone of the adjacent tooth; therefore, the papilla height depends on the interproximal bone peak of the adjacent tooth.[20]

From a biological point of view and with regard to tissue stability over time a provisional crown is not mandatory. This is based on the observation that the papillae adjacent to single-implant restoration present similar volume two years after definitive restoration insertion, regardless of the type of the abutments used (healing cap or interim restorations).[21] However, waiting for the papilla to rebound creates immediate esthetic problems and complicates the predictability of the definitive restoration.

Interim crowns are also valuable in diagnostics with regard to the future peri-implant soft tissue esthetics as well as to the ideal shape of the final crown. Therefore, it is highly recommended to use provisional crown in esthetic sites. Implant therapy in the anterior maxilla is considered an advanced or complex procedure and requires comprehensive preoperative planning and precise surgical procedure based on a restoration driven approach. [14] The highly esthetic zone of the premaxilla often needs both hard (bone and teeth) and soft tissue restoration. As a consequence, maxillary anterior single-tooth replacement is often a challenge, regardless of the experience and skill of the dentist.[22]

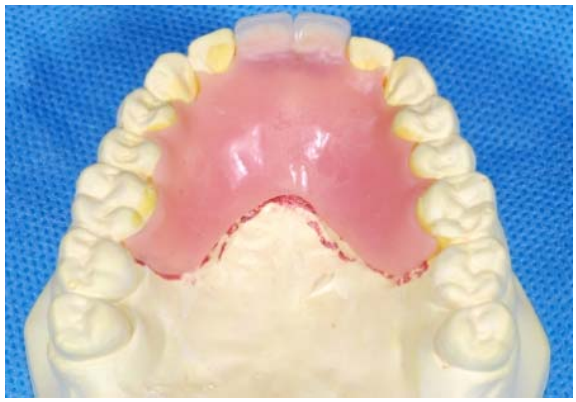

Figure 3. In this removable partial provisional denture palatal undercuts on the surrounding teeth provided retention.

Table 1. Types of interim prostheses in implant dentistry

	Type of support	Prosthesis type
Removable	Tissue-supported	Partial acrylic denture
	Tooth-supported	Essix appliance Partial denture with occlusal rest
Fixed	Tooth-supported	Archwire-supported pontics Resin-bonded pontics Metal or fiber-reinforced resin bonded FPD Wire-retained resin-bonded FPD Conventional interim FPD
	Implant-supported	Transitional implant-supported Definitive implant-supported

3. Improving the bone quality

The pretreatment prosthesis may be used to progressively load bone to improve its strength. A pretreatment prosthesis to improve bone quality is most always used in D3 or D4 bone supporting implants before the fabrication of final restoration. Interim acrylic restorations that gradually load bone for progressive loading may consider a pretreatment prosthesis.[22]

4. Communication tool

Interim restorations are useful as a communication tool between members of the treatment team consists of the restorative clinician, implant surgeon, laboratory technician, and the patient. The clinicians may use information such as shade, crown and soft tissue contours from the interim restoration to the laboratory. Interim implant-retained restorations also allow the patient to visualize and evaluate the end restorative result, thus assisting in acceptance and/or guiding of modifications required for the definitive restoration.[17] In most conditions, two or three months are needed for tissue to stabilize around the custom abutment and provisional restoration. During this time the restorative dentist can modify contours as needed to satisfy the patient's phonetic and esthetic demands.

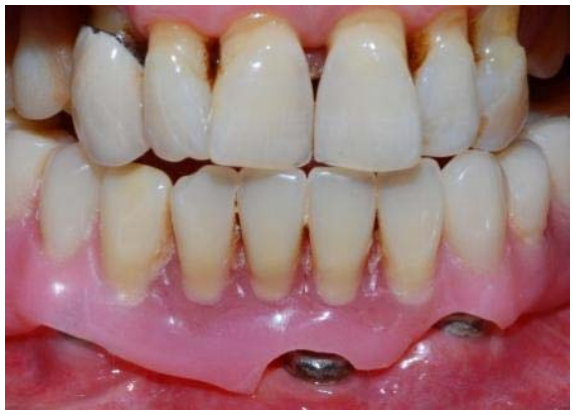

Figure 4. The removable interim denture should be passive over the implant site.

Types of the Interim Restorations in Implant Dentistry

Interim restorations in implant dentistry can be in the form of removable or fixed prostheses (Table 1). Removable interim prostheses are generally tooth and/or soft tissue borne. Fixed interim restorations can be supported by adjacent teeth or implant. These restoraions can be fabricated chairside, or in the laboratory on working casts; or as a combination of indirect-direct technique, where a provisional shell is fabricated before the patient's appointment, reducing chairside time.

Interim restorations may be constructed prior to tooth extraction, during socket healing, prior to implant placement, or during osseointegration period. These restorations could also be constructed after implant loading, allowing maturation of peri-implant soft tissue, and during construction of the final prosthesis.[17]

Advantages and disadvantages of various types of interim restorations, as well as their fabrication guidelines will be discussed in this section.

Interim removable prostheses

Removable partial acrylic dentures are most common interim prostheses used during post-extraction and throughout the implant treatment. (Figure 3) They are simple to construct, relatively inexpensive, and easy for the surgeon or restorative dentist to adjust and fit. For patients who may require multiple procedures of extraction, soft and hard tissue augmentation, and implant placement, interim removable prostheses may be modifed quickly by adding or reducing acrylic resin to adjust with ridge anatomy changes with minimal cost. However, they may reduce the effectiveness of any additional surgical bone and gingival augmentation procedures used to optimize the implant site.[17]

Care must be taken to prevent the gingival portion of the provisional partial denture from contacting the healing soft tissue or an exposed healing abutment. (Figure 4) Soft tissue borne prostheses which are used during healing may cause uncontrolled implant loading and lead to implant exposure, marginal bone loss, and/or failed integration. Provisional dentures are often adjusted to minimize contact with the healing implants. A removable interim restoration may transmit micromotion to the implant site; have a negative effect on the volume yields from hard and soft tissue site-development procedures. In certain instances, a removable interim restoration may contribute to failure of such grafts via the same mechanism that cause implant

failures.[23] Removable interim prostheses are not recommended if vertical grafting is planned. Even when relieved the potential exists for intermittent pressure on the graft, which lead to resorption.

Removable interim prostheses may also depress the interdental papillae of the adjacent teeth, and compromise the esthetic.[22] Removable prosthesis with a cast framework and occlusal rests can prevent rotation and loading of the soft tissue during function.

Interim removable prostheses, often called "flippers," are commonly used during single-implant therapy. They are simple to fabricate and easy to seat. In young and growing patients who are still not old enough for implant placement, the adjustable nature of interim removable prostheses again facilitates modifications. However, removable appliances are bulky, interfere with speech, initiate an inflammatory soft tissue response from the acrylic base, and are frequently broken or destroyed. Implant or graft integrity may be compromised if passivity of fit cannot be maintained. It may be difficult to prevent pressure from the pontic on a fresh surgical site. Patients with inordinately strong gag reflexe are often unable to wear removable prostheses that partially cover the palate. For those patients with minimal distance between the implant platform and the opposing dentition, the thin connector area is prone to fracture, and repeated repairs during the interim period can be frustrating for both patients and dentists. Acceptable esthetics may be difficult to achieve with interim removable appliances. The denture teeth used in removable prostheses can be modified to match the shape of the corresponding tooth, but the limited shade selections may no closely approximate the adjacent natural dentition. During initial periods of integration or after hard and soft tissue augmentation, removable appliances should remain passive over the implant site, which may necessitate an unsightly gap between the ridge and neck of the denture tooth. After initial soft tissue healing, the tooth can passively contact the ridge to impart a more natural appearance. It is best to eliminate any flange in the region of the pontic, thus giving the patient and the dentist a more realistic approximation of the result. Healing abutments on the implants may be facially inclined or too long to be completely hidden by a removable appliance in the esthetic zone. A facially angled healing abutment may be reduced on the facial aspect with a carbide bur and polished, allowing the pontic to contact the ridge slightly facial to the modified healing abutment. A long-healing abutment can be replaced with one that is nearly subgingival, allowing the pontic to seat directly over it and slightly into the soft tissue of the ridge. When needed, extra retention can be added by using interproximal wrought wire ball clasps. Ball clasps placed between premolars and molars are not objectionable to most patients.[24]

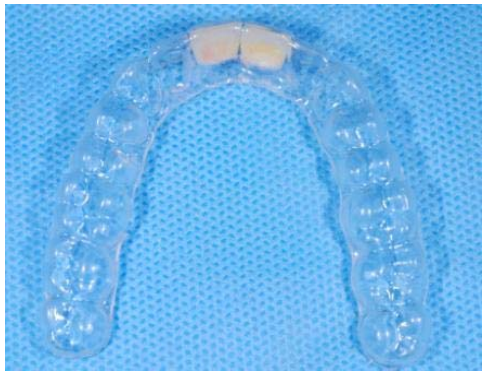

Figure 5. An Essix appliance replacing maxillary central incisors.

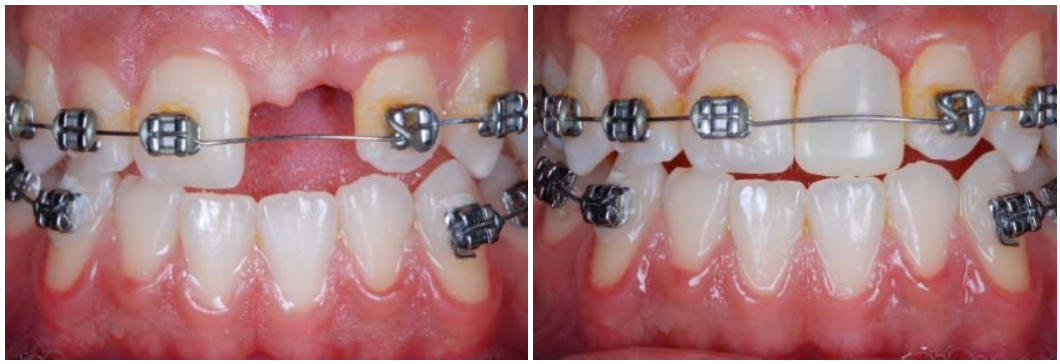

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Figure 6. Archwire-supported pontics, a. Preoperative facial view, b. Central incisor replaced with archwire-supported pontic.

Tissue conditioning materials can be used after implant surgery under a removable prosthesis, and during healing period. These materials may respond to the swelling and tissue changes immediately after soft tissue reflection. At the suture removal appointment, the tissue conditioner is replaced with a soft liner. This material remains soft during extended periods and is less likely to load the implant.[22]

Vacuum-formed appliance (Essix appliance)

An alternative removable prosthesis to tissue-borne provisional restorations is vacuum-formed appliance. Dentists using vacuum-formed matrices to retain prosthetic teeth as temporary appliances often refer to them as Essix retainers (Figure 5), although true Essix retainers (Raintree Products, Metairie, LA, USA) are made with specific copolyester sheets that are reported to be stronger and more durable than typical plastic sheets.[24] Vacuum-formed appliances were suggested as temporary prostheses for missing anterior teeth,[25] and most recently recommended as interim prostheses for implant patients.[26] An Essix appliance may be used as a removable prosthesis in limited interocclusal space or deep anterior overbite cases. This prosthesis is made from an acrylic tooth attached to a clear vacuform material on a cast of the diagnostic wax up. This prosthesis provides protection for the underlying soft tissue and implant during the healing phase. Limitations of this provisional restoration include its inability to mould the surrounding soft tissue, and in patients who lack compliance, it can cause rapid occlusal wear through the vacuform material.[17]

One type of Essix appliance is especially useful in the immediate postoperative period. On occasion, the clinical crown of the extracted tooth is modified as the pontic. This type of interim restoration can be fabricated on the day of surgery and adjusted by the surgical team to ensure tissue support or stress free healing at the surgical site.[23]

For making an Essix retainer after obtaining an impression to create a master cast the selected acrylic denture teeth will be set on the stone cast. A mesiodistal trench 4-mm wide and 3-mm deep is then cut into the palatal or lingual surface of the denture tooth to create a mechanical lock. The tooth is fixed to the cast with acrylic resin. Wax is not a suitable alternative because it will melt during heating. The Essix interim restoration is then fabricated using the MiniSTAR device (Great Lakes Orthodontics, Tonawanda, NY). Various thickness

of Invisacryl or Bioacryl translucent material are available to suit the needs of individual patients. A plastic sheet, usually 0.030-inch thick, is thermoformed over the cast and trimmed. It is important to smooth and polish all the edges after final trimming of the material. Esthetic results can be as good as those of interim removable partial dentures (RPDs), primarily because unsightly clasps are unnecessary.[24] Unlike with interim RPDs, in vacuum-formed prostheses, pressure on surgical sites is easily avoided because they are tooth retained and supported. Essix interim restorations are used only on a temporary basis until initial healing is achieved, after which they are replaced by a more definitive interim restoration. Advantages of using modified Essix interim restoration include excellent esthetics and phonetics and a relatively low cost. Disadvantages of this type of interim restoration are the obvious interference with effective mastication, and occlusal wear of the appliance.[23,24]As with other removable appliances, vacuum-formed prostheses are not as comfortable as fixed alternatives.

Interim tooth-supported fixed prostheses

Provisional restorations that are fixed to the adjacent teeth or that completely eliminate the possibility of soft tissue contact may be more beneficial for implant integration and soft tissue maintenance. Tooth-borne or fixed provisional restorations may also satisfy patients' esthetic, functional and psychological demands. [17]

Archwire- supported pontics

Orthodontic brackets and archwire on several teeth adjacent to the implant site with an attached acrylic pontic can be used as a fixed tooth-supported provisional restoration. (Figure6) The archwire/resin retainer can be removed and reattached between the different surgical and prosthetic stages. In addition they can be used to guide the surgeon during grafting procedures and as a template for the final restoration.[24]

Resin-bonded pontics

Extracted natural teeth,[27] denture teeth or ceramic pontics,[28]adhesively bonded to adjacent etched tooth surfaces can be used as provisional implant prosthesis. (Figure 7) Sometimes small retentive grooves within enamel on the adjacent teeth can be used to increase retention of the pontic.[17] These-bonded pontics are usually indicated for short-term use, particularly if there is insufficient time to make or prescribe other options. Because laboratory involvement is not required, bonded-teeth provide an immediate solution. Esthetic results may be inferior due to the bulk of composite resin in proximal spaces needed to retain the pontic. If an extracted tooth is used, additions to the gingival aspect are difficult, which also compromises esthetics. [24]

Provisional resin-bonded prostheses are not commonly used on posterior teeth because an occlusal rest is needed which is difficult to obtain without tooth reduction. If substantial tooth preparation is required, patients would be better served with RPDs, vacuum-formed retainers, or implant-supported provisional restorations. [24]

Cast-metal or fiber-reinforced resin bonded FPD

Cast-metal resin-bonded FPDs, originally developed as conservative options for definitive tooth replacement, are used frequently as provisional prostheses for implant patients. In young patients with congenitally missing teeth who have not reached sufficient skeletal maturity for implants, cast-metal resin-bonded FPDs are ideal interim prostheses. [24] Resin-bonded FPDs are retained and supported by adjacent teeth, and thus will remain passive over the implant site and not interfere with implant integration or soft tissue healing; since they are fixed appliances, they are unlikely to be misplaced or damaged.

In regards to esthetics, achieving optimal results can be difficult with cast-metal resin-bonded prostheses. Thin or translucent teeth are unable to mask the palatal metal retainers, thus lowering the value of the adjacent teeth, and proximal metal margins may be visible. [24] This type of provisional is difficult to reuse throughout the implant procedures as the bond strength between the metal retainer and the enamel can be unpredictable during removal and reattachment between procedures.[17] Furthermore, the laboratory costs are relatively high for a short-term appliance, retention and removal are unpredictable, and modification of a ceramic pontic during ridge maturation is difficult.[24] Fiber reinforced adhesive prostheses provide acceptable esthetics, but are usually destroyed upon removal. (Figure 8)

Wire-retained resin-bonded FPD

Resin-bonded pontics with customized composite resin tooth can be reinforced by 30-gauge, half round wire. A full-arch impression is made of the unprepared teeth, a shade is selected, and the prosthesis is made in the laboratory. The technician adapts the wire to the palatal surfaces of the teeth on the cast adjacent to the edentulous space. A composite resin tooth is then attached to the wire. The palatal surfaces of adjacent teeth are etched. Flowable composite resin is injected onto the etched surfaces; the wire is embedded into the resin, which is then polymerized with a curing light. Removal of the prosthesis is easy and predictable. Once the resin is detached from the wire, the prosthesis debonds and the remaining composite resin is polished from the tooth surfaces. [24]

Conventional interim FPD

Acrylic resin provisional prostheses are used routinely to protect abutment teeth and provide temporary replacements when using conventional FPDs. When teeth adjacent to implant sites require complete coverage restorations, interim FPDs are convenient and predictable restorations. (Figure 9) They can be seated immediately after implant placement without risk of compromising the implant site. [24]

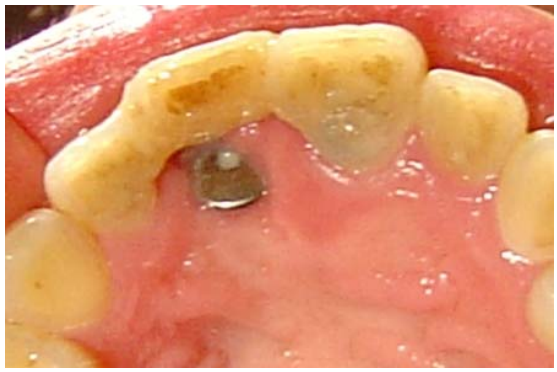


Figure 7. Extracted natural teeth adhesively bonded to adjacent teeth can be used as provisional prosthesis.

Figure 8. Fiber-reinforced resin bonded FPD.

For short term use, chairside restorations made from self-curing materials, such as bis-acrylic resin, provide esthetically acceptable results at a low material cost. Chair side interim resin materials have limited shades available and their color darkens over several weeks which limit their esthetic potential. However, for long term use such as implant treatment and during the healing phase, laboratory-processed prostheses are more durable and shades can be customized to individual patients needs, althogh the cost will be increased. [24] Like other tooth-supported provisional restorations, implant sites can be maintained without pressure on gingival tissues. These provisional prostheses can easily be modied by reducing or adding appropriate material when alterations are necessary to accommodate the anatomy of the implant site. [24] Disadvantages of these types of provisional restorations for long term use include fracture, loosening, and the possibility of abutment teeth recurrent caries. [24]

In some cases, a staged extraction and implant placement approach can be used.[17, 24] In this technique, the hopeless teeth that occupy implant sites are extracted while the remaining failing teeth are used to support a fixed provisional restoration. Usually, natural abutments with poor prognosis are used as interim abutments and can be extracted when the implants have integrated. The tooth-supported provisional restoration is then converted to an implant-supported provisional restoration. This technique is often used in a full arch situation, where the patient's dentition is failing due to periodontal disease. This interim prosthesis is more costly for the patient and may have adverse consequences for implant integration in cases that retained abutment become infected. [29]

Transitioning the patient from teeth to an implant-supported restoration is more difficult than replacement of missing teeth with implants because precise planning will now include timing of extractions, method of retaining fixed provisional restorations, manipulation of remaining teeth to enhance implant receptor sites, and many other considerations. [29]

Implant-supported interim prostheses

Transitional implant-supported interim prostheses

In extended partial edentulous areas where there are no or limited natural abutments to support a provisional restoration or in cases where all the remaining teeth must be extracted, one or more transitional implants may be used.[30] These transitional implants are loaded immediately and support the provisional restoration. They can be used to support fixed restorations. In cases where it is not possible to place a sufficient number of immediate provisional implants to support fixed interim restoration, these provisional implants can be used to stabilize a removable prosthesis, thereby reducing the possibility of transmucosal loading to the underlying surgical sites.[17,23]

Immediate transitional implants may be used while hard or soft tissue grafts are healing or during osseointegration of simultaneously placed definitive implants. Provisional implants can be used as part of a natural tooth–implant combination to eliminate anterior cantilever.[31]

These small diameter transitional implants are placed adjacent to the definitive implants in a distribution that allows the support of fixed interim restoration until the definitive implants have achieved osseointegration, allowing transition to fixed implant-supported interim restoration.[23]

Transitional implants enable undisturbed healing of definitive implants site and fulfil the patients demand for immediate function and esthetics. [29] The primary function of transitional implants is to absorb masticatory stress during the healing phase, ensuring stress-free maturation of the bone around the submerged implants.[32,33]

Most importantly, immediately after the surgical phase, patients experience the benefits of implant dentistry and fixed prosthesis. The transitional implants are particularly useful in situations where bone quality is not adequate for immediate loading of the definitive implants but the patient requests a fixed transitional prosthesis. For complete arch prostheses, a sufficient number of transitional implants should be placed to allow survival of the prosthesis if one or two implants fail. [29]

Transitional implants are an effective way to generate esthetic transitional prostheses. Patients can return to their daily activities with a fixed restoration and avoid social embarrassment.

In most instances removal of the transitional implants is a simple procedure performed under local anesthesia. One disadvantage of using transitional implants is the need for frequent follow up visits. Follow up of three week intervals is recommended to monitor the stability of the interim prosthesis and health of the bone and soft tissues around the provisional implants.[23]

Despite numerous advantages of transitional implants they still should be used with caution. Occasionally the volume of bone used for transitional implants placement may be of strategic value for the definitive implants and there are high risks of fibrous tissue formation or bone resorption because of immediate loading of transitional implants. Also, if a definitive

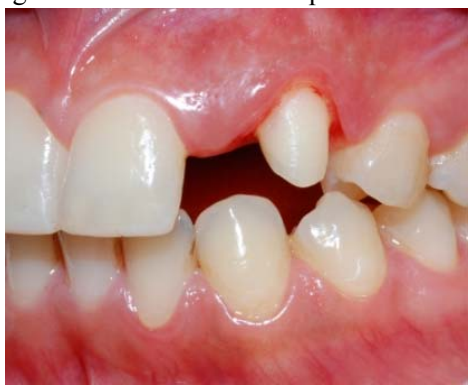
implant fails, the alternate site which is occupied by transitional implant would be unavailable. [29]

Definitive implant-supported interim prostheses

The need for provisionalization with implant-supported fixed prostheses may be increasing. Acrylic resin fixed provisional restorations are frequently used for immediate or early occlusal loading of implants.

Loading protocol of implants

Interim restorations may be constructed prior to tooth extraction, during socket healing, prior to implant placement, or during osseointegration period. Interim restorations could also be constructed after implant loading, allowing maturation of peri-implant soft tissue, and during construction of the final prosthesis.



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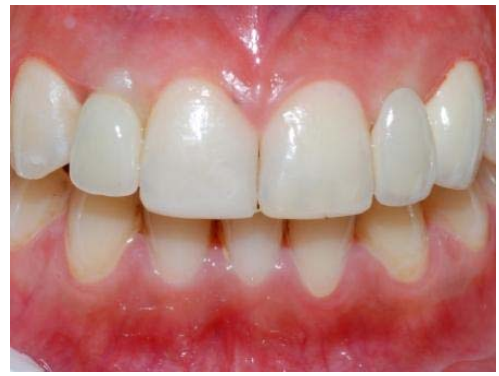

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Figure 9. Tooth-supported FPD as the provisional restoration, a. Prepared tooth, b. Interim FPD.

In recent years, confusion has been evident with terminology of loading protocols in implant dentistry. Most of these terms were defined in conference on immediate and early loading that was held in Spain in May 2002. The modified definition are presented here:[34,35]

Conventional loading: The prosthesis is attached in second procedure after a healing period of 3 to 6 months.

Early loading: A restoration in contact with the opposing dentition and placed at least 48 hours after implant placement but not later than 3 months afterward.

Immediate restoration: A restoration inserted within 48 hours of implant placement but not in occlusion with the opposing dentition.

Immediate loading: A restorative placed in occlusion with the opposing dentition within 48 hours of implant placement.

Delayed loading: The prosthesis is attached in a second procedure that takes place some time later than the conventional healing period of 3 to 6 months.

In edentulous mandible, the immediate loading of implants with an overdenture or fixed restoration in the interfrominal area with rigid fixation and a cross arch stabilization is a predictable and well-documented procedure; provide that a relatively large number of implants are placed. The early loading of implants (splinted or unsplinted) in the edentulous

mandible is not well-documented procedure. Immediate or early loading of implants with fixed prostheses in the edentulous maxilla is not well documented.[35]

Using immediate provisional technique has several benefits for patient as well as members of the treatment team. The practitioner benefits include reduced number of post operative visits and maintenance of the interim restorations. Immediate loading is a very effective way of transitioning patients from teeth to a complete full arch implant-supported restoration. Immediate provisionalization offers the patient improved comfort and function during the implant healing period compared with a conventional denture.[36] Complications that may arise from wearing complete dentures during the period of osseointegration included loose dentures, fractured prostheses, sore spots, and periodic provisional relines.

The decision to immediately restore or load dental implants is usually made during the treatment planning phase. The treatment can only be confirmed clinically at the time of implant placement with appropriate assessment of implant stability, bone quality, and general site health. Primary stability of these implants is crucial in the decision for immediate provisionalization which depends on proper surgical technique and type of bone. [37-39]

There are many factors to be consider in loading of implants such as the number of implants used, bone quality and quantity, the position of implants, the type of definitive prosthesis, the physical design of the implant, the type of occlusion, the nature of opposing arch, and finally the decision of the treatment team.[40] Current litreture on clinical factors that should be consider before application of immediate restoration or loading is summarized below: [34, 39-42]

1. Clinical stability of implants should be achieved (minimum insertion torque 35-50 Ncm). Neugbauer et al[43] confirmed that only when torque applied to the implant during insertion was more than 35 Ncm in immediate loaded implants, a higher degree of bone formation can be shown in comparison to unloaded implants.
2. The bone quality and quantity should be adequate. A minimum bone height of 10 mm is desirable and adequate bone quality (type I or II) are ideally required.
3. There should be an adequate number and distribution of implants to provide cross arch stabilization.
4. Splinting of implants is recommended where possible to reduce the mechanical load applied to the implants.
5. Reduce cantilevers by using appropriate number of implants and optimizing implant distribution. Cantilever should be minimized to one premolar.
6. Limiting and distributing occlusal contact in centric occlusion or maximum intercuspation. Removing all excursive contacts from the interim restorations.
7. Limiting the off axis loading.
8. Accuracy of fit and passivity of restoration.
9. Sufficient mesial-distal, buccal-lingual, and interocclusal space should be present for making an anatomic and rigid interim restoration. If the space is less than 6 mm or if the opposing occlusion interferes with the provisional restoration, then a 2-stage technique is recommended rather than the immediate provisionalization method.
10. Where possible, interim restoration should be remain in place through the healing phase, allowing adequate healing of the hard and soft tissues in contact with implants and prosthesis.
11. Patients with parafunctional habits may not be ideal candidates.

Ganeles and Wismeijer[44] stated that most publications concerning immediate or early loading of implants indicated that implant survival was comparable to the results with conventional loading protocols. Chiapasco[45] indicated that there is limited histological data to support reliability of immediate loading under various clinical conditions. Such unreliability reduces possibility of widespread use of immediate or early loading of implants in all clinical situations. The use of immediate or early loading of fixed implant-supported prostheses in the maxilla is not supported by sufficient data to consider this treatment as routine, although preliminary results seem to be encouraging.[40]

Important prosthodontic considerations during the immediate loading phase include the protection of the achieved primary stability and the establishment of normal emergence profile. Protection of primary stability is achieved by preventing micromotion (approximately 150 μm).[46,47]Micromotion can be prevented by splinting multiple adjacent implants, by adjusting occlusion to avoid centric and eccentric contacts, and by allowing only a soft diet for the first 2 months.[48]

Furthermore, immediate provisional restorations can be useful in preserving the facial soft tissue. A one year prospective study[49] showed that only 0.5 mm of the free facial gingival recedes around implants that are immediately placed and loaded, in contrast with the approximately 1 mm recession documented in the literature for the delayed approach.[50] Therefore, immediate provisional restorations can effectively support both the papillae and the facial gingival tissue. This, in combination with the preservation of the hard tissue due to immediate placement, can lead to the long term preservation of the soft tissue.[51]

Preservation of the hard and soft tissue that exist around natural teeth while transitioning to implants is easier and more predictable than losing these elements following tooth extraction then trying to rebuild them. Favorable socket architecture can occur only in fresh extraction sockets. These sockets should have intact bony walls with no defects or signs of inflammation. Therefore the techniques that involve immediate extraction, immediate placement, and immediate loading or restoration have been advocated.[29]

Restorative dentist can fabricate the provisional restoration at chairside or before implant placement. Chairtime for the dentist and patient is less when the provisional restoration is fabricated before implant placement.

Risks of immediate loading are perceived to be higher during the first week following the insertion of implants. The bone interface is actually stronger on the day of implant placement compared with three months later, although this is location-dependent.[52] The risk of immediate loading is implant failure and if the remaining distribution of implants does not allow proper stabilization of the provisional restoration, implant failure can necessitate use of a removable prosthesis. Failure also leads to additional appointments and greater chairtime for the clinician, which increases costs. Patients must be aware of the risks of failure prior to selecting immediate loading protocol.[29]

The advantages of immediate restoration are obvious; however, the application of immediate or early load may pose an increased risk of implant failure in single-tooth situations. The parameters for achieving and maintaining equal success rates of osseointegration for single teeth are not fully known. Sufficient data are available to support the concept of immediately restored and loaded implants in single-tooth situations in the esthetic zone using many implant systems and protocols.[52-54] It must be mentioned that most of these studies and patient treatment reports are carried out by experienced surgeons and restorative dentists with optimal resources for complex treatments. The risk of immediate

load in a single tooth situation should be obvious to the clinician. The risk of failure is a costly one that has both biological and financial repercussions.[29]

The decision to utilize immediate, early or conventional loading protocols should be based on careful evaluation of the patient, and on each individual situation. Important factors that may influence the clinician's choice for an appropriate treatment protocol with regard to loading can be divided into five categories. [35]

1. Quantity and quality of scientific documentation supporting the treatment approach; a loading protocol is considered well-documented when clinical studies with at least 5 years follow-up have been published in peer-reviewed journals. A procedure is considered moderately documented when only short to medium ranged clinical studies (1-3 years) are available. Procedures are scientific not documented when no clinical studies or only case reports have been published.
2. Patient benefits associated with the treatment approach; reduced treatment time, patient comfort, enhances soft tissue conditioning, shaping and maturation compared to conventional loading protocols can be considered beneficial to the patient.
3. Risk for complication of the treatment approach; the early or immediate loading can be associated with an increased incidence of complications, including bone and soft tissue loss and most importantly early implant failure.
4. Treatment difficulty; there are some factors related to restorative treatment which can increase the complexity of treatment such as skeletal jaw relationship, symmetry in the anterior maxilla, available space and the position of the implants.
5. Cost-effectiveness of the treatment approach; where possible, patients should be provided with cost effective treatment options that optimize outcomes and control the risk of complications.

Clinician has the option to either cement or screw retained the final implant restorations.[55,56] Similarly the implant-supported interim restorations can be cement or screw-retained. There are advantages, disadvantages and limitations for each option and it is important to understand their influence on the final prostheses. The decision whether to cement or screw-retained a provisional or final implant restoration would be dependent on the clinical situations and clinicians' preference towards the method of fixation.

Cement-retained implant-supported interim prostheses

Cement retention for implant restorations is an acceptable alternative to screw retention.[56] The advantages of cement-retained restorations include enhanced ability to develop esthetics and occlusion because of the absence of screw access holes, enhanced ability to achieve passive fit for frameworks, reduced complexity of clinical and laboratory procedures and less mechanical complications. [22, 56]

Definitive or temporary abutments can be used to support the interim restorations. Most implant companies have prefabricated definitive abutments for cement-retained restorations. These abutments come in various heights to allow proper space for porcelain of definitive crown. They also have a slight taper and some kind of grooves or flat surfaces providing resistance and retention form for the overlying restorations. The abutments are torqued onto the implants, left in situ and an abutment level impression will be used to transfer the abutment position to the master cast. A plastic protection cap, usually cylindrical in shape,

may be cemented on the prefabricated abutment until the delivery of the final prosthesis.[17] This technique is often used by clinicians in non-esthetic regions of the mouth. But in the esthetic zone and when patient has high expectation regarding esthetic treatment outcome and presents thin, highly scalloped gingival biotype and prosthetic-guided soft tissue is needed to enhanc emergence profile, the provisional crown is highly recommended.[14]

Esthetic provisional restorations can be constructed on the definitive abutments during the period between impression and prosthesis delivery.[24] The provisional restorations are usually made from a prefabricated custom shell (Figure 10) (prefabricated preformed acrylic crowns; vacuform template from the diagnostic wax up; hollowed out denture tooth; or even a hollowed out decoronated clinical crown). These custom shells will be relined using self or light cured resins intra-orally to capture the shape of the abutment, and then completed extraorally to fit the implant restorative margins. To facilitate treatment, the crown form can be waxed up, or selected, sized, and trimmed in advance to fit the edentulous site on the study cast.[17]

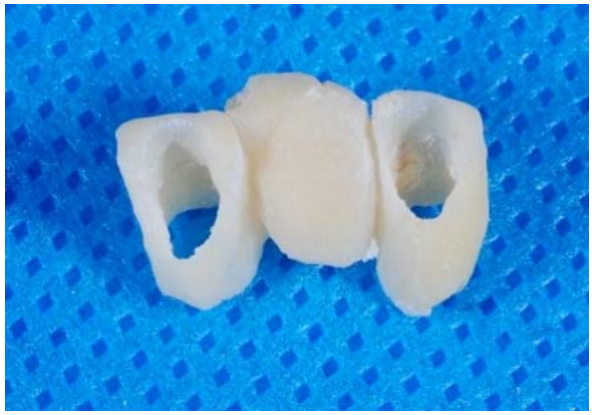


Figure 10. Customized shell for implant-supported interim prosthesis.

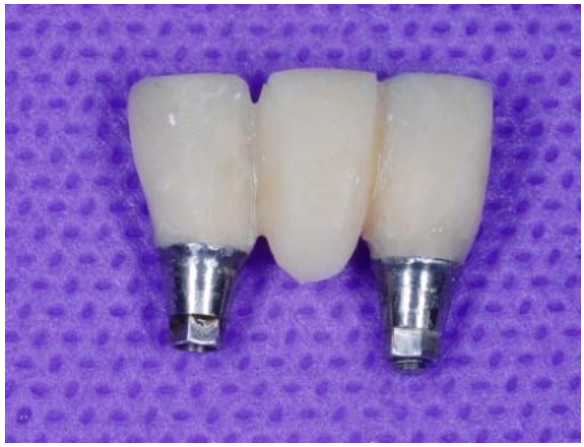


Figure 11. Completed implant-supported interim prosthesis on temporary abutments.

Alternatively implant-retained provisional restorations can be supported by a temporary implant abutment (Figure 11). Temporary abutments which are made of titanium or plastic are effective, easy to prepare, and less costly than a definitive abutment. In addition these temporary abutments would allow a machined connection at implant shoulder, and

customized cement margin that can be modified to allow a slightly subgingival restorative margin for ease of cement removal. Care should be taken during the cementation procedure where the crown margin is placed deep subgingivally, especially in the anterior aesthetic region of the mouth. It can be difficult to access deeply placed implant shoulder, and excess residual cements are difficult to clean and may cause peri-implant inflammation.[57] Immediately following implant placement or upon second-stage uncovering, a temporary abutment is secured to the implant. This abutment can be prepared and customized using diamond bur to form the finishing line with accessible level placed just below the gingival margin. Correction of any angulation problems to retain the provisional crown can be made. Reduction and preparation of a temporary abutments may be completed intraorally on an integrated implant. However, extraoral abutment preparation is necessary on an immediately placed implant to avoid disruption of initial implant stability and contamination of the site with debris from the temporary abutment.[24] The temporary abutment is reseated and the screw is gently hand tightened. A cementable provisional crown is then constructed using conventional crown and bridge technique. Interim restoration can be made from a vacuum or silicone matrix on a preoperative cast or on an ideally contoured waxing of the replacement tooth. A prefabricated crown is also an acceptable option.

It is advantageous to adapt the shape of the provisional crown at chair side. This allows the establishment of the ideal shape, size, and contour in a single step or in multiple steps. It can be done by subtracting or adding temporary materials. To modify the shape of the crown, one to three conditioning steps are necessary; depending on the design, emergence profile, and the quality of the mucosa. Within six to eight weeks, this process leads to the final soft tissue contour. [58]

Screw-retained implant –supported interim prostheses

Although the advantages of cement-retention are appealing, the indications, as well as the benefits of screw retention cannot be underestimated.[59] Screw retention is prefered when submucosal implant shoulder placement is greater than 3mm subgingivally, or in cases when interarch space is significantly increased or decreased, and when multiple misaligned implants support long span restorations.[60]

Advantages of the screw-retained provisional restorations include; a more simplified design, ease of adjustments, delivery and retrieval. Further more, complications of the cemented provisional restorations, such as incomplete removal of the temporary cement and loss of retention of the provisional, are avoided with a screw-retained provisional design.[61] Another advantage of using screw retention is elimination of the rough surface created at the crown abutment junction by providing a highly polished surface which facilitates tissue healing. Since the screw is only hand-tightened to prevent applying extra torque to the implant during provisional crown removal, screw loosening is a possible complication that could occur during the healing period which might require retightening and additional visits to the dental office. Another disadvantage of the screw-retained provisional restoration relates to the fact that the retaining screw access hole can compromise the esthetics of the provisional when it emerges through the facial or incisal aspect of the provisional. However, the screw access hole can be easily masked with restorative composite, and the esthetics of the provisional restoration should not be significantly compromised. [61]

For a screw-retained provisional crown, a hole must be placed in the matrix, providing access for screw removal prior to complete setting of the temporary resin (Figure 12). The vacuum-formed or silicone matrix is filled with temporary resin, seated, and then removed before complete setting of the material. Because the soft tissue will quickly collapse around the temporary abutment, a void will remain between the gingival crest and the subgingival implant margin. After removal, appropriate temporary material is added to fill the void, and the restoration is contoured. The subgingival contours of the restoration are modified by adding or subtracting resin until the soft tissue profile is optimal.[62-65] Increasing or decreasing pressure on the fixed amount of soft tissue present with the provisional restoration will subtly influence soft tissue levels.[66]

When implants are well positioned and screw access opening are located favorably screw-retained provisional restorations can be fabricated intraorally using autopolymerizing acrylic resin or composite. Improper alignment of implants compromises both esthetics and function due to unfavorable positions of screw access openings.

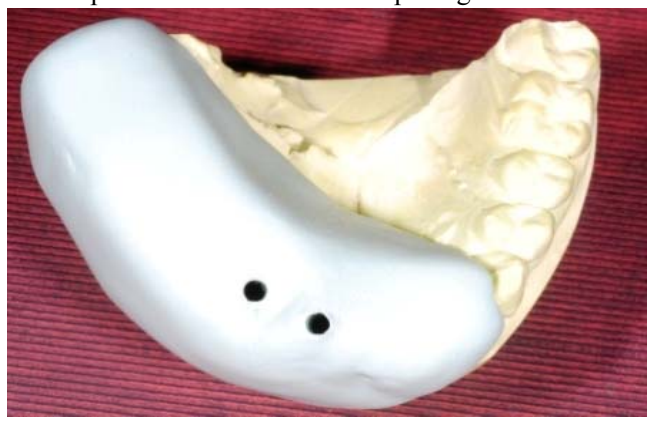


Figure 12. The holes in the matrix for screw access of the abutments.

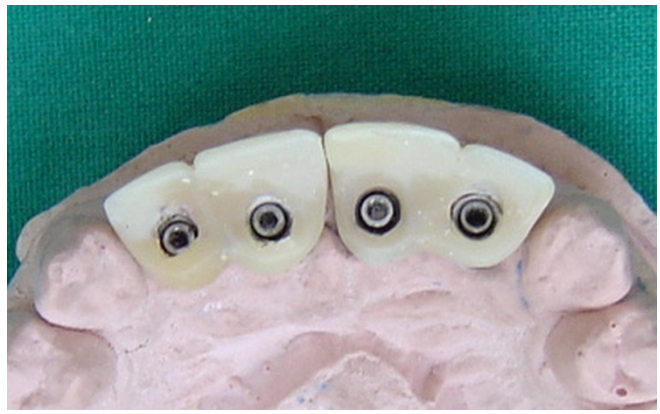
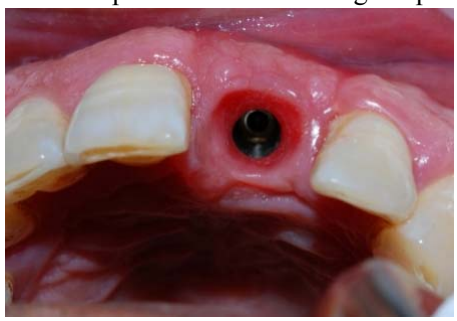


Figure 13. Screw-retained interim implant-supported prosthesis.

After final polishing and addition of a resin glaze, the provisional restoration can be screwed into implants (Figure 13).

An alternative to a chairside implant provisional restoration is a laboratory-processed restoration.[67] An index of implant position is made at the time of implant surgery with

autopolymerizing resin or fast set hard polyvinyl siloxane impression material (J. Morita, Irvine, CA). This procedure involves attaching a fixture level impression coping to the implant and registering its three dimensional position relative to the adjacent dentition. After removing the index from the patient's mouth an implant analog is attached to impression coping of the index. For seating of the index on the master cast the edentulous site is prepared to receive the implant analog. Subsequent to seating the index, the analog is secured to the cast with quick-set plaster or acrylic resin. The result is a working model that contains a replica of the implant as recorded at the time of surgery. In most instances the laboratory either casts or mills a custom abutment that incorporates proximal rise to ensure that the restorative margins are located in the superficial aspect of the peri-implant sulcus circumferentially. A provisional implant restoration is then made on the cast by the dentist or the laboratory technician and delivered at the time of implant exposure. Because an implant-level provisional restoration actually emerges from the sulcus, it provides the highest potential for optimal esthetics during the provisional stage of implant treatment.[23,24]



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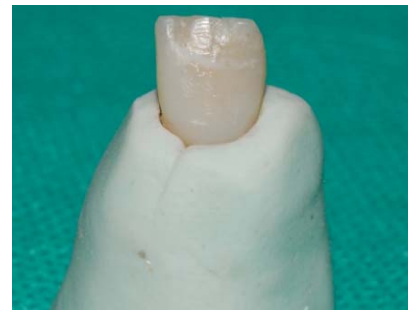

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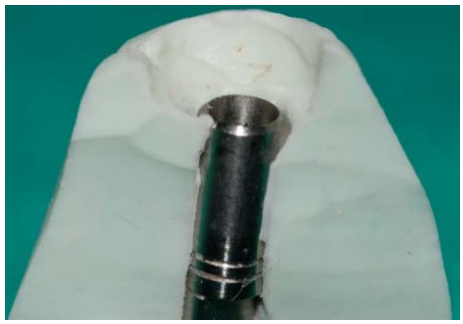

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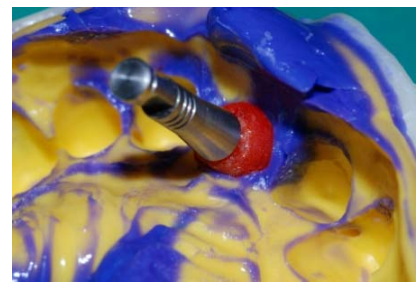

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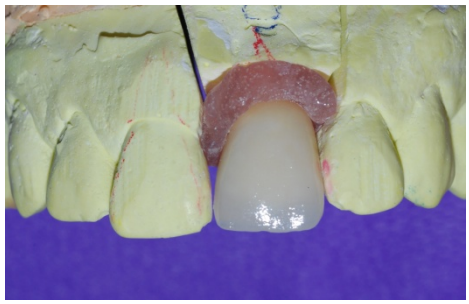

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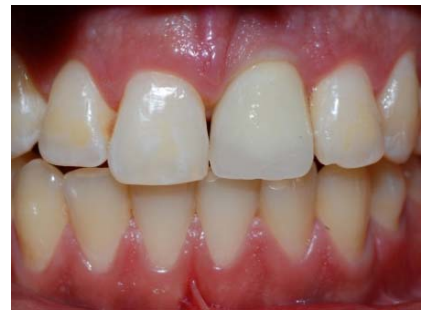


14h

Figure 14. Transferring the peri-implant soft-tissue emergence profile from a provisional to a final prosthesis. a. Peri-implant tissue moulded with provisional prosthesis, b. Interim restoration and attached implant analog inserted into impression material, c. Precise copy of cervical shape of provisional restoratin is captured within the silicone, d. The gap between the silicone and the impression cap filled with auto polymerizing resin, e. Individualized impression cap screwed into the implant , f. Definitive impression, g. Definitive master model precisely mimics the shape of the peri-implant mucosa, h. Definitive restoration.

Developing an optimal emergence profile with interim implant-supported prostheses

Proper emergence profile of an implant-supported prosthesis is important for hygiene, gingival health, and appearance.[68] Provisional implant restorations for development of emergence profile are indicated primarily in the esthetic zone, between maxillary canines. Implant-retained provisional restorations have been demonstrated to be an effective mean to temporarily restore single implants following integration and uncovering by facilitating the development of the soft tissue prior to definitive restoration.[21, 69-72] An implant-supported provisional restoration may be the best way to establish the optimal restorative design of the definitive restoration. The vertical length of the subgingival portion of the restoration is particularly important because guided gingival growth is indirectly proportional to the submergence depth of the implant.[73]

Maturation and stabilization of the peri-implant mucosa around a provisional crown take place within the first three to twelve months after insertion.[74-76] It is recommended that the provisional crown remain in situ for at least three months.

At this time the restorative dentist can modify contours as needed to satisfy the patient's phonetic and esthetic demands.

When the desired shape and emergence profile are achieved it should be transfer to the master cast. This may be accomplished with a custom impression coping. The customized impression coping allows the clinician to capture the moulded soft tissue with the appropriate emergence profile onto the master cast. The following technique can be used for transferring the peri-implant soft-tissue emergence profile from a provisional to a final prosthesis. (Fig14 a-h)

1. Remove the screw type provisional restoration with desire emergence profile from the patient's mouth. (Figure 14a)
2. Screw the interim restoration onto an implant analog (048.124, ITI Dental Implant system, Straumann AG, Waldenburg, Switzerland).

3. Insert the implant analog and attached interim restoration into putty type silicon material (Speedex; Coltene AG, Altstatten, Switzerland) to capture the shape of the crown's cervical portion. (Figure 14b)
4. After the setting of the silicon, remove the crown by loosening the occlusal screw. A precise copy of it's cervical shape is captured within the silicone. (Figure14c)
5. Seat a screw-retained impression cap (048.090, ITI Dental Implant system, Straumann AG) on the analog and tighten its screw.
6. Fill the gap between the silicone and the impression cap with auto or light polymerizing resin (Pattern Resin; GC America, Alsip, Ill). (Figure 14d) The result is an individualized impression cap that exactly mimicks the temporary crown's cervical portion. This individualized impression cap not only prevent the peri-implant mucosa from collapsing when the temporary crown is removed, but it also provided ideal support for the mucosa during impression taking and capture the ideal emergence profile within the impression.
7. Screw the individualized impression cap into the implant in the patient's mouth. (Figure 14e)
8. Make an impression with a custom tray and elastomeric impression material.[77] (Panacil, Kettenbach, Eschnenburg, Germany) (Figure 14f) Make the face-bow and interocclusal record and transfer the cast to articulator.[78]
9. Inject a mix of polyvinyl siloxane soft tissue simulating material (Gi-Mask; Coltene/Whaledent Inc., Mahwah, NJ) around the implant analogs in the impression and allow the material to polymerize. Pour a mix of type V dental stone (Prima Rock; Whip Mix Corp., Louisville, KY) into the impression to make a cast. The result will be a definitive master model that precisely mimics the shape of the peri-implant mucosa, and transfer the diagnostic findings and past clinical procedure to the dental technician. Therefore, it is used for the fabrication of the emergence profile of the cervical third of the crown. (Fig 14g) This cast will be used for the fabrication of the definitive restoration in the standard way. (Figure 14h)

Materials for Interim Fixed Restorations

The characteristics of an ideal interim material are as follows:[10]

- Good handling properties: adequate working time, easy molding, rapid setting time.
- Biocompatibility: nontoxic, nonallergic, nonexothermic.
- Dimensional stability during polymerization.
- Ease of contouring and polishing.
- Adequate strength and abrasion resistance.
- Good appearance: translucent, color controllable, color stablility.
- Good acceptability to patient: nonirritating, odorless.
- Ease of adding to or repairing.
- Chemical compatibility with the interim luting agents.

As yet, an ideal interim material has not been developed. A major problem still to be solved is dimensional change during polymerization. These materials shrink and cause

marginal discrepancy[3,79-81], especially when the direct technique is used. Also the currently used resins are exothermic and not entirely biocompatible.

Interim restorative materials can be divided into the following four groups according to composition:[3,10] polymethyl methacrylate (PMMA), polyethyl or butyl methacrylate, micro-filled bisphenol A-glycidyl dimethacrylate (Bis-GMA) composite resin, and urethane dimethacrylate (lightpolymerizing resins). The overall performances of the groups are similar, with no material being superior in all categories. Choosing a material should be based on optimally satisfying the requirements or conditions crucial for the success of the treatment. For example, materials with the least toxicity and least polymerization shrinkage should be chosen for a direct technique. Alternatively, when a long span prosthesis is being fabricated, high strength is an important selection criterion.[10, 82] Clinicians select a product based on factors that include ease of manipulation, cost effectiveness, esthetics, strength, and marginal accuracy.

Materials composition [10]

The material used for fabrication of an interim restoration consists of pigments, monomers, filler, and an initiator, all combining to form an esthetic restorative substance. The pigments are incorporated by the manufacturer so that the set material appears as much like natural tooth structure as possible, with a variety of shades available. Although each of the other ingredients plays a role in the handling, setting, and final properties of the interim restoration, many important characteristics of the material are determined by the primary monomer. The ability of this monomer to convert to a polymer allows the material to set into a solid that is durable enough to withstand the oral environment for the necessary interim period.

Free radical polymerization

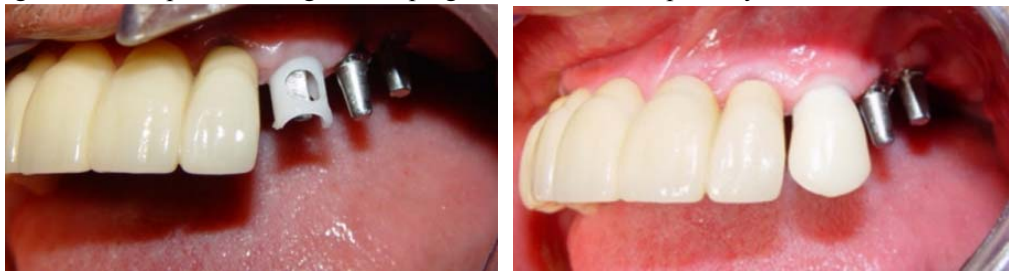
The polymerization process leads to chemical, mechanical, dimensional, and thermal changes that affect the successful use of these materials in dentistry. Because monomers may be unpleasant or even harmful biologically, the chemical conversion of monomer to biologically inert polymer is desirable. Also, if the polymerization process is not properly initiated or if it is prematurely terminated, the resultant restoration may not have adequate mechanical properties and may fail easily or quickly. However, because the density of the polymer is inherently and often substantially greater than that of the monomer, a dimensional contraction occurs during polymerization. The polymerization reaction is exothermic, which causes the material to become hot before it loses its fluidity, and so an additional contraction occurs on cooling of the restoration. If a direct technique is being used, the heat of reaction can cause irreversible damage to nearby pulpal tissues, which may already have been thermally insulted during cavity preparation.

Properties associated with the monomer

The various monomers exhibit different initial and setting characteristics and result in polymers with significantly different properties (i.e., viscosity before setting, exothermic heat of reaction, dimensional change on setting, and strength). In general, the greater the size of the monomer molecule, the less is the exothermic heat of reaction on setting and the lower the physical strength of the set mass.

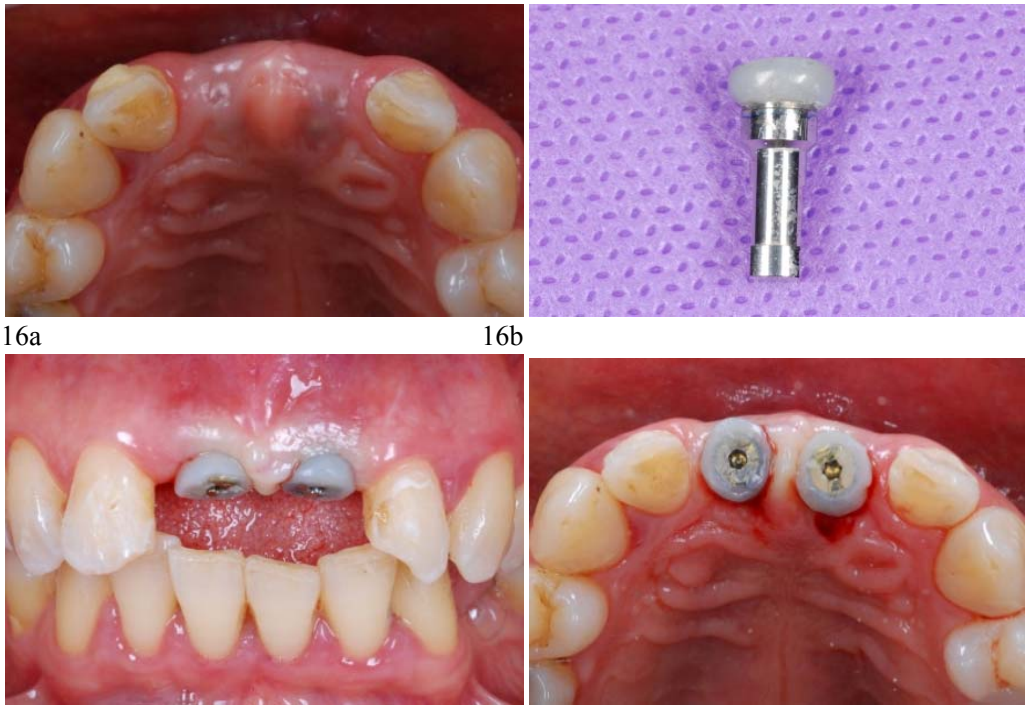
Filler

Although the primary properties of an interim restorative material are determined by the monomer or monomers involved, a decrease in the less desirable setting and mechanical properties is accomplished mainly through the filler. An increase in filler content reduces the relative amounts of exothermic heat and contraction while increasing the strength of the set material. However, too much filler can lead to insufficient handling characteristics before setting, and this impedes mixing and shaping, and it introduces porosity in the set restoration.



15a 15b

Figure 15. a. Cutting impression cap. b. Adding resin to provisional restoration to improve gingival contour and emergence profile.



16a 16b

16c 16d

Figure 16. a. The implants should be uncovered, b. Light cure composite resin added around the healing cap, c. Customized healing abutments in the patient's mouth, d. Occlusal view.

The two most common types of materials utilized for the fabrication of a provisional restoration include acrylic and composite resins. Both can be successfully utilized for the provisional fabrication, and there is no clear clinical superiority of one class of material over

the other. It is obvious that a large number of provisional material brands exist in the dental market. However, between the group and within the group, clinical performance data is not available, and it appears that choice of the specific material is empirically based.

Composite resins (Bis-acryl)

The most popular material for interim restorations today is bis-acryl resins. Many brands are available in the dental market. Examples are Protemp 3 Garant (3M ESPE, St. Paul, Minn.), Integrity (Dentsply Caulk, Milford, Del.) and TempSpan(Pentron, Wallingford, CT)

The advantages of bis-acryl materials are:

- Low heat generation during setting;
- Delivered through a syringe;
- Can be smoothed and polished;
- Can be characterized by modifying color;
- Minimal shrinkage allows good fit;
- High flexural strength.[82]

However, there are some disadvantages:

- High cost compared to other materials.
- Some of the materials are difficult to repair.
- They can not be used for relining the prefabricated acrylic crown.

Polymethyl methacrylate

Acrylic resins were the main interim restorative material before introducing the bis-acryls. In many circumstances this material is still useful. The most well-known brand is Jet (Lang, Wheeling, Ill.). The advantages of polymethyl methacrylate (PMMA) are the following:

- Relatively good color stability over a few weeks;
- Can be smoothed and polished;
- Can be characterized;
- Low cost;
- Easily repaired.

Disadvantages are the following:

- Highly exothermic reaction; the high temperature generated during the setting of PMMA can be traumatic to the dental pulp if not dissipated with cool water and air during the polymerization stage.
- Shrinkage is relatively high.
- The odor of the material is objectionable to many patients.

Polyethyl methacrylate (PEMA)

PEMA are similar to PMMA but have some positive and negative differences. Well-known brands are Trim II (Harry J. Bosworth Co., Skokie, Ill.) and Snap (Parkell, Farmingdale, NY). Advantages of PEMA include:

- Lower exothermic reaction than PMMA, but higher exotherm than bis-acryl resins;
- Can be smoothed and polished;
- Can be characterized;
- Low cost;
- Easily repaired.

Disadvantages of PEMA are:

- Bodily discoloration over a two-week period;
- Odor of the material is objectionable to some patients;
- Weaker than PMMA and bis-acryl materials.[82]

Techniques for Making Implant-Supported Interim Restorations

I. A method of providing a provisional restoration for an ITI solid abutment.[83]

Procedure

1. Make a preliminary irreversible hydrocolloid (Alginoplast; Heraeus Kulzer, Dormager, Germany) impression and form a stone cast (Moldano; Heraeus Kulzer) of the solid abutment. Complete a diagnostic wax-up of the anticipated final restoration over the stone abutment.
2. Make an index of diagnostic wax-up with putty-type impression material (Rapid; Coltene AG, Altstatten, Switzerland).
3. Place an ITI impression cap over the abutment to engage the implant shoulder (Figure 15a). With a sharp surgical blade, cut away any part of the cap that interferes with contours of the diagnostic restoration.
4. Apply petrolatum (Vaseline; Tyco Healthcare, Montreal, Quebec, Canada) on the abutment and surrounding gingival tissues. Mix desired tooth color auto-polymerizing resin and fill in the index.
5. Place the index over the trimmed impression cap and its abutment. When the resin has set, remove the provisional restoration and add resin to improve gingival contour and emergence profile (Figure 15b).
6. Adjust the occlusion and polish. The impression cap will engage the undercuts beneath the implant shoulder and the resin provisional will fit the flat surface of the abutment, so there is usually no need to use provisional cement.

II. Prosthetic-guided soft tissue healing with customized healing abutment

Procedure

1. Uncover the implants (Regular platform, Nobel Replace; Nobel Biocare, Yorba Linda, Calif) with appropriate soft tissue punch (32Z2002, Nobel Replace; Nobel Biocare). (Figure 16a)
2. Measure the thickness of soft tissue and screw a healing abutment (29439 Nobel Replace; Nobel Biocare) with the same height on an implant replica (29500, Nobel Replace; Nobel Biocare).
3. Add light cure composite resin (Filtek Supreme; 3M ESPE) around the healing cap. Try the customized healing abutment until the correct emergence profile achieved.(Fig 16b-d)

III. Making an interim restoration with prefabricated acrylic crowns

Procedure

1. Screw the proper temporary abutment (24180, Astra Tech Implants; Mölndal, Sweden) into the implant (24512, Astra Tech Implants) and prepare it to have enough space for provisional crown. (Figure 17a,b)
2. Remove the prepared temporary abutment from the patient's mouth and apply some opaque resin on it. (Figure 17c)
3. Make a hole in the lingual surface of a prefabricated acrylic crown to have access for temporary abutment screw. (Figure 17d)
4. Fill the perforated crown with acrylic resin and put it on the prepared temporary abutment in the patient's mouth. Keep the access hole free of the resin with wax.
5. Unscrew the crown and attached and tighten the abutment on fixture replica (22397, Astra Tech Implants).
6. Fill the gaps between the gingival part of the crown and abutment with acrylic resin. (Figure 17e)
7. Add or cut the gingival portion of the crown to achieve proper emergence profile. Finish and polish the restoration margin and screw the abutment and interim prosthesis into the implant. (Figure 17f)

IV. Converting the patient's existing denture into screw-retained provisional fixed hybrid prosthesis.

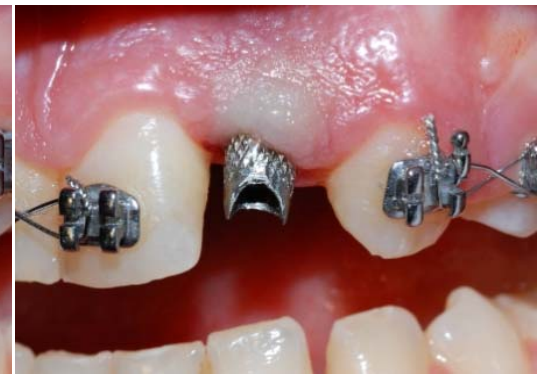
Procedure

1. Screw impression copings (29488-92-94, Nobel Replace; Nobel Biocare) onto the implants (Regular platform, Nobel Replace; Nobel Biocare) and modify patient's existing mandibular denture to accommodate the impression copings. (Figure 18a,b)
2. Make an open tray impression with polyvinyl siloxane material. (Fig 18c)

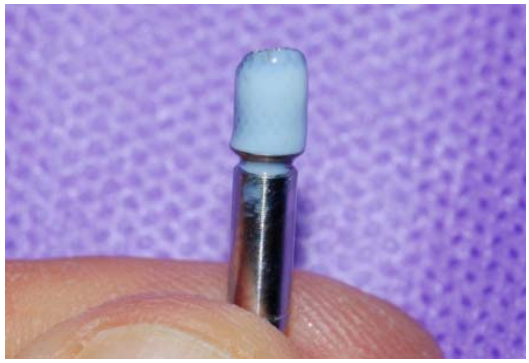
3. Pour the cast with type III stone while the implant replicas (29500, Nobel Replace; Nobel Biocare) are screwed into the impression copings embedded in the impression material.
4. Make an index from the position of the denture teeth with putty-type impression material. (Figure 18d)
5. Place temporary abutments (29035-37-39, Nobel Replace; Nobel Biocare) onto the implant analogs, prepare them to accept the perforated denture and paint them with opaque resin. (Figure 18e)
6. Place the denture on the stone cast with the aid of index and attach the temporary abutments to denture with auto polymerizing resin. (Figure 18f)
7. Remove the excess denture base material from the tissue surface of the denture, finish and polish the denture. (Figure 18 g)
8. Screw the interim prosthesis to the implants. (Figure 18h,i)

This technique may also be used in immediate or early loading implant protocols using chairside technique.

17a

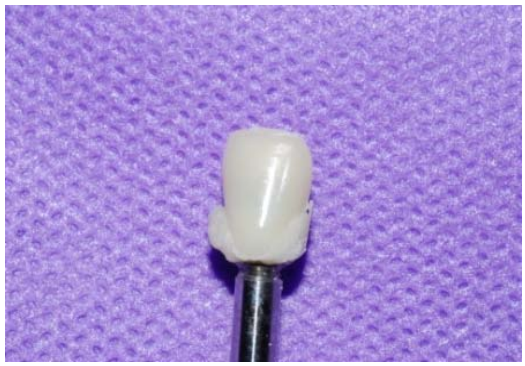


17b



17c

17d


17e

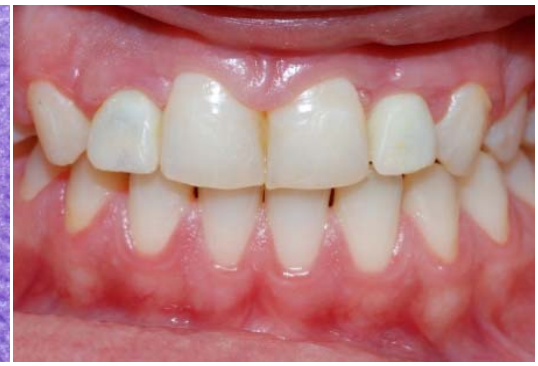
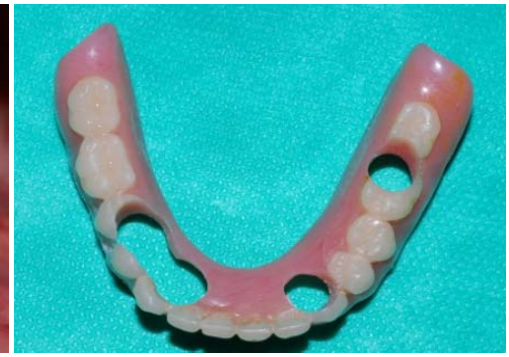
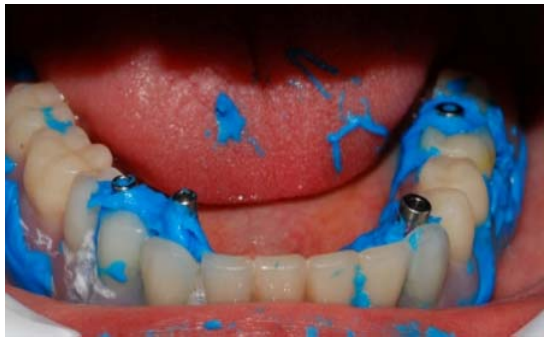
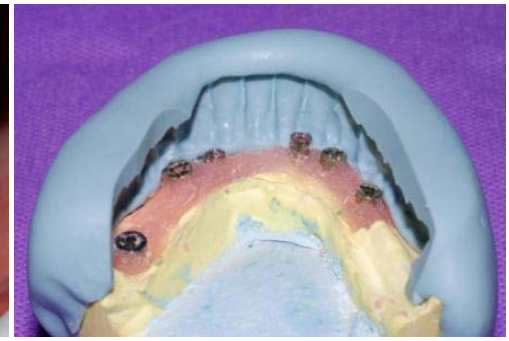

17f

Figure 17. a, b. Temporary abutment screwed into the implant and prepares to have enough space for provisional crown, c. Opaque resin applied on the prepared temporary abutments, d. Hole in the lingual surface of a prefabricated acrylic crown for accessibility of abutment screw, e. The gaps between the gingival part of the crown and abutment filled with acrylic resin, f. Screw-retained provisional fixed prosthesis.

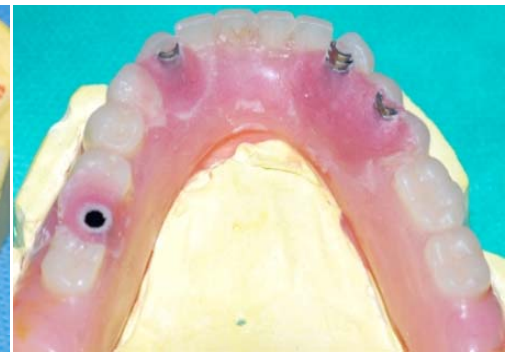

18a

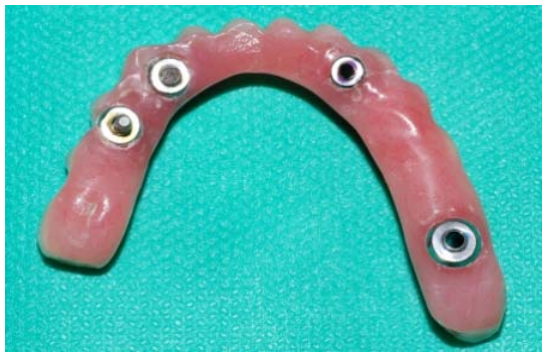

18b


18c


18d


18e


18f


18g

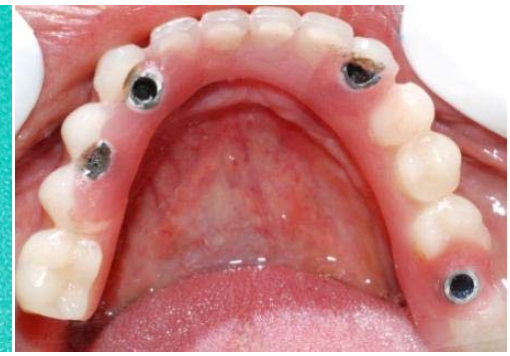

18h

Figure 18. (*Continued*)

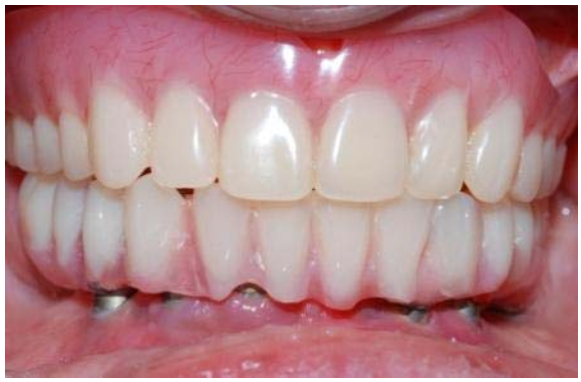

18i

Figure 18. a. Impression copings screwed into the implants, b. Patient's existing mandibular denture modified to accommodate the impression copings, c. Open tray impression, d. Index from the position of the denture teeth, e. Temporary abutments prepared and painted with opaque resin, f. The temporary abutments attached to denture with autopolymerizing resin, g. Tissue surface of the denture, h,i. Screw-retained hybrid interim fixed prosthesis in the patient's mouth.

CONCLUSION

This chapter discussed the importance of the interim prostheses in implant dentistry. Different types of interim restorations and interim materials were also discussed. The provisional phase is one of the most critical stages in implant therapy. Available options for interim implant prostheses include various types of removable prostheses, tooth-supported fixed prostheses, and implant-supported provisional restorations. The restorative dentist should consider eight criteria in selecting the most appropriate type of interim prostheses including the esthetic potential, patient comfort, treatment time, cost, occlusal clearance, ease of removal, durability, and ease of modification. The expectations of the patients in implant therapy are high for both function and esthetics. The restorative dentist should choose the provisional restoration which is most suitable for a specific patient's situation and satisfy the patient's expectations. With using favorable interim prosthesis patient realize the benefits of implant therapy and accept the extensive implant restorations.

Construction of provisional restoration may take up more time and effort but they may save time and expense at subsequent appointments, hence resulting in better definitive restorations.

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Chapter 16

DENTAL IMPLANTS IN ORAL CANCER PATIENTS

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ABSTRACT

The treatments of oral cancer prevent many patients from bearing conventional prostheses. These patients are, thus, candidates for oral rehabilitation with osseointegrated implants. Anatomical and histological therapy-induced changes decrease their success rate.

Ablative surgery leads to anatomical modifications, with loss of keratinized tissues, loss of bone and dental anchorage.

Reconstructive surgery aims to restore the oral anatomy but the quality and the volume of soft tissues do not always allow a functional prosthesis. The use of microvascular flaps for reconstructive surgery has transformed prosthetic rehabilitation after large ablative surgery. Their poor resorption and their success allow the use of dental implants supporting a fixed or movable prosthesis.

The most important adverse effect of external radiotherapy concerns bone, as it decreases its healing potential through hypocellularity and hypovascularization, which lowers the success rate of the osseointegration.

The factors of osseointegration which we can control are the site of implantation, the waiting period between radiotherapy and the implant surgery, the irradiation dose and the conditions of loading. Animal studies and clinical series gain widespread backing: osseointegration is possible in radiated bone and in fibula, scapula or iliac grafts, but it takes a longer time to obtain an intimate contact between the implant and the bone. The implant surgery can be planned before or after radiotherapy. The success rate decreases with radiation doses around to 50 to 60 Grays. The preferential sites for implantation are the anterior mandible or maxilla, which present a lower dose of radiation and an easier access (less concerned by the limitation of the mouth opening).

The success rate of dental implants in microvascular flaps is around to 80-90%; nonetheless there is a difference between the rate of osteointegration and the percentage

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of loading, due to the new anatomy and the orientation of the implants. Moreover the management of soft tissues is often difficult.

This will be avoided by the use of local or locoregional grafts of keratinized tissues and the planning of the implants placement thanks to surgical guides, which development is the next evolution in the rehabilitation of oral cancer patients.

INTRODUCTION

Oral cancers and their treatments lead to major alterations of the oral anatomy and physiology, which prevent many patients from receiving conventional prostheses.

The tumour quickly extends through the epithelium, the conjunctive tissue and the bone. Ablative surgery removes the tumour and a surrounding margin of normal tissues to ensure complete resection. The anatomy of the mouth is severely altered and oral functions are modified. Radiotherapy, which is usually given after surgery, causes histological modifications of both soft and hard tissues.

The combination of treatments results in poor prosthesis stability and retention, which makes the patients candidates for implant-retained oral rehabilitation. Nonetheless, side effects also affect bone osseointegration. Irradiation dramatically decreases bone vascularisation, resulting in a reduced success rate of osseointegration. The risk for osteoradionecrosis must be considered by the implantologist and special precautions are required.

A. SEQUELAE OF ORAL CANCER TREATMENTS: SURGERY

I. Anatomic Considerations

Ablative surgery has anatomical and functional consequences for rehabilitation.

Tumours of the mobile tongue are surgically treated by glossectomy. The suture of the tongue to the oral mucosa leads to direct abutment of the tongue to the gingiva, decreased tongue mobility and obliteration of the gingivolabial and gingivolingual sulci. Surgical resection of a cancer of the lips or of the cheek impairs the prosthetic foundation and compromises both function and aesthetics.

Pelvectomy is often associated with mandibular resection; the procedure is called pelvimandibulectomy. In case of mandibulectomy, the craniofacial anatomy is restored by means of a myocutaneous flap. The anatomical sequelae of the procedure are similar to those of partial glossectomy. In patients undergoing interruptive surgery, the mandibular arch can be restored by using an osteomyocutaneous flap.

The maxilla is generally involved in patients with gingival or palatal tumours, or secondary to ethmoïdal, sinusal or cutaneous carcinomas [1]. Ablative surgery results in oro-sinusal communication, which causes severe dysfunctions. The cavity is easily covered by a prosthesis or by insertion of a simple plate with an obturator. Reconstructive surgery can be proposed [2] as second-line treatment, but it is ineffective for local tumour control and follow up, and results are seldom satisfactory. Moreover, the adaptation of a prosthesis is very uneasy in case of surgical reconstruction.

The placement of a conventional prosthesis is often possible to restore the maxilla, but it is not suitable for the correction of post-surgical mandibular defects. Primitive tumours of the mandible are rare but the bone is prone to invasion by cancers of the gingiva, of the floor of the mouth or the intermaxillary commissure.

Interruptive and non interruptive mandibular surgeries must be clearly distinguished.

Non interruptive mandibular surgery is used for patients with gingival or pelvibuccal carcinomas, without bony invasion, involving the symphysa or the lateral part of the horizontal ramus of the mandible. Loss of bone volume leads to bone weakening, increased risk of fracture and vertical discrepancy with the residual mandibular arch and the remaining teeth. [3]. Reconstruction is provided by myomucosal flap transfer; one of the most frequently used method is the FAMM (*facial artery musculomucosal*) flap [4,5,6].

Interruptive mandibular surgery can also involve the anterior or lateral parts of the mandible, rarely the condyle or the temporo-mandibular joint. Anterior resections are quite systematically reconstructed since the residual mandibular stumps are deviated inward, leading to major functional troubles. Use of osteomyocutaneous flaps taken from the fibula or the iliac crest is recommended. Lateral mandibular interruption causes lateral deviation of the remaining arch and retroposition of the chin, due to muscular retraction. The residual hemimandible is deviated to the ill side. Reconstructive surgery can be proposed, using either a myocutaneous or an osteomyocutaneous flap. Musculocutaneous flaps like pectoralis major or dorsalis major flaps can restore soft tissues but do not correct lateral deviation. Osteomyocutaneous flaps allow reconstruction of both soft tissues and the mandibular arch and restore pseudo-physiological mandibular movements.

II. Functional and Occlusal Consequences

Partial or subtotal glossectomy may impair tongue mobility. Contralateral hypertrophy is frequent; it offsets the hypofunction, but precludes any prosthetic rehabilitation. Moreover, it is always associated with sensitive and motor nerve damage. Speech troubles are unavoidable.

Prosthetic rehabilitation is possible in only 1 out of 2 patients after non interruptive pelvimandibulectomy. Only 20% of the patients recover correct chewing function [7].

Mandibular non interruptive surgery has few occlusal consequences. A severe vertical discrepancy is generally reported between the site of the tumour and the remaining mandible. Moreover, flap reconstruction methods like FAMM flap allow the restoration of non keratinized, thick and mobile oral soft tissues. These tissues reduce the retention and stability possibilities of the future prosthesis. They are also fragile and therefore susceptible to prosthetic traumas. However, the prosthetic rehabilitation remains possible.

Interruptive mandibular surgery leads to severe dysfunctions. The chin is deviated and retropositioned. The remaining mandible is deviated inward. Masticatory cycles are unpredictable, slower and non reproducible [1]. Patterns are no longer drop-shaped but asymmetrical, with increased lateral freedom. Maximal intercuspidal position is instable, with approximately 50% loss of masticatory force [8]. Occlusal forces transmitted to the bone are less effective and much less evenly distributed. Labial incompetence is frequent, leading to salivary incontinence. Troubles are observed during mouth opening and closing and at swallowing [2]. Non physiologic position and function of the remaining temporo-mandibular join tend to cause local inflammation and pain. Preservation of the temporo mandibular joint and of the coronoid process on the operated side seems to limit troubles; the best result is

usually obtained after restoration of the mandibular arch by an osteo-musculo-cutaneous flap [9], a procedure which decreases lateral deviation. Nonetheless, biomechanical conditions remain specific and evolve during the healing process and throughout functional mandible recovery [10]. Flap reconstruction involves the placement of 10 to 16 mm of bone height and soft tissues. The grafted tissues are much thicker than the gingiva, they are not fixed to the bone by the periosteum and not keratinized, and they generally do not allow the reconstruction of the vestibule. Vestibuloplasty is often necessary for reconstruction of the labio-gingival and gingivo-lingual sulci. Thinning of the flap is often required. Reychler [11] has observed abnormal occlusal relationships between the maxilla and the mandible after mandibular reconstruction, a vertical discrepancy between grafted and non grafted zones, and a lack of vestibule and granular soft tissue at the implant recipient site, due to a lack of keratinized tissue.

The oro-sinusal communication created by ablative surgery of the maxilla leads to difficulties in swallowing, speech and mastication, and to nasal regurgitation of solids or liquids. Immediate correction of the problem is mandatory.

III. Aesthetic and Psychological Consequences

In the mandible, the most serious aesthetic sequelae are caused by peripheral ablative surgery:

- After surgery for labial or vestibular tumours: microstomia and lack of soft tissue laxity (lips and cheeks).
- After lateral interruptive surgery: flattening of the mandibular angle, laterodeviation of the chin towards the operated side, retrognathia, retrusion and tightening of the lower lip.

In the maxillary, surgery is often associated with loss of the oral mucosa of the cheeks, causing lagophtalmos, epiphora and flattening of the cheekbone.

These aesthetical consequences are associated with speech and swallowing troubles and may lead to social isolation. Very simple everyday activities like visiting or phoning friends or going to the restaurant become insurmountable obstacles. The quality of life of oral cancer patients is thereby severely altered.

Surgery results in severe alterations of the oral anatomy. Prosthetic rehabilitation must take different problems into account:

- loss of mandibular support,
- lack of vestibule and/or gingivo-labial sulcus,
- cicatricial bridles,
- non keratinized tissues,
- reduced prosthetic corridor,
- decreased tongue mobility.

Stabilisation, retention and suspension of the prosthesis are compromised in a large number of patients. Mechanical retention aids, like endosseous implants, can be used to improve functional and aesthetic rehabilitation.

B. Sequelae of Oral Cancer Treatments: Radiotherapy

Radiotherapy consists in using ionising radiations to cause cell death. External radiotherapy is frequently used for treating oral cancer patients. The ionising particles used may be electrons, photons or ions. The ionisation of intracellular water molecules leads to the creation of free OH- radicals which are toxic to the DNA. Since the healing potential is lower for tumour cells that for healthy cells, the cell-killing effect is more important on the tumour site. Nonetheless, healthy cells are also altered by the irradiation. To preserve normal surrounding tissues, new techniques allowing a better delineation of the target volume, conformational radiotherapy and intensity-modulated radiotherapy, have been developed. The usual dose delivered to oral tumours ranges between 55 and 70 Gy, by means of 1.8 to 2 Gy fractions given 5 days a week over a period of 5 or 6 weeks).

Adverse effects of radiotherapy on healthy tissues are unavoidable and, because of their persistence over time, it is necessary to consider previous treatments when planning dental rehabilitation.

I. Masticatory Muscles

Retractile myositis appears progressively in the weeks following radiotherapy. It evolves into fibrosis, possibly associated with a limitation of the mouth opening if mecanotherapy is not provided early [12]. The limitation is increased when patients receive irradiation to the masseter, the pterygoïd or the digastric muscles [13].

II. Skin and Oral Mucosa

Epithelitis appears after 3 to 4 weeks of radiotherapy, which corresponds to a dose of 30 to 40 Gy. The skin is erythematous, with desquamation zones, like burned skin. The hair follicles are destroyed, leaving the irradiated zones smooth and hairless. The depilation is permanent.

On the oral mucosa, mucositis appears at doses above 10 Gy (corresponding to 5 to 8 days of radiotherapy). A diffuse erythema is observed, with sensations of burning and dryness of the mucosa. Above 40 Gy, multiple ulcers appear in the treatment area, promoting secondary infections. At the end of the irradiation, fibrosis leads to decreased soft tissue elasticity. The oral mucosa remains sensitive to any contact [14, 15, 16].

Radiotherapy compromises the microvascularization of the oral mucosa and of the gingiva. Collagen aggregates are produced, and the deposits cause vascular thrombosis and ultimately ischemic tissue necrosis [16]. The mucosa is atrophic, less flexible and more sensitive to irritation; this hypersensitivity is exacerbated by the hyposalivation induced by radiation exposure. These adverse effects appear for doses above 50 Gy.

Hypogeusia is a common complication after the 3rd or 4th week of irradiation; it is due to local alterations of the taste buds and decreased salivary excretion. This complication is reversible and normal taste function is restored about 2 months after the end of treatment.

III. Salivary Glands

Damage to the salivary glands occurs early in the course of treatment, even if the clinical onset of symptoms is belated. From 10 to 20 Gy, irradiation induces histological

modifications of the serous acini. Effects on the mucous acini are seen at higher doses. Three types of alterations are observed on the glandular parenchyma [13]:

- formation of thick sheathes of conjunctive tissue,
- intralobular sclerosis,
- fibrosis.

These alterations lead to decreased salivary production and qualitative modifications.

Ten to 12 weeks after irradiation at 50 to 70 Gy, the number of serous acini decreases, the collector canals are dilated and a distortion of the acini is observed, which leads to atrophy and fibrosis [16]. From 60 Gy and 6 months after irradiation, a fibrosis and a dilatation of the excretory canals of the mucous acini are observed.

Salivary pH and HCO3- rates are decreased, whereas sodium, natrium, chlorine, calcium, magnesium and salivary protein concentrations are increased [16]. These alterations cause increased saliva viscosity, associated with a spumous and sometimes coloured aspect, an environment conducive to the development of microorganisms.

Partial recovery is possible over a period of 18 months to 3 years for doses lower than 50 Gy.

The clinical consequences of radiotherapy to the salivary glands are xerostomia, speech and swallowing disorders, increased sensitivity of the oral mucosa and tongue, risk of decay on the residual teeth (which can be prevented by fluorine prophylaxis) and increased risk of opportunistic infections (candidiasis, for instance).

IV. Bone

Bone is particularly sensitive to radiation; this material is 1.8 times denser than soft tissues and thus absorbs more ionizing radiation [12, 16, 17, 18].

The effects of radiotherapy on bone have been studied in animals. Marx [19] has described the « 3H » triad: hypoxia, hypocellularity, hypovascularisation.

Granström [20, 21] has shown that, for doses higher than 11 Gy, the formation of new bone is reduced by 65 to 75% and the regenerative potential of cells is altered for many generations. Microscopically, the number of osteocytes and osteoblasts alive after radiation is low; and the secretion of extracellular matrix proteins by residual cells is decreased [22]. The apoptosis of osteoblasts begins at the dose of 20 Gy. Osteoclast lysis continues, thus upsetting the formation/resorption balance. The mitotic index of osteoprogenitor cells at the growing ends of bone is decreased and the cellular infrastructure is altered [22]. Vascularisation is altered in endothelial cells and in the vascular wall as a whole [24]. Small arteries are more sensitive to radiation than venules. Acellular structures and collagen are retained in the intima and media and the vascular walls become thicker, which causes stenosis and an increased risk of thrombosis. Some authors think that cellular alterations occur secondary to vascular alterations [25]. For others, alterations of the osteoblasts appear first. A study on mice [26] has shown an effect of radiations on osteoblastic growth factors implicated in bone remodelling and healing, which induces an inhibition of osteoblast secretion, without any relation to hypoxia. This dystrophy, even if asymptomatic, weakens the bone and increases its sensitivity to trauma or infection, because of decreased healing potential. Although a slight

recovery of the healing potential can sometimes be observed after longer periods, alterations are permanent.

Nonetheless, bone has limited healing potential: neoangiogenesis has been observed 10 weeks after irradiation at 65 Gy, indicating the presence of a residual osteogenic potential.

The susceptibility to radiations is higher in the mandible than in the maxilla. This can be explained anatomically, since the vascularization of the mandible is terminal, whereas the maxilla is surrounded by a rich and dense vascular plexus that allows substitute vascularization.

The most dreaded complication on bone is osteoradionecrosis (ORN). Exogenous osteitis develops in previously irradiated bone and is characterized by necrosis and exposition of the bone which, without treatment, leads to extension and fracture. Clinical features are pain of variable intensity, homolateral otalgia, suppuration, limitation of mouth opening, and sometimes orostomy. Radiological features are a radioclear, inhomogeneous, eroded bone, with loss of trabeculations and inconstant bone sequestration. ORN is more frequent on the mandible that on the maxilla and generally develops on the lingual side of the horizontal branch of the mandible, in the molar zone. Its frequency is evaluated from 1 to 35% according to the different authors [27].

To explain ORN pathophysiology, Dambrain [28] refers to the « 2I » theory, infection and ischemia, which is not far from the "3H" combination of hypoxia, hypocellularity and hypovascularisation described by Marx [19]. Nonetheless, infection appears secondary and is not the cause of necrosis. Infection of the necrotic bone occurs very quickly after exposition to the oral microflora. Recently, a theory based on the fibroatrophy of the irradiated bone has been exposed. According to this theory, the irradiation would generate a progressive fibrosis of the concerned tissues associated with decrease of the reparation potential [29, 30].

Aggravation factors are as follows:

- Treatment dose, fractionation, dose staggering [31], association with other therapies like interstitial radiotherapy or chemotherapy,
- Tumour site and volume, and bone involvement,
- Initial dental status: the presence of residual teeth increases the risk of osteoradionecrosis,
- Alcohol and tobacco intoxication,
- Clinical status of the patient.

ORN can appear early (within 6 months following the end of radiotherapy) or more often later (but it usually appears later). It is either spontaneous or induced by a trauma, generally of dental origin (extraction, prosthetic wound, placement of dental implants).

Depending on the stage of the ORN [27], the treatment can be medical (antibiotics) or surgical (curettage or mandibular resection –either interruptive or non interruptive).

Hyperbaric oxygen therapy (HBO) can also be proposed to prevent or cure ORN and reduce hypoxia. Its aim is to increase the partial pressure of oxygen in the tissues by administration of pure oxygen to the patient placed in a hyperbaric chamber. The most important effects of HBO are:

- decrease of the oedema and vasoconstriction,
- maintenance of tissue oxygenation,
- increased fibroblast proliferation and collagen production,

- enhanced osteoblast and osteoclast function.

HBO increases osteoclastic activity and osteoblastic synthesis, thereby stimulating osteogenesis. According to Johnsson et al. [32], it helps increase post-radiotherapic bone maturation.

The indications for HBO are:

- the need for increasing the radiosensitivity of cells before radiotherapy,
- the prevention and treatment of ORN,
- for some authors, the preparation of implantation in irradiated bone.

The contra-indications are:

- previous thoracic surgery or pneumothorax,
- lump infections or diseases,
- arterial hypertension,
- epilepsy,
- recent auricular surgery,
- sinusitis,
- claustrophobia.

Complications of HBO are tympanic rupture, decompression injury, and gaseous embolism.

The treatment consists in the administration of 100% O2 at 2.4 atm during 90 minutes with 5 minute-breaks every 20 minutes, once or twice a day.

The widespread use of HBO should not be recommended regarding the paucity of controlled trials and the lack of unified assessment of the improvement of symptoms. It is commonly used as an adjunct to surgery for the treatment of osteoradionecrosis.

C. Implantology in Irradiated Bone

Anatomical conditions do not always permit functional prosthetic rehabilitation; lack of dental anchorage, dysfunctions of the tongue or lips and reduction of the prosthetic corridor may affect the stability of the prosthesis.

Intraoral endosseous implants provide firm bony anchorage for the prosthesis and make it able to resist adverse occlusal and muscular forces. Some indications, such as the treatment of totally edentulous patients after interruptive mandibular surgery, seem self-evident; implants are crucial for stabilization of the prosthesis and consequently of the residual hemimandible [33].

However; the management of radio-induced histological alterations of bone and soft tissues requires some special precautions.

I. Specificity of Osseointegration in Irradiated Bone

General considerations

Even if anatomical conditions may complicate the implant surgery, the main limiting factor is actually the radio-induced weakening of the bone.

Implantation in radiated bone has not always been considered clinically useful. In 1987, Benoist [34] contra-indicates implantation in irradiated areas. But the clinical demand and the advances of radiotherapy have led many clinicians to use these techniques.

The first models developed for osseointegration in irradiated bone date back to 1986 [35, 36]. The peri-implant reactions observed in rabbits were decreased bone formation, increased bone resorption, and a decreasing number of capillary vessels. A unique dose of 15 Gy has been reported to decrease bone formation by up to 72%.

Johnsson et al.[32] have shown that peri-implant bone healing increases with an increased waiting period between radiotherapy and implantation. Most of the authors agree that a waiting period of 8 to 12 months is necessary before dental implantation [36].

In 1998 [37], implantation and loading were tested in beagle dogs irradiated at 40, 50 and 60 Gy. All implants were lost in the group irradiated at 60 Gy, showing that failure and peri-implant bone resorption increase with the dose of radiotherapy. In 1999, a histologic study in a dead patient showed that similar osseointegration results are obtained in irradiated and in non irradiated bone [38].

Other animal studies [22, 23] have shown that a smaller torque is needed to unscrew an implant in irradiated than in non-irradiated bone.

Verdonck et al. [39] have observed an increased bone mineral density 3 months after irradiation, whereas vascularisation is decreased, which corroborates the hypothesis of bone sclerosis. This explains the decreased implant stability and increased rate of failure reported in irradiated bone.

A summary of current knowledge by Ihde et al. [40] confirms that:

- The risk of loosing an implant is 2 to 3 times higher in irradiated than in non irradiated bone for intra-oral implants and 12 times higher for extra-oral implants;
- The dose of radiotherapy influences osseointegration; better results are obtained with doses under 50 Gy;
- Implantation can be done before or after radiotherapy, with a waiting period of at least 4 months. If the implant is placed before radiotherapy, the scattering effect is weaker when high energies are used. An area of about 1 mm around the implant is involved.
- The mandible is the best site for implantation.
- The type of implant does not influence the success rate.
- Hyperbaric oxygen therapy has not proved effective. Some authors have reported that 20 sessions before and 10 HBO sessions after implantation increase the quality of healing and shorten the healing period. For others, HBO efficiency is highly questionable.

For patients receiving dental implants, Larsen [41] proposes to administer 20 sessions of HBO before implantation and 10 afterwards. According to Chen et al. and Johnsson et al.

[42], HBO enhances bone formation and bone-implant contact. Granström [20] reports 53.7% failure without HBO and 13.5% with HBO. On the other hand, some authors do not recommend the use of HBO as it does not seem to increase success rates: 97.5% success without HBO for Andersson et al. [43] and 99% for Eckert [44]. Furthermore, Keller [31] advises against the use of HBO for dental implants on the ground that the same result can be obtained with a longer waiting period.

Nowadays HBO is not systematic and there is no consensus on its use in patients undergoing dental implantation. Nonetheless, its general indication for the treatment and prevention of ORN remains valid.

Success rates

The most difficult task, in both clinical series and everyday practice, is to evaluate the dose of radiotherapy at the site of implantation. Most authors have presented series of dental implants in bone « presumably » irradiated because since the precise dose at the implantation site is impossible to establish.

The rate of osseointegration is excellent, not far from that of non–irradiated tissues, ranging from 62.5 to 90% [45, 46, 47, 48]. It is clearly dose-dependent and severely decreases with doses higher than 60 Gy.

In the mandible, success rates are about 87.6% for Watzinger et al.[49], 94% for Arcuri et al.[24] and 97.8% for Andersson et al.[43]. For Wagner et al.[50], the success rate for osseointegration is 97.9% at 5 years and 72.8% at 10 years. The overall success rate reported on microvascular free flaps is quite similar [51, 52].

In the maxilla, results are rather different: Niimi et al.[45] have observed 1.8% failure in the mandible and 28.8% failure in the maxilla. Rates of 4.2% failure in the mandible and 23% in the maxilla have also been reported in another study [46]. Nonetheless, because of the very limited number of implants placed in irradiated maxilla, results are not statistically interpretable.

The good success rate obtained on microvascular free grafts can be explained by the fact that, in most cases, the vascularisation of the graft is safe and not altered by radiotherapy since radiotherapy is given before grafting.

Few studies have considered the conception and the success rate of the prosthesis itself [40, 53, 54].

Protocols for implantation

The implantation protocol is quite similar for irradiated and non-irradiated patients. Nonetheless, patient selection in case of previous irradiation is much more important. Contra-indications to the procedure are:

- poor prognosis,
- poor general status,
- recurrence of the tumour,
- secondary localization or metastases.

Remission must have been observed for at least 6 to 12 months and the radiation dose should not exceed 55 to 60 Gy.

If clinical and radiological examinations are similar to those in non irradiated patients, the following points must be given particular attention:

- degree of mouth opening (as radiotherapy induces fibrosis of the masticator muscles),
- mobility and sensitivity of the tongue,
- oral functions,
- salivary flow,
- number and status of residual teeth,
- addictive habits; most head and neck cancer patients are addicted to tobacco and alcohol. Weaning is recommended and must be maintained for candidates to implantation.

The final decision for treatment is multidisciplinary; head and neck surgeons, oncologists and radiotherapists should be involved in the decision-making process.

The surgical phase itself is generally performed under general anaesthesia to decrease the risk of infection and to preserve vascularisation. Local or loco regional anaesthesia can be proposed in some cases.

After implant placement, systematic antibiotic prophylaxis is used until complete mucosal healing. The prosthetic phase is started 4 to 8 months after surgery [50].

Considerations about radiotherapy and dental implantation

In most series, implants are placed after completion of curative treatment, including surgery and radiotherapy. Implantation is therefore performed in more or less irradiated bone, (according to the site of implantation and the area and dose of irradiation). To enhance the healing potential, authors recommend a longer waiting period after the end of radiotherapy. According to Marx and Johnson [25], the number of alterations in both soft tissues and bone is high in the first 6 months after radiotherapy, decreases until 18 months after the procedure, then starts increasing again afterwards. Most authors recommend a waiting period of at least 6 months (6 to 24 months) after radiotherapy [16, 45].

In several series, implants have been placed before radiotherapy. The question that arises is whether irradiation of metallic elements poses a risk for surrounding tissues and for osseointegration.

Two situations should be considered:

- either the patient has had implants for a long time and these implants are fully osseointegrated at the time of radiotherapy,
- or implant placement has been planned during ablative surgery or between ablative surgery and radiotherapy.

Implantologists may question whether it is worth taking off pre-existing implants before radiotherapy. Indeed metallic elements cause the reflection and diffusion of radiation, which modifies the dose distribution. These secondary emissions must be considered when implants are within the radiation field.

Thatcher [55] has shown that Cobalt-60 radiation is increased by 25% in front of the implant and decreased by 15% behind the implant, which could cause necrosis in front of the implant and under irradiation of malignant tissues placed behind the implant. The overall increase of dose at 1 or 2 mm from the implant or more is negligible. For other authors, the dose increase is 15% [56].

For implants placed long before radiotherapy, osseointegration is not compromised if it is complete at the time of radiotherapy and if the thickness of the tissues is sufficient, but the radiotherapist must account for the presence of implants in the radiation field. Suprastructures should be removed during radiotherapy, and implants be re-submerged, especially in case of important rehabilitations with metallic structures [57, 58].

If implant placement is planned between ablative surgery and radiotherapy or during ablative surgery, a waiting period should be observed. Immature bone is more sensitive to radiation; therefore a waiting period of at least 6 to 8 weeks should be established. Nonetheless, in spite of increased bone resorption and weakness of the peri-implant zone, success rates are quite good: 87.5% success in the mandible for Granström [57], 75 to 90 % in other studies.

The main advantage of the method is a shorter time to oral rehabilitation after treatments; the con is that patient prognosis is not known at the time of implantation.

II. Limits of Implantology in Oral Cancer Patients

1. Site of Implantation and Radiation Dose

The success rate is dose-dependent and is more important in the mandible than in the maxilla.

Most published clinical series fail to indicate the dose received at the implantation site. Some do not even mention the initial diagnosis which could help estimate the radiation dose. Others include implants placed in non irradiated bone, which increases the global number of implants considered (and probably the osseointegration success rate as well).

According to Colella et al.[59], no implant failure is to be feared at irradiation doses lower than 45 Gray. Smolka et al. [54] have observed early complications in 41.5% of the 56 patients studied, only in those who had been irradiated.

2. Evaluation of the Prosthetic Result

Studies on the prosthetic outcome of implantation and the restoration of oral function are quite recent and the results reported raise many questions:

- In a series of implants placed after microvascular fibula flap reconstruction, Iizuka et al. [53] report the functional loading of 23 implants out of 37, in spite of a 100% osseointegration rate. No explanation is given for the unloading of one third of the implants.
- In a French study [52] of dental implants on free fibula flaps, 3 implants out of 70 were found not loaded in spite of good osseointegration. The authors explain this result by the bad positioning of the implant or the flap, which would have

led, if loaded, to repetitive local trauma of the mucosa and to unsatisfactory prosthetic control.

- Smolka et al. [54] demonstrate a statistically significant difference between implant success rate (92%) and prosthetic success rate (42.9%). This difference could be due to poor patient cooperation, local or regional recurrence of the cancer, implant loss or unfavourable intermaxillary relationships. They conclude by suggesting the necessary evolution of both reconstructive surgery and implant surgery.

3. Management of Soft Tissues

Inflammation of peri-implant soft tissues is frequent in oral cancer patients. Gingivitis seems to be more frequent in irradiated tissues. Operculation is reported in 17% of the patients, possibly associated with local necrosis of the soft tissues in 50% of the cases [60]. Werkmeister et al [61] have observed a rate of complications in soft tissues of 28.6% for implantation in irradiated bone and 8.3% in non-irradiated bone. It can be explained by the paucity of keratinized tissue which is weakened by radiotherapy and by mouth dryness. Moreover, reconstructive flaps consist of mobile, non keratinized tissue, non adherent to the underlying bone, which renders them particularly sensitive to mechanical inflammation. Flap hypertrophy is common, especially in non irradiated flaps or in young patients, and can be treated by CO2-laser resection.

4. Marginal Peri-Implant Bone Loss

An increased marginal bone loss is observed in irradiated areas when compared to non irradiated bone. The loss ranges from 2 to 9 mm over a period of 3 years after implant surgery [49]. The marginal bone loss is a predictive factor for future implant failure which can occur as late as 2 to 5 years after implant loading.

5. ORN and Implants

The risk for developing an ORN during the placement of oral implants is certainly under evaluated in the literature. Twenty years ago, some authors used to contra-indicate implantology because they thought that the benefit-risk balance between implantation and functional enhancement and the risk of ORN was unfavourable. Although this view is now unquestionably outdated, some studies still report cases of ORN after implant placement [14, 49, 60]. Wagner et al. [50] have reported a case of ORN appearing 5 years after failure on 5 implants, and estimate the risk at 1.6%. Other authors consider that this figure is underestimated and propose a rate of 5%. Esser et al. [60] have reported 2 cases of ORN after mandibular implantation. Whereas some authors advocate the use of HBO to minimize the risk of ORN and to increase the healing potential of the irradiated bone [30, 61, 62], others consider that the benefit-risk balance is insufficient to recommend HBO in these patients [29, 50].

CONCLUSION

Osseointegration in irradiated bone is possible provided that some precautions are taken. However, some problems remain, such as the management of soft tissues and the elaboration of the prosthetic planning which is not always easy. Improving anatomo-physiological features in order to facilitate rehabilitation is essential for both soft and hard tissues. The procedures involved include tissue reconstructive surgery and tissue engineering.

Modifications to the surgical procedures could lead to decreased trauma, compensation for cellular alterations and for radio-induced histological lesions in the tissues.

If a 60 Gy radiotherapy is not a definitive contra-indication to implantology, the management of soft tissues is a major problem in oral rehabilitation [63]. Hyperplasia leads to decreased success rates of prosthetic rehabilitation [53]. Pre-implantation soft tissue surgery, such as flap thinning by removal of adipose and connective tissues, provides better volume and anatomical background for implantation of the prosthesis. The lack of keratinized tissues can be corrected by epithelial grafts [64] taken from the palate, 6 to 10 months after the placement of the implants.

As for bone modifications, orthognatic surgery is not indicated in many patients because of age or radiotherapy. On the other hand, when the free flaps are too thin, two options can be considered:

- Vertical osteogenic distraction: the method, which is based on the regenerative potential of bone, is used as an alternative to double-barrel graft to compensate for vertical discrepancies between the graft or the resection site and the residual mandible [65, 66]. The bone height obtained is compatible with implant placement.
- Double-barrel grafts [67]: the superposition of two fragments of fibula makes it possible to increase bone height and reduce vertical discrepancies. The implants should transfix both fragments for optimal stability.

Osteoinductive molecules like bone morphogenic proteins or stem cells are currently being developed [68, 69, 70]. Animal studies show that interruptive loss of substance can be repaired with the association of a collagen membrane, EZ Cure®, and biphasic calcium phosphate granules coupled with autologous stem cell injection [71].

Evolution of surgical implant procedures toward minimally invasive surgery could decrease the operative trauma, which would minimize the risk of developing an ORN. The development of real preoperative strategy and planning, coupled with new medical imaging technologies, is expected to allow:

- the planning of implant surgery, permitting to avoid non loaded implants because of unsuitable implant position or direction,
- a more precise prevision of future prosthetic interventions,
- the development of minimally invasive surgery, with limited periosteum impairment of the irradiated bone,
- a shorter operative time,
- a reduced risk of infection and necrosis.

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Chapter 17

EMERGING THEMES: A COMMENTARY ON INTERPROFESSIONAL TRAINING BETWEEN UNDERGRADUATE DENTAL STUDENTS AND TRAINEE DENTAL TECHNICIANS

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ABSTRACT

This commentary will detail emerging themes of personal and professional interest to the authors concerning the development of shared learning between undergraduate dental students and trainee dental technicians. Collaboration between health care professionals is not a new phenomenon. Interprofessional training and working practices in health care are well established in such fields as nursing and social care. Indeed, in practical terms, the maintenance of health demands such a wide range of expertise that it is highly problematic for any single health profession to deliver care in isolation. However, interprofessional education presents many challenges: the participants inevitably bring with them their essential differences, culturally, technically, vocationally, and of course, professionally. As a result, barriers exist at many levels. In some training contexts, this has led to cultural developments that collectively work against effective interprofessional

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collaboration, including the formation of rigid professional boundaries, the persistence of stereotypes and limited knowledge about each others' roles due to the isolation of professionals during training. For interprofessional learning to be successful, prejudices must be broken down, and there must be willingness among all involved to engage in the process.

Our research addresses such issues and discusses possible structural solutions, examining in particular a programme of interprofessional curriculum development.

INTRODUCTION

High standards of both technical and clinical work in dentistry are important to the comfort and health of patients and to the function and appearance of their mouths. The clinical and technical aspects of any procedure are complementary and, while distinct, should not be seen as separate. Ultimately the skill of both dentists and technicians reflects the quality of their education, training and experience. A successful outcome to treatment for any patient depends on two or more people working together. The effectiveness of the team is limited both by the standard of the least skilful of its members, as well as by the degree to which they co-operate (Nuffield Foundation, 1993).

Historically the working relationship between dentist and dental technician has always rested on a basis of communication by written prescription with little face to face dialogue. Both parties made assumptions, based on their own experience, about the other's approach to the needs of the end user, the patient. Unsurprisingly, this sometimes unreliable means of communication resulted in a variable quality of service (Davenport *et al,* 2000) and a body of literature exists describing the unsatisfactory interface between clinician and laboratory (Leith *et al,* 2000, Schneider, 2000, McGarry *et al,* 2004, Tamamoto, 2005). Some of this highlights the poor nature of prescription patterns which contravene the European Union Directives for the fabrication of oral prostheses (Lynch *et al,* 2003, Grey *et al,* 2004). The training of dental technicians in the United Kingdom has normally been structured in such a way that the trainee dental technician has only minimal contact, if any at all, with clinical undergraduates during their training. This segregation can lead to difficulties when dentist and technician meet in daily practice. It is essential therefore that a dialogue between technician and dentist takes place in a way that allows the expertise of both to be used to achieve the best possible outcome for the patient (Davenport *et al* 2000, McGarry *et al* 2004). Closer integration between trainee dental technicians and undergraduate dental students during their otherwise disparate courses of training has been suggested (Reeson *et al*, 2005) as a solution to actual and perceived difficulties in the quality of provision of dental health. Others too regard dental technician training without exposure to clinical dentistry as incomplete (Barrett *et al,* 1999).

For effective team work to take place, the team members should have a clearer understanding of one another's role than has previously been the case (Nuffield Foundation, 1993, General Dental Council (GDC), 1997, 2001, 2002, 2004). In the light of this it seems sensible to conclude, that bringing together the combined training and experience of both clinician and technician in other words, undergoing interprofessional training will bring greater benefit to the patient (Challoner, 2002, Warden, 2002, McGarry *et al*, 2008). Such interprofessional education is the focus of this commentary.

Interprofessional education is a challenge as the participants inevitably bring with them their essential differences and as a result many barriers exist. A major barrier as Ginsburg &

Tregunno (2005) point out is the strong professional cultures that hinder realization of effective and widespread collaborative practice in the wider health context. Historically, health professionals have been established to function as specialised groups, each with their own assumptions, beliefs, identities and practices that constrain the ways that they interpret and act on problems (D'Amour *et al*, 2005; Oandasan *et al,* 2005). This has led to many cultural developments that collectively work against effective interprofessional collaboration, including the formation of rigid professional boundaries (Glen *et al,* 2002), the presence of stereotypes (Hind *et al,* 2003) and limited knowledge about each others' roles due to the isolation of professionals during training (Reese *et al,* 2001; Drinka *et al,* 2000). Therefore, it is important to acknowledge the risk of entrenching negative attitudes rather than fostering good ones, or at least questioning existing beliefs. For interprofessional learning to be successful, prejudices must be broken down, and there must be willingness among all involved to engage in the process. Of course, one of the aims of interprofessional learning is to remove such prejudices, thus allowing effective collaboration.

The body of evidence reported in the literature is primarily concerned with nursing, medical and associated professionals and students. There are few studies that include dental students particularly where learning occurs with dental care professions (DCP). Cannavina et al (2000) undertook a study to identify the level of shared learning on a Bachelor of Medical Science in Dental Technology (BMedSci) course at the University of Sheffield, School of Clinical Dentistry. A summative evaluation of the course was carried out, using semi-structured nominal group interviews. BMedSci students, BDS students and recent graduates were questioned and their answers analysed to identify shared learning activities. The results revealed that different levels of shared learning opportunities occurred within the different departments which delivered the course modules. Shared learning such as group work and learning from one another's experiences was viewed favourably by the students. The authors felt it has the potential to maximise the use of resources and offered the opportunity for developing an integrated dental team.

Reeson & Jepson (2005) examined whether the training of dental technicians should be linked with that of the dental undergraduate. This involved third year trainee dental technicians and third year undergraduate dental students working together to provide complete dentures for a patient within the formal undergraduate course in complete denture construction. The trainee technicians also attended a series of lectures relevant to this course alongside undergraduate dental students.The outcomes of the exercise were evaluated by means of focus groups, observations and semi-structured interviews. Results indicated that both trainee dental technician and undergraduate dental student benefited to some extent from closer collaboration during training.

Morison et *al* (2008) investigated the attitudes of dental and DCP students to inter professional education (IPE) and highlighted some of the barriers to developing programmes for these students. Undergraduate dental students, student hygienist's along with student nurses took part in the study. Two questionnaires, the Readiness for Interprofessional Learning Scale (RIPLS) and a dental roles and responsibilities questionnaire were distributed. The results show that undergraduate dental students and DCP students had a positive attitude to IPE as a means to improve teamwork and communication skills but there are potential obstacles as demonstrated by the differing perceptions of each of the three groups about the roles of the other. Some aspects of practice, involving personal care and advice to patients, were regarded by all groups as a shared role but the dental hygiene students regarded

themselves as having a shared role in several tasks identified by dental and dental nurse students as the sole role of the dentist. Dental hygiene students in this study did not see their role as primarily to support the dentist but more as a partner in care. Professional identity and its development are issues that must be considered by dental and DCP educators developing IPE initiatives.

Ross *et al* (2009) investigated the impact of teamworking on the knowledge and attitudes of final year dental students. The aims of this study were to relate final year UK dental undergraduates' experiences of teamwork-related training to their knowledge of the clinical role of dental hygienist-therapists, and their views of the clinical roles of dental care professionals. The study suggests that acceptance of non-dentists providing patient care lags behind the comparable situation within the primary care medical team. The authors suggest that if we are to succeed in the delivery of a modernised dental care system, it is crucial that dental education promotes awareness and acceptance of the professional status and ability of DCP colleagues.

Interprofessional teaching is in itself seen as one way of modelling the interdisciplinary skills required of the next generation of health professionals. The use of common curricula across health professions similar to the GDC's Developing the Dental Team (2004) will help in the development of a common worldview including common values, language, and perspectives. Interprofessional learning has been said to improve communication and trust between different professions by improving collaborative skills, thereby enhancing professional relationships and facilitating more creative and integrative responses to healthcare. O'Neill *et al* (2000) argue that meaningful interprofessional learning experiences can better prepare students for encountering the complexities of real-life interprofessional problems in the work environment. Interprofessional learning and education deals with the knowledge, skills and attitudes (that is, competency) required for collaborative interprofessional learning and clinical education. It provides students with the knowledge of the contribution of other disciplines, the skills to seek out, communicate with and work with other professionals, and the ability to value such contributions.

However, there is still a need to provide evidence that shared learning does have the impact on practice and the benefits to patient care that it purports to (Mattick *et al,* 2003; Barr *et al,* 2005). In particular, there is still a lack of evidence in relation to the effects of pre-qualification programmes (Page *et al,* 2004; Zwarenstein *et al,* 2004; Barr *et al,* 2005). Moreover, debate continues as to which elements of the undergraduate curricula benefit from health students learning together, whether the setting in which it occurs is important, and whether benefits can be sustained in the longer term (Finch, 2000; Freeth, 2001).

Whilst the National Health Service in the United Kingdom currently promotes interprofessional teamwork as a means of providing more patient-centred care (Department of Health, 1998a, 1998b, 2000), this approach to health care is not without problems. Difficulties have been identified with shared learning between health professionals (Arlton *et al,* 1990, Ling *et al,* 1990, Zungolo, 1994). It has been found that professional and organisational barriers such as previous single-disciplinary training, differing educational experiences, and professional socialisation can negatively impact on the shared learning process (Ling *et al* 1990).

However, it is noted that such barriers can be overcome and students can share these differences and use them constructively in their learning (Zungolo 1994). Health care professionals, by virtue of their training and professional socialisation tend to have

professional allegiances that cut across institutional allegiances (Handy, 1993). Status, identity and a sense of role security may be maintained by marking boundaries between professional groups that are defined as 'them and us', with negative consequences for collaboration (Turner, 1991, Brooks *et al,* 2002).

Membership of a professional group is said to form part of a person's self concept Ellemers *et al* (1999), which helps explain why perceived threats to that group, or to membership causes hostility towards others (Spears *et al,* 1997, Hind *et al,* 2003). The creation of professional identities is part of the socialisation process of health professionals, a process which begins with undergraduate education (Harter *et al,* 2001), but which continues in the workplace. A study of nurses, for example, suggested that their mentors (senior nurses) had more of an impact on their professional identity than their undergraduate training. Doctors are also said to model their professional behaviour on their mentors (Anspack, In Conrad *et al,* 1990). As Apker & Eggly (2004) note *"Research indicates that the occupational identity doctors develop during training has critical implications for their future professional relationships"* (p 414). This socialisation process quickly develops into professional boundaries and territories (Beattie, 1995). Interprofessional rivalry, tribalism and stereotyping are known to operate (Mandy *et al,* 2004, Braithwaite, 2005) as is turf protection. These have significant influence on the ability of team members to work in multidisciplinary fashion, as professionals struggle to come to terms with differences in values, language, and worldviews (Becher, 1994). Add to this the differing accreditation and licensing regulations, which act as barriers to cross-disciplinary learning, then what has occurred, is the dominance of role over the meeting of patients' needs (Greiner *et al,* 2003).

The stated objectives of multidisciplinary teamwork and interprofessional practice, including the sharing of power as well as expertise, mean that this can be perceived as a threat to professional and personal identity, although a number of authors would argue that genuine collaborative practice actually leads to the empowerment of all the health professionals involved (Sullivan 1998, Cowan *et al,* 1994). There may be for example strong professional identities, creating barriers that have long been impermeable. Atwal 2002, Barr *et al,* 2005, Reynolds 2005 suggests the concepts and processes involved in interprofessional working are complex and can be influenced by many variables, such as a lack of understanding of, and respect for, different team roles, rivalry and the influence of team hierarchies. There are historical and social-status tensions between dentist and dental technician which unlikely as they are to be resolved without radical change must also be taken into consideration. Relatively low pay and low status have been problems shared by many dental technicians (Bower *et al,* 2004), who are aware that dentists are *'reluctant to accept technicians as equal partners'* (Nuffield Foundation, 1993 p73).Therefore, it is important to acknowledge the risk of entrenching negative attitudes rather than fostering good ones. For interprofessional learning to be successful, prejudices must be broken down, and there must be willingness among all involved to engage in the process. Skilful, collaborative interaction with colleagues from diverse professional backgrounds requires not only respect for each others' roles but an awareness to adopt a holistic approach to treatment, and sound communication skills (Carpenter, 1995; Harbaugh, 1994; Leaviss, 2000; Mathias *et al,* 1997).

The past strong emphasis on dental laboratory techniques in the training of dentists has changed. Today there is no expectation that dentists will ever have to carry out their own laboratory work. In fact, laboratory instruction for dental undergraduates has fallen in the United Kingdom over the last 25 years by 75% (Reeson *et al,* 2005). The current

undergraduate dental student in the United Kingdom will by the end of their training, have spent a relatively short time on laboratory work compared otherwise to the dental technician in training. This reduced experience limits the ability of dentists to partner with dental technicians. The claim is that reduced laboratory instruction for dental students is achieved at the expense of repetitive laboratory exercises rather than a reduction in the relevant techniques covered. The aim is to produce 'laboratory aware' rather than 'laboratory competent' dental students. New materials and technologies will increase the need for dental technicians to be highly educated to provide support to dentists. This is especially true for procedures such as the newest computer-aided design/computer-aided manufacturing technologies, which will demand greater cooperation between dentists and dental technicians. With dental students having little or no experience with many of the newest materials and techniques, dental technicians will be the source of information for general dentists. A greater familiarity with laboratory work should lead to better understanding of the technical difficulties faced by the technician, which in turn may lead to clearer communication and more thoughtful prescribing by the dentist.

McWhinney (1997) informs us there are a number of possible reasons why trainee dental technicians and undergraduate dental students might resist change. For example, they both might regard change as a threat to their sense of competence. Being comfortable with the status quo, they might fear that they will fail at new tasks. Bringing about change can be difficult; Scott & Jaffe (1990) inform us that *'people do not normally change their behaviour simply by being given information'* (p.33). Others are not always willing to take on board our own ideas good or otherwise.

If we are to bring about change, others must be encouraged to take ownership of the change itself. Partly this is due to Ajzen & Fishbein's 'Theory of Reasoned Action' in that people rationalise their behaviour according to how they think they are behaving. It is far more common for people to change because of the support and encouragement given to them, in order to modify behaviour and values. Change, in any organisation can be difficult to bring about. Prejudice and entrenched working practices do not always facilitate change. Thus the agent of change runs the risk of *'upsetting the boat'*, and in an organisation such as the dental profession, barriers to change can be difficult to overcome. Interprofessional learning has the potential to improve the effectiveness of team working between healthcare professionals and ultimately the quality of patient care. It does, however, require a high degree of commitment from all those involved in its implementation and requires careful planning and organisation. Barriers that could be encountered, both in terms of practicalities and student attitudes, need careful consideration prior to the implementation of interprofessional learning, as an ineffective programme could potentially further ingrain negative stereotypes.

Increasingly, educational initiatives in which students from different health care professions follow a common curriculum for part of their course are seen as an important means of fostering mutual respect, understanding one another's role, and effective skills for working collaboratively in the multidisciplinary teams that they will encounter in the clinical context. Leaviss (2000) investigated whether a two-day interprofessional course (including students of medicine, radiography, nursing, dentistry, occupational therapy and physiotherapy) in the final year of training, had influenced their practice on qualification. Respondents felt that their greater understanding of the professions had led to more appropriate referrals, increased their awareness of profession specific skills, and improved understanding of professional pressure and of holistic care. However, participants also

reported negative professional attitudes which had not been changed by the interprofessional experience. In Reeves *et al's* (2002) evaluation of an interprofessional training ward, focus group findings indicated that communication and team work skills had increased, but from practice observations, the researchers noted that medical students were not as engaged in team duties as the nursing, occupational therapy and physiotherapy students. Most students felt that the intervention had been too short (two weeks) to form any lasting interprofessional effect, but on questioning a year later they reported that the training ward had given them a valuable insight into the roles of other professions and of interprofessional working. In a longer intervention over a seven week period, Lindqvist *et al* (2005) organised experimental group meetings between professionals to discuss interprofessional learning and working, finding afterwards that they were significantly more likely to view each others profession as being 'caring than students in a control group.

It appears from these studies that students are generally positive about the concept of interprofessional learning and that there is a greater understanding of other professions and an improvement in communication and team work. Effective interprofessional education (IPE) requires much more than students or practitioners from different professions simply listening together in shared lectures (Freeth *et al*, 2002). Participants need to work actively together on tasks for which they take joint responsibility. Therefore IPE arguably requires close attention to team-building among students (Gilbert *et al*, 2000).

Social psychological theory and evidence suggests that group climate and cohesiveness can have major influences upon participants' behaviour, productivity and commitment within groups (Napier *et al,* 1999). Group climate tends to be characterised by the presence of engaged, avoidant or defensive behaviours (e.g. Stockton *et al,* 1992). A defensive group climate in which participants feel judged, or in which they believe there are hidden agendas, tends to inhibit open sharing of ideas (Johnson *et al,* 2000). Respectful behaviours help to promote a positive group climate which in turn tends to encourage full participation and good quality decision-making (Mayer, 1998). Poor cohesion is another feature of group dynamics that can diminish the productivity of a group. It is usually manifested by fragmentation, cliques and dislike among some group members. Groups that suffer from poor cohesion, not surprisingly, usually generate little commitment from their members (e.g. Wech *et al*, 1998). Any difficulties in forming cohesive groups during educational sessions (including any failure to settle interprofessional conflict) may encourage students to develop negative attitudes towards joint working in the clinical setting.

A few studies suggest that interprofessional educational experiences can enhance participants' understanding of teamwork, although participants have mostly been professionals already working in the clinical setting. For example, Nash & Hoy (1993) reported that GPs and nurses believed that a three-day residential workshop on the interprofessional delivery of terminal care had enhanced their capacity to work collaboratively. Gilbert *et al* 2000, Hilton & Morris 2001 found that students were found to be highly motivated when given opportunities to participate in shared interprofessional learning experiences. Hilton & Morris (2001) found for example that physiotherapy students rated positively the opportunities that they had for interprofessional working whilst on placement. A study of final year students of occupational therapy, physiotherapy and other allied health professions found that two-day interprofessional workshops had a positive influence on participants' understanding of teamwork (Parsell *et al*, 1998). Nevertheless, there have been rather few studies of undergraduate students engaging in IPE. A recent

review of published evaluations of IPE has shown that less than 30% of the studies selected for the review had addressed the experiences of pre-qualification students, and that most of those had been concerned with students in the later phases of their courses, usually in the placement setting (Freeth *et al*, 2002).

CONCLUSION

There have been very few qualitative studies of students' experiences, and also very few that follow-up students over time to determine whether any of the attitudes and skills learned in IPE are taken into the clinical setting. According to Freeth *et al* (2002), the various studies reviewed suggested that learners are generally very positive about their experiences of IPE. However, there is relatively little information about how students learn through interaction with peers and tutors during IPE, particularly the processes whereby students apply skills and attitudes acquired in university-based education to collaborative team working in the practice setting. According to Freeth *et al* (2002), the various studies reviewed suggested that learners are generally very positive about their experiences of IPE.

Currently there is a paucity of research into shared learning between trainee dental technicians and undergraduate dental students. To address these shortcomings in the current literature, the authors of this commentary are currently involved in a study which will explore two main research questions:

- Does shared learning promote a better understanding of professional roles and effective interprofessional teamwork, and how far are these aims successfully accomplished?
- What are the students' experiences and perceptions of their team working skills; group dynamics and impediments for future team working in both a clinical and laboratory setting?

Adopting a qualitative methodology, the overall goal of this study is to understand trainee dental technicians and undergraduate dental students' experiences of these shared learning opportunities together with any perceived barriers to learning in the workplace. As with most qualitative based studies, this study is designed to examine a small group of individuals' lived experiences in a specific context (Rossman *et al,* 2003).

The particular and unique characteristics of this setting and the selection and number of the participants are recognized in that they may therefore limit the transferability of the findings. A single and specific context will be studied, i.e., a Dental Teaching Hospital in the United Kingdom. Furthermore, the small group of participants have been selected purposefully as they are students currently enrolled on a recognized training course within a University Dental School/Hospital.

At this stage of the research, any attempts to generalize the data would be premature. Initial findings reflect a selection of interesting data from the early analysis. Therefore, as such, this may be regarded as background work which appears to be confirming much of the existing literature. However, a number of themes are emerging which offer clues on students' early attitudes to their professional socialisation and status, and also how they view collaborative working. Early data suggests;

- Both undergraduate dental students and trainee dental technicians are pragmatic in their expectations of shared learning. In particular, they regard the exercise useful in terms of communication and understanding each others' roles.
- Additionally, the students also regard the processes of shared learning as having a positive impact on future interprofessional teamwork.
- Students' views are similar, some of which are traditional, stereotypical views of their own professional status. Initially, these findings are consistent with the related literature.
- There is little evidence arising from the data to support issues of power and perceived social and interprofessional hierarchies.

The above conclusions offer an early insight into the experiences of the students. Since they are preliminary conclusions it is unwise to place too much emphasis on them as definitive at this stage of the research.

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Chapter 18

ARE METAL-FREE CROWNS TRULY MORE AESTHETICALLY PLEASING?

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ABSTRACT

Current concerns about facial aesthetics play major roles in our lives. In dentistry, it is increasingly common for patients to search for aesthetic restorations because the desire to have a good-looking smile is prevalent in today's society. Due to the easy access to information through various media, patients are able to constantly monitor the growing trend of aesthetic dental materials. New alternatives for aesthetic prosthetic resolutions, including all-ceramic crowns, are available on the market, and persuasive manufacturer's advertisements suggest to both professionals and patients that these materials have superior qualities. With the promise of optimized aesthetics by the elimination of the metal in the metal-ceramic fixed prostheses, a new reality has become part of the daily routine. Several manufacturers and experts emphasize that all-ceramic crowns allow light transmission, which demonstrates improved aesthetic properties when compared to metal-ceramic crowns. In daily practice, dental clinicians should be aware of the real optical benefits provided by these restorations. While the light reaches the buccal face of crowns towards the lingual, clinicians should consider the following: is the total transmission of light through an all-ceramic crown really important? Additionally, will it really result in a more aesthetic appearance? The aim of this chapter is to present to readers with the various properties of all-ceramic crowns by comparing them, in a critical way, to the clinical reality of dental professionals.

INTRODUCTION

The word "ceramics" comes from the Greek "keramos" meaning clay. The first evidence of ceramic was discovered in the excavation of the Nile Valley in Egypt and was dated as 13,000 years old. Since the tenth century, the technology of art in ceramics, which have a strong internal structure and very white color, has been dominated by China, and only arrived

in Europe in the seventeenth century, aptly named "china table." Despite the enormous effort to copy the composition of Chinese porcelain, it was not until 1717 that the Europeans discovered the secret of the Chinese art. Ceramic was made from three basic components: kaolin (China clay), silica (quartz) and feldspar (a mixture of silicates of aluminum, potassium and sodium).[1]

Fifty-seven years later, the Frenchman Alexis Duchateau was dissatisfied with total prosthetic teeth made of ivory, and, after verifying the durability and resistance to staining and abrasion of ceramic home appliances, he decided to use this material in new prostheses. Thus, with the help of Nicholas Dubois de Chemant, the art of ceramics transformed dentistry and continues its transformation today.[2]

The application of porcelain in the confection of a jacket crown for restoration of weakened teeth was first performed by Land in 1886; after 1916, this procedure gained wider acceptance.[3]

For many years, the pure porcelain jacket crown was the only type of metal-free crown. Made of porcelain applied on a matrix of a thin layer of platinum or adapted to the model or die abutment of the prepared tooth, this type of crown preceded the ceramic-metal crown and was indicated for the prosthesis unit.

In 1950, leucite was added to the porcelain formulation to increase the coefficient of thermal expansion and permitted mixtures with gold-based alloys used for the manufacture of full crowns and fixed partial dentures (PPFs).[2]

Although the total metal crown has been used for many years to restore weak teeth, the growing need for the aesthetic stimulated a change in these crowns by covering it with a facet of porcelain. Thus, in the 1960s, the technique of porcelain cast to metal was developed.[4]

In 1965, McLean and Hughes[5] developed a hollow crown of porcelain with an interior core of aluminized porcelain that contained 40 to 50% of aluminized crystals to block the spread of cracks. This inner core, which enhances the restoration, is present in a layer of conventional porcelain and is two times more resistant than the jacket crown of porcelain.

A major advantage of the dental ceramic known as dental porcelain is its ability to simulate the natural tooth, largely due to its inherent optical quality and resistance. The increasing esthetic demand dictated by modern society has spurred enormous scientific development to improve the physical and chemical properties of dental ceramics.

In 1976, a new technique was introduced to further enhance the resistance of the jacket crowns on alumina.[6] For this, a sheet of platinum was used with a layer of tin oxide, which promotes the union between porcelain and platinum.

Recently the concern for aesthetics in general has had a great impact on our lives. The dictionary defines aesthetic as receptive and appreciative of dedicated beauty or refinement, and it represents a factor directly influenced by culture and self-image. With regard to dentistry, aesthetic requirements have become more present in daily life as the need for a harmonious smile became a prerequisite for living in society. The current concern is focused on obtaining dental prostheses and restorations that have color parity with the original elements.

Given this trend, toward the end of the twentieth century, several innovative systems were introduced to permit the preparation of metal-free ceramic restorations. Since then, several ceramic systems have been developed with the aim of improving the physical and mechanical properties of ceramic materials. With the advent of the metal- free prosthesis, a

new reality has become part of the daily routine with the promise of optimizing aesthetics by removing the metal present in metal-ceramic fixed prostheses.

In modern society, harmonic restorations are so important that patients' demand for aesthetic results is very strong, requiring professionals to constantly upgrade to meet this need.

Although dentists have always been aware of the importance of dental aesthetics, the media has put more emphasis on this branch of the profession in recent years and, millions of dollars are spent annually on cosmetic dentistry. Along with a very significant group of professionals, many patients have been directly influenced by media and manufacturers in the decision to replace metal-ceramic crowns with metal-free crowns with the great promise that the aesthetic problems of those crowns will be solved. This overvaluation of the aesthetic superiority of the metal-free crowns dictated the vast majority of its major economic engagement. This overvaluation must be carefully examined, and many questions arise, including: Are these systems really more aesthetic than metal-ceramic prostheses? Have systems of metal-free fixed prostheses begun to replace systems with metal infrastructure? Do we really believe everything that the manufacturers claim about their materials?

Manufacturers know that the smile is considered one of the biggest attractions of the human being and understand that many people are willing to invest time and money to improve it. Thus, this chapter aims to stimulate the critical mind of the reader to consider the aesthetic characteristics of metal-free fixed prosthesis systems compared to systems with a metal infrastructure.

CLASSIFICATION OF CERAMICS

Over the past decade, there was a considerable increase in materials for producing pure porcelain restorations. Each of these materials used a different approach with the aim of improving aesthetic and mechanical properties, and they can be classified according to their technical procedures and different chemical compositions.[7,8]

Conventional or feldspathic porcelain has been used in metal ceramic prostheses for more than 30 years and is made of leucite crystals dispersed in a matrix of silicate glass,[9] having the basic components of feldspar, quartz and kaolin.[10] Feldspar is the main component of feldspathic porcelain, comprising 75 to 85% of the final product. It melts at about 1300 ºC and becomes a part glass, part crystalline material called leucite. Quartz is used in the form of pure crystals of silicon (SiO_2) that generally remain unchanged from its crystalline form during the cooking of porcelain, producing a frame that considerably increases the resistance of the restoration. It constitutes about 12 to 22% of the total volume of porcelain. Finally, the kaolin or clay is about 3 to 4% of the total volume and is a hydrated silicate of alumina that serves as a binder to keep the form given by the technician before being placed in the oven. Despite having excellent properties, feldspathic porcelain are highly friable and therefore cannot be used without being attached to a metal coping because their flexural strength is only 60 to 70 Mpa.[10]

Feldspathic porcelain can be used in making metal ceramic crowns, facets in ceramic, pure porcelain crowns and inlays in porcelain[11,12] and may be used alone or in association with other systems in which a feldspathic porcelain covers a china aluminized porcelain or aluminized glass, which gives it greater resistance to fractures. Because it displays excellent

translucency characteristics and color similar to the natural tooth, feldspathic porcelain is often used as a coating.[10,13]

The elimination of metal to obtain more natural-looking restorations has been the impetus propelling the entire development of the composition and techniques used in the development of different types of ceramics, with the goal of maximizing both aesthetics and strength. Ceramic systems have undergone many changes from the hollow feldspathic porcelain crowns to the current systems, which are reinforced by alumina, zirconium and leucite.

In an attempt to increase the strength and versatility of ceramic crowns, the innermost layer of most current ceramic systems consists of a ceramic coping of high flexural strength receiving layers of low-melting-temperature porcelain to construct the final restoration. Methods for the strengthening of ceramic systems include, among others, the use of ceramics reinforced by leucite, such as IPS Empress (Ivoclar, Vivadent), the IPS Empress 2 reinforced by lithium disilicate, systems based on alumina copings such as In-Ceram Alumina (Vita Zahnfabrik) and Procera AllCeram (Nobel Biopharm), and those composed of a mixture of alumina oxide, zirconium, In-Ceram Zirconium and Procera All-Zircon.[9,14-16]

The In-Ceram system, developed by SADOUN in 1988, employs the technique of "slip casting" for the preparation of a ceramic coping whose main component is aluminum oxide infiltrated with glass powder. Through the technique of "slip casting", a ceramic powder of fine particles (4 μm) with high alumina content is mixed with a special liquid. This mixture is applied to the model and duplicate by capillary forces, moisture is absorbed, and the particles are clustered on the model, thus forming a dense and firm structure. It is then subjected to sintering for 11 hours at 1140 ºC, resulting in a crystalline structure with a white-opaque appearance and low resistance. Next, the coping is sintered and infiltrated with molten glass through a second cooking, which gives it high strength and translucency.[17] To cooking, a special coating of porcelain, the Vitadur Alpha (Vita), is applied in the conventional way.[15,18]

In-Ceram is the most resistant metal-free ceramic system available, with a three- to four-fold increase in flexural strength. This system requires a special furnace (In Cerammat) developed for this type of porcelain, and the sintering and burn of the matrix glass takes around 16 hours. Due to the high concentration of alumina, the final product is very opaque, which is inappropriate for external use and should be covered later by porcelain with better aesthetic properties (Vitadur Alpha). When used properly, this cover provides a satisfactory and acceptable margin.[19]

In addition to the In Ceram Alumina, two other types of In Ceram were placed on the market: In-Ceram Spinell and In-Ceram Zirconium. The Zirconium is indicated for use in dental unit crowns and implant-supported and fixed prostheses for up to three elements because these prostheses have more resistance due to the incorporation of zirconium oxide.[19]

In-Ceram Spinell contains magnesium oxide (MgAl2O4), which improves the translucency of the final restoration by providing 40% greater light transmission, but is 20% less resistant than In-Ceram Alumina.

This system has advantages including greater resistance to fracture, excellent marginal fit and good aesthetics; disadvantages include high cost, the perceived opaqueness of the gum paste and the 0.5 mm thickness of the coping.[20]

Launched in 1990 after development by the Wohlwend Innovative and Dental Institute, Zurich University, the IPS Empress system is based on the principle of leucite crystal

dispersion in which porcelain is injected and is found in the form of pre-fabricated tablets.[9,15,17] This system offers great promise for all ceramic restorations because it demonstrates good fluorescence and durability and lower abrasion to opposing teeth versus any other available porcelain.[21]

It uses the technique of heat, pressure and the principle of the lost wax confection of coping. The wax pattern is included in a special refractory coating, a ring composed of paper. Pre-fabricated chips are placed inside the mold, which is transported to a special furnace that injects ceramic into the mold under heat and pressure.[15] After undergoing cooling, the supply line is cut and the coping structure may then be marked based on the corresponding bodies of the porcelain enamel and dentin. With the first version of the IPS Empress, the restoration was made entirely with the source material rather than using a porcelain coating.[17] The aesthetic was impaired, although its use is advantageous when little space is available.[22] The IPS Empress 2 is the second generation of this technology and has a substrate comprising lithium disilicate (60% of volume) embedded in a glassy matrix.[17] IPS Empress and IPS Empress 2 systems differ both in microstructure composition and properties, and therefore have different indications. The first is only for crowns, inlays and onlays, and the second version has expanded its indication to prostheses fixed to three elements.[22] The IPS d.SIGN (IVOCLAR), a vitreous porcelain of fluorapatite, can be used with the IPS Empress 2. The process of sintering porcelain produces coverage by apatite crystals similar to the structure of the natural tooth, and the structure of ceramic fluorapatite is more similar to the tooth's structure than feldspathic porcelain for metal ceramic crowns.[23]

Optec is a system of Jeneric Pentone in which the restorations are obtained through successive burns on refractory swapped in order to offset the contraction. In the ceramic process of the glass, there is an increased load of leucite, which improves the quality of ceramic restoration.

The technique of DICOR differs from conventional techniques by handling the porcelain with brushes and ceramic pastes and the use of refractory dies. DICOR is worked as gold, through the technique of closing or loss wax.

DICOR is a restoration that produces greater translucency and appears to produce a good aesthetic due to somite. However, the occlusal adjustment or wear by chewing can remove the extrinsic paint, demonstrating the milky glass without color and changing its aesthetic characteristics. This ceramic material is suitable for making facets, hollow porcelain crowns and inlays.

The introduction of computer-aided design systems to restorative dentistry was a great innovation in recent years.

Since the early 1980s, researchers and clinicians have sought new methods of ceramic restoration fabrication that encompass strength, color stability, better wear characteristics and accuracy of adjustment, regardless of the dental arch region. The Procera AllCeram system, launched in 1986 by Anderson & Oden[21] in cooperation with Nobel Biocare and Sandvick Hard Materials AB and originally developed for infrastructure titanium, continues to expand its range of utilization.[21] This new system provides opportunities to enjoy advances in the clinical performance of ceramic restorations.

By employing CAD/CAM (Computer-Aided-Design/Computer-Aided-Manufacturing) technology, which manufacturer infrastructures in titanium and alumina, a special porcelain, AllCeram (Ducera Dental), has an expansion coefficient compatible to the material base. The coping with the high alumina content (99%) is sintered and highly compact.[21] There are

two possibilities for its use. According to the structure of the coping (titanium or alumina), it is either Procera AllTitan or AllCeram. The Procera AllTitan system is indicated for crowns, fixed prostheses on implants and manufacture of custom abutments on implants, while the Procera All Ceram is recommended for dental crowns and implant-supported and fixed prostheses of three elements. In 1998, the abutments for implants were introduced to the market, and, currently, the great promise of this system rests in the possibility of making custom or specific abutments to be used on implants, either in titanium or sintered alumina.[21,22]

Using the Procera system requires a laboratory with a special scanner connected to a computer program with the Procera system and a modem to transfer the information to a central laboratory in Sweden. The procedure is performed by scanning the abutment die through a tip of sapphire which "reads" the shape of the abutment die and applies minimal pressure to it. Scanning is performed over 3 to 5 minutes by rotating the spiral movements of the sapphire tip. In total, 30,000 measurements are taken and scanned in a single preparation. The outer contour of the future coping is then calculated on the computer screen in three dimensions (Technology CAD). Measurements are transferred to the central Procera laboratory via modem, where a turning machine controlled by computer (CAM technology) produces a second model 20% larger than the original in order to offset the expected 15 to 20% contraction which occurs during sintering of aluminum oxide.[21,22,23] Highly pure aluminum oxide powder (99.5%) is pressed on the replica of the model with high pressure, resulting in a very dense and homogeneous texture without porosities. Before sintering, the external coping is made under the control of the computer, a process that takes several minutes. Then, by sintering at temperatures above 1600 ºC, the coping acquires the dimensions of the original preparation.[21,22,23]

The coping takes between 2 to 3 days to be sent to the laboratory site, where it is reviewed on the thickness of the walls (which should not be less than 0.5 mm) and adaptation. The Procera coping is then covered with porcelain, which may be AllCeram (Procera All Ceram Ceramics, Dental Ducera), which was developed especially for the system with a coefficient of thermal expansion compatible with the coping. Moreover, AllCeram has fluorescence properties that contribute to the restoration of a more natural appearance.[21,23]

The Procera AllCeram system has the potential to be the most resistant material, a characteristic that is attributed to its high content of alumina (99% compared with 85% content of In-Ceram).[16] Wagner and Chu,[24] evaluating the flexural strength of three metal free ceramic systems, reported a higher statistically significant difference for the Procera AllCeram system (687 MPa), compared with In-Ceram (352 MPa) and IPS Empress (134 MPa).

The CEREC system uses a CCD-chip camera similar to a handheld intra-oral camera to see and capture the image of the preparation, a computer monitor, and a milling machine with three axes of rotation. The program then enters the printing and an optical inlay or onlay is designed specifically for the computer, making it unnecessary to perform casting or prosthetic laboratory. The material is presented in blocks of porcelain in six different shades, and, after selecting the color and placing the block of porcelain in the device, a restoration is made in approximately seven minutes. The restoration of porcelain is then carefully adjusted and cemented in preparation, carved and polished intraorally. The major drawback of this system is the marginal end of the restorations.

The Celay System (Vivadent) uses a wear technique to produce ceramic inlays and onlays based on a one-track pantographic from an inlay or onlay made from a precision molding material directly on the prepared tooth. The material is then subjected to a prototyping process of polymerization or photopolymerization before being removed from the tooth and is then carved into a homogeneous block of ceramics in one of two shades. This system can also be used as an indirect process in which a molding is done, and an abutment die is developed in the laboratory. The composite resin for the manufacture of the prototype is placed in an abutment die used to represent the restoration, which is brought to the system for matching. These restorations can be characterized and glazed before cementation, or carved and polished after intraoral cementation.[21]

PROPERTIES OF CERAMIC SYSTEMS

Ceramic systems have limitations based on the limitations of the machine to reproduce the design of the restoration. With CEREC, the inability of the disc cutting in the chisel angle makes it essential that the dentist who uses this system is trained specifically in cavity design. The Celay, however, is incapable of duplicating a long bevel with perfection, and a thin chamfer is extremely counter-indicated. Moreover, these systems require a high investment, which increases the cost of such restorations.

In 1996, Wagner and Chu[24] demonstrated the superiority of the Procera system's flexural strength for the In-Ceram and IPS Empress, obtaining results of 699.4 + 58 MPa for Procera, 450.1 + 120 , 4 MPa with the In-Ceram and 184.8 + 86.1 MPa with the IPS Empress. However, in 1998, Neiva et al.[25] found no significant differences in fracture resistance between these systems, although the process has resulted in lower levels of resistance to fracture. In the same year, Strub & Beschindt evaluated the fracture resistance of five different ceramic systems before and after receiving pre-load in an artificial clinical oral environment.[26] These systems included In-Ceram, IPS Empress (for technical characterization), IPS Empress II (for the technique of stratification), Celay feldspathic crowns and In-Ceram crowns, and the results showed that the simulation of artificial aging reduced the resistance to fracture of all groups, with significant differences between the mode of fracture of the samples before and after aging. The results were similar in all groups.[26]

The marginal fit is also an important clinical criterion because it is directly associated with the retention of plaque and periodontal health. In 1997, Sulaiman et al.[27] evaluated the adaptation of ceramic crowns of In Ceram, IPS Empress and Procera AllCeram during the various stages of the process (obtaining coping after the application of porcelain and after finishing). There were differences in the ceramic material used; the In Ceram system has greater marginal discrepancy, around 160.66 μm, which is above the acceptable levels of disadaptation (120 μm). The best results were obtained with the IPS Empress (62.77 μm), followed by Procera (82.88 μm).

DISCUSSION

Dentistry, both technically and scientifically, has been experiencing great changes in recent years, especially in the field of cosmetic dentistry. With the constant advancement of adhesive techniques that combine conservative preparations of cavities with the excellent retention and preservation of remaining tooth structures, cosmetic dentistry has aroused great interest among practitioners of dentistry. While dentists have the option of direct and indirect restorations in aesthetic solutions for their patients, they, in turn, are expecting metal-free restorations that are durable and that provide an attractive smile.

Metal-ceramic restorations have been developed as a means of reducing the risk of fractures through a metal substructure that strengthens porcelain. A thin metallic collar on the vestibular cervical region, a proximal pole and a metal lingual collar are recommended to receive the porcelain and serve as a basis for enhanced metal substructure, preventing its deformation during the porcelain burning cycles[28] and serving as a support to the fragile coating of porcelain which undergoes traction or shear force that can worsen the prognosis when the thickness of the porcelain is less than 1.0 mm or greater than 2.5 mm.

The metallic collar can become a disadvantage to aesthetics by producing a line or a dark shadow under the gingival tissue, making it visible when there is gingival recession. In addition, it can expose the opaqueness in this region where the porcelain is less translucent because of contour corrections and can also promote the accumulation of plaque on the rough surface that cannot be polished or finished.[29] However, is this really true? (Figure 1)

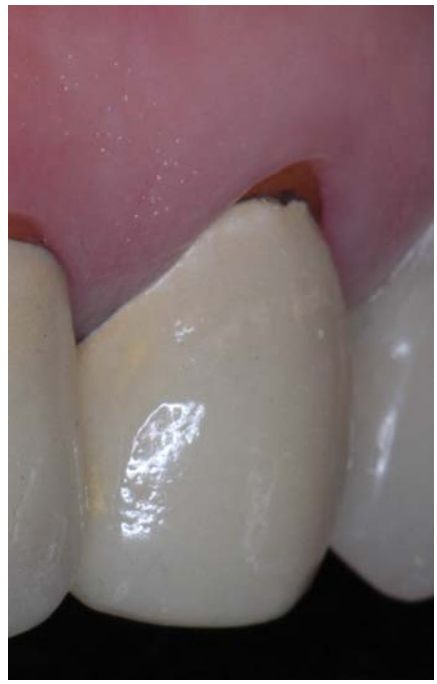

Figure 1. The darkness of the root becomes a disadvantage to aesthetics by producing a line or a dark shadow under the gingival tissue, making it visible when there is gingival recession. The main problem is not the presence or absence of metallic collar. The line union between the crown and root is a disadvantage both in metal-ceramic or metal-free crowns.

When a preparation is done for the exact dimensions of wear (1.6 mm at least - 0.4 mm for metal and 1.2 mm porcelain), all these problems of thickness are solved. The same happens when the preparation in aesthetic regions is taken to 0.5 mm subgingival. (Figure 2)

In cases of thin gingival tissue, patients may have a good indication for aesthetic metal-free prostheses, thereby preventing the view of the metal collar by transparency through the gums that cannot be seen when the root is darkened, in which case it will be seen by translucency through the gums.

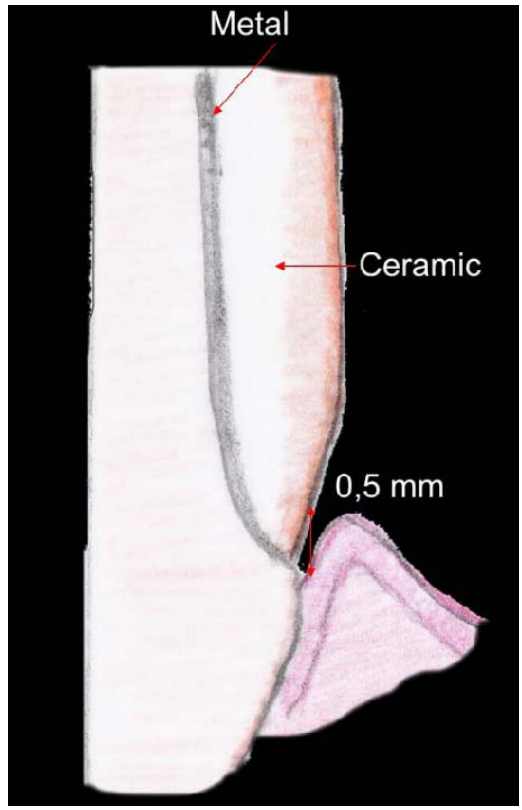

Figure 2. Scheme of ideal preparation and ideal thickness of metal and ceramic. The prepare must be located 0,5 mm subgingival.

Great care must be taken to indicate the use of metal-free prostheses justifying that, over the years, it may produce gum recession and the appearance of the metal collar. The appearance of the union between the root and the metal-free line is even more aesthetically displeasing than the metal band itself. (Figure 3)

In recent years, the demand for greater aesthetics has renewed the interest in metal-free crowns and led to the development of new techniques that use swapped refractory, making it more accessible to a larger number of laboratory technicians.[4]

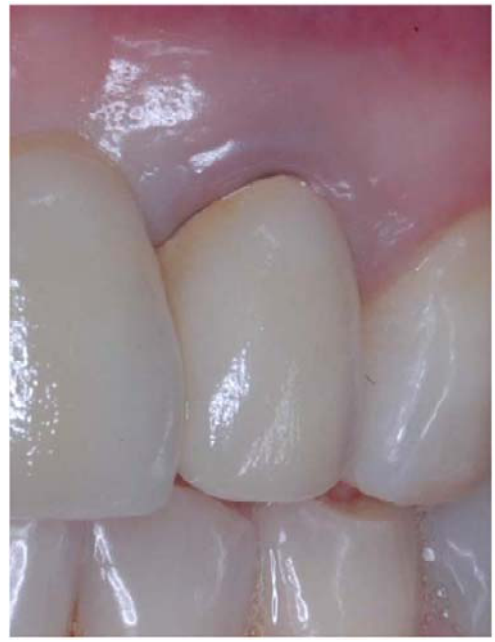

Figure 3. The appearance of the union between the root and the metal-free line.

Figure 4. The lights is crossing through the metal-ceramic crown.

These restorative procedures can be fully controlled if both the clinician and the technicians are familiar with the basic principles of natural oral aesthetics. Both the dental aesthetic and the gingival tissues work together to bring a balanced, harmonic smile. A defect in the surrounding gingival tissues or poorly prepared tooth may not be compensated by the quality of restoration.

Dental aesthetics can be defined as the science of copying or harmonizing or working with the natural, showing the art imperceptible. In the case of porcelain, this is the most remarkable stage, where man can imitate nature with the utmost perfection, giving to the artificial tooth the characteristics of natural tooth.[10] However, this is only possible with the metal-free prosthesis?

Many professionals and most of the manufacturers and sellers of metal-free systems answer this question saying that the metal-free systems are much more aesthetic than the metal-ceramics prosthesis. It is challenging to convince them that the lights cross through the metal-free prosthesis like the natural tooth, which is not the case with metal-ceramic prosthesis. (Figures 4 and 5)

Figure 5. The lights is crossing through the metal-free crown.

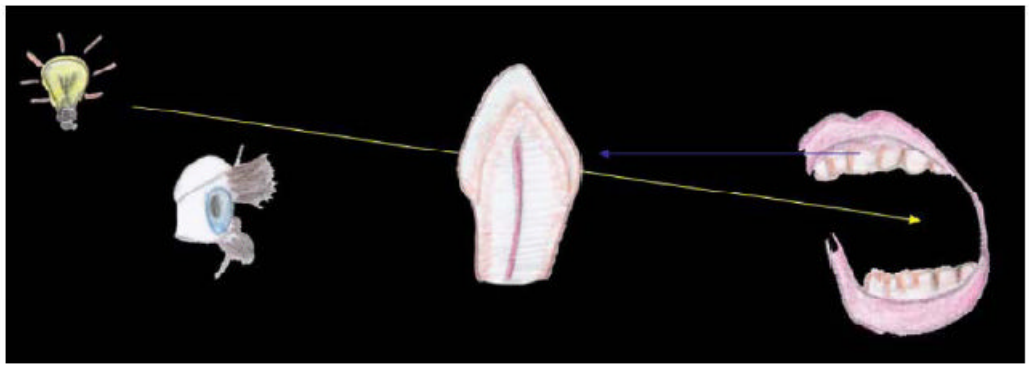

Figure 6. First path of light propagation. Origin outside of the mouth goes to inside of the mouth.

Let us think together. What is the path traveled by light? Note the two schemes and answer what is the correct path of light propagation. (Figures 6 and 7)

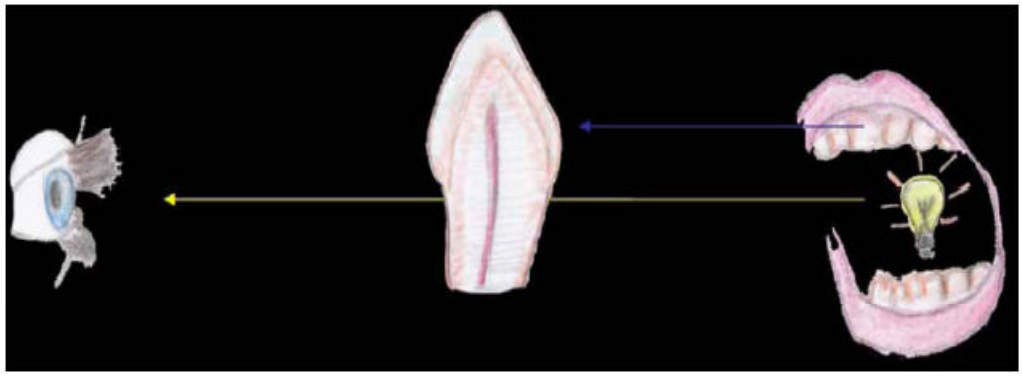

Figure 7. Second path of light propagation. Origin inside of the mouth goes to outside of the mouth.

If we think a little, we conclude that light always has its origin in the object and moves toward our eye, where the color is processed by rods and cones. Thus the characteristics of color and brightness, in our case of the tooth, are determined. When a picture shows the light transmission through a metal-ceramic and a metal-free prosthesis, we can see the characteristics of light passing through them. We either have a flashlight at the throat of the patient or lie down and the person stands over us with the neck tilted back. (Figures 8 and 9)

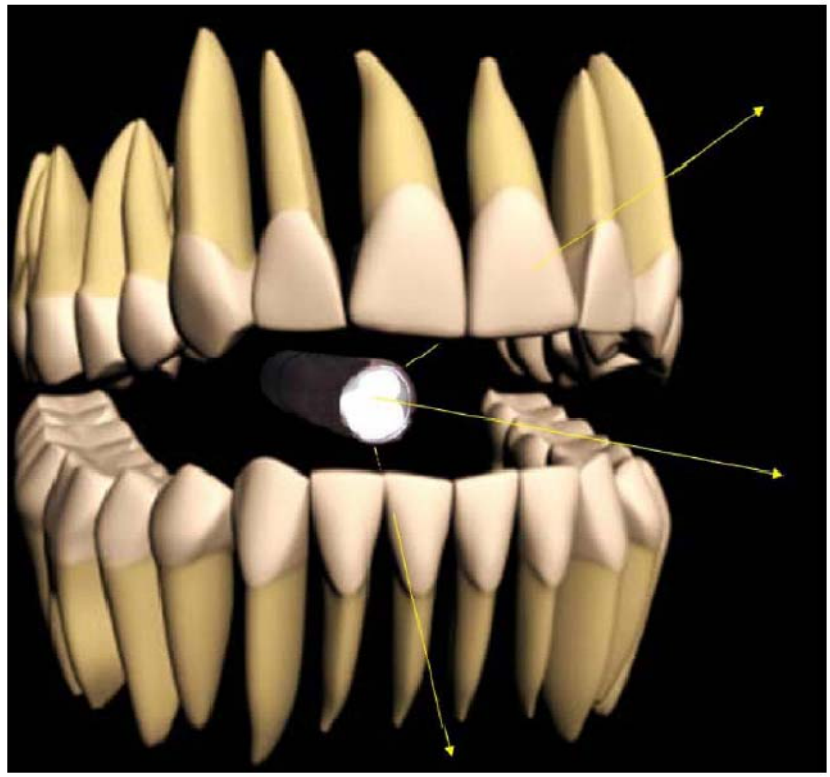

Figure 8. A Flashlight at the throat of the patient.

If the reader does not agree, please try to determine which of the two crowns below are metal-free or metal-ceramic. (Figures 10 and 11)

Are you sure about that? Absolutely? Answer with sincerity.

This work was done by the same laboratory technician at the same time, with the same thickness of ceramic coating and infrastructure.

To complicate matters a bit, the patient RCA, female, 42 years of age, appeared in the dental office complaining that she was dissatisfied with the aesthetics of her anterior teeth. Without any more details, tell me how many metal-free and metal-ceramic crowns exist in the six anterior teeth? (Figure 12)

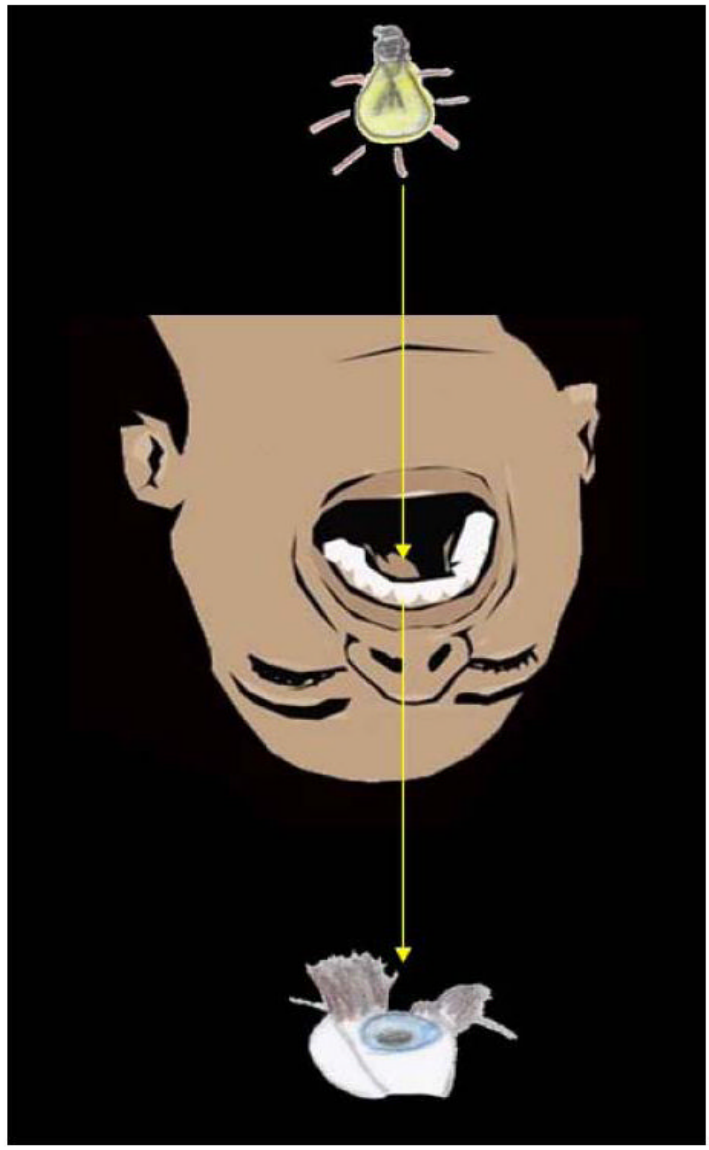

Figure 9. Patient lie down and the person stands over us with the neck tilted back.

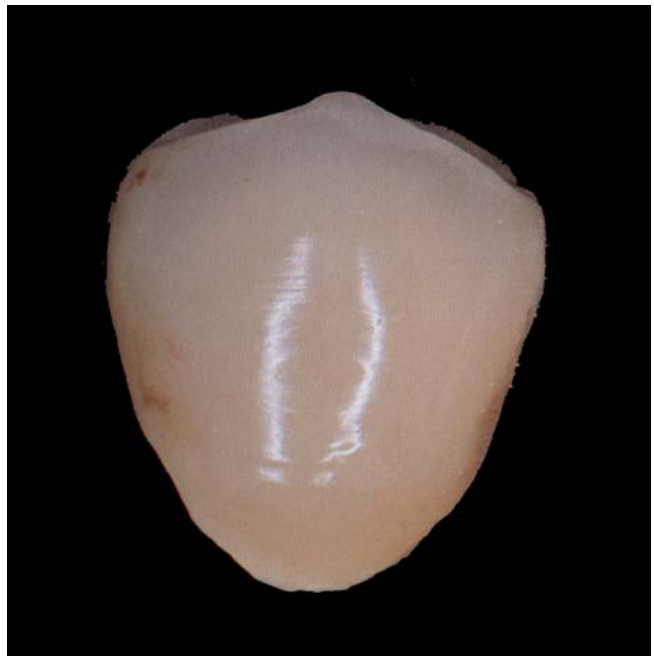

Figure 10. Metal-Free or metal-ceramic crown?

Figure 11. Metal-Free or metal-ceramic crown?

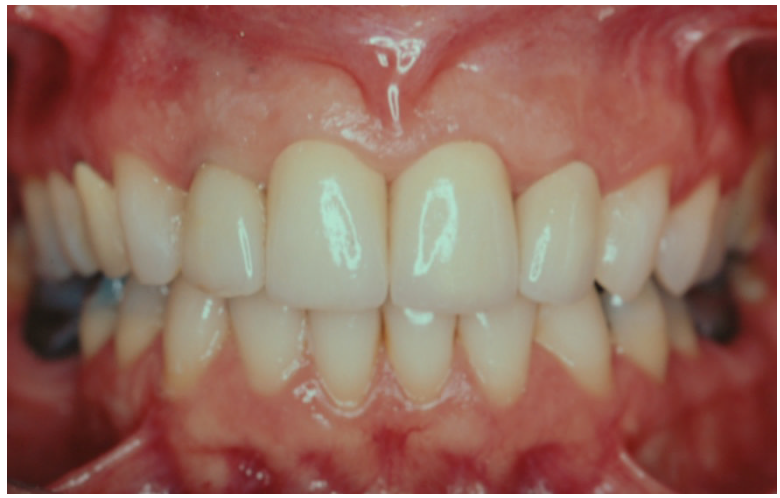

Figure 12. How many metal-free and metal-ceramic crowns exist in the six anterior teeth?

Is this question difficult? Can the reader answer with certainty how many and what types of crowns are displayed in this photograph? Please recall that the crowns were made only once and made by two different laboratory technicians; they have not been made repeatedly to try to hide the differences between the materials.

Initially, aesthetic rehabilitation was proposed with facets on teeth 11, 12 and 21 and a metal-free crown on tooth 22. However considering the lack of remaining enamel, which does not indicate this treatment, the proposed resolution was to use metal-free crowns. At the end of planning, for purely didactic reasons, there are 4 full crowns (3 metal-free crowns and 1 metal-ceramic crown) among the six anterior teeth. Where are they? Please identify them. (Figure 13)

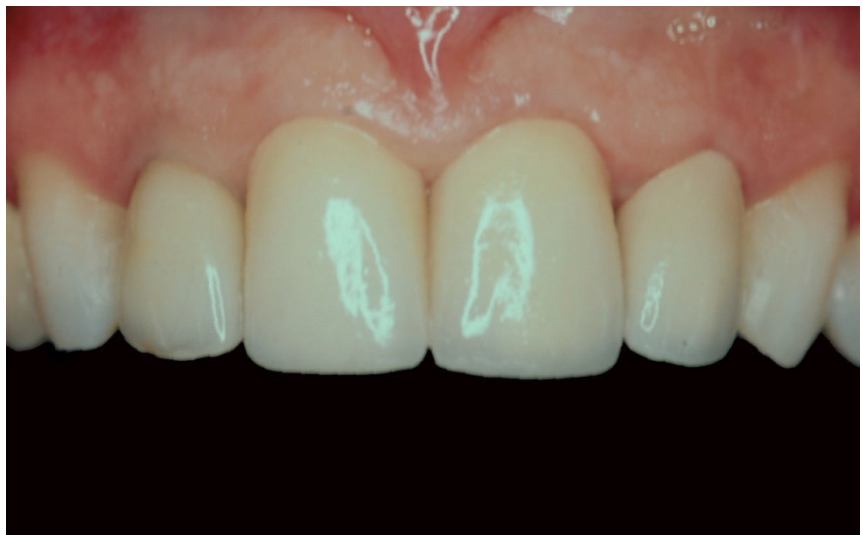

Figure 13. Which of them is metal-free?

Are you sure about your choice? If the metal-free crowns are much more aesthetic than the metal-ceramic crowns, why can the reader not identify them easily? This task should be even easier because there is no saliva and the size of the crowns is increased hundreds of times in this photograph. (Figure 14)

To complicate matters further, among the metal-free crowns, there are a Procera crown, a In-Ceram crown and a Empress II crown. To facilitate the decision, the Empress II is installed about the metal post and core. Now can you see the difference? Does the reader agree that the metal-free prosthesis are much more aesthetic than metal-ceramic crown? The response to the two cases is located at the end of the chapter. (Figures 15 to 30)

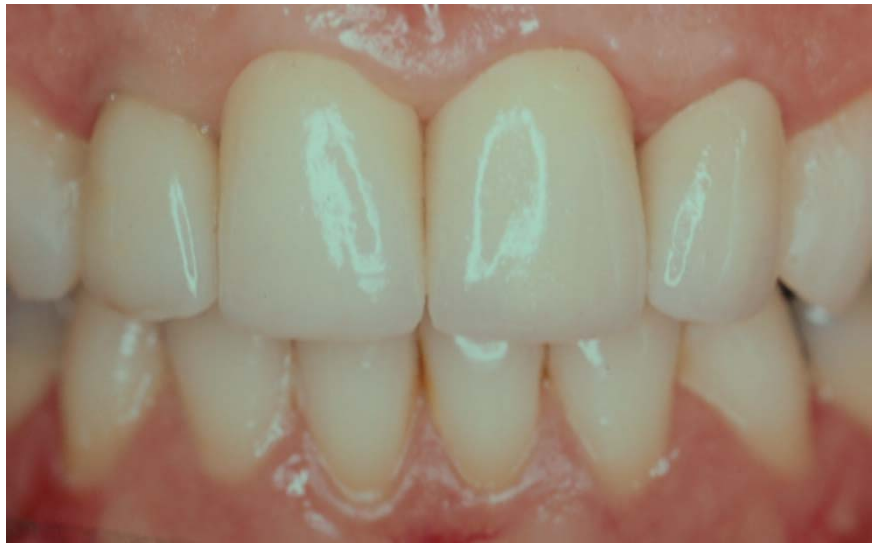

Figure 14. Identify metal-free crowns.

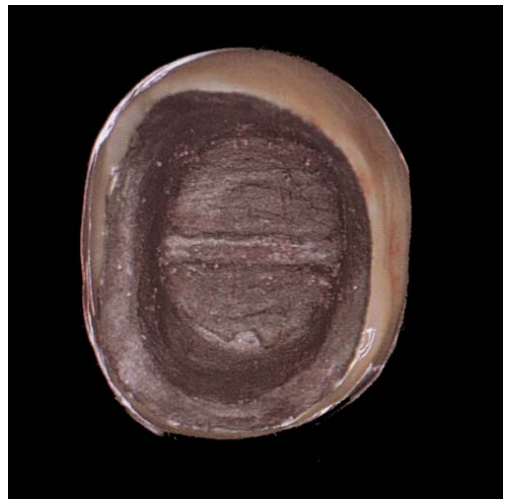

Figure 15. Metal-ceramic crown.

Figure 16. Metal-free crown.

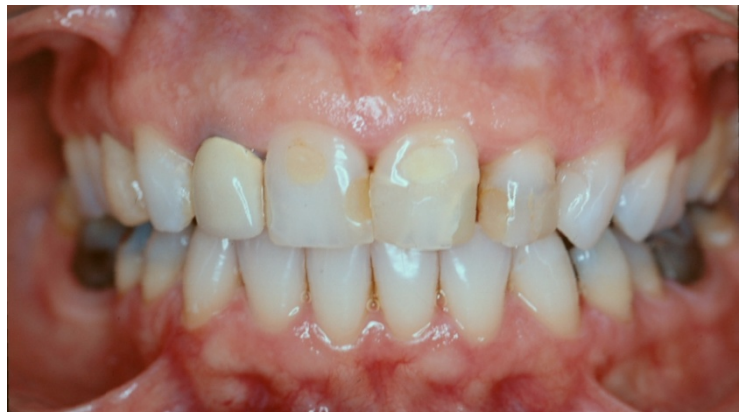

Figure 17. Before the beginning of the treatment.

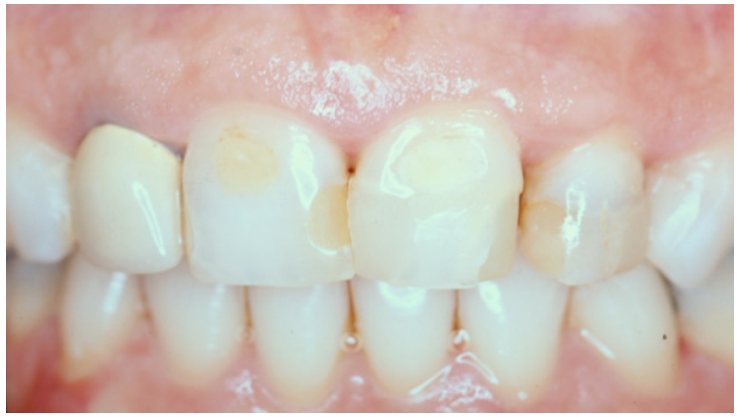

Figure 18. Three anterior teeth (11,21 and 22) restored with composite resin and 12 restored with metal-ceramic crown.

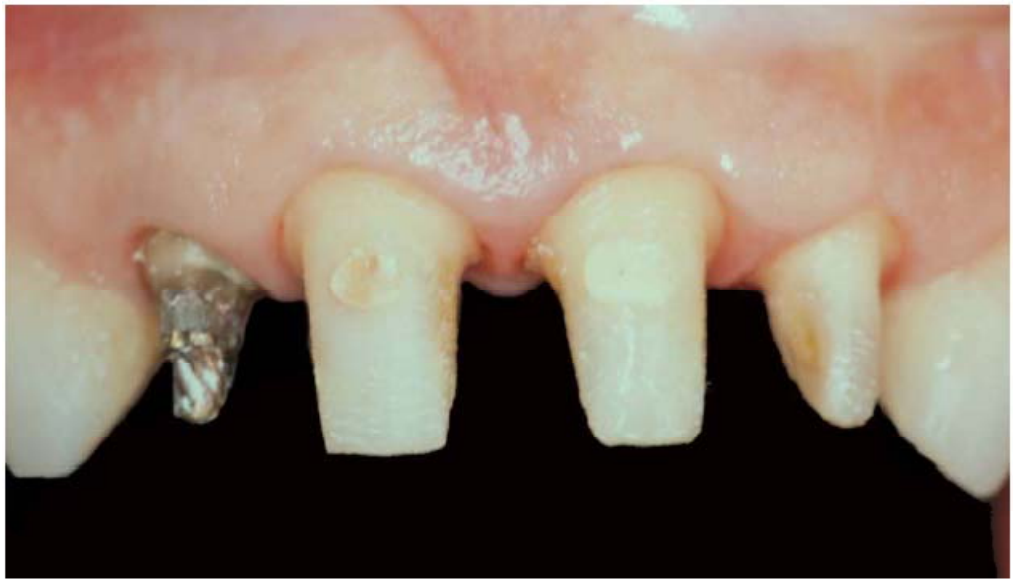

Figure 19. Prepared teeth.

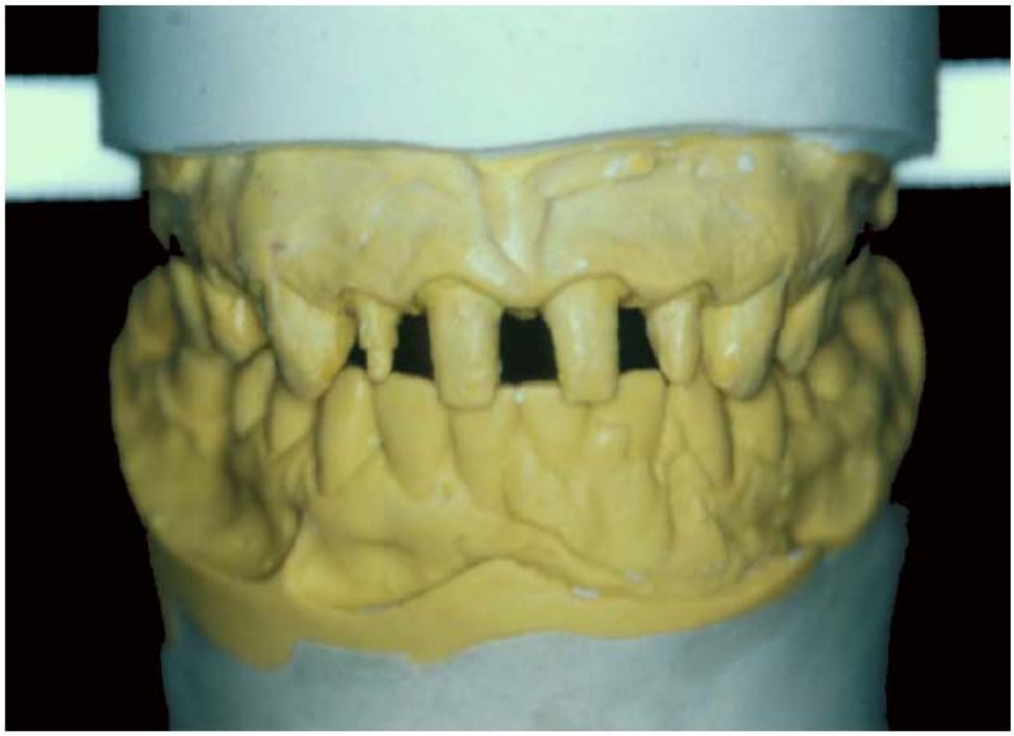

Figure 20. Plasters models on semi adjustable articulator.

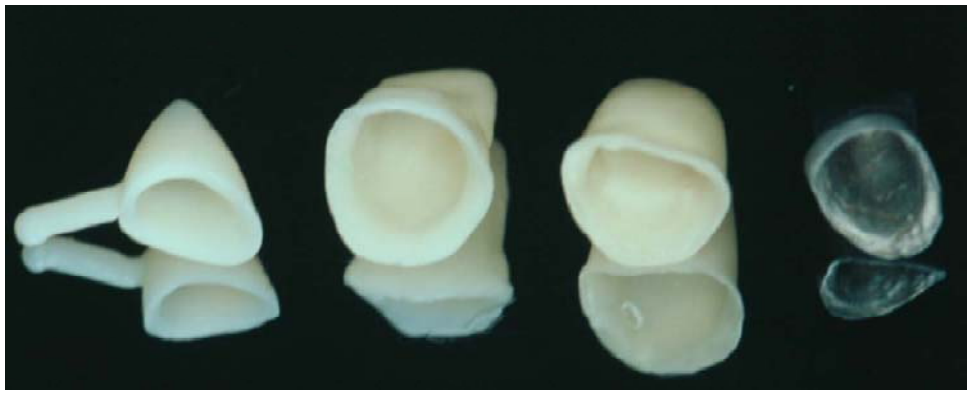

Figure 21. Infrastructures.

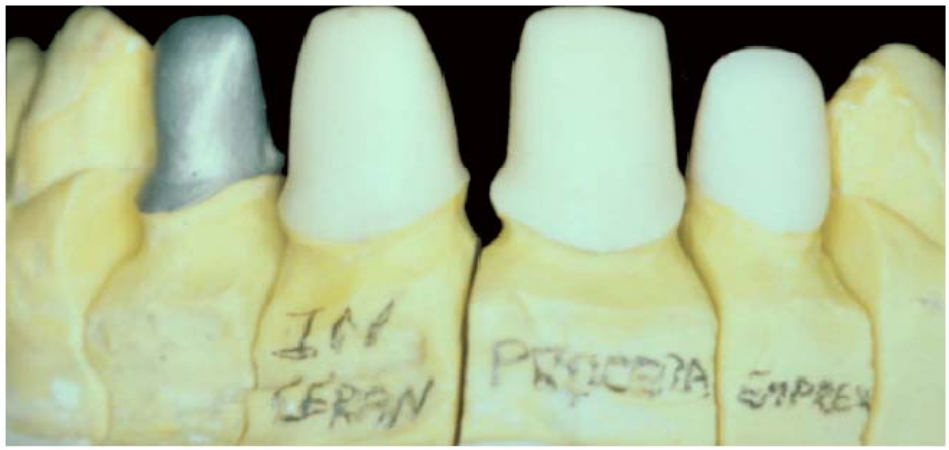

Figure 22. Metal, In-ceran, Procera and Empress infrastructures.

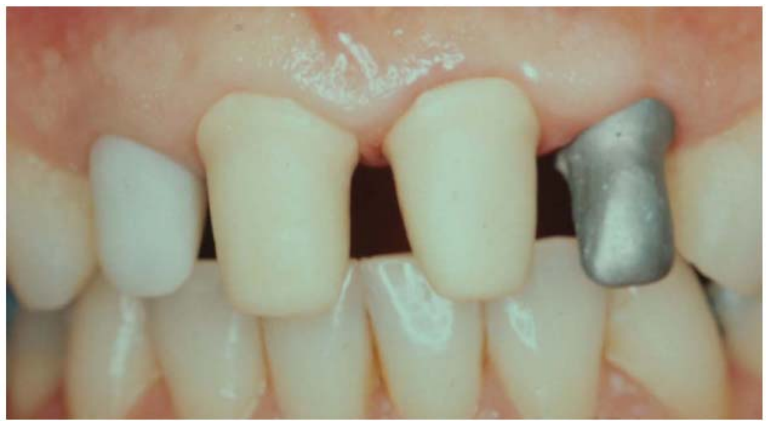

Figure 23. Adjustments of infrastructures.

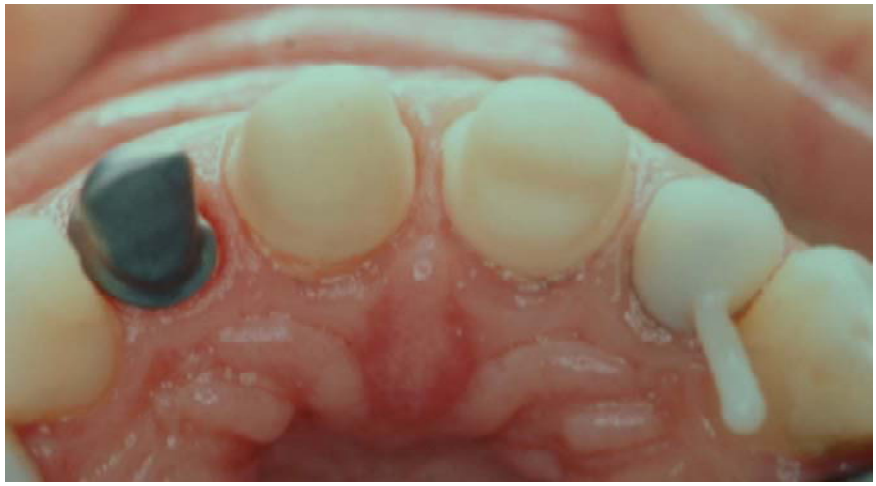

Figure 24. Palatal view of the infrastructures.

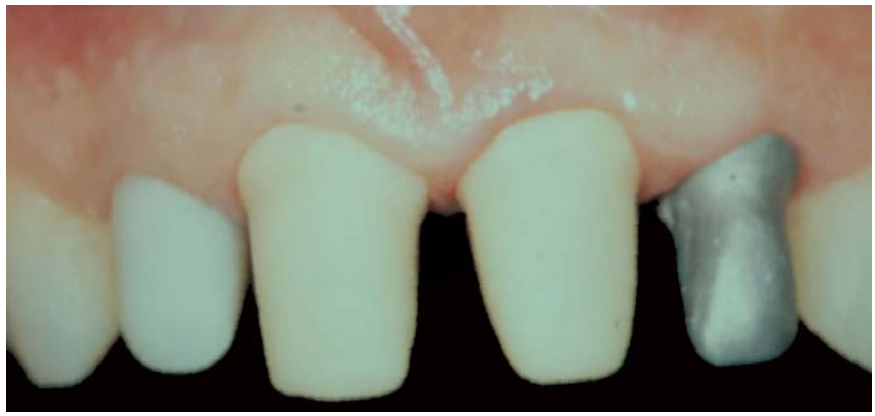

Figure 25. Vestibular view of infrastructures.

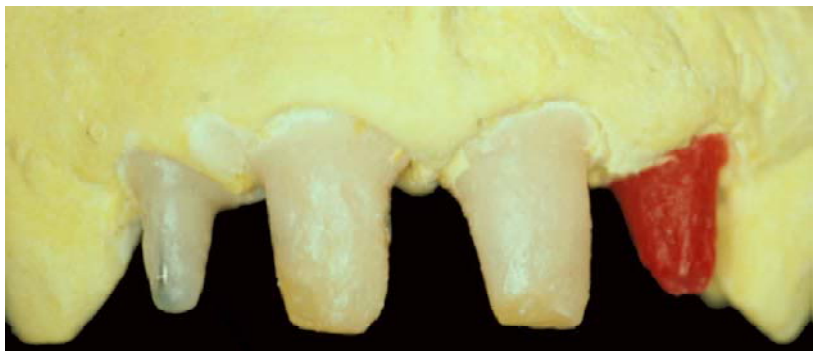

Figure 26. Red resin to metal-ceramic crown and tooth color resin to metal-free crows (The red resin hinder the adjustment of color in the metal free crowns).

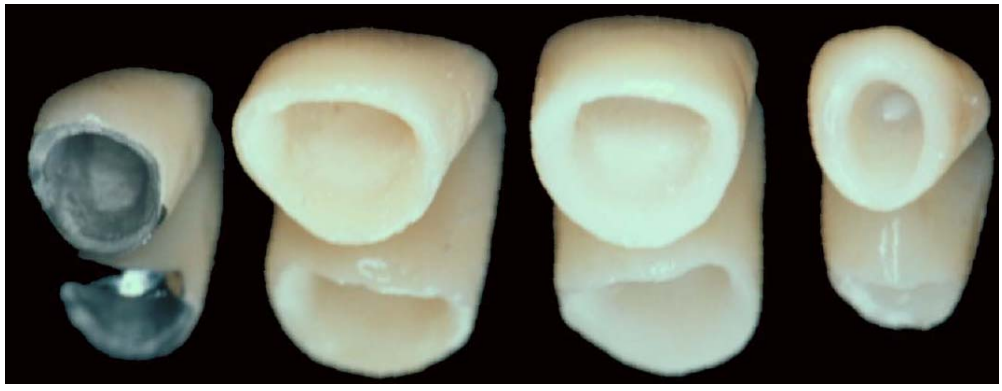

Figure 27. The metal-ceramic and metal-free crowns.

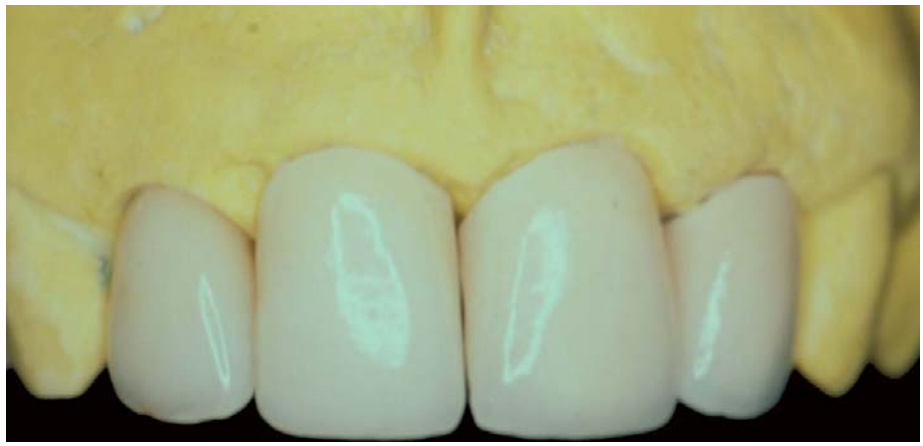

Figure 28. The metal-ceramic and metal-free crowns on plaster models.

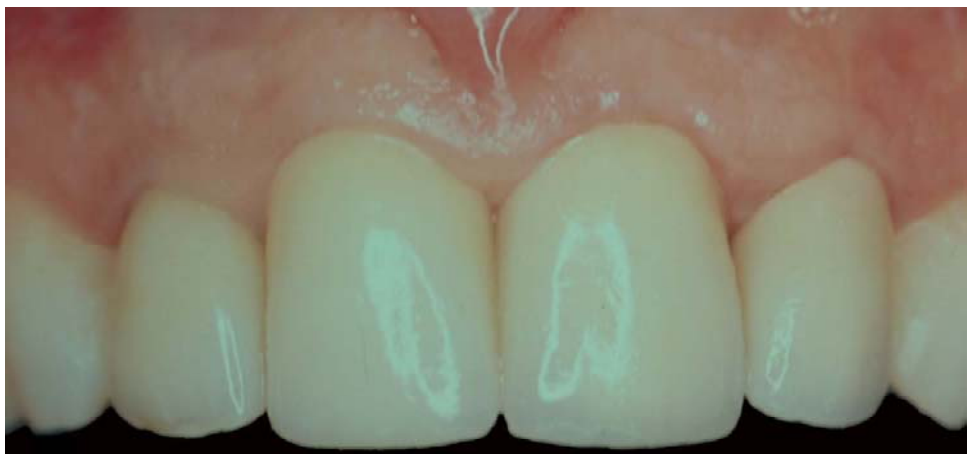

Figure 29. Crowns cemented.

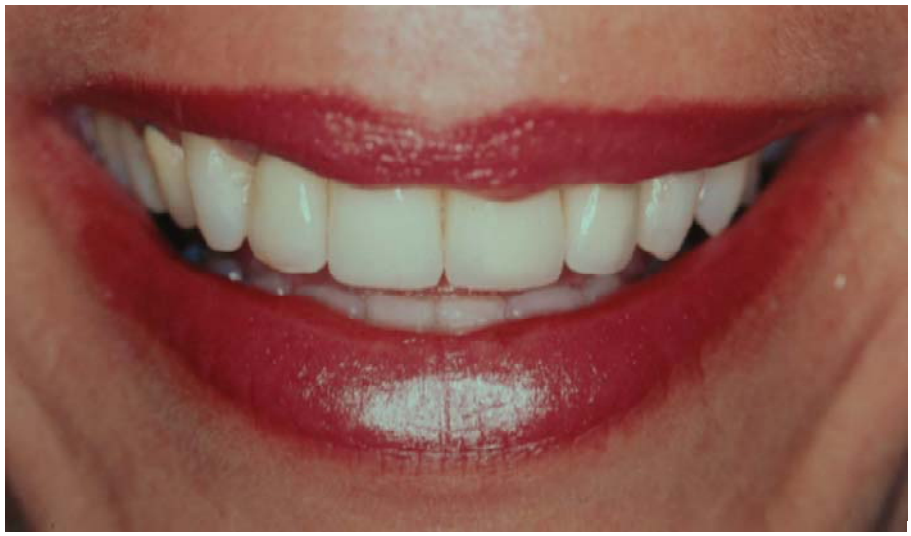

Figure 30. Final aspect of the crowns. The perfect smile.

CONCLUSION

The most modern treatment in dentistry is prevention, with oral hygiene care and professional maintenance. The best prosthesis in the world, made by the best dentist, together with the best prosthetic, can never compare to our natural teeth.

In aesthetics, as well as in implantodontology, professional concerns have replaced good sense. Dentists have performed inappropriate procedures in oral rehabilitation to the point of extracting healthy teeth for installing implants and "more aesthetic teeth." They justify this by saying that, in the near future, the implants can no longer be performed because the patient may lose bone and a satisfactory aesthetic cannot be achieved. Thus, we should think together: If there is a possibility of losing bone and it could be impossible to install implants and "more aesthetic teeth," why do these professionals not extract their own teeth, prophylactically preventing the loss of bone and the possibility of being without "aesthetic teeth"?

Thus, we must improve our knowledge and get new information every day so that we can carry out plans of treatment that are in accordance with the wishes of the patient as well as ethics and common sense. Today the world is, unfortunately, supported by money and driven by the media, which promote images of what is considered the most beautiful. Manufacturers try every minute to convince us that their product is the best, the most aesthetic, the strongest.

Remember that the only way we can really be sure of what is useful or not and what we can use or not is through knowledge based on scientific evidence. Only serious science and serious researchers, not financial interests, can bring what all professionals and patients are really looking for: the perfect smile.

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Chapter 19

PLACING AND LOADING IMPLANTS (WHEN & HOW?)

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INTRODUCTION

Immediate loading of dental implants is an international demand.

The field of dental implants is currently in a dilemma concerning whether to load implants immediately or after what's defined as a "healing period".

In both situations, a common factor is always a measure of success, namely osseointegration.

Osseointegration can be easily defined as: Direct implant-bone interface. Through the years many definitions were introduced,[1] but it all falls within the above mentioned sentence.

However, in comparing implant-bone relations, authors did compare it with ankylosed teeth. Such a relation is in favor with implants because it lacks the main problem of ankylosed teeth, namely, root resorption.

Achieving osseointegration involved several factors. Most authors related this to health, implant materials, surfaces, asepcis, minimum trauma, primary stability and a healing undisturbed period.

Maintaining osseointegration became a strongly studied issue involving atraumatic surgical technique, the amount, type of prosthetic loading and to a lesser degree, oral hygiene levels.

IMPLANT PLACEMENT TECHNIQUES

Predictable formation of a direct bone-to-implant interface is one treatment goal in implant dentistry. [2] The two-stage surgical protocol established by Branemark et al to accomplish osseointegration consisted of several prerequisites, including: [3]

1. Countersinking the implant below the crestal bone.
2. Obtaining and maintaining soft tissue covering over the implant for 3-6 months.
3. Maintaining a minimally loaded implant environment for 3-6 months.

The primary reasons cited for the submerged, countersunk surgical approach to implant placement were: [4, 5]

1. To reduce and minimize the risk of bacterial infection.
2. To prevent apical migration of the oral epithelium along the body of the implant.
3. To minimize the risk of early implant loading during bone remodeling.

After this procedure, a second-stage surgery was necessary to uncover these implants and place a prosthetic abutment. Predictable long-term, clinical rigid fixation has been reported after this protocol in both completely and partially edentulous patients. [4, 5] A two-stage approach with a 3- to 6- month healing period is recommended for the "conventional" osseointegration technique with oral implants. This may induce inconvenience and discomfort for patients, and so, immediate loading protocols are preferable. [13]

During the last 15 years, several authors have reported that root form implants may osseointegrate, even though they extend above the bone and through the soft tissues during early bone remodeling. [6, 7]

This surgical approach has been called a one-stage or nonsubmerged implant procedure because it eliminates second-stage implant uncovering surgery. As a result, the discomfort and inconvenience of, and time required by the surgery and suture removal are eliminated. In addition, the soft tissue is more mature before fabricating the final prosthesis. [2]

This should not be confused with immediate loading as implants do not have to be loaded immediately. [8]

IMMEDIATE IMPLANT PLACEMENT

Timing of implant placement significantly influences clinical bone healing around implants.

Implants can be placed at the time of tooth extraction (immediate), after several weeks (delayed), or after complete healing (late). [9]

Immediate-Immediate Implant Placement

Implant placement at the time of extraction has become an acceptable treatment option. [10] Time, cost, and morbidity are reduced, and the prosthetic solution is also eased for the benefit of the patient. [11]

Early implantation may preserve the alveolar anatomy, and the placement of a fixture in a fresh extraction socket helps to maintain the bony crest. [12]

Advantages of extraction with simultaneous replacement include the maintenance of vertical dimension, elimination of reline procedures and inter-denture therapy, and potential improvement of soft tissue healing. [14]

Osseointegrated dental implants have proven predictably successful when appropriate guidelines are followed. The Branemark's technique includes a period of up to 12 months for post-extraction bone healing. [15]

This delay, combined with the inevitable ridge resorption following extraction may contribute to several problems. The two common difficulties are: insufficient available bone for ideal implant placement and, prolonged treatment time. [15]

Immediate implant placement after tooth extraction is becoming a common procedure in implant-supported oral rehabilitation. However, lack of primary full flap closure can jeopardize final results. [16]

A surgical approach that would enable predictable primary soft tissue closure over implants placed into fresh extraction sockets is described and evaluated. This technique is based on a rotated deep split thickness palatal flap (RSPF) containing periosteum and connective tissue, covering the implant and/or a barrier membrane. [16]

This approach offers a predictable treatment approach in achieving complete soft tissue coverage, while allowing for healing of bony defects in immediate implantation procedures. [16]

Another technique that does not require any incisions during immediate implant placement is described. No barrier membranes were used and the sole grafting material used was autogenous bone chips. Full soft tissue coverage was achieved 1 week to 2 months post-implantation. Clinical osseointegration was achieved with minimal gingival recession and papillae preservation. [17]

Another technique using small autogenous bone chips (from bone adjacent to implant sites) were grafted into the defect between the implant and the socket walls when needed. Closure of the wound was obtained by coronal repositioning of the flap, and no membranes were used. [18]

Care was taken to minimize hematoma formation under the flap during healing by part-time use of removable prosthesis with thick soft linings after implant surgery. At second stage surgery, mucoperiosteal flaps were apically repositioned for maximum attached gingival width and to reconstruct the vestibule. [18]

Immediate post-extraction implant placement often deals with two major problems: maintaining the initial stability of the implant(s) and preventing soft tissue ingrowth during the healing period. Both problems may lead to the loss of the implants. [19]

Primary socket closure in immediate implantation may be difficult, due to the opening left by the extracted tooth. However, immediate implants can succeed without primary flap closure. [17]

Delayed-Immediate Implant Placement

The best results were obtained with delayed implantation. Delaying the placement of immediate fixtures allows for the elimination of associated infective processes, the achievement of maximum osteoblastic activity that theoretically could help the osseointegration process and complete wound covering. [20]

The delayed immediate placement of fixtures has a good short-term prognosis with bone regeneration occurring around the defect without the use of barrier membranes or bone substitutes.[20]

IMPLANT LOADING CONDITIONS

Misch et al presented accurate terminology to avoid this confusion. These terminology were: [2]

Immediate Occlusal Loading

Immediate occlusal loading within two weeks of implant insertion.

Early Occlusal Loading

Occlusal load to an implant prosthesis between 2 weeks and 3 months after implant placement. The actual time may use the number of weeks in parentheses (i.e. early [5weeks] occlusal loading).

Non Functional Immediate Restoration

An implant prosthesis in a partially edentulous patient delivered within 2 weeks of implant insertion with no direct occlusal load.

Non Functional Early Restoration

An implant restoration delivered to a partially edentulous patient between 2 weeks and 3 months after implant insertion.

Delayed Occlusal Loading

Occlusal loading to an impart restoration more than 3 months after implant insertion.

Two-Stage Delayed Occlusal Loading

The soft tissue covers the implant after initial placement. A second stage surgery after 3 months exposes the implant to the oral environment, after which the implant is loaded.

One-Stage Delayed Occlusal Loading

The implant is positioned slightly above the soft tissue during the initial implant placement. The implant is restored into the occlusal load after more than 3 months.

The success rate of immediately loaded implants is similar to that obtained in the case of delayed loading, after osseointegration has taken place. In contrast, this method shortens dental rehabilitation times with relevant satisfaction for patients. [21, 22]

Factors That May Influence the Success of Immediate Loading Include[23]

1. Patient selection.
2. Bone quality
3. Required implant length, design and surface characteristics of the implant.
4. Surgical skill.
5. Atraumatic surgical technique.

On histological and electron microscopical analysis of the implant-bone interface of immediately loaded implants, a direct bone apposition on the implant surface as well as the attachments of cells and matrix proteins in the early loading phase was reported. [24]

Protocols for immediate loading strive for an increase either of primary stability, which can be achieved either through an optimized implant form or implant surface, or an optimized surgical preparation of the implant bed. [3]

Moreover, through the modification of the implant surface, as acceleration of the bony healing is intended to achieve an earlier osseointegration and therefore a faster acceptable secondary stability for successful loading. [3]

PROBLEMS FACING IMPLANTS LOADING CONDITIONS

Protocols of overdentures immediately loaded on two implants in the lower canine region seem to have two main problems concerning**:**

1. Soft tissue healing and response to surgery.
2. Bone healing.

(1). Concerning Soft Tissue Healing and Response to Surgery

Second stage surgery in single or multiple implant cases is currently a simple procedure. Nevertheless, complications arising from inappropriate handling of the soft tissues, particularly during exposure of several implants, can lead to poor cosmetic and/or functional results. [25]

A very simple technique of cutting the gingiva and soft tissues covering the implants' coronal aspect with a circular blade is called "punch technique". [25]

Briefly, once the screw of the implant is located through the gingiva in the usual manner (either with the aid of the surgical guide used at the first stage surgery or using an explorer),

the round cutting device is pushed through the gingival tissues following the long axis of the implant. [25]

The shape and size of the instrument enables the surgeon to cut only as much soft tissues as necessary to expose the cover screw, clean around the implant, remove the cover screw, and place the appropriate abutment. [25]

So, in second stage surgery, punch technique provides a mean for implant placement that greatly reduces associated surgical morbidity and increases patient acceptance. The benefits of avoiding a mucoperiosteal flap increases final esthetic outcome and may result in reduction of crestal bone loss. [26]

(2) Concerning Bone Healing

Alveolar and residual bone has cortical and trabecular components. Cortical and trabecular bone may be modified by modeling or remodeling. [27]

Remodeling, or bone turnover, permits the repair of bone after trauma or allows the bone to respond to its local mechanical environment. The bone is most often lamellar but during the repair process may become woven bone, so it may respond rapidly to surgical trauma. [28]

Lamellar and woven bones are the primary bone tissues types found around a dental implant. Lamellar bone is organized, highly mineralized, is the strongest bone type, has the highest modulus of elasticity, and is called load-bearing bone. [28]

By comparison, woven bone is unorganized; less mineralized, is of less strength, and is more flexible (lower modulus of elasticity). Woven bone may form at a rate of up to 10 micron per day. [28]

The two-stage surgical approach to implant dentistry permitted the surgical repair of the implant to be separated from the early loading response by 3 to 6 months. The surgical process of the implant osteotomy preparation and implant insertion cause a regional accelerated phenomenon of bone repair around the implant interface. [29]

As a consequence of the surgical placement, organized, mineralized lamellar bone in the preparation site becomes unorganized, less mineralized woven bone of repair next to implant. [28] At 4 months, the bone is still 60% mineralized, organized lamellar bone. [30]

However, this has proven to be sufficient in most bone types and clinical situations for implant loading. Therefore, a rational for immediate loading is not only to reduce the risk of fibrous tissue formation (which results in clinical failure) but also to promote lamellar bone maturation to sustain a continued occlusal load. [2]

The immediate implant loading concept challenges the conventional healing time of 3 to 6 months of no loading before the restoration of the implant. Often, the risk of this procedure is perceived to be during the first week after the implant insertion surgery. [31]

In reality, bone in the macroscopic thread design of implant is stronger on the day of the implant placement compared with 3 months later, since there is more mature bone in the threads of the implant. [31] However, the cellular connection of the implant surface condition does not yet exist. [2]

On the day of surgery, there is residual cortical and trabecular bone around the implant. When the implant is inserted, it has some contact with this prepared bone. Early cellular

repair is triggered by the surgical trauma and begins to form an increased vascularization and repair process to the injured bone. [2]

Woven bone formation by appositional bone growth may begin to form as early as the second week after insertion at a rate of 30 to 50 microns per day. The implant-bone interface is weakest and highest risk of overload at approximately 3 to 5 weeks after surgical insertion, since the implant-bone interface is least mineralized and unorganized during this time frame. [2]

Roberts reported a devitalized zone of bone for 1 mm or more around the implants as a result of the surgery. [30]

One method for decreasing the risk of immediate occlusal load is to have more vital bone in contact with the implant interface by decreasing the surgical trauma at implant placement. [2]

Causes of surgical trauma include thermal injury and mechanical trauma that may cause microfracture of bone during implant placement, which may lead to osteonecrosis and possible fibrous and granulation tissue encapsulation around the implant. [32]

Ericksson and Albrektsson reported bone cell death at temperatures as low as 40ºC.[33] Sharawy et al reported that the amount of heat generated in the bone next to the implant drills was dependent on their design and revolutions of the drill. [34]

The temperature next to the drill ranged from 38ºC to more than 41ºC from a 37ºC baseline and requires 34 to 58 seconds to return to base line. The Two implant drill systems tested with internal cooled drills cut at a higher temperature than the two implant drill systems with external irrigation. [35]

Other factors relates to heat generated within bone while drilling include the amount of bone prepared, drill sharpness, depth of the osteotomy, variation in cortical thickness, and the temperature and solution chemistry of the irrigant. [36]

The implant-bone interface will have a larger zone of repair when the implant is significantly compressed against the bone. [37] The implant should be non-mobile upon insertion, but excess strain within the bone from additional torque and space filling may also increase the risk of microdamage at the interface. [2]

A proposed protocol for immediate load has been to insert the implant within the bone from 45 to 60 Ncm. [38, 39] This concept helps ensure that the implant has relatively rigid fixation in good quality bone. However, the additional torque used to ensure or evaluate fixation of an implant in bone may actually result in pressure necrosis and/or increase the strain magnitude at the interface, and therefore, increasing the amount of damage and remodeling, which would decrease the strength of the implant-bone interface. [2]

Misch et al suggested a method to decrease microstrain and the associated remodeling rate in bone by providing conditions to increase functional surface area to the implant-bone interface. The surface area of load may be increased in a number of ways, i.e., implant number, size, design and body surface conditions. [40]

Histological Background

Piatelli et al evaluated bone reactions and the bone-titanium interface in early loaded implants in monkeys compared with unloaded implants in the same arch several months after immediate loading. [41]

A later study by the same group demonstrated greater bone contact percentage after 8 months. However, loaded implants have less marrow spaces and more compact bone. [41]

A later study by the same group demonstrated greater bone contact in immediately loaded implants at 9 months. No fibrous tissue was found at the interface. After 15 months, both unloaded and immediate loaded implants were compared, and loaded implants exhibited greater (almost twice) direct bone contact at the interface. [42]

In particular, early loaded screws demonstrated thicker lamellar and cortical bone than unloaded implants. This suggests that early occlusal loading may enhance bone remodeling and further increase bone density compared with unloaded implants. [43]

Randow et al evaluated the bone interface clinically after 18 months in an immediate-loading situation and reported a direct bone-implant interface. [44]

Ledermann observed histologically similar findings in a 95 years old patient who had immediate-loaded bar-connected overdenture in function for 12 years. Thus a long-lasting direct bone-implant contact relationship appears possible. [45]

A NEW CLINICAL APPROACH

Placement Timing

A NEW CLINICAL APPROACH offers a solution for this dilemma. This approach is based on that atraumatic surgical technique by gradual drilling and increasing the cutting speed which will decrease the cutting force and specific energy. Accordingly, the heat generation will decrease avoiding thermal bone necrosis that may influence bone healing and implant fixation. [46]

Rationale for New Approach

Perrone reported that bone healing after osteotomy passes through three stages: [47]

1. Inflammation (granulation tissue)
2. Fibrous tissue phase.
3. Maturation phase

The fibrous tissue phase was chosen to be definitely an acceptable implant bed configuration since it shows irregular collagen formation and revascularization. Moreover, at the second week, maximum resorption is complete at the margins of the bony defect and by the third week, rapid formation of new trabecular bone to repair the defect begins. [47]

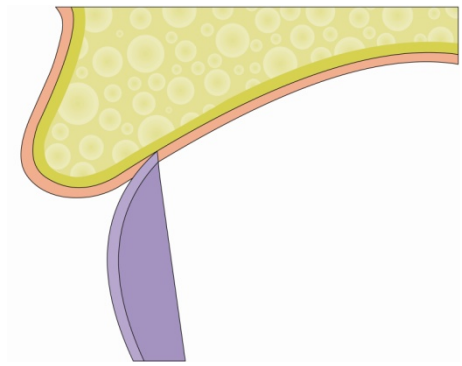

Figure1. Palatal full-thickness mucoperiosteal flap.

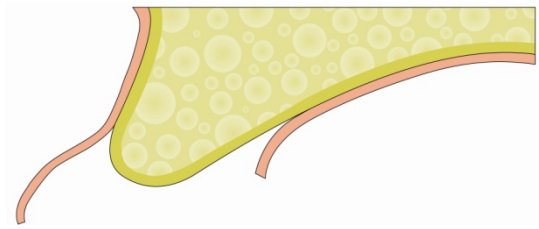

Figure 2. Mucoperiosteal elevation in a labial direction.

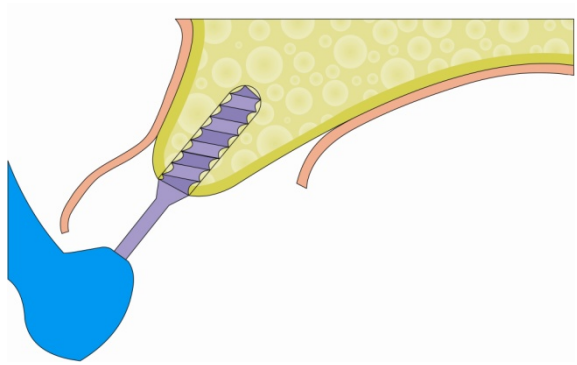

Figure 3. Bone Drilling (i.e., implant osteotomy preparation).

Atraumatic heat-free insertion of implants at this stage and before maturation gives a primary stability enables the immediate loading of implants. [46]

Accordingly, it seems logical to try avoiding most of the factors resisting ideal osseointegration combined with immediate loading, namely, heat generation, edema and soft tissue problems.

1. Palatally situated full-thickness mucoperiosteal flaps reflected to preserve the neighboring interdental papillae. This approach was used to allow for optimal drilling of bone and preparing the site for implant insertion (Figure. 1 and 2)
2. Bone drilling performed according to the manufacturer's instructions as follows (Figure 3): pilot drill, 2-mm twist drill, and final drill according to the implant diameter.

3. Closure of the flap on an empty (blood filled) socket, giving time for the socket to heal from trauma and heat (Figure 4)
4. Two weeks later, a punch technique [48] is performed for preserving soft tissue. A tissue punch is used to go through the covering mucosa at the desired level of gum margin (Figure 5)
5. Slight curettage of the socket and irrigation with saline .
6. Using the hand wrench for implant insertion, a minimal amount of heat generation is anticipated on implant insertion (Figure 6)
7. Immediate abutment placement and temporary crowning with the desired emergence profile. (Figure7 and 8)
8. Three to six weeks later, final impression and fabrication of final restoration (Figure 9)

*In a research performed by the author, a total of 30 implants were successfully osseointegrated after 12-months follow-up. Indirect, digitalized standardized periapical radiographs were performed immediately after loading, and after 6 and 12 months, respectively. Bone level changes showed an insignificant amount of bone resorption in all cases.

Immediate loading after delayed implant insertion showed successful results because of several factors. The use of a gradual drilling (i.e., in reaming and relatively increasing the drill rpm) will decrease the cutting force and specific energy (i.e., total work required to prepare the osteotomy). Accordingly, both the heat generation and trauma will decrease, avoiding thermal bone necrosis and excessive bone microfractures that may influence bone healing and implant fixation. [32]

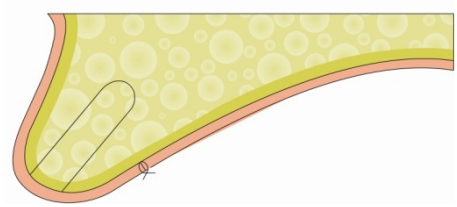

Figure 4. Flap closure on an empty (blood-filled) socket

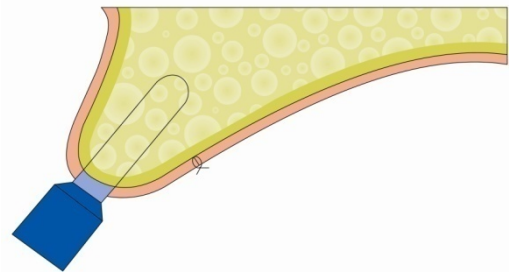

Figure 5. Tissue punching after 2 weeks.

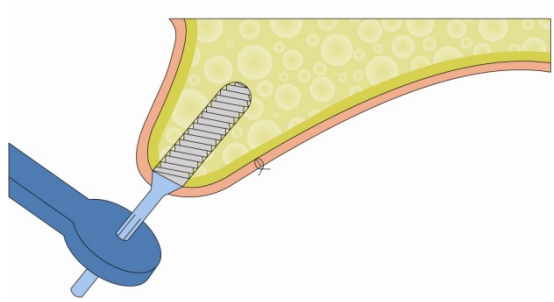

Figure 6. Implant insertion using a hand rachet wrench.

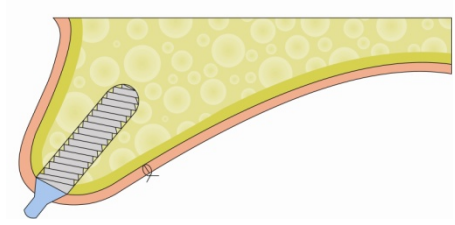

Figure 7. Immediate abutment placement.

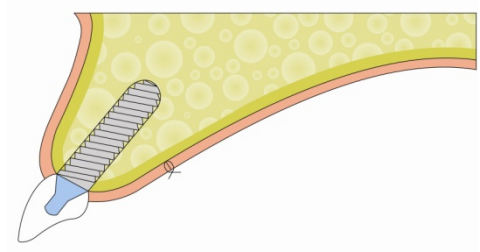

Figure 8. Immediate restoration with temporary crown.

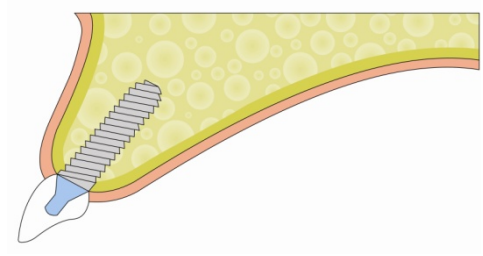

Figure 9. Final restoration in place after 3-6 weeks.

The implants were inserted at the fibrous tissue phase after 2 weeks of osteotomy because this stage shows irregular collagen formation and revascularization. This is definitely an acceptable implant bed configuration. Moreover, at the second week, the maximum bone resorption is complete at the margins of the bony defect. By the third week, rapid formation of new trabecular bone occurs to repair the defect.

Early woven bone formation by apposition growth may begin to form as early as that suggested for implant insertion. [2] At the fibrous tissue phase, primary stability of implant

insertion was achieved. The created primary stability is mandatory to enable the immediate loading of implants. [46] Using a flap approach for implant osteotomy is definitely a preferred approach than the punch technique.

The Punch Technique

From the surgical point of view, flap reflection is the most ideal approach for implant placement. However, the punch technique provides a method for implant placement that highly reduces associated surgical morbidity, and it increases patient acceptance. [48] The benefits obtained include: [49]

- No sutures needed to adjust the gingiva around the abutment.
- Excessive undermining of the gingiva is avoided as in cases in which an extensive incision is used to expose several implants.
- Minimal bleeding.
- Gingival attachment around the abutment is rapidly and completely achieved.
- Postoperative pain, discomfort, swelling, or tenderness is unusual.
- Cosmetic appearance and functional properties of the gingiva around the abutment are improved.
- Natural look and firm attachment are achieved.

CONCLUSION

The presented timing of the implant insertion (i.e., second week after preparing the implant osteotomy) opens the road for future research work in preparing implant beds. Offering a relaxed healing implant bed ready to receive a fixture is obviously preferable than inserting a fixture in a traumatized and heated site. The presented technique may start a new way of relating implant failure, especially with immediate loading, to the condition of the implant bed. While dealing with soft tissue, using the tissue punch is definitely a positive step for offering maximum esthetics.

What a dilemma if we come to think about it. A question of what is right and what is wrong appears. However, in science different and contradicting issues will continuously be there. The dental world is now heading towards immediate loading.

The author started thinking about the human bone. Bone is not a piece of wood, it is alive and interactive. It is full of blood and blood is life.

Our way of thinking questions the difference between placing an implant in a piece of wood and in living bone. We think highly and respectfully of bone. We question the validity of the conventional protocol of implant insertion and loading.

Finally, we hope that our ideas would add to the ongoing efforts of solving the big dilemma of dental implantation.

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Chapter 20

CLINICAL SUCCES OF ALVEOLAR BONE DISTRACTIONS FOR IMPLANT REHABILITATION: REVIEW ARTICLE

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Vertically deficient alveolar ridges represent a challenge for appropriate prosthetic rehabilitation. Nowadays osteointegrated implant use is the most popular treatment modality in such cases. A variety of techniques have been used to establish a suficant osseous tissue for supporting dental implants Onlay grafting and guided bone regeneration, are the frequently used surgical procedures. Distraction osteogenesis is an alternative augmentation technique providing the sufficient enlargement of the bone and soft tissue volume for the placement of dental implants. The purpose of this chapter was is to define the techniques and compare the complications and implant survival rates in localized alveolar deficiencies reconstructed by alveolar distraction osteogenesis.

RESULTS

Autogenic onlay bone grafts show unpredictable bone resorption.. increased morbidity is also expected due to the needness of harvesting bone from intraoral or extraoral sites. The use of GBR guided bone regeneration to increase vertical bone dimension is very limited. Aditionally it has high risks of membrane exposure, infection and limited vertical regeneration because of insufficient vascularization.. Alveolar ridge distraction appears be an effective technique for the placement of implants in the severely vertical alveolar bone resorption considering treatment outcome ,implant survival and surgical complications

Increasing the alveolar vertical dimension shows six general ways: guided bone graft augmentation, onlay block grafting, interposition alveolar bone grafting, alveolar distraction

osteogenesis, iliac corticocancellous augmentation bone grafting, and sinus bone grafting. When bone grafting is used for this purpose, the morbidity is greater and some bone resorption is expected. Alloplastic materials are not suitable for implant placement.[1,2]

Distraction osteogenesis (DO) is a bone generation technique that involves progressive bone fragment elongation within the gap created by an osteotomy. Following von Langenbeck's first description of DO in 1869, this concept was explored in the long bones and later in the craniofacial region.[3]

The Russian orthopedic surgeon Gavriel Abramovich Ilizarov (1921–1992) is the father of modern distraction osteogenesis. Ilizarov explored the possibility of monofocal, bifocal, and trifocal callotasis in transport osteogenesis and established two basic principles of DO: the law of tension-stress and the influence of mechanical load and vascular supply. Following the basic principles of Ilizarov, DO was subsequently applied in the mandible, maxilla, and vertically deficient alveolar ridge.[3]

Using a canine model, Snyder *et al*. were the first to adapt the limb DO principle to the craniofacial skeleton, in 1973. Their success was a considerable technical accomplishment that ignited the field of craniofacial DO and created the momentum for numerous experimental surgical models.[4]

This chapter gives basic surgery rules of the distraction osteogenesis and common failure reasons of the procedure . Also give literature review of clinical outcome

SURGICAL TECHNIQUE

Distraction osteogenesis procedures can be performed under general anesthesia or sedation with local anesthesia depending on the complexity of the surgery or the comfort of both the surgeon and patient.

The buccal cortex in the region of the defect is exposed through a horizontal vestibular mucoperiosteal sulcus incision . The borders and level of the incisions should be planned according to the defect borders and planned osteotomy lines (Figure1a, Figure1b). The transport segment must remain attached to vital tissue. The palatinal or lingual mucosa should not be detached from the alveolar bone that supplies blood to the distraction chamber. In addition, the mucoperiosteal flap should not be extended too far toward the alveolar crest so as not to disturb the blood supply to the transport segment, to allow successful healing.

The osteotomy lines can be planned after considering three-dimensional (3D) computed tomography (CT) and 3D models. The lines can be marked with a small round bur and completed with osteotomes. While using the chisel, the surgeon's other hand should be positioned palatally or lingually, to prevent damage to the mucoperiosteum. The osteotomies can also be performed with either micro-saws or Piezosurgery devices under saline irrigation(Fig 2a, 2b). After completing the osteotomies, the surgeon should check whether the transport segment is mobilized fully. If the surgeon rushes to mobilize the segment before completing all of the osteotomy cuts, or the transport segment is too thin, unplanned transport segment fractures will occur, endangering the procedure. In such cases, the procedure should be stopped and postponed until the unplanned fracture has healed. The horizontal osteotomy should leave a minimum of 4 mm of bone to prevent resorption of the transport segment during distraction. The angles of the vertical osteotomies are vital for the success of distraction. The osteotomy walls must be slightly divergent (inverted trapezoid-shaped) to

avoid friction between the parts while the bone fragment is moved upward. Avoid making the osteotomy walls with vertical angles smaller than 90° to prevent undercuts . [5]

Figure 1a. illustration of anterior vertical bone deficiency for implant aplication

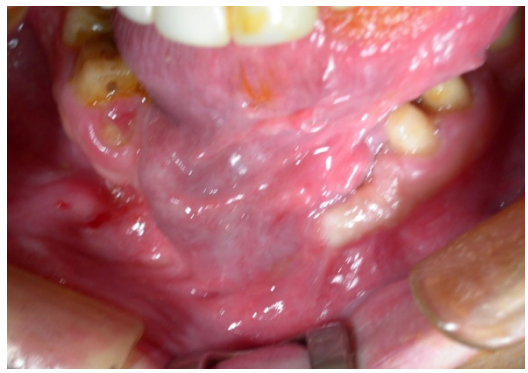

Figure 1b. clinical view of a severe anterior vertical bone defect due to the car accident

For safe distraction osteogenesis, the device needs to be activated during surgery to observe whether it is working properly. Proper vector assessment is fundamental for distraction and is affected by the pulling effect of the thick keratinized palatal mucosa and genial muscles. To save time, all of these surgical steps can be tested and plates can be adapted before the actual procedure using models. The stability of the distractor device should be ensured in order for it to withstand the soft-tissue forces encountered during distraction. This vector should be controlled throughout the distraction process. During distraction, tilting of the transport segment can be controlled by prosthetic or orthodontic wire stoppers to guide the distractor rods. Excessive drilling and screws can both reduce the blood supply of the transport segment and chamber.

Figure 2a. ostetomy of the segment with micro saw

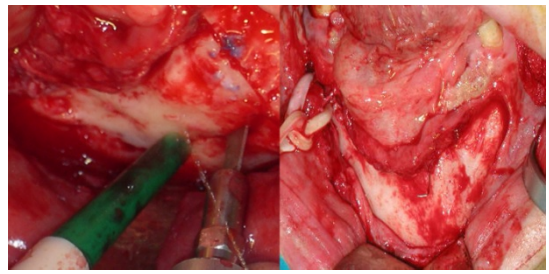

Figure 2b. İntraoperative view of the osteotomy of the segment

The choice of distractor should be adequate to meet the treatment needs, and it should not be too large or too small. [6]

CURRENT PROTOCOLS IN DISTRACTION OSTEOGENESIS

The *latency period* is the waiting period after surgery. This period is the interval between the osteotomies and the start of distraction (Fig 3a, 3b). Although the latency period can range from 0 to 7 days , it is most commonly 5 to 7 days, during which soft callus forms [7,8,9]. The latency period allows the formation of a blood coat and fibers rich in type 1 collagen between the base and transport segments. The latency period is followed by the *activation period*. During this stage, bone lengthening is activated at a constant rate (Figure 4a). Histological studies indicated that the proliferation of bone-forming cells during distraction osteogenesis is affected by the rate of distraction. Activation rates slower than 0.3 mm/day do not stimulate cell proliferation effectively. Distraction rates of 0.3–0.7 mm/day result in increased cell proliferation. It has been claimed that a distraction rate of 0.7 mm/day is the optimal for cell proliferation. Distraction rates exceeding 1.3 mm/day cause tissue damage and necrosis.[10] During the *consolidation period* (Fig 5a, 5b, 5c), the device is left in place for approximately 6–8 weeks to allow the bone in the distraction gap to mature and stabilize the segment.[11,12] There are many opinions regarding consolidation and the timing of *implant insertion*. Even when implants are placed during the early stage of consolidation, osseointegration is achieved without significant disruption of the degenerative process. Early implant application 6 weeks after distraction is thought to decrease relapse during consolidation. Osseointegration requires 6 months.

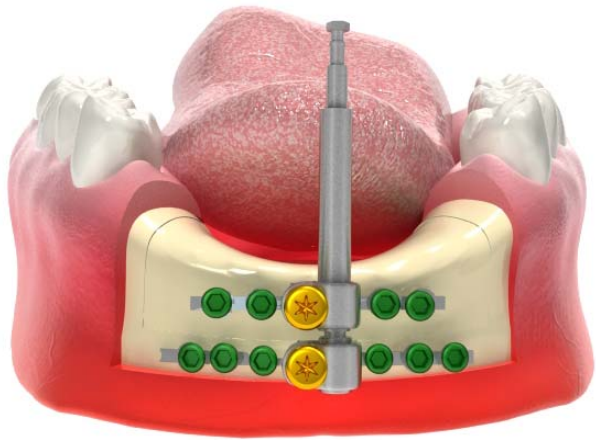

Figure 3a. Placement of the distraction device

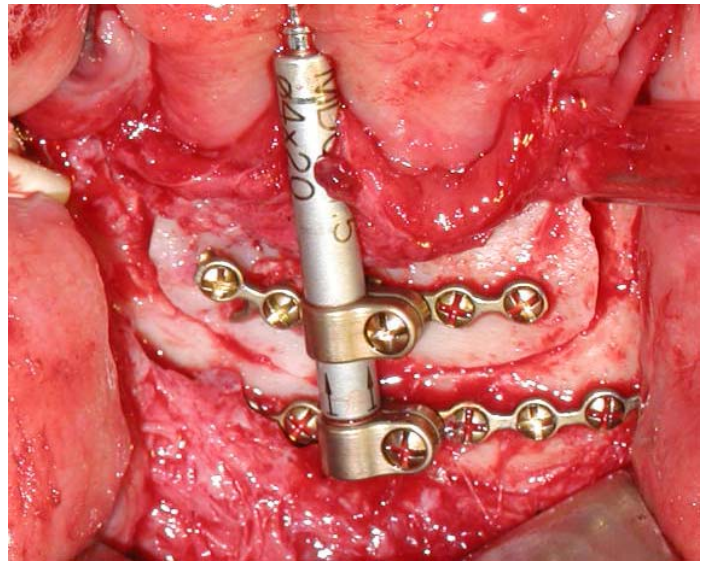

Figure 3b. fixated distraction device during surgery

ADVANTAGES OF ALVEOLAR DISTRACTION

Autogenous bone grafting and distraction osteogenesis are the most frequently used augmentation methods for moderate to severe alveolar deficiencies. Although favorable results with both methods have been published, discussion regarding the ideal treatment model is ongoing. A few studies have described the complications and benefits of using autogenous bone grafting and distraction osteogenesis. [13-15]

There are many advantages of distraction osteogenesis over other vertical augmentation methods for reconstructing lost bone in terms of the donor site morbidity, bone quality, bone quantity gained, and bone resorption important to achieve better implant anchorage and esthetically functional prosthetic reconstruction.[16]

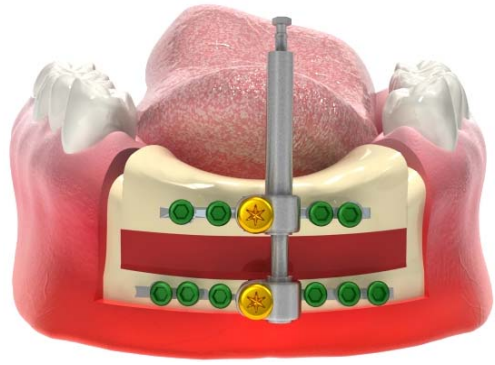

Figure 4a. Activation of distraction chamber

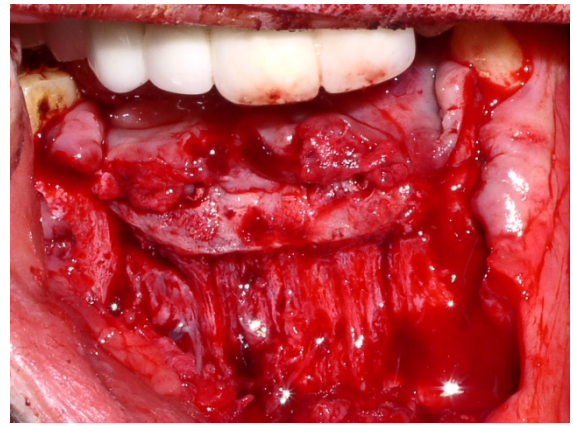

Figure 4b. Operative view after Removal of the device

In the posterior mandible, the use of other vertical augmentation procedures such as onlay or inlay bone grafting and guided bone generation with titanium-reinforced polytetrafluoroethylene (PTFE) membrane-supported grafts, has had some success. Distraction osteogenesis is most helpful in the anterior alveolar region, giving a better esthetic outcome for the dental arch.[17،18]

The extensive loss of bone and teeth in the anterior maxilla presents a complex problem for reconstruction of esthetic such as long unnatural crowns , especially in patients with severe bone loss in the horizontal and vertical planes. [19]

The use of autogenous block bone grafts in bone regeneration procedures for alveolar ridge augmentation can be limited by *donor-site morbidity* and complications seen in the recipient side.[20] Distraction osteogenesis avoids the donor site morbidity associated with bone grafting and the complications at the recipient site.[21]

Although short implants can be used to compensate for vertical bone deficiency, the survival and esthetic problems of implants depend on the *crown root ratio*. Also short implants are anchored only in the superior cortex, which compromises their load-bearing capacity. To obtain the ideal vertical hard tissue, distraction osteogenesis is a good choice for overcoming this problems.[22]

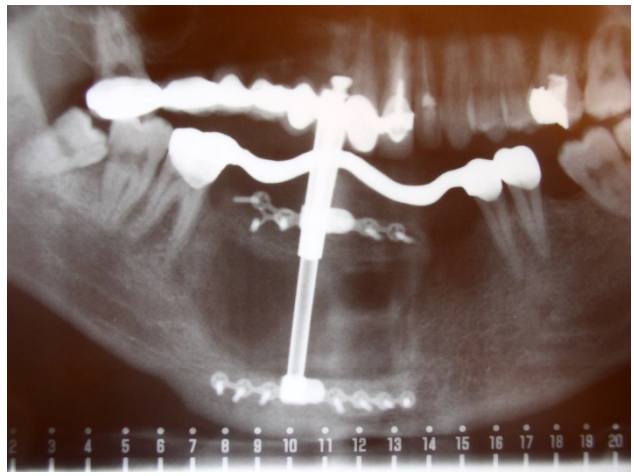

Figure 5b. Clinical aparance of the newly augmented bone with distraction. 20 mm new verticalbone was gained

With bone graft augmentation of severe alveolar defects, there is frequently a need to advance a local flap to cover the bone graft; this requires a secondary alveoloplasty to create attached mucosa at the crestal area around the implants. By contrast, vertical distraction osteogenesis has the advantage of simultaneous distraction of both bone and soft tissue together, and the original preoperative attached mucosa remains at the crest. [21,23]

Bone grafting creates much more immediate tension during the heightening of the alveolar process by using block or particular bone grafts. By contrast, alveolar ridge distraction stretches the neurovascular bundle gradually and gently, preventing stenosis.

The main limitation of severe defects is that there is not enough soft tissue to cover a large bone graft adequately. Distraction osteogenesis results in the genesis of the soft tissue necessary to cover the newly augmented bone.[24]

One of the significant factors in long-term implant success is the peri-implant soft tissue. The absence of attached gingiva may increase the risk of peri-implantitis, which is a common reason for late implant loss.[24] Maintaining keratinized tissue around dental implants decreases the risk of peri-implantitis and increases the survival rates. Most of the resorbed alveolar bone needs free keratinized tissue grafting after autogenous bone grafting. Studies show that more keratinized tissue is lost with onlay bone grafting, as compared to distraction osteogenesis.[25]

One important disadvantage is that the technique takes time and the patients must be monitored closely during the activation period. Good patient cooperation is critical for the success of this procedure.

Common Failure Reasons and Complications during and after Distraction

Distraction osteogenesis can fail or complications can occur during the operation, such as malfracturing, incomplete fracture of the transport segment , nerve damage, stability and the vector problems of the distractor , during activation such as pain, infection, and premature consolidation or during post-distraction periods after removal of the distraction device such as relapse , malunion and persistent nerve damage.

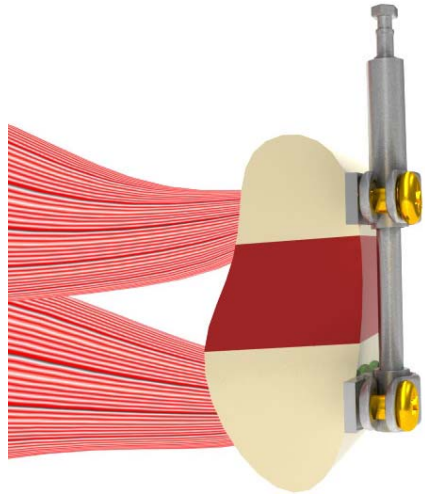

Figure 6. Radiological view of the augmented defect after consalidation before the removal of the device

Aronson *et al*. identified four categories of failed distraction osteogenesis: (a) ischemic fibrogenesis caused by arterial disruption; (b) cystic degeneration related to obstructed venous return; (c) nonunion, which was categorized as device fixation failure; and (d) late buckling or fracture, which is usually caused by premature removal of the fixation.[26]

Incomplete fracture of the segment is a well known main reason of the failure of distraction osteogenesis. To prevent the complication of incomplete fracturing, the corticotomy procedure should be performed cleanly. A complete osteotomy at the distraction site is needed, instead of a green-stick fracture. A green-stick fracture will cause difficulties during the movement of the transport segment. It may cause fracture of the device or the fixation plates. In some cases, green-stick fractures cause severe pain, as the segment does not move during activation. Checking the mobility of the segment by activating the distractor intraoperatively is the best way to overcome this problem.[27]

Immediate distraction, starting the day of surgery, should be avoided to prevent *wound dehiscence*, which would expose the regenerating bone to the oral environment. A guideline for starting distraction is 4 to 7 days after the osteotomy surgery. Dehiscence can be minimized by smoothing the sharp edges of the transported fragment. It is treated by resuturing the soft tissues.[28,29]

Altougth infection via the distraction rods rarely seen problem, surgeon should not neglect this situation. The mucosal epithelium results in it lining an open tract in the oral cavity, such as along the distractor rod. Epithelialization can lead to chronic infection and loss of the distractor and regenerated bone. To reduce the risk of infection, the distractor should be removed as soon as possible after consolidation is complete, ideally 6 to 8 weeks after finishing distraction. Distractor left in for more than 8 weeks may have small amount of purulent discharge from the rod track. Local irrigation, improved oral hygiene, and the administration of antibiotics are sufficient to control distractor-dependent infections.[6,23,27]

Slow distraction rates, incomplete fractures, or mechanical problems during distraction may result in premature consolidation. If this occurs, the procedure must be repeated.

Fractures of the transported or basal bone can be prevented by using very sharp blades to make the osteotomy fine without applying over forcess. If an unplanned fracture occurs, distraction should be canceled or evaluated for a osteosynthesis. The volume of the transported segment must be sufficient for appliance fixation, and there should also be sufficient bone volume to minimize the risk of resorption of the segment.[30] Complete resorption of the transport segment following attempted distraction has been reported and, most likely, resulted from vascular compromise. This can be overcome by overcorrecting the defect by around 2 mm.[23]

Distraction device instability and Deviation from the correct distraction vector decrease the clinical success of distraction. A good preoperative evaluation of the bone density and 3D distraction device model used is needed to solve this problem. Also pull effect of genia lmuscle should be considered to avoid deviation from the correct distraction vector (Fig 6)

There are limitations to distracting the anterior maxillary and mandibular alveolus because of the resistance of the thick keratinized palatal mucosa and lingual genial muscles. The thick keratinized palatal mucosa cause palatal deviation, while the genial muscles pull the transport segment lingually. Palatal or lingual inclination of the newly generated bone will prevent the surgeon from inserting implants with the correct bucco-lingual angulation. In such cases, additional horizontal bone augmentation is necessary to obtain ideal implant rehabilitation.

A number of strategies have been developed to overcome vector problems: secondary bone grafting can be considered during removal of the distractor; the vector of the rods can be adjusted while fixing the device; a temporary prosthesis can be used as a mechanical barrier to stop rod deviation.

Anatomic considerations may cause technical difficulty with the reconstruction of alveolar atrophy of the posterior mandible with distraction osteogenesis. Mandibular osteotomies should be performed carefully to avoid *nerve injury*, especially with severe vertical alveolar bone loss, because a complete bone fracture may occur. The osteotomies should be carried out at a safe distance from the mandibular nerve, and the osteotomies should not compromise the continuity of the mandible. If the bone above the inferior alveolar nerve is less than 6 mm high, distraction should be avoided.[10]

Neurological injuries can be prevented with correct localization of the osteotomy and the placement of retention screws based on CT evaluation. If such complications are seen radiographically, the screws should withdraw immediately. Most of the neurological complications due to pressure or stretching recover if the mandibular nerve has not been damaged severely by the drills and osteotomes.

In a series of 70 patients clinical study (140 sites), 23 patients (33 sites, 23.6%) had altered sensation in the distribution of the mental nerve when tested with sharp and blunt stimuli after distraction. Only two patients (3sites, 2.1%) still had hypoesthesia 12 months after removing the distraction devices.[27]

Relapse, Resorption and Per Imlant Bone Lost

Oda *et al*. demonstrated that the alveolar ridge can be augmented successfully using distraction osteogenesis in a histological and radiographic study of adult dogs. They also demonstrated the integration of implants within both the transported segment and the newly generated bone.[7] The authors noted that there was minimal bone resorption following

distraction, with a lower infection rate. Clinical studies report implant survival rates of 97% after vertical alveolar distraction versus 83.8% with interpositional or onlay bone grafting.[31] The new bone created by distraction in the maxilla and mandible is stable and predictable.[21]

Resorption of the transport segment and sinking of the implants into the cancellous bone as a result of masticatory forces are the main causes of relapse. Pressure over the transport segment during bone maturation resulting from either early loading of the implants or using temporary removable dentures enhances the resorption rates of the distracted bone.[7]

Since the regenerated bone is immature during the lengthening and consolidation period, pressure over the mucosa of the distracted bone, such as the pressure caused by temporary dentures, should be avoided to prevent relapse. To prevent the implant from sinking into the base segment while the newly generated bone is not hard enough, the supporting plate should be left in after distraction. Conversely, repeated mucoperiosteal elevation, such as while removing the device and inserting implants, is another reason for relapse and resorption of the new bone. .[7,21]

In order to prevent fracture or resorption of the transported alveolar segment, care should be taken not to make it too small. The transported segment should be at least 5 mm high to allow connection with plate and screws. [10]

The reported resorption rates after implant loading are consistent with that in native bone: a mean bone resorption of 1.93 mm at the 3-year follow-up with a 94% implant success rate and 1.4 mm after 4 years with a 94.2% implant success rate. Bianchi *et al*. reported 1.4 mm of bone resorption at the end of the consolidation period just before implant placement after obtaining a 10-mm vertical bone gain, compared with the preoperative situation (mean value 17.6%).[32] Polo *et al*. reported 15.6% resorption after the consolidation period[33] and Hwang claimed 20% resorption during consolidation.[34] Conversely, Garcia *et al*.[35] and Klug *et al*. [36] found no relapse from the end of the distraction phase until the time of implant placement.

In most studies, the occurrence of relapse ranging from 15–20% indicates that 20–25% overcorrection should be included when performing alveolar distraction osteogenesis to compensate for any resorption. Recent studies regarding Peri-implant bone loss around dental implants placed after distraction osteogenesis shows various results. Polo WC et al. observed 1.9 mm/year bone resorbtion whith a high implant survival.[37] On the other hand Perez-Sayans M et al. found that vertical bone resorption around implants placed in distracted alveolar bone is similar to that seen around implants placed in nondistracted bone.[38]

Autogenic onlay bone grafts show unpredictable bone resorption.. increased morbidity is also expected due to the needness of harvesting bone from intraoral or extraoral sites. The use of GBR guided bone regeneration to increase vertical bone dimension is very limited. Alveolar distraction osteogenesis was shown to be an effective vertical augmentation method which requires careful planning and close patient fallow up. Regarding its clear advantages over conventional bone grafting technique distraction osteogenesis appears be an effective technique for the placement of implants in the severely vertical alveolar bone resorption considering treatment outcome ,implant survival and surgical complications

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Chapter 21

BIOMECHANICS OF ORAL IMPLANTS

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BONE STRAIN: IMPLICATIONS FOR ORAL IMPLANTS

Bone is responsible for many different functions in the body, including structural support, mineral storage, and physiological functions such as the formation of blood vessels. It is an extremely complex tissue that changes its form, mass and internal structure under load. The understanding of functional adaptation of bone tissue to mechanical stimuli has been mostly founded by Julius Wolff, in his greatest treatise, *Law of Bone Transformation* [1]. According to Wolff (1892) "Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws." The trajectorial theory of the organization of the trabeculae in the cancellous bone was the core of Wolff's Law, where he also proposed that the pattern and orientation of trabeculae were transformable with alterations in loading pattern and that, in equilibrium, trabecular patterning represented the average regime experienced by the bone [2]. During the past decades, there has been extensive research addressing the verification of Wolff's law. Currently, it is known for certain that many basic tenets of Wolff's phenomenocigal approach are incorrect and his assertion was unable to explain the nature of these changes mathematically and verbally. Nevertheless, the idea that mechanical stimuli influence the architechture of bone has gained wide acceptance. In 1917, Thompson [3] stated in his classic treatise, *On Growth and Form*, that the general bone adaptation is dependent on load-induced mechanical deformation, namely, strain. In the 20th century, it was Evans [4] who had undertaken the first attempt to quantify strains engendered in bone by means of strain-gauges, although his unpublished data were far from the truth. Following the introduction of the strain-gauge technique after two decades [5,6], it became possible to measure *in vivo* bone strains under physiologic and/or artificial loading conditions. Undisputedly, the technique

made a great contribution to our current knowledge on mechanobiology of bone, as it would be impossible to understand comprehensively strain-related bone response without direct quantification of strains on bones of different species.

The adult shape and mass of bones are influenced by the functional strains induced within the tissue. After maturity, the basic effect of functionally-related bone remodeling is to regulate bone mass. The adaptive remodeling response of bone is stimulated by dynamic loads and static loads have negligible or no effect, because fluid can only be moved through bone by cyclic loading and relaxation, and lead to shear stresses [7-10]. It may be assumed that at a higher strain rate, fluid velocity and consequent shear stresses will increase. However, osteogenic response saturates quickly in response to mechanical loading, and cells require a recovery period to reestablish their mechanical sensitivity before they can fully respond again to their mechanical environment [11]. There is an abundance of scientific evidence supporting that strain magnitude, strain rate, strain energy density, piezoelectric currents including streaming potentials and fluid shear flow play as the ruling factors for bone adaptation [9,10, 11-16]. Dynamic loading within physiological strains increases bone mass and induce osteogenic activity, but disuse of bone leads to negatively balanced remodeling and a decrease in bone mass. Indeed, in an individual skeletal element, bone resorption may take place as a specific response to overloading, immobilization or disuse, or as a generalized response to decreased loading, such as weightlessness or bed rest [17-20].

According to Frost, hypertrophy in the periosteal envelope is stimulated when 2000 με is exceeded within the tissue. For load-bearing bones of a variety of animals, strain levels between 2000-3000 με can be obtained under vigorous exercise and are similar [21]. It may be estimated that, the threshold for bone remodeling may be species-independent and similar for all bones. Strain-gauge and overloading experiments, however, have shown that strain levels and distributions which would be acceptable for one location, could be unacceptable for another and lead to adaptive remodeling in others [10,22]. Indeed, the functional strain levels are different at different locations throughout the skeleton, i.e, strain values among locomotor elements [23] and even within each element [24] are quite different. The latter idea may be much more realistic, as even the physiological strains in the bones of the masticatory system are different [25].

In the context of oral implants, strain gradients that guide bone modeling and remodeling processes and whether bone adaptation mechanisms are driven by site-specific strains are unknown for humans. The reason is that *in vivo* strain measurements in bone surrounding implants have not been performed by means of tissue-integrated biosensors. Nevertheless, it is known for certain that the highest strain gradients, regardless of implant design, occur around the apical part and the neck of implants. Because the apex of implants is located deep in the bone, clinical complications do not arise. The implant neck, however, is surrounded by cortical bone, which is supposed to carry more load than the body of the implant. At present, biomechanical evaluations for oral implants are usually performed by finite element analyses, based on “assumptions” or implemented *in vivo* data from animal experiments. As for finite element analysis technique, the idealization of the physical problem to a mathematical model requires certain assumptions that lead to differential equations governing the mathematical model, and since the procedure is numerical, it is imperative to verify its predictive accuracy. The results of finite element analysis studies on oral implants may be questionable, because only a few studies have implemented *in vivo* strain data obtained from humans into finite element models. Another approach is to use strain-gauge analysis at the “abutment level”.

However, strains at the abutment level can never mirror the biomechanics of marginal bone and therefore its clinical value is very poor. Hence, one of the responsibilities of the discerning prosthodontist is to design studies to evaluate *in vivo* mechanocoupling of oral implants and to quantify actual *in vivo* strains around implants in humans. Because strain-gauge analysis is time-consuming, gauges cannot be placed at the bone-implant interface, the chemicals used for conditioning of bone surface as well as the bonding agent may lead to adverse tissue effects, the technique has some practical limitations. However, fresh human cadaver studies conducted under well-controlled loads may provide valuable information not only for the determination of functional strains around natural teeth but also around implants supporting fixed and removable prostheses.

OSSEOINTEGRATION AND DENTAL IMPLANT DESIGN

Osseointegration of oral implants made of commercially-pure titanium has been defined as the structural and functional bone contact with the load carrying implant or functional ankylosis [26,27]. This is a time-dependent process. Oral implants undergo a cascade of biological events at the bone-implant interface leading to bone tissue differentiation and osseointegration. As a sequel of intimate and rigid bone-implant contact at the ultrastructural level, the bone surrounding implants must function without a stress-reducing element at the interface, such as a periodontal ligament, which exists around natural teeth. The maintenance of this relationship involves continuous remodeling activity at the bone-implant interface.

When a titanium implant is installed in a bone socket, the body recognizes the material as foreign, and consequently, an inflammatory reaction is elicited, resembling normal wound healing regarding cell recruitment and persistence [28,29]. At the fresh bone-implant interface, the surgical intervention results in both direct and indirect damage to the tissue. The direct damage is immediate destruction of bone, soft tissue, blood vessels, and nerves. The indirect damage results from formation of the blood clot and haemostatic mechanisms that seal off the blood supply to tissues adjacent to the site of injury. Ossification as well as osseointegration is closely related to progressive vascularization of the callus or the tissue-implant interface [30-32]. According to a hypothesis put forth, the mature interface of a commercially-pure titanium implant to living tissues constitutes a hydrated titanium peroxy matrix and the formation of this matrix is unique to titanium, as other possible transition metals have too low solubility of their peroxy complex or too low stability of the complex [28]. Importantly, titanium and its main oxide layer TiO_2 does not impair vascularization [31-33]. The process of “fracture healing” commences with the proliferation of pluripotential mesenchymal tissue at the interface, resulting in callus formation. The healing mechanism proceeds by stages of tissue differentiation resulting in ossification and osseointegration, which then remodels to form lamellar bone.

The exact mechanism by which mechanical stimulus directs the healing process of a bone fracture or bone-implant interface is not fully understood. The first theory on fracture healing, proposed by Perren and Cordey, included only the effects of longitudinal strains rather than direct strains in all three orthogonal directions and shear strains that may reach the limit before longitudinal strains. According to Carter and his collaborates, dilatational and octahedral shear stress history played a decisive role on differentiation of mesenchymal tissue into bone, cartilage or fibrous tissue [34]. Carter and his collaborates believed that low strains

permit intramembranous bone formation, low to moderate magnitudes of tensile strains and hydrostatic tensile stresses may stimulate intramembranous ossification, high tensile strains stimulates fibrous tissue formation, and tensile strains together with hydrostatic compressive stresses cause development of fibrocartilage tissue [35-37]. Recently, Claes and Heigele [38] proposed a new quantitative hypothesis for the differentiation of callus tissue, developed from experiments on healing ovine osteotomies. By using axisymmetric finite element models, callus stress and strain environments were calculated, and the types of tissue formation arising from various mechanical environments were observed from histological specimens distributed over time [39]. However, in that study only controlled compressive movements at an interfragmentary gap predefined by a transverse osteotomy and constrained against natural torsion and bending movements were evaluated during weight bearing. In the context of oral implants, it is not known whether any of these theories may be implemented into numeric analyses, particularly using poroelastic models, as bone adaptive response seems to be site-specific.

One of the critical elements influencing the long-term uncompromised functioning of an oral implant is its design. The rationale for designing endosseous implants is based either on biological requirements, where biocompatibility and ameliorated tissue healing are the primary goals or biomechanical needs, where controlling local tissue strains in the vicinity of implants is the main focus [40,41]. Suprisingly, considerable emphasis has been given on implant designing with the biological approach so far, although biomechanical factors appear to be predominant on implant survival. Because oral implants always penetrate the integument of the body with the tissues, it has been crucial to develop an “ideal” hybrid implant surface promoting bone apposition on the implant body, while also granting an efficient and biological seal at its neck. Essentially, the rationale behind developing rough implant surfaces was to allow bone cells to migrate into the scaffold and while in this environment, experience osteogenic strain levels in the vicinity of implant to promote bone-implant interlock. Indeed, several *in vivo* studies have identified implant surface geometry as a design variable significantly influencing long-term clinical outcome, although recent studies suggest that implant design does not have any impact on long-term marginal bone reactions. Once an implant is placed, the coagulum and thereafter the initial connective tissue in the bone defect [42-44] can transfer low-magnitude functional loads and stimulate bone that is not contacting the implant, even when the implant is sleeping [45]. If the implant surface is rough, the total area used to transfer functional/parafunctional forces to the bone increases, and lower tissue strains can be achieved in the vicinity of the implant. Because rough-surface implants also provide better mechanical interlock with bone in comparison with machined-surface implants [46,47] implants with machined/turned surfaces have an inherent potential of experiencing debonding with bone or bone resorption due to stress-shielding. Stress-shielding may be an important factor leading or contributing to marginal bone loss around functioning implants particularly within the first year of oral function.

Alterations in biomaterial surface morphology and roughness have been used to improve tissue response and the mechanical properties of the bone-implant interface. Although the results are encouraging, there is a large inconclusive literature on their clinical effects. In a study conducted by Carr and co-workers [48], commercially pure titanium, titanium alloy, and TPS implants placed in baboons after 6 months of healing demonstrated that bone-implant contact and percent bone area in maxilla (50.8, 43.6%) was lower than the mandibula (60.8, 52.6%). The biomaterial analyses, however, revealed no significant differences. In a

comparative histometric analysis of bone implant interface between a rough titanium surface and smooth implants in low-density human jawbone after 3, 6, and 12 months of submerged, undisturbed healing, the rough implant had significantly higher bone contact in comparison to the smooth implant [49]. In the clinical stiuation, however, comparative clinical studies on different implant designs have reported similar levels of annual bone loss and prosthetic complications [50-52]. It is also assumed that the possible reason why some implants does not reach a steady state at the same time as others in the same mouth are not dependent particularly on the implant design, but the local bone anatomy and the surgical technique [53]. However, the macrogeometry of the implant, independent from the implant-abutment junction, do affect the biologic outcome. For example, the annual bone loss for single tooth Branemark implants with a conical collar design demonstrated higher amount of bone loss than self-tapping and standard implants [54,55]. Another reason for bone loss may be the smoothness of this implant surface at its neck, leading to stress shielding [54]. This explanation may be correct due to remarkably high marginal bone levels found around Astra Tech implants, which are provided with retention elements at implant collar [56]. Nonetheless, comparative studies on TiO_2-blasted and machined Astra Tech implants also did not reveal significant differences in bone loss after 2 and 5 years [57,58]. Therefore, in spite of the elaborate claims made, a conclusion in strict scientific sense cannot be drawn due to the lack of clear evidence on the superiority of one surface texture or implant design over another. Overall, it seems that implant design does not have a great impact on time-dependent marginal bone reactions [59,60].

THE BONE-IMPLANT INTERFACE

1. Mechanical Predictors of Interface Healing and Primary Implant Stability

Throughout life, the remodeling of bone tissue provides the exquisite lamellar microarchitecture of cortical and trabecular bone for scar-free healing and regeneration of injured bone. The regeneration process of bone covers four overlapping phases, namely, the inflammation phase (haemostasis), the soft callus phase (osteoconduction), the hard callus phase (*de novo* bone formation) and the remodeling phase. The peri-implant bone healing takes place as distance and contact osteogenesis and results in a 100-400 nm-thick interface in humans [61,62]. The relatively slow regeneration of peri-implant cortical bone relies exclusively on lamellar remodeling, while the generation of trabecular bone involves remodeling of existing lamellar trabeculae and rapid formation of new trabeculae by the recruitment of new populations of osteogenic cells within the healing zone. The processes of osteoconduction and *de novo* bone formation constitute contact osteogenesis [63]. The advance of contact osteogenesis on implant surface considerably alters the interface mechanical properties and therefore, it would be appropriate to define osseointegration as a time-dependent and living process. Indeed, an up to 12-week animal study [44] showed that the process of bone formation started as early as in the first week. The primary woven bone at implant surface that included trabeculae of woven bone was replaced by parallel-fibered and/or lamellar bone and marrow. The bone next to the implant threads became resorbed and

replaced with newly formed viable bone in the first two weeks. One-year follow-up of implants placed in animals showed a time-dependent increase in bone implant contact as well as removal torque values (from 10 to 88 N.cm) [64]. This implies that the advancement of osseointegration is coupled by increase in the stiffness of the interface and decrease in micromotion. From a macroscopic biomechanical point of view, osseointegration was defined as "a fixture is osseointegrated if there is no progressive relative motion (micromotion) between the fixture and surrounding bone and marrow under functional levels and types of loading for the entire life of the patient and exhibits deformations (strains) of the same order of magnitude as when the same loads are applied directly to the bone" [65]. Therefore, the mature bone-implant interface is actually "expected" to have the mechanical behavior of "bone tissue". However, the cement lines in bone tissue including that at the bone-implant interface have been identified as fragile components, and the tensile or shear strength of this tissue never approaches the strength of bone [66]. This clearly shows why it is essential to control load at the interface.

It is generally presumed that a threshold of tolerated micromotion, somewhere between 50-150 μm exists [66,67], although micromotion levels less than 150-200 μm have also been reported not to jeopardize commencement of osseointegration [68,69]. The level of osseous micromotion of current implants has never been a clinical problem under conventional loading, but it is vital to control micromotion in early and immediate loading protocols. Retrieved immediately loaded dental implants in humans, which were deemed successful in clinical and radiographic examinations, showed histomorphometric bone-implant contact of approximately 40-75% [70-72]. When immediate loading of an implant is considered, the problem becomes more complex with regard to the implant-bone interface than conventional loading. The implant placed and loaded in the freshly prepared bone socket is not in contact with bone at the ultrastructural level, but kept in place solely by the initial mechanical torque-tightening of the implant. Moreover, an immediately loaded implant in an extraction socket should have intraosseous stability comparable or higher and micromovement values approaching to or preferably lower than an immediately loaded implant in a surgically-prepared bone socket to achieve uneventful osseointegration. Following placement, the bone defect around the immediate implant is occupied by coagulum and a fibrin network derived from plasma of damaged blood vessels in the socket. The coagulation process is primarily important to arrest hemostasis. Yet, the physical properties of the coagulum, i.e., platelet contractile forces, elasticity modulus and fracture strain of the fibrin network, effects of cyclic strain on angiogenesis and osteogenesis, and rheological properties of the clot and granulation tissue are only a few of the critical biomechanical factors having a delicate interplay at the cellular level. Ideally, to obtain an optimum force transmission around an immediately loaded implant in an extraction socket, the cyclic strain amplitudes within the differentiating tissue must fall into the physiologic tolerance threshold of that tissue, where the loading history of the implant will rule tissue differentiation.

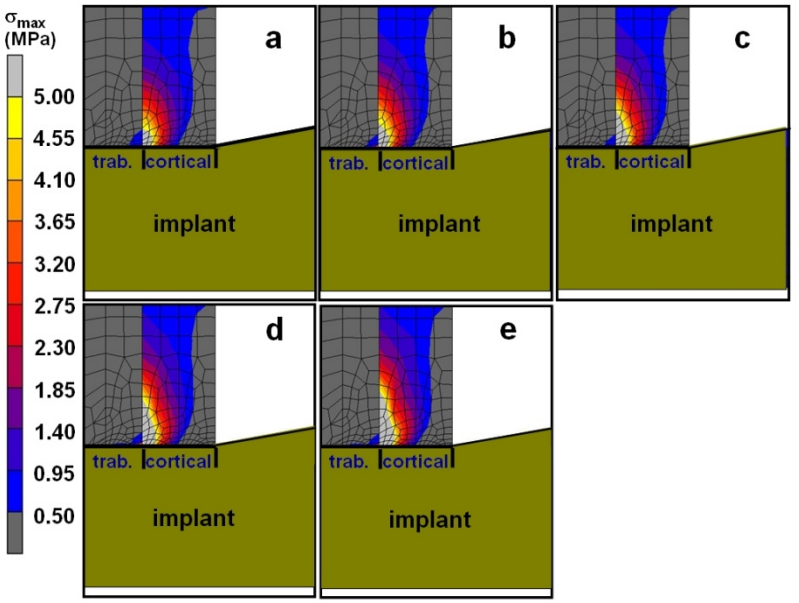

Figure 1. Time-dependent changes in maximum principal stress distributions around an implant supported by cortical and trabecular bone. (a. Loading after osseointegration; b. At 3 months of loading; c. At 6 months of loading; d. At 9 months of loading; e. At 12 months of loading.)

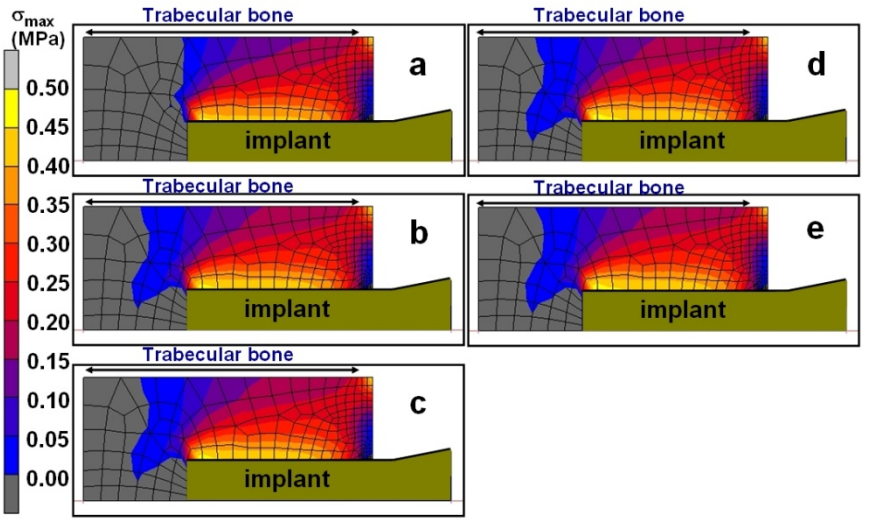

Figure 2. Time-dependent changes in maximum principal stress distributions around an implant supported only by trabecular bone (a. Loading after osseointegration; b. At 3 months of loading; c. At 6 months of loading; d. At 9 months of loading; e. At 12 months of loading.)

A finite element study showed micromotion levels of 152 µm and 284 µm and corresponding stress values of 37 MPa and 30 MPa, for immediately loaded (500 N at 75º inclination) Nistatan® and Xive® implants, respectively [73]. When numerical analyses have been undertaken in a time-dependent manner (i.e., by implementing the *Stanford Theory*), Eser and colleagues observed almost constant levels of micromotion for Straumann® implants

as a squeal of 75 N cyclic daily loading up to 1 year [74]. More importantly, the time-dependent increase in stresses (maximum principal stress and minimum principal stress) in the marginal zone of the implant surrounded with cortical bone support was higher than those supported solely by trabecular bone (Figures 1 and 2). Higher strain energy density around implants having cortical bone support might indicate apposition and increase in interface stiffness and decrease in micromotion, whereas lower strain energy density around implants supported solely by trabecular bone could be associated with skeletal tissue loss [74] (Figure 3 and 4). Based on the remodeling mechanism described in the strain-energy theory, the bone system induces, adjusts, responses, circulates, until the bone material distribution satisfy the optimization criterion of uniform strain energy distribution, which is the normal state of bone [75]. Osteocytes within the trabeculae has been considered as strain energy density sensors, signaling Basic Multicellular Units (BMU) of osteoclasts and osteoblasts at trabecular surfaces to add or remove net bone mass [76]. Huiskes and colleagues used dynamic loading variables (strain energy density rate) that activate osteocytes in the bone matrix to transfer osteoblast bone-formation stimuli to trabecular surfaces, through the canalicular network. They found that coupling between osteoclast and osteoblast activities in remodeling was governed implicitly by the mechanics, through strain energy density concentrations around resorption cavities [77,78]. The total strain energy density distribution around implants, therefore, represents the regions where there is resorption and apposition.

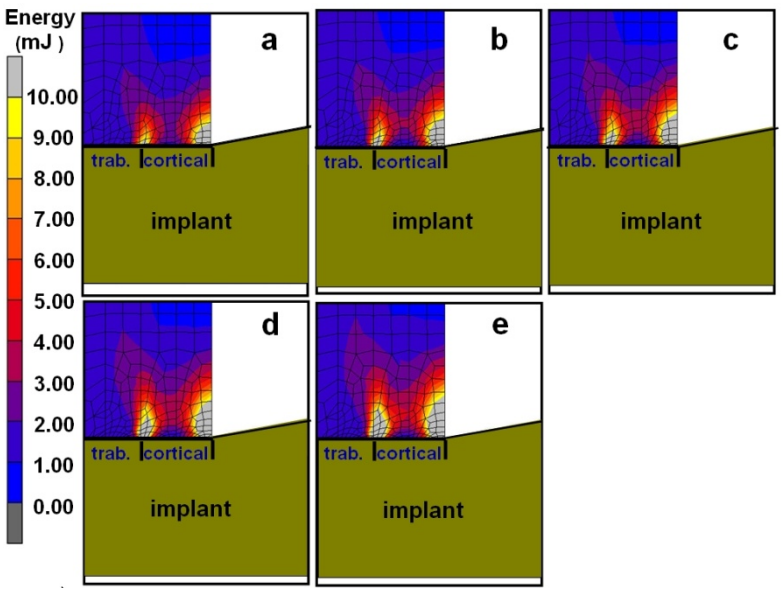

Figure 3. The strain energy distribution around the implant for the model with 1 mm cortical thickness. (a. Loading after osseointegration; b. At 3 months of loading; c. At 6 months of loading; d. At 9 months of loading; e. At 12 months of loading.)

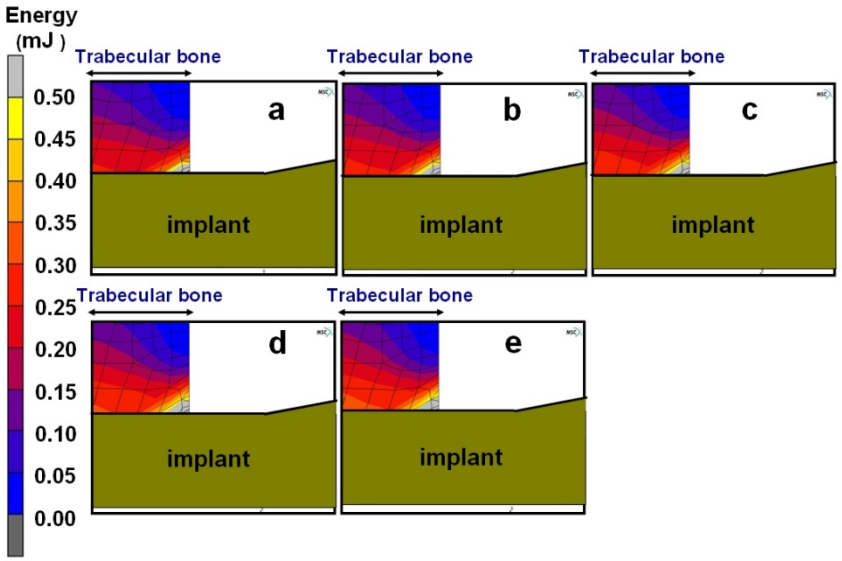

Figure 4. The strain energy distribution around the implant for the model with 0.5 mm loss of trabecular bone. (a. Loading after osseointegration; b. At 3 months of loading; c. At 6 months of loading; d. At 9 months of loading; e. At 12 months of loading.)

When a parallel-sided and a tapered implant is compared under 100 N immediate oblique load for up to 1 month by implementing the *Stanford Theory* [79], the maximum and minimum principal stresses in cortical bone were found to be higher around the tapered cylindrical implant. Stresses in the trabecular bone were higher around the parallel-sided cylindrical implant. Strain energy density around both implants increased in cortical bone, slightly decreased in trabecular bone (Figure 5, and 6), and higher values were obtained for the parallel-sided cylindrical implant. Displacement values slightly decreased in time in x-axis, and an initial decrease followed by a slight increase was observed in the y-axis. This outcome is in line with the observations of Chou et al. [80], who stated that threadless implants tend to develop a softer bone in their periphery in contrast to threaded implants, where a substantial increase in bone density is predicted at the tips of the threads. Chou et al [80], however, could not discriminate the effects of implant design on the bone remodeling process for conventional loading, as the differences between the implants were low. In the study by Eser et al. [79], the displacement values of both implants decreased in x-direction suggesting that the interface stiffness also had a time-dependent increase. This is strongly supported by clinical findings, as the stability measurements for immediately loaded implants show increasing levels [67,81]. Resonance frequency analyses of different implants in animal studies have also showed an increase in stability over time [82,83]. Likewise, a recent numerical simulation by Wintera et al. [84] suggested that the load-bearing capacity of an immediately loaded implant substantially increases up to 300 N during the first 24 weeks and then tends to reach a steady state due to significant increase in ultimate torque and ultimate shear stress of the bone-implant interface. Their predictions are in line with the findings of Eser et al. [79], who suggest mechanically-mediated apposition of bone associated with decrease in axial displacement, increase in strain energy density, (and elastic modulus) particularly in cortical bone in the early stages of healing.

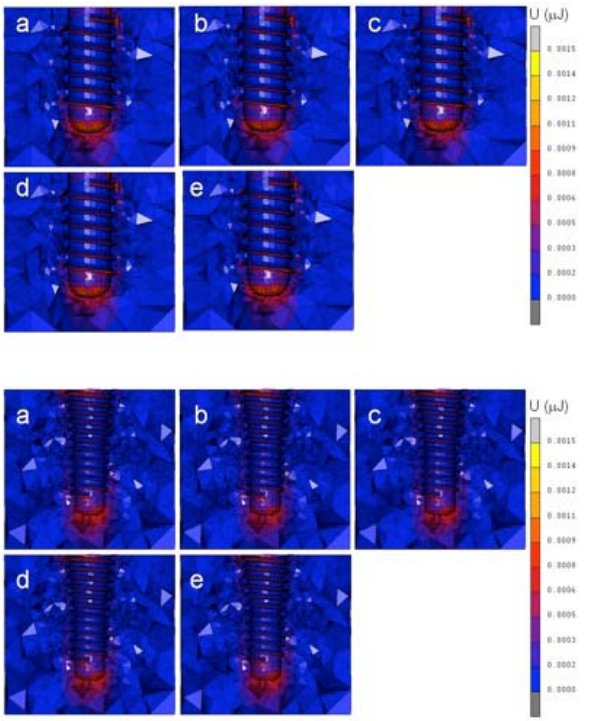

Figure 5. Contour Plots for the strain energy density (µJ/mm^3) on cortical bone for parallel-sided (up) and tapered (down) implants at placement (a) and after 1-week (b), 2-weeks (c), 3-weeks (d), 4-weeks (e) under l00 N oblique loading.

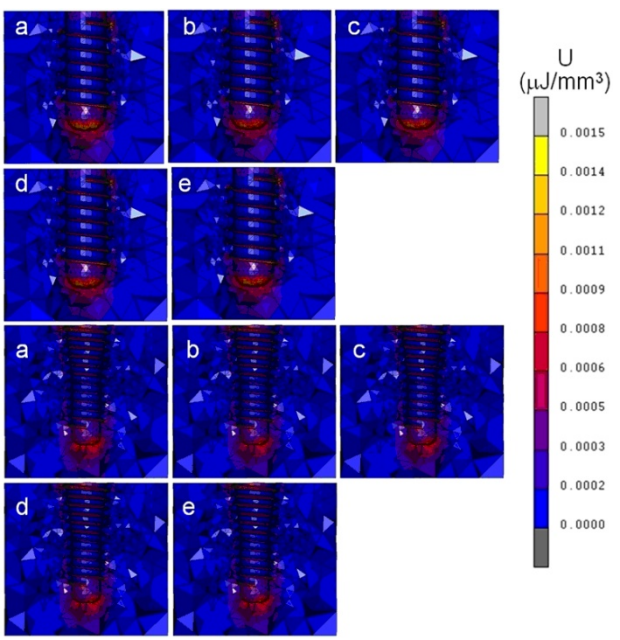

Figure 6. Contour Plots for the strain energy density (µJ/mm^3) on cortical bone for parallel-sided (up) and tapered (down) implants at placement (a) and after 1-week (b), 2-weeks (c), 3-weeks (d), 4-weeks (e) under l00 N oblique loading.

From a clinical aspect, quantification and monitoring the interface healing or integrity is an important task, especially in treatments involving early or immediate loading. Albrektsson et al. describe a number of factors that contribute to the achievement of osseointegration. Factors such as a suitable host, biocompatible material, careful surgery following a specific protocol, and an appropriate healing time are discussed. Primary implant stability is one of these prerequisites for achievement and maintenance of osseointegration [85]. It depends exclusively on the mechanical engagement of an implant with bone of the osteotomy, but this stability decreases with time as remodeling of the surrounding bone takes place. Secondary implant stability is the result of osseointegration, occurring after formation of new bone in the area adjacent to the implant. Unlike conventional implant sockets, which provide bone implant contact from the apex to the collar of the implant, a site-specific three-dimensional bone defect is always created at the marginal bone level of immediately placed implants in extraction sockets, which extends apically more than a few millimeters in most cases. This situation dramatically influences primary implant stability. For such implants, an uneventful bone healing phase can be completed without using a barrier membrane, as the "jumping distance" is usually below 2 mm and the initially empty bone defect immediately becomes occupied with a coagulum and a granulation tissue that is replaced by a provisional matrix in the early stages of healing [44]. Nonetheless, the dynamics of skeletal tissue healing around implants is somewhat different in comparison with bone formation in tooth extraction sites. Since there is a delicate interplay between bone resorption in contact regions and bone formation in contact-free areas near implants [44], the biomechanical capacity of the healing bone is much lower than a functionally adapted one. Hence, if immediate loading is considered for immediate implants, it is extremely crucial to achieve high initial intraosseous stability.

A number of devices and techniques have been developed to assess primary and secondary implant stability. The Periotest (Siemens Gulden- Medizintechnik, Bensheim, Germany), initially developed to measure the damping characteristics of natural teeth, has been used to evaluate implant stability [86]. The force used to insert a dental implant is described as cutting-torque or insertion torque [87,88]. In truth, the reverse torque test, a method based on unscrewing (the opposite of the cutting-torque) the implant from bone, had already been developed before cutting-torque measurement has been introduced. This method has been extensively used to quantify the torsional strength (critical threshold for torsional failure of the interface) of bone-implant contact in experimental animal studies [64, 89].

Meredith [90] introduced resonance frequency analysis, another noninvasive method used previously in construction engineering. Resonance frequency analysis, based on continual excitation of the implant through dynamic vibration analysis makes use of a transducer connected to an implant, which is excited over a range of sound frequencies with subsequent measurement of vibratory oscillation of the implant. This technique causes the implant to vibrate, while at the same time analysing implant motion, and provide information as Implant Stability Quotient (ISQ) on a scale from 1 to 100. ISQ is a numeric descriptive presentation of implant stability determined by the device (Osstell®, Integration Diagnostics, Gothenburg, Sweden) but the values obtained are not directly correlated with any specific physical parameters.

Recent studies show that implant design has a great impact on initial stability in bone [91-94]. Timing of measurement (the amount of interface healing) also has a great impact on the numeric value of primary stability [82,83]. While no gold standard exists for accurate

quantification of implant stability, the level of correlation between current methods is apparently a matter of debate. Several investigators have questioned the correlation between resonance frequency analysis with cutting-torque [95-98] and removal torque measurements [91,99], as well as bone mineral density and trabecular bone volume and pattern factor [96], and histomorphometry [98,100]. These problems eventually do not allow correct quantification and establishment of the critical threshold of primary implant stability. Therefore, in treatments involving immediate or early loading, there is not any reliable numeric value that could guide the clinicians.

2. Mechanical Aspects of Marginal Bone Loss around Implants

Many biomechanical and/or biological factors have been identified as causes of time-dependent marginal bone loss [101]. According to Albrektsson and co-workers [102], the annual bone loss after the first year of function should not exceed 0.2 mm and bone loss between 1-year and 3-years should be no more than 0.2 mm. This phenomenon implies that there is a clinically-acceptable 1 mm physiologic bone loss threshold in the first year, but this needs to be followed by a tendency toward reaching a plateau in the upcoming years [103,104]. Nevertheless, there may be an ongoing remodeling of marginal bone around individual implants and longer periods might be required to reach a steady state. Marginal bone resorption could also be induced by surgical trauma or bacterial infection [105] and essentially, this biologically-driven marginal bone loss has been unquestionably well-demonstrated in animal studies [106-108]. The consequences of bacterial pathway regarding onset of peri-implant infections has been also explored [109]. Current scientific evidence on overloading-induced bone resorption is, however, inconclusive. While some studies report bone loss [110-112], others report no loss [113-115].

The process of converting physical forces into biochemical signals and integrating these signals into the cellular responses is referred to as mechanotransduction. In response to mechanical and biochemical signals mediated by osteocytes [116-118], coordinated actions of osteoclasts and osteoblasts are believed to be in charge for bone modeling and remodeling at the cellular level. This observable fact implies that bone cells including those in the peri-implant region can perceive and respond to mechanical stimuli by regulating their biological and biochemical activities. Considering the strain gradients in bone tissue, the functional strain levels appear to be dissimilar at different locations throughout the skeleton, whereas peak strains in load-bearing bones are quite similar [119]. For example, maximal in vivo strains in tibia of humans have been found to range from around 1,200 με (principal compressive strain) to about 1,900 με (maximum shear strain) [120]. According to Frost [121], bone mass will change when induced absolute peak strains in “a” bone fall either below or above the “physiological window” (approximately between 200–1500 με). When this information is employed to dental implants, it has long been recognized that the implant-bone complex should be stressed within a certain range for physiologic homeostasis and optimum functioning, where strains within the skeletal tissue would range between 200-1500 με [121,122]. In the event of overloading and marginal bone loss, general idea has been that “high stress” concentrations arise at the interface and, according to well-supported hypotheses [123,124], strain fields that exceed 1500 με in bone tissue may stimulate bone resorption, jeopardizing implant survival. It should, however, been taken into account that these data have been obtained from conventional strain-gauge analyses that provided global strains

occurring in an area determined by the measuring grid of the gauge [120-124]. Recent evidence show that experimentally determined bone matrix strains around osteocyte lacuna resulting from macroscopic strains of approximately 2000 με (0.2%) can reach levels of over 30.000 με (3%) over fifteen times greater than the applied macroscopic strain, possibly due to local inhomogeneities in the bone matrix and strain concentrating effects of the local microstructure [125,126].

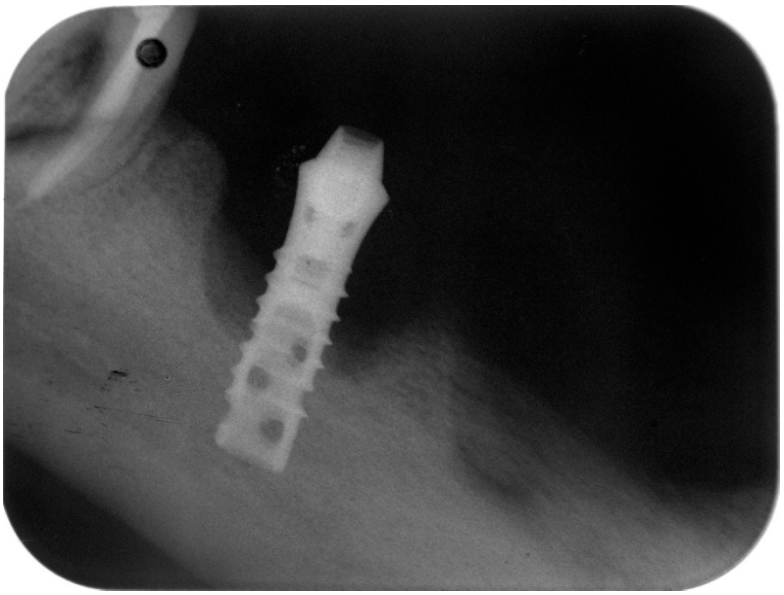

Figure 7. Radiogaphic view of a hollow-screw implant presenting excessive crater-like bone loss.

Modeling is not only bone grow-mate *per se* is prominent during growth but also likely to be effective in modulation of bone architecture and mass when the mechanical environment changes. Thus, under circumstances of bone strains exceed a "modeling threshold", in which minimum effective strain (global strains) centered near 1500 με [121,122], mechanically controlled formation drifts invoke to increase bone mass and strength [127]. This, in turn, could possibly explain why stable marginal bone levels were found around implants subjected to occlusal overload [113-115]. Owing to deterioration in quality of adult bone, bone remodeling replaces itself continually for the maintenance of eligibility at biomechanical level. Trabecular bone may perforate and remove trabeculae, while cortical bone increases porosity and decreases cortical width during remodeling [127]. In addition, natural bone turnover is disturbed and even complicated with site preparation during implant surgery. Changes in mechanical environment of peri-implant bone are associated with various occlusal loading protocols. In the event, progressive or excessive marginal bone loss occurs (Figure 7), the biomechanical behavior of the load-carrying implant is dramatically influenced by the amount of support loss and whether or not cortical bone exists. A finite element study [128] on progressive bone loss around implants showed that under vertical and oblique loading, principal stresses decreased as with the increase in bone resorption. With progressive bone loss and under oblique load simulations, displacement and equivalent of elastic strain increased considerably in trabecular bone contacting the implant neck. The presence of cortical bone contacting a load-carrying implant, even in a bone defect, improved the

biomechanical performance of implants in comparison with only trabecular bone support as a sequel of progressive marginal bone loss.

FACTORS INFLUENCING LOADING OF THE BONE-IMPLANT INTERFACE

In this section, we will discuss some of the main biomechanical factors that are believed to have an influence on marginal bone reactions and/or prosthetic outcome of implant treatment. Several factors have been identified to create peri-implant stress fields (Table 1). When evaluating the biological effects of an applied load, it is essential to determine its source. An implant-supported prosthesis may be under the influence of external (functional or parafunctional forces) and/or internal (internal or external preload) forces. Qualification and quantification of these forces on implants and in bone is required to understand the in vivo behavior of these devices.

Table 1.

Biomechanical factors
Bone (location in the dental arch, density, cortical bone thickness
Implant: Design, length, diameter, number, location, and angulation of supporting implants
Prosthesis: Type (fixed versus removable or freestanding versus tooth-implant), design of the prosthesis, material (?), superstructure fit
Patient-oriented: Age and sex of the patient, condition of the opposing arch (prosthesis versus natural dentition), location, direction and magnitude of applied occlusal forces on the prosthesis stiffness of food, mandibular deformation

1. Bite Force

In dentate patients, the maximum biting force varies between individuals and different regions of the dental arch [129,130]. Occlusal forces are somewhat decreased due to age-related deterioration of the dentition. Present evidence based principally on static force measurements indicates that, the average biting force is 100–150 N in adult males, and males have higher biting force than females [129]. Raadsheer [131] reported 545.7 N and 383.6 N maximal voluntary bite forces in men and women, respectively, and the maximum biting forces measured in men and women were 888 N and 576 N, respectively. Dentate patients have 5-6 times higher bite force than complete denture wearers [132]. Patients with implant-supported fixed prosthesis have a masticatory muscle function equal to or approaching to that of patients with natural teeth, or with tooth-supported fixed partial dentures [133]. Placement of a mandibular fixed implant-supported prosthesis in complete denture wearers improves masticatory function and the magnitude of bite force [134-136]. Haraldson and Carlsson [136] measured 15.7 N for gentle biting, 50.1 N for biting as when chewing, and 144.4 N for maximal biting in patients treated with implants. In another study, Carr and Laney [137] reported maximum bite forces between 4.5-25.3 N before and 10.2–57.5 N after three months of treatment with implant-supported prosthesis, and emphasized that, the amount of increase was dependent on the duration of being edentulous. Forces on implants are also dependent on the location of the implant in the dental arch. Mericske-Stern and Zarb [138] investigated

occlusal forces in a group of partially edentulous patients restored with implants supporting fixed partial prostheses and measured an average value of maximum occlusal force lower than 200 N for first premolars and molars and 300 N in second premolars.

The application of an external load on an implant-supported prosthesis induces stresses within the entire load-bearing system and stress reactions in the supporting bone which are theoretically the same in magnitude, but in opposite directions. During clinical loading of an implant, the direction of forces almost never coincides along its central long axis, providing an absolute axial loading. Quite the contrary, the occlusal force is applied at different locations and frequently, in a direction that creates a lever-arm, which causes reacting forces and bending moments in the bone. Bending moment is the force times the orthogonal distance between the force direction line and the counter-acting support. The longer the distance, the greater will be the bending moment. Accordingly, the fraction of force transmitted to implants and the induced stresses are dependent particularly on where the load is applied on the prosthesis. For instance, considering that two vertically placed implants supporting a fixed prosthesis is axially loaded from the middle, equal load partitioning is expected between implants. If the load is applied only on one implant, it will bear the entire load with a potential apical movement. Cantilever loading will result in a dramatic increase in load transferred to the implant neighboring the cantilever [139]. Hence, it is imperative to establish equilibrium between acting and counter-acting forces. During functional loading, however, implants may not always reach this vital requirement and may fail.

2. Bone Density

The amount of available bone, cortical bone thickness, and bone density are important factors influencing primary stability, peri-implant stress fields, and implant survival. Bone quality types 1 and 4 are found much less frequently than types 2 and 3 [140]. Quality 2 bone dominates the mandible, and quality 3 bone is more prevalent in the maxilla, although variations in density exist in each region. Both anterior and posterior jaw regions are often characterized by types 2 and 3 bone. The anterior mandible has the densest bone, followed by the posterior mandible, anterior maxilla, and posterior maxilla [141]. From a biomechanical point of view, it is reported that implant survival is directly proportional to peri-implant bone mass and that 70% histomorphometric bone-implant contact appears to be sufficient to withstand functional forces [142,143]. Truhlar and co-workers [144] reported that among 2,131 implants, quality 1 bone experienced the greatest implant failure rate, whereas quality 2 and 3 bone had the lowest incidences of implant failure. According to Bahat [53], the quality and quantity of bone do not have a crucial effect on implant survival, but the surgical techniques are more important. Numerical simulations suggest that the increase in bone density improves the mechanical properties of the interface. Implants are demonstrated to have less micromovement, increased initial stability, and reduced stress concentrations in high density bone [145,146]. In addition, the existence and thickness of cortical bone explicitly favors the peri-implant stress field as well as primary implant stability [91, 128, 147].

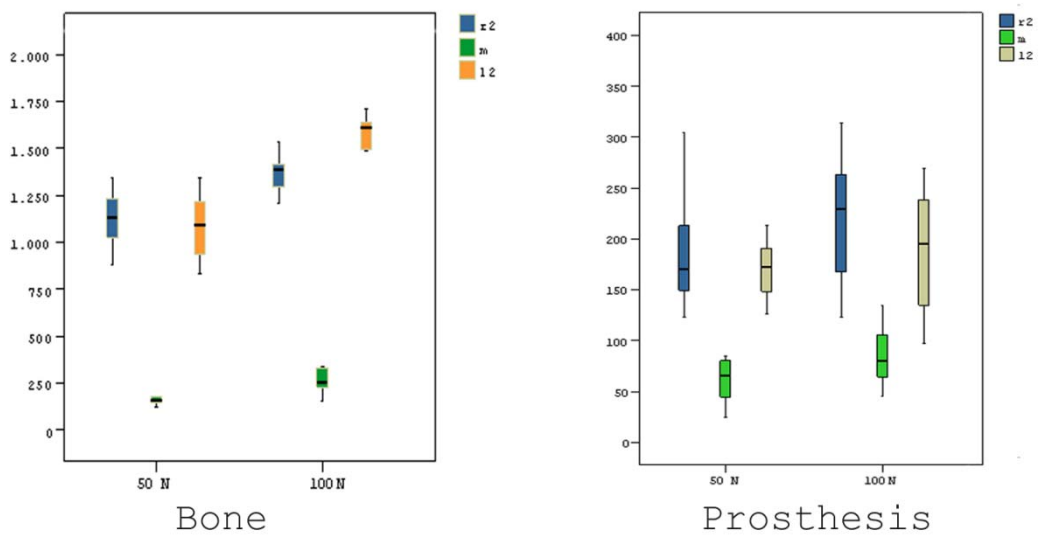

Fig 8a. Box-plot and whisker diagram of "absolute" strain magnitudes on bone and prostheses for three implant supported scenario. R2: implant at right canine region; M: implant at the mandibular symphysis; L2: implant at left canine region.

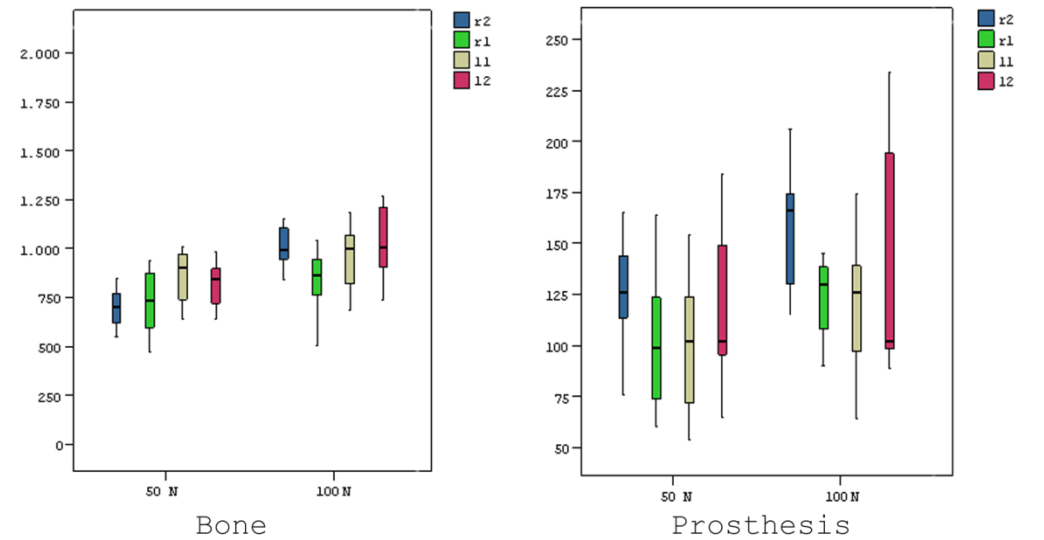

Figure 8b. Box-plot and whisker diagram of "absolute" strain magnitudes on bone and prostheses for four implant supported scenario. R2: implant at right canine region; R1: implant at right canine region; L1: implant at left lateral region; L2: implant at left canine region.

3. Supporting Implants

The impact of implant design is explained elsewhere in this chapter. Therefore, this part will focus on length, diameter, number, and angulation of supporting implants. Studies show that the increase in number, length and diameter of implants improve the biomechanical behavior of implants, particularly under bending forces [148-151]. To understand the impact of the number of supporting implants, a good example is to consider the evolution of fixed prosthesis treatment options in the mandible. Rehabilitation of completely edentulous mandibles by means of 5 to 6 implants in the symphysis area to support one-piece fixed prostheses was originally proposed by Brånemark and co-workers. This approach, based on an undisturbed skeletal tissue healing phase of 6 months for uneventful osseointegration, was considered as a rule until the same author found that clinical outcomes of 4 to 6 implant-supported mandibular fixed prostheses were comparable [152]. As a drastic shift from the conventional 6-month bone healing phase, early-immediate loading of 5 to 6 implants in the mandible was then introduced. Almost simultaneously, Brånemark and co-workers [153] proposed a modification of his initial approach to allow immediate loading. The supporting three specific implants were connected to a standard framework incorporating denture teeth delivered to the patient on the day of implant surgery. The clinical experience with the Novum System® seems promising [154,155], although neurosensory disturbances can occur during surgery [155]. In search for the least number of supporting implants for a fixed mandibular prosthesis, De Bruyn et al. [156] early-loaded 3 regular platform Brånemark implants in the symphysis area by means of a temporary fixed prosthesis. They observed an implant failure rate of 10% within the first year and concluded that this approach led to a less favorable clinical outcome. More recently, Malo et al. [157] introduced the "All-on-four" concept for immediate loading of fixed prostheses in the mandible using 4 regular platform implants in the symphysis area and reported a success rate of 96.7% after 3 years. In this approach, the distal implants are tilted toward distal to decrease length of distal cantilever. It should be noted that tilting of supporting implants do not have any negative effect on marginal bone reactions and implant survival [151,157]. A prospective clinical trial and in vivo force measurements on Novum System implants revealed that, the amount of crestal bone loss around distal implants was not promising [158]. This fact relies on load partitioning among implants and there seems to be a critical threshold of the number of supporting implants. Indeed, bone strains around immediately-loaded 3 implants supporting a fixed prosthesis are remarkably higher than the 4 and 5 implant-supported scenario [139] (Fig. 8a and b). Overall, these clinical and experimental data suggest that the more the supporting implants, the safer the treatment may be.

4. Prosthesis Type

Another important factor is the type and design of the prosthesis. In cement-retained implant restorations, the occlusal surface is devoid of screw holes and the occlusion can be developed that responds to the need for axial loading. Screw-retained fixed prosthesis or overdentures, however, are subjected to off-set loads that cause an increase in bending moments [159,160]. Nevertheless, the information on this issue is scarce and inconclusive. A comparative in vivo study on axial and bending moments on maxillary implants supporting a screw-retained fixed prosthesis or an overdenture revealed that, force application on an overdenture resulted in lower compressive force, but higher bending moments on abutments

during function when compared to a fixed prosthesis [159]. Mericske-Stern and collaborators [161] also registered forces on implants supporting one-piece full-arch fixed prosthesis and bar-retained overdentures in the maxilla. They concluded that, the type of prosthesis did not have a determining effect on force pattern. Unlike implant-retained/supported mandibular overdentures, treatment of the edentulous maxilla by implant-supported overdentures is challenging. It is complicated by inherent problems, such as reduced bone quality and quantity, divergent implant axes, and off-set positioning of denture teeth, which increase bending moments on implants more than mandibular fixed prostheses and overdentures [99, 128, 162]. Since the positioning of denture teeth frequently creates an anterior or labial cantilever, which acts as a long lever-arm, high bending moments are created on maxillary implants. This situation may explain why implant survival rates are significantly lower in the maxilla, particularly with overdenture treatment.

5. Superstructure Fit

Absolute passive fit between fixed prosthetic retainers and implant abutments has been extensively discussed to reduce biomechanical complications in the treatment of edentulism by means of oral implants. The phenomenon of passive fit refers to achieving an ideal relationship between these components upon prosthesis connection/cementation, where zero microstrain is induced within the prosthesis or in any implant component in the absence of an applied external load [163,164]. Research has shown that lack of passive fit depends on several factors. There is a consensus that screw-retained superstructures are more prone to experiencing misfit than cement-retained superstructures, as the latter can be adjusted clinically to decrease misfit-induced strains [164]. The machining tolerance (lack of precision fit) of implant components vary between implant systems and lead to inevitable misfit at the outset of prosthetic treatment [165]. As misfit of superstructures create initial stress/strain on implants, mechanical complications such as fracture of the prosthetic framework or veneering material and fracture or loosening of occlusal and/or abutment screws may be observed upon functional loading [166,167]. Several attempts including application of advanced technologies such as computer numeric controlled manufacturing and laser welding have been undertaken so far to achieve passively-fitting superstructures [168,169]. Yet, none of these attempts has been able to meet the absolute passive fit criteria.

The importance of accurate impression making has also been emphasized in achieving passive fit. Splinting of impression copings with acrylic resin or the use of non-splinted impression copings with specific modifications have been applied [170-173], although none have succeeded in obtaining passive superstructure fit. At present, correct three-dimensional transfer of implant or abutment positions from the mouth to working casts is the objective, coupled with attention dedicated to obtaining "optimum" fit between the implant and the superstructure during fabrication. From a biologic perspective, the idea that passively-fitting superstructures are a prerequisite for the maintenance of osseointegration depends on the fact that the bone-implant interface allows a very limited movement of 10 μm [171]. Despite its mechanical disadvantages, the prosthesis misfit does not seem to have any negative effect on marginal bone loss around implants [174,175].

EXPERIMENTAL BIOMECHANICAL OUTCOMES *VS* CLINICAL EXPERIENCE

One of the most critical goals in biomechanical assessment of load-carrying bone implants is to qualify and quantify *in vivo* peri-implant stress and strain in humans. Once this is established, a correlation between stress/strain and the cascade of biologic events that occur in skeletal tissue must be made relative to interface healing and/or peri-implant bone loss. Photoelastic stress analysis, two and three dimensional finite element stress analyses (FEA), two or three dimensional mathematical (geometric) analyses, and strain gauge analysis (SGA) have been extensively used to assess the biomechanical loads on implants. The major shortcoming of *in vitro* biomechanical stress analysis methods is the necessity to drive certain assumptions or to use materials, which frequently do not simulate the complex nature of living tissues. Methods based on finite element analysis have been used to calculate, rather than measure tissue stress and strain [176]. One of the major purposes of the 3-D FEA technique is to solve physical problems or to determine the effectiveness or behavior of an existing structure or structural component subjected to loads. The idealization of the physical problem to a numeric model requires certain assumptions that lead to differential equations governing the mathematical model. The accuracy of assumptions or the data implemented in the mathematical model are very important, as it governs the mechanical behavior of the tested structure in the virtual environment.

The application of SGA to dental implants is based on the utilization of electrical resistance strain gauges and its associated equipment, and provides both in vivo and in vitro measurement of induced strains under static or dynamic loads. Under an applied force, a strain gauge measures the mean dimensional change where it is bonded or embedded. The configurations of strain gauges often used for implant biomechanics are uniaxial and/or rosette, and are usually bonded to the implants, abutments, rigid connectors of a prosthesis in clinical experiments, and on bone in *ex vivo*/*in vitro* experiments [159,161,162,173].

In general, photoelasticity demonstrates the quality, quantity and distribution of force in an object by fringe patterns that appear as a series of successive and contiguous different-colored bands (isochromatics) in which each band represents a different degree of birefringence corresponding to the underlying stress in the tested part. The contour of an isochromatic fringe is determined by the flow of stresses in that particular region and represents equal differences in principal stresses. Hence, the color of each band uniquely identifies the birefringence, or fringe order (and stress level), everywhere along that band. The tint of passage is a dividing zone between red and blue in the first-order fringe and red and green in the second-order fringe, indicating first and second fringe orders, respectively. Beyond this point, a repetition of pink and green colors is observed and each transition indicates a new fringe order. The number of fringes indicates the stress or strain magnitudes, and the fringes being close to each other demonstrate higher stress concentrations at that region. This technique has been extensively used as a descriptive tool to evaluate the force transfer characteristics and load partitioning of oral implants supporting fixed and removable prosthesis.

Studies on the consistency of numeric models and their coherence with biological data are scarce [177] and the level of agreement and consistency between different engineering methods, especially on quantified stress/strain remains a topic debate [45, 178-180]. As

inconsistencies could arise due to differences between experimental conditions, interpreting the results of animal studies and extrapolation of those findings into humans must be performed with caution and skepticism. In previous studies, the application of 3-D FEA and SGA to in vitro animal and human experiments have been stated to reveal mutual compatibility and agreement between the techniques [178, 180]. However, a comparison between linear 3-D FEA with in vitro SGA showed that SGA measurements were higher than the 3-D FEA for both numeric models [45]. Iplikcioglu and his colleagues found that the strains on the neck of an implant body, as measured by non-linear FEA, were almost two-fold higher than the comparable data obtained by in vitro SGA, and mutual agreement was found for measurements on a solid abutment and acrylic resin where the implant body was embedded [179]. This result was attributed to differences between techniques in quantifying the bending moments on the neck of the narrow diameter Straumann dental implant, which possesses high deformation under lateral loading [181]. In addition, the differences between these studies [45,180] and previous work [178-180] were a consequence of the nature of experimental conditions and the objects (solid vs. non-solid) or sites where measurements were undertaken. For example, Keyak and co-workers [180], had bonded strain gauges on the cortical bone surface of human femur, where comprehensive numerical simulation was redundant, and Baiamonte et al. [178] had bonded strain gauges on two implant abutments, that resembled a solid-like structure. Therefore, it was not surprising to discern fundamental differences in measurement of bending moments by nonlinear-FEA and in vitro SGA on the neck of a narrow-neck implant.

Although these approaches may seem “academic” rather than observing clinically the outcome of treatments, the main reason for using these engineering techniques and comparing their agreement is to predict or analyze accurately current/future treatment outcomes involving implants. Their predictive capacity and the outcome could sometimes be exaggerated and probably misleading. For instance, biomechanical studies on passive fit have suggested that its achievement was critical, and its lack could lead to mechanical problems and even implant failure [163,164]. So far, no clinical publications exist supporting this claim. Likewise, it has long been pronounced that implants should be loaded axially and implant positioning [182], and loading position or even the application of off-set (non-axial) loads could lead to more marginal bone loss [183,184]. Today, it is known that implant positioning, such as staggered implant placement, has no clinically-proven advantage or disadvantage, implants could be placed in angulations probably more than 30 degrees and could be subjected to off-set loads without any risk of failure [151]. It seems that many of the critical factors that are believed to jeorpardize implant survival will presumably be considered safe in the future.

KEYNOTES TO TREATMENT PLANNING

1. Background

Under- and post-graduate education is becoming more important for incorporation of the dental implant application principles, which have rapidly developed and established with the scientific facts especially in the last two decades, into clinical practices. In this sense, the studies of organizations such as EAO (European Association for Osseointegration), AO

(Academy of Osseointegration) and ITI (International Team for Implantology) and their collaboration with prestigious scientific journals are remarkable in spreading correct clinical practices. In this context, the consensus reports and supplemental issues published by the above-mentioned organizations are highly active, and more importantly, such publications take as a guide the evidence-based patient treatment which was initiated in 1967 at McMaster University (Hamilton, Ontario, Canada). The purpose of this is to create case-specific treatment planning based on the scientific knowledge for optimum clinical practice. In order to achieve such purpose, the first stage includes arrangement of the existing scientific knowledge systematically for clinical practice. In this section, the basic approaches and normative definitions that can be taken as a guide in the treatment of missing teeth with dental implant-supported dentures are presented.

As a result of the experimental studies conducted to eliminate functional, aesthetic, and psychological problems arising due to the lack of retention and/or stabilization of mucosa-supported mandibular total dentures, osseointegrated implants has dramatically affected current prosthetic principles. Therefore, use of intraosseous oral implants in treatment of total edentulism has covered a large portion of contemporary clinical practices today. Additionally, they are also used in order to support maxillofacial prostheses and provide anchorage in orthodontic treatments in addition to their utilization to support dental prostheses. It is clear that the need for osseointegrated implants in clinical practice is the prosthetic treatment of missing teeth and loss of related soft/hard tissue. However, it should be remembered that osseointegration of dental implants following surgical placement into bone with defined guidelines is essential for successful treatment. In conclusion, from a wider perspective of the clinical application of dental implant treatment, there exits two different disciplines: prosthetic and surgical application.

2. Rationals in Planning

As the relationship between implant and bone is better understood scientifically [26,27], the implant systems have been continuously developed commercially based on the restorative expectations. Dental implants are becoming a routine in treatment of total and partial edentulous arches and even single missing tooth cases. Patients ask for prosthetic treatment of aesthetic and functional problems due to missing teeth, and apply to specialists. Although the clinician is supposed to create an optimum prosthetic planning to meet the demands and expectations of the patient, the primary goal in a successful treatment is to determine the type of the implant-supported denture to be delivered to the patient at the end of the treatment. In absence of universally accepted classification of implant-supported dentures, approach based on prostheses type may be acceptable. Therefore, implant-supported dentures are either fixed or removable regardless of the edentulism. To enhance the understanding, dentures for partial edentulism and single-tooth implant crowns are further divided into aesthetic and functional based on the priorities of the requirements. However, as the prosthetic treatment of all the teeth in the dental arch and related soft/hard tissues are realized for total edentulism, aesthetic and function should be considered equally.

3. Complete Edentulism

Mandible

Implant-supported dentures were initially proposed for extremely resorbed totally edentulous mandible. Swedish working group originally referred to screw-retained one-piece fixed dentures supported with five to six osseointegrated dental implants [26]. Available bone for dental implant placement in mandibular posterior region is usually limited due to resorption and restricted to mandibular canal as a anatomic landmark. Therefore, it is proposed mostly to place the implants in anterior region of mandible between mental foramen. The long-term successful outcomes of such denture design have been scientifically documented, and today this design is used based on the choice of the clinician. As well as missing all teeth, this denture design also replaces the loss of hard and soft tissue. For this reason, the term hybrid denture is commonly used for this kind of denture design. In this design, the denture is totally retained with dental implants without any soft tissue supported. Therefore, intraoral forces are distributed in bone around the implants. The denture consists of a one-piece framework, which holds the missing teeth and lost related soft/hard tissues with bilateral distal cantilevers. As the denture is one-piece in design, the adaptation between the denture and implants, and their impact has been studied over the years.

In this regard, location of dental implants, clinical practice carried out particularly in impression making and laboratory procedures including material selection are addressed several times in scientific studies. Hypothetically, the importance of passive fit is emphasized especially for the maintenance of the bone around the implant. However, studies have shown that it is not possible to ensure an absolute passive fit [163,164]. On the other hand, it is still not known to what extend misfit lead to loss of osseointegration. Nevertheless, it is known that in adaptation level which may arise due to laboratory stages has been minimized with the development of CAD/CAM technology in recent years. Another issue to discuss with this design is that most of the information on the amount of cantilever extension is the one anticipated from the in vitro studies and mathematical calculations since basic scientific studies are seriously limited due to ethical reasons. However there exits a bundle of scientific knowledge based on short/long-term follow-ups. In the light of these clinical outcomes, even 4 implants have been used along with the “all on four” concept [157].

When the edentulism period is relatively shorter, the available bone in the posterior area is more likely to receive implant placement. In such cases, posterior implants would reduce the possible negative effects of cantilever extension particularly on the prosthetic reconstruction including screw loosing/fracture and implant fracture. When posterior edentulous segments are involved into treatment planning in terms of implant placement, it is crucial to design the dentures as multiple individual short span fixed partial dentures. Another reason for this planning is to avoid possible hazardous effect of the deformation due to mandibular deformation during functional movements. Thus it is highly suggested not to use posterior implants bilaterally to support one-piece fixed dentures because of increased risk factor that would cause loss of osseointegration of posterior implants. Therefore, in cases when implants can be placed in posterior area of totally edentulous mandible, the number and the distribution of implants supposed to support fixed denture should be planned accordingly. In addition to all, use of implants in posterior region would likely improve function as the

chewing center would shift to molar occlusion compared to premolar occlusion as in one-piece cantilevered fixed denture design.

Treatment of long-term edentulism with implant-supported removable dentures, called as overdentures, is one of the suggested denture designs that have been successfully documented over the years. Furthermore, two implant-supported overdenture is accepted as the minimum treatment for mandibular total edentulism. Implant-supported overdentures are specifically recommended for the cases in which there is a physiological and/or pathological soft and hard tissue loss. In similar cases, less complicated clinical/laboratory procedures, improved aesthetic outcome and easiness in hygiene and decreased complication risks can be listed as its advantages compared to the fixed dentures with hybrid fixed denture design. Likewise, two or four implants can be placed in between the mental foramen, and implants are either connected to each other with a bar or stand free to support overdentures. Implant supported overdentures are naturally implant and mucosa supported. The load participation between implants and mucosa to a large expand depends on the number of implants used and the applied retention type [99]. Implant support increases as well as the retention and stability of overdentures with the increased number of implants and connected with a bar design. There are numerous retentive element types used between implants and overdentures basically depending on the implant preferred implant system. Critical point in planning of the retention type is to establish a prosthetic path of insertion regardless of the implant angulations in bone. Otherwise, free-standing implants with angulation problems that interfere with the path of insertion of the overdenture would require frequent maintenance for the attachments.

Maxilla

Prosthetic planning of total edentulism in maxilla is completely different from mandible. While the choice of denture type, fixed or removable, for the clinician in totally edentulous mandible basically depends on the available bone and economical, decision making for maxillary total edentulism should based on evaluation of certain diagnostic factors. The vertical and horizontal position of the maxilla in relation to intra-oral factors (e.g. mandible) and extraoral factors (e.g. esthetic parameters) plays an important role in the design of implant-supported dentures. Patients frequently ask for implant-supported fixed dentures to fullfill their esthetic expectations and functional requirements. The clinician should also stick to esthetics and function in planning but not in the way of patients in terms of denture type. To establish a successful treatment, the desires of patients for fixed dentures in all conditions rationally conflict with the principles of clinical practice. Patients allude that fixed dentures is key to esthetics and function. However, to create a retentive and stable denture design is the priority for the clinician in planning that already covers esthetics and function. In other words, the clinician should record, evaluate and analyze the extra- and intra-oral conditions which would be key to success in decision making. Accordingly, maxillo-mandibular relation should be recorded, the opposite dentition should be evaluated and the dento-labial analyzes of maxilla should be carried out. After completion of a complete dental history, the patient and the clinician should together discuss the conditions. Involvement of patient in decision making particularly in treatment of edentulous maxilla is helpful. If the reason of teeth loss is primarily dental for the patients who became totally edentulous not so long in the past or planned to become totally edentulous, frequently implant-supported fixed dentures are planned. In such cases, as the soft and hard tissue loss is mostly minimum, the fixed denture

would end in acceptable, but slightly changed teeth morphology with particularly increase in servico-insical dimension due to replacement of soft tissue. Correct implant placement in three dimensions in accordance to natural teeth position is critical to achieve a pleasing esthetic outcome. Posterior occlusion with opposing mandibular arch would be a guide to decide in distal extension of occlusion. Ending with first molar occlusion is scientifically accepted with regards to function. On the other hand, implant placement in posterior region would be determined by the maxillary sinus. In extreme anatomical restrictions, placement of implants into premolar region to support one occlusal unit distal cantilever can be accepted for selected cases. Although there is not consensus regarding the number of implants to support fixed denture, use of six to eight implant is common. But it is highly suggested to plan with multiple short-span fixed dentures supported with two implants. It is, therefore, required to increase the number of implants to be placed. When the number of implants is reduced because of economical reasons to support one-piece fixed denture, certain compromises have been accepted in fabrication (e.g. adaptation) and maintenance (e.g. porcelain fracture) of prostheses. However, alternatively one-piece structures constructed with CAD/CAM systems may be used to improve the fit of prostheses with six implants. There are numbers of suggestion regarding the implant distribution but not an agreement on one alternative. However, while bilateral central incisor, canine, premolar and molar tooth are preferred for multiple short span fixed dentures, there are also other alternatives for the anterior region. For instance, implants bilaterally placed in lateral incisor or canine region may be acceptable in planning.

For the patients that have been totally edentulous for a long time, remarkable amount of hard and soft tissue replacement should be needed. Because of anterior bone resorption in palatal direction, there will be a distinct difference between edentulous ridge and the anterior teeth to be placed. Therefore to meet with esthetic expectations, the denture should replace certain amount of hard and soft tissue loss. Other approaches such as hybrid type of fixed dentures, would fail in point of esthetics. Patients that are becoming totally edentulous due to loss of periodontal support, present with similar requirements described above for long-termed total edentulism. Therefore for cases that there are remarkable disparity between the planned teeth position and edentulous ridge, implant supported removable dentures (overdenture) are essential for dento-labial esthetics. In such planning, it is recommended to use at least four implants bilaterally placed in the lateral and premolar tooth area. Minor modifications can be made according to available bone in placing the implants. In design of overdenture, the goal is to create a prosthetic path of insertion from the different implant angulations inevitably occurred due to anatomy of edentulous maxilla. In this sense, connecting the implants with a bar is the commonly applied approach. When implants are concerned to support individually, customized abutments with telescopic design should be fabricated. As it is not possible to establish a single path of insertion using prefabricated attachments for four implants, a long term functional overdentures can not be made. Due to lack of distinct scientific interpretation so far, use of two implants to support maxillary overdenture remains as a popular approach.

In addition to all above discussions made separately for totally edentulous maxilla and mandible, the dentition of the opposite arch and age of the patient should be considered in terms of number and location of implants to placed.

4. Partial Edentulism and Single-Tooth Missing

Functional Area

Treatment planning of partially edentulous posterior region is basically based on evaluation of occlusion with opposite. Primarily, the treatment should be cover replacement four occlusal units in each quadrant. However shortened dental arch concept or distally cantilevered one occlusal unit designs could be applied because of anatomy that limits placement of implants distally or the opposing dentition ending with first molar tooth. Besides those modifying factors, normative treatment planning refers the application of two implant-supported splinted and/or free-standing crowns for missing two adjacent teeth. Also, terminally implant and tooth supported three unit fixed partial denture is a scientifically accepted alternate solution only for selected cases. For missing three occlusal units, two implant supported three unit fixed partial denture with a central pontic is a routine in practice. Alternatively two adjacent implants may support a mesial or distal cantilevered three unit fixed partial dentures for the same cases. Placement of three adjacent implants to support three missing posterior should be considered when the available bone is compromised. When four posterior teeth is going to replaced, planning three implants to support four unit fixed partial denture is optimum but for selected cases two terminal implants might be a treatment of choice.

Regardless of the information that occlusion would seriously affect due to the teeth movements in the relevant area, patients rarely apply to dentists for the treatment of single tooth missing. It is highly recommended to treat the relevant area with implant-supported crowns before the occlusal relation is disrupted. In general, if the anatomic conditions are convenient, application of one implant-supported crown is the normative approach.

Aesthetic Area

It is primarily aimed to meet the esthetic expectations of the patients in treatment of partially edentulous and single tooth missing maxillary anterior region. Although use of implants in maxillary anterior region is challenging due to high esthetic expectations, partially edentulous maxillary anterior region is more demanding compared to single-tooth missing cases. For missing adjacent two teeth, use of two implants is only appropriate two central incisors. In other cases in which missing two teeth are located unilaterally relative to midline, placement of one implant to support two unit fixed partial denture with one cantilevered pontic should be treatment of choice. Otherwise esthetic outcome of two adjacent implants not covered by midline would not be satisfactory. Therefore, it highly suggested to replace unilateral missing lateral and canine teeth with one implant supported two unit fixed denture. If more than two teeth are missing, it is recommended to distribute the implant supports leaving one or two pontic spaces between them. In addition to multiple missing teeth, cases with extreme loss of soft and hard tissues are extremely difficult to manage with both surgically and prosthetically. Such cases need care of expert clinician in the field.

Replacement of single tooth missing cases is relatively less demanding compared to partially edentulous anterior region. Nevertheless, comprehensive evaluation is vital to meet with esthetic demands. Mainly available bone would drive the treatment planning particularly in terms of surgical placement of the implant. From prosthetic point of view, correct placement in all direction is essential.

Implant-supported fixed dentures are either cement or screw retained. Cement retention is preferred due to easiness in fabrication, low complication rate, better fit of restoration and cost effectiveness. However, screw retention may be indicated when the restoration margin should be located deep in mucosa, the interocclusal space is limited and there is a severe implant angulation problem. Even existence of these, CAD/CAM based customized abutments could be used for cement retention. Therefore cement retention is becoming more widely used for implant supported fixed dentures.

5. Roadmap in Clinical Practice

After completion of all required steps to treatment planning, the final decision should be made with the patient and clinician. Every aspect of the treatment should be discussed in detail with the patient. Next is to determine on the surgical and prosthetic procedures to be followed. Accordingly, there is a wide range of options that are scientifically approved for clinical practice. All alternatives in clinical practices are defined on timeline to shorten the total treatment period. However decision making on which procedure to follow should be based on patients' convenience. Therefore, clinician should always keep in mind that the preferred procedure would provide apparent advantages for the patient not to the clinician. As defined earlier in treatment planning, two main clinical practices involve the surgical placement of implants into bone and prosthetic definition of loading conditions. Surgical and prosthetic clinical practices are independent from each other but closely related.

Prosthetic loading conditions are defined according to the time passed following implant placement and occlusal relation with the opposing dentition of the delivered immediate or permanent dentures. Dentures not in functional occlusal contact with the opposite dentition completed within 48 hours following the surgical placement of implants are referred as immediate restoration, and that are in functional occlusal relation is referred as immediate loading. Dentures that are delivered with occlusal contact within three months following implant placement is defined as early loading. Dentures of three to six months after implant placement are defined as conventional loading; whereas, the subsequent stages have been defined as delayed loading.

Definitions of loading conditions are based on scientific facts to reduce or eliminate edentulous period. To achieve osseointegration that can shortly be defined as functional ankylosis is still the key for clinical success of implant-supported dentures for all defined loading conditions. However, there is a critical difference for the immediate restoration/loading and early loading conditions that are evolved to shorten the treatment time. Under the immediate restoration/loading condition, mechanical stimulation is somehow proposed to transform the mechanical bone to implant appears directly after surgical implant placement into biological bone to implant contact. In other words, under the immediate restoration/loading condition, frequently osseointegration is created by applied intraoral load with the delivered dentures. Control of the mechanical load to remain within reliable limits of bone is vital for osseointegration. As of today, even though there is some information available for the increased load amount within the reliable limits in terms of clinical practices, it's not quantitatively known. It is clearly known that the most important advantage of this loading condition is the elimination of edentulous period in functional terms. The anticipated advantage which is frequently mentioned but which does not have a distinct difference compared to the traditional practices is to optimize the soft tissue recovery especially in

esthetic area. However, the clinician should keep in mind that immediate restoration/loading is kind of challenging which in turn increases the risk of failure in achievement of osseointegration. Additional cost and chair time due to replacement of immediate denture with the permanent after osseointegration are other known disadvantages.

What is anticipated for the early, traditional and delayed loading conditions is to creation of intraoral forces over the implant-supported permanent dentures following osseointegration. Therefore, there is a philosophical deviation from the immediate restoration/loading. Reduced time compared with conventional loading condition, that is to say early loading condition is completely related to the biological stimulation of the bone around implants with rough surface specifications at micro level. Increasing preference in clinical practice of early loading condition is associated with the development of implant surface features that ensure biological bone to implant contact in a shorter time. Therefore, the risk factors in the early loading condition are limited to what is known to ensure osseointegration. However, it is undoubtedly a risk factor to select an early loading condition with the implant surface features which are not scientifically documented sufficiently. Even though edentulous period is not eliminated with early loading condition, its reduction to a significant degree and placement of permanent denture are considerable advantages. Definition of delayed loading condition by extending the known time for the conventional loading condition is related to the optimization of the periimplant bone tissue which is regenerated with advanced surgery techniques and biomaterials.

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Chapter 22

TAPE CASTING IN THE DEVELOPMENT OF DENTAL BIOMATERIALS

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ABSTRACT

Tape casting is one of the main methods used in the microelectronics industry to ensure precise thickness control and consistency in the manufacture of flat ceramics. This method uses specially formulated slurry comprising base material powder, binder, plasticizer, and dispersant that can be cast on a moving carrier surface. The major advantage of tape casting is that the thickness of the ceramic sheet can be adjusted precisely by varying the gap between the blade (the so-called doctor blade) and glass surface. Fabrication by tape casting therefore offers advantages that enable the preparation of dental biomaterial sheets. Thus, there are potentially a wide range of dental applications.

A number of studies have already reported new tape-casting fabrication methods for dental biomaterials such as bone substitutes and scaffolds for bone regeneration and dental ceramic materials for prosthetic restoration. Several studies have shown the tape-casting preparation of scaffolds for bone-tissue engineering. Studies by the present author have resulted in the development of a fiber-reinforced ceramic material that is suitable for a new type of dental prosthesis. These studies have demonstrated that tape casting is a useful technique in preparing new dental biomaterials.

This article is an overview of the development of various dental biomaterials fabricated by tape casting.

Keywords: Tape casting; Dental biomaterials; Scaffolds; Dental prostheses; Fiber-reinforced ceramics

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INTRODUCTION

Tape casting is one of the main methods currently used in microelectronics for manufacturing flat ceramics with precise thickness and consistency [1–6]. Since 1952 when Howatt (US Patent No. 2 582 993) obtained a patent for tape-casting [1], applications have been developed in a wide variety of industries, such as microelectronics, photovoltaic cells, solar applications, laminated composites, and rapid prototyping. Shimokawa et al. [4] reported the use of tape casting in preparing ceramic sheets composed of Al_2O_3. Likewise, Fukui et al. [5] fabricated $La_{0.9}Sr_{0.1}Ga_{0.8}Mg_{0.2}O_3$ thin film as an electrolyte by tape casting. In addition, Arabatzis et al. [6] pointed out that tape casting can be employed as a fast, non-energy-consuming procedure for preparing ceramic sheets with good uniformity and reproducible properties. Thus, tape casting by means of a doctor blade system has the advantage of not requiring expensive fabrication facilities.

The procedure for preparing flat ceramics by tape casting using a doctor blade machine is summarized in Figure 1. Generally, the tape-casting process consists of several steps including slurry formulation, vacuum defoaming, casting, drying, lamination, and sintering. Each step influences the final properties, and failures in one step cannot be corrected in one of the following steps. The process of tape casting starts with the ball milling of the base material, and continues with the mixing of the powder with other components such as binder, plasticizer, and dispersant to obtain a homogeneous suspension called a slurry. On the basis of the powder properties of the base material to be tape cast, a suitable ratio of binder / plasticizer / dispersant must first be selected. Kim et al. [7] reported that the mechanical properties of alumina-glass dental composite prepared by tape casting were influenced by the ratio of alumina powder, binder, and plasticizer. Suzuki et al. [8] prepared needle-shaped hydroxyapatite filters for ion exchange by tape casting, and found that the best aqueous slurry for tape casting was a combination of 20 mass% hydroxyapatite, 15 mass% binder, 15 mass%, plasticizer, and 1 mass% dispersant in their system. Previous studies in our laboratory [9,10] determined the best base material / binder / plasticizer / dispersant mixture ratio to be 50 / 8 / 1 / 0.2. It is very difficult to tape cast a slurry if the slurry has a high viscosity. Conversely, a slurry with low viscosity cannot produce a sheet with uniform thickness. Therefore, it is important to determine a suitable ratio of binder / plasticizer / dispersant. In addition, it is important that the powder properties of the base material such as particle size, size distribution, and morphology are well controlled by ball milling because the particle size and morphology determines the amount of binder, plasticizer, and dispersant needed to prepare a slurry for tape casting.

A doctor blade system is filled with slurry formulated as shown in Figure 2. Tape casting is performed using a batch caster. The casting head and doctor blade traverse over a stationary floating glass slab, discharging slurry onto the surface. Figure 3 shows a green alumina sheet prepared by tape casting in our laboratory. The sheet has uniform thickness and is highly flexible, enabling free-form shaping. The major advantage of tape casting with a doctor blade machine is that the thickness of the ceramic sheet can be adjusted precisely by varying the gap between the blade (the so-called doctor blade) and the glass surface, enabling accuracy of up to 10 μm. Köbel et al. [11] prepared an overcoat of $Bi_2Sr_2CaCu_2O_x$ thick film on polycrystalline MgO substrate by tape casting. They varied the tape thickness from 100 to 1000 μm and evaluated the effect of tape thickness on the electrical properties of the ceramic

sheets. Tok et al. [3] found that a higher degree of accuracy for the tape thickness can be obtained by casting at a lower speed. They stated that an optimal relationship between the casting speed and blade height is needed to achieve an accurate thickness of the fabricated sheet.

By applying tape casting to dental technology, a number of studies have reported new fabrication methods for dental biomaterials such as bone substitutes or scaffold materials for bone regeneration as well as dental ceramic materials for prosthetic restoration. In general, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAP] and tricalcium phosphate [$Ca_3(PO_4)_2$, TCP] are calcium-phosphate-based ceramics that are widely used as scaffold materials for bone-tissue engineering because of their good bioactivity and osteoconductivity. Presently, there are fabrication methods for calcium phosphate scaffolds that involve freezing [12], hot pressing [13], spark plasma sintering [14], and three-dimensional fabrication [15]. Several studies have shown the tape-casting preparation of scaffolds for bone-tissue engineering. Gough et al. [16] prepared a bioactive glass disc by tape casting and investigated the primary attachment of osteoblast-like cells to tape-cast bioactive glass discs. Zhang et al. [17] prepared an HAP sample by tape casting and reported that it exhibited excellent properties for cell attachment and proliferation. These authors noted that tape casting can be effectively used for biomedical applications.

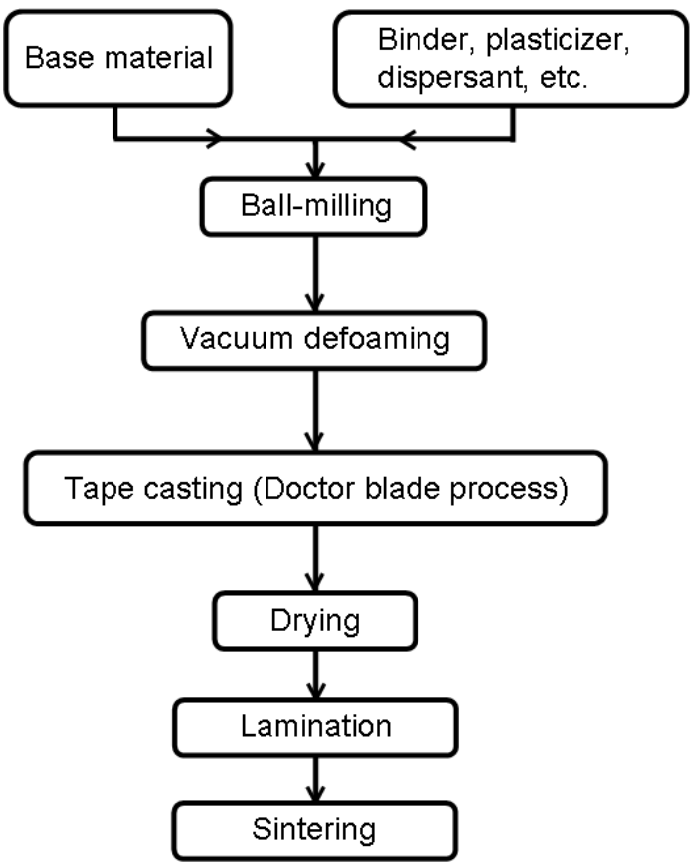

Figure 1. Flowchart of the tape-casting process.

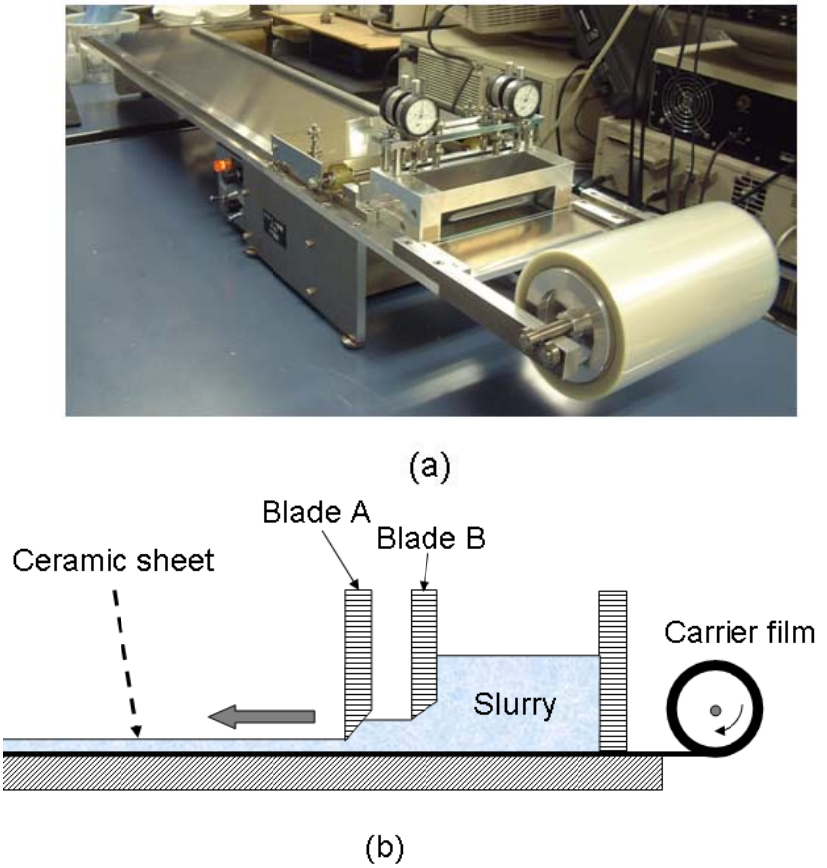

Figure 2. Tape casting with a doctor blade machine. (a) Overview of the doctor blade machine; (b) schematic drawing of the doctor blade system.

Dental ceramics have been commonly used as esthetic restorative materials for crowns, bridges, and fixed partial dentures. Furthermore, the increased use of ceramics for restorative procedures and the demand for improved clinical performance have led to the development and introduction of several new ceramic restorative materials and techniques [18,19]. A method for the fabrication of dental ceramic materials by tape casting has been reported [7,20]. Especially in our laboratory, a tape-casting method for the fabrication of an alumina fiber-reinforced alumina-based ceramic material as new dental ceramic for prostheses has been developed [9,21]. The resultant ceramic material fabricated by tape casting was an all-ceramic material composed of alumina fiber reinforcement and alumina-based ceramic. This fabrication method helped to reduce sintering shrinkage and improve the flexural properties of fiber-reinforced ceramic when compared with unreinforced ceramic. These studies demonstrated that tape casting is useful in preparing new dental biomaterials.

From the above discussion, fabrication by tape casting has advantages that enable the preparation of dental biomaterial sheets with uniform thickness, and such ceramic sheets have potential use in dental applications. Therefore, this method has been used extensively in the preparation of new biomaterials in dentistry.

This article provides an overview of the development of various dental biomaterials using the tape-casting method.

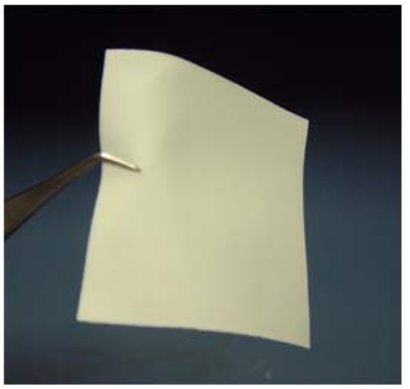

Figure 3. Photograph of an alumina sheet prepared by tape casting.

Scaffold Materials for Bone Regeneration

Calcium phosphate ceramics such as HAP and TCP are widely used as bone substitutes or scaffolds for bone regeneration because of their excellent biocompatibility, osteoconductivity, and bone-bonding properties. Generally, calcium phosphate ceramics can take many forms, including powders, granules, pellets, dense and porous ceramics, and cements. However, the brittleness and insufficient strength of calcium phosphate limits its application to physiologically non-load-bearing bone lesions. To overcome the shortcomings of calcium phosphate, composites of calcium phosphate and resorbable polymers such as polylactic acid were prepared as scaffold materials for bone-tissue engineering [22–24]. In addition, researchers attempted to prepare HAP composite in sheet form [25,26].

The preparation of calcium phosphate sheets as biomaterials for bone-tissue engineering by tape casting has also been reported. Krajewski et al. [27] prepared an HAP-based sheet from formulated slurry containing HAP, organic binder, and plasticizer by tape casting. The authors demonstrated that an HAP-based green sheet can be cut easily with scissors and perforated before sintering to obtain various shapes. Likewise, our laboratory prepared β-TCP green sheets by tape casting [10]. The β-TCP sheet obtained is highly flexible, allowing twisting and free-form shaping, and is easily trimmed with scissors. In addition, the stress–strain curve of the β-TCP sheet has nonlinear behavior in a tensile test, whereas ceramic materials generally have a linear relationship between stress and strain owing to their brittleness. These reports indicated that fabrication by tape casting has the advantages of enabling the preparation of bioceramic sheets with precise thickness and not requiring expensive fabrication facilities. However, calcium phosphate green sheets such as HAP and β-TCP are flexible, and thus they are not stable in body fluids and have excessively rapid dissolution. If calcium phosphate sheets are to be suitable as biomaterials, a sintering process is required.

It is well known that the physical and chemical properties of calcium phosphate ceramics are influenced by the sintering process and additives. Wang et al. [28] reported that hardness and Young's modulus of TCP ceramics increases with sintering temperature, but that Young's modulus decreases when transformation from β to α-TCP occurred, even though hardness increases. Suchanek et al. [29] found that β-$NaCaPO_4$ is an effective sintering

additive for HAP, in that it enhances sinterability without decomposing the HAP and leading to the formation of other undesired phases.

In the case of tape casting for biomedical applications, Clupper et al. [30] reported the effect of sintering temperature on the *in vitro* bioactive response of tape-cast and sintered bioactive glass-ceramics. Moreover, Clupper and Hench [31] characterized the microstructure of silver-doped tape-cast bioglass and reported that silver doping had the effect of decreasing the sample porosity.

It is well known that porosity control is important both in terms of its effect on mechanical properties and as a mechanism for enhancing bonding to bone tissue [32,33]. In particular, the pore size or spaces between particles must exceed 100 μm for bone cell ingrowth to occur [34]. In applying tape casting, the porosity, pore size, and pore shape are controlled. Arita et al. [35] prepared HAP sheets with controlled porosity starting from a mixture of dicalcium phosphate [$CaHPO_4$] and calcium carbonate [$CaCO_3$] powders. The authors indicated that the sintering process can control the porosity of HAP prepared by tape casting.

The structures of living tissues show are not homogeneous and have natural functional gradients. Thus the optimized structure for scaffolds should have similar functional gradients to regenerate the natural tissue. Functionally graded materials (FGMs) as new materials have recently attracted much attention. The concept of functionally graded structures was applied to develop biomedical applications [36], and the wide variety of methods used to process functionally graded biomaterials has recently been reviewed [37,38]. Werner et al. [32] used tape casting to produce HAP scaffolds with porosity and pore-size gradients. Here, graded porosity was achieved by the multilayer casting of HAP tapes with controlled pore structure. Initial *in-vitro* cell compatibility of functionally graded HAP materials was demonstrated. The design of FGMs offers potential applications in bone-tissue engineering.

In most studies reporting the use of tape casting, HAP was used as the calcium phosphate for bone-tissue engineering. The low biodegradation rate of HAP associated with inherent biological stability remains a hurdle because it frequently prevents natural bone ingrowth for extended periods. On the other hand, it is known that TCP is more resorbable than HAP is in biological environments. Thus, TCP is a promising candidate because it is more readily dissolved in a biological setting. Recently, TCP sheets have been developed by tape casting in our laboratory [10,39–42]. The sintering temperature is generally an important factor in bioceramics because it determines the phase transformation and degree of sintering, which in turn affect the physical and chemical properties of bioceramics [28]. Therefore, we investigated how the sintering temperature affects scaffold characteristics such as grain size, porosity, bioactivity, and mechanical properties in previous works. Knowledge of the bioactive and physical properties of TCP materials gained in the previous works enables us to control the final scaffold characteristics.

From the above reported results, we see that biomaterials fabricated in processes consisting of several steps, including tape casting and sintering, have potential use in a wide range of biomedical applications by the control of processing parameters, and they are expected to be useful as tailor-made biomaterials that can be designed for different applications and/or requirements.

Dental Ceramic Materials for Prosthetic Restoration

Dental ceramics are commonly used as esthetic restorative materials for crowns, bridges, and fixed partial dentures. Metal ceramics have been especially popular for restorations in fixed prosthodontics [43]. However, the metals used in metal-ceramic restorations potentially have allergic or toxic reactions within soft or hard tissues. Therefore, there is extensive clinical research into developing metal-free restorations [44] or metal-ceramic restorations using pure titanium with a high degree of biocompatibility [45]. In particular, several new ceramic restorative materials and techniques have been developed to date. For example, various kinds of new ceramics, such as castable ceramics [18] and computer-aided designed/manufactured ceramics [19], which were introduced into dentistry in the 1980s, have shown considerable potential as restorative materials.

There have been several reports on ceramic materials prepared by tape casting for dental prostheses. Oh et al. [20] investigated the properties of glass-infiltrated alumina tape produced using a water-based solvent. The fracture toughness and flexural strength of glass-infiltrated alumina tape was observed to be 4.6 MPa $m^{1/2}$ and 498 MPa respectively. The authors concluded that the glass-infiltrated alumina core produced from water-based alumina tape-casting composites could possibly be used as an all-ceramic crown system in a fixed partial denture because its flexural strength exceeds 300 MPa. In another application for dental prostheses, titanium sheets have been produced by tape casting [46]. The mechanical properties of the titanium sheets such as hardness, flexural strength, and tensile strength significantly increase with sintering temperature. It thus seems possible to apply sintered titanium to dental prostheses.

Tape casting utilizes specially formulated slurry composed of ceramic powder, binder, plasticizer, and dispersant that can be cast on a moving carrier surface. Generally, the binders, as additives to the slurry, influence the physical properties of the prepared ceramics [7,47]. Kim et al. [7] investigated the effect of the ratio of alumina powder to organic additives, including binder and plasticizer, on the mechanical properties of alumina-glass dental composites prepared by tape casting. They reported that the mechanical properties depend closely on the mean alumina particle spacing in the tapes, which can be manipulated by the mass fraction of alumina / (alumina + organic additives). Furthermore, a cobinder consisting of polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), and gelatin was used to prepare aqueous-based alumina tapes for three-dimensional forming [48]. The strength, flexibility, and adhesiveness of the aqueous-based alumina tape were optimized using a cobinder with a composition of 89.5 mass% PVP, 10 mass% PVA, and 0.5 mass% gelatin.

Studies by the present author have resulted in the development of an alumina fiber-reinforced alumina-based ceramic material for use as a new type of dental prosthesis, fabricated by tape casting. [9,21]. The fiber-reinforced ceramic is an all-ceramic material composed of alumina fiber reinforcement and alumina-based ceramic. The results of the studies showed that the shrinkage and flexural properties of fiber-reinforced ceramic exceed those of unreinforced alumina, hence demonstrating the positive effects of fiber reinforcement [9]. The effects of sintering temperature on flexural properties of an alumina fiber-reinforced alumina-based ceramic have also been investigated [21]. Fiber-reinforced ceramics fabricated by tape casting in our laboratory have potential in clinical applications such as crowns, bridges, laminate veneers, and dental ceramics for many types of dental prostheses; however, the clinical applications of fiber-reinforced ceramics have not been investigated in detail.

Therefore, the clinical applications of fiber-reinforced ceramics to dental prostheses such as crowns and bridges should be further investigated.

CONCLUSION

This article reviewed the use of tape casting in preparing dental biomaterials such as scaffolds for bone regeneration and dental ceramics for prosthetic restoration. Dental biomaterials introduced in this review have several distinct advantages because of their superior producibility and excellent properties. Tape casting could potentially become an important technique for applications in dentistry, and the application of tape casting to dental biomaterials should be developed further.

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Chapter 23

A NOVEL STEREOLITHOGRAPHIC SURGICAL TEMPLATE IN COMPUTER-AIDED IMPLANTOLOGY: METHODS AND APPLICATIONS

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ABSTRACT

The complications caused by improper implant placement pose a significant challenge in implant dentistry. In order to improve the precision of placement, there is a trend towards computer-aided oral implantology, especially the application of CT-derived surgical templates. Actually, there are three types of surgical guides, i.e., bone supported, mucosa supported, and tooth supported, reported in literature. As far as conventional clinical cases are concerned, these kinds of templates might be relatively stably placed on the underlying tissue such as jawbone or mucosa. However, with regard to some complex cases involving severely resorbed edentulous cases, clinical experience demonstrates that fixture of the surgical guides is not so stable due to unsatisfactory match between the templates and receptor sites. Aiming to solve this problem, a novel bone-tooth-combined-supported surgical guide is introduced in this study. With the use of a 3D-laser scanner, more detailed surface information at the level of the dentition can be obtained. Then, fusion of laser scanned dental occlusion data and CT data is realized through image registration technique. On the basis of this fusion data and the information obtained from preoperative planning software, a 3D computer model of this kind of bone-tooth-combined-supported surgical guides can be designed and finally fabricated via stereolithography technique. Because this approach is achieved using both laser scanning and CT imaging, thus improving the fit accuracy and reliability of this sort of surgical guides. Their applications in two severely resorbed edentulous cases show that the average distance deviations at the coronal and apical point of the implant were 0.66 mm (range: 0.3 to 1.2) and 0.86 mm (range: 0.4 to 1.2), and the average angle deviation was 1.84° (range 0.6°to 2.8°), therefore proves that the novel combine-supported templates are superior to the conventional ones. However, more clinical cases will be conducted to demonstrate its feasibility and reliability.

Keywords: Computer-aided surgery, Oral implantology, Preoperative planning, Stereolithographic template

1. INTRODUCTION

The complications caused by improper implant placement pose a significant challenge in implant dentistry [1-4].CT-derived surgical templates (guides) enable clinically significant improvements in accuracy, time efficiency, and reduction in surgical error, benefiting the patient, surgeon, restorative dentist, and laboratory[7].Nowadays, several CAD/CAM systems for preoperative planning and the fabrication of this kind of surgical templates have been developed commercially available, including:

(1) SimPlant, Materialise, Leuven, Belgium [3, 4, 6, 7]
(2) NobelGuide, Nobel Biocare, Yorba Linda, CA [8, 9]
(3) ImplantMaster, I-Dent Imaging, Ft. Lauderdale, FL [11]
(4) Med3D, med3D AG, Zurich, Switzerland [10]

The templates dictate the location, angle, and depth of insertion of the implant, so as to provide a link between the planning and the actual surgery by transferring the simulated plan accurately to the patient. Actually, there are three types of surgical guides, i.e., bone supported, mucosa supported, and tooth supported [3, 4,7]. As far as conventional clinical cases are concerned, the template might be relatively stably placed on the underlying tissue such as jawbone or mucosa. However, with regard to some complex cases involving severely resorbed edentulous cases, clinical experience demonstrates that fixture of the surgical guides (especially for bone-supported or mucosa-supported ones) is not so stable due to unsatisfactory match between the templates and receptor sites. Problems will occur since even a slightest angular error may result in significant positional errors at the end of the tool trajectory [20].

The purpose of this study is to introduce a novel bone-tooth-combined-supported surgical guide for implant placement. With the use of a 3D-laser scanner, more detailed surface information at the level of the dentition can be obtained. Then, fusion of laser scanned dental occlusion data and CT data is realized through image registration technique. On the basis of this fusion data and preoperative planning information, a 3D computer model of this kind of bone-tooth-combined-supported surgical guides can be designed utilizing special software and finally fabricated via stereolithography technique. The hypothesis is that this approach is achieved using both laser scanning and CT imaging, thus improving the fit accuracy and reliability of this sort of surgical guides.

2. MATERIAL AND METHODS

2.1. The Preoperative Planning Software

In response to the requirement of oral implantology, we built a software called CAPPOIS (Computer Assisted Preoperative Planning for Oral Implant Surgery). The original CT data of the patient can be imported to CAPPOIS for 3D-Reconstruction and preoperative surgical planning. First of all, a threshold and region growing combined method is adopted for the

segmentation of the object bone tissue so that a 3D cranio-maxillofacial model is reconstructed through the marching cubes algorithm[21] and triangular surface decimation method[22]. Then, a panoramic curve can be manually drawn following the curvature of the jaw bone on one of the imported axial CT image slices. On the basis of this panoramic curve, the series of panoramic images and cross-sectional images are reconstructed. Then, the anatomical landmark points on these 2D images or the 3D cranio-maxillofacial model can be located so that the distance between any two points and the angle among any three points can be calculated. In addition, bone density can be measured by locating a certain area on the 2D images, and the bone volume measurement for maxillary defects can also be conducted. According to the above-mentioned information concerning the relevant anatomical structures, prosthodontists can select a certain type of virtual implants and place them into the ideal areas. The virtual implants will be rendered on all of the 2D/3D views; therefore, the type, number, size, position, and orientation of the implants can be determined explicitly by iterative optimization taking into account prosthetic requirements and available local bone. An example for a preoperative plan with CAPPOIS is shown in Figure1.

The key technology of the software involved some algorithms in the field of medical image processing and computer graphics. The major algorithms included DICOM file parsing, Image segmentation and 3D- visualization[21,22], Spline curve generation, Multi-planar reconstruction, Spatial search and 3D distance computing[23], Cutting[23], Volume measurement[24,25], etc. For each of algorithms, we developed a set of dynamic link libraries (DLL) using Microsoft Visual C++, as well as the Visualization Toolkit (VTK, an open source, freely available software system for 3D computer graphics, image processing, and visualization etc., http://www.vtk.org/) and Insight Toolkit (ITK, an open-source software toolkit for performing registration and segmentation, http://www.itk.org/) via object oriented programming methodology. This basis can be extended by virtually any new approach or algorithm, which then becomes seamlessly integrated into the method set of the preoperative planning software framework. The aim is to provide well-defined levels of abstraction (the hiding of implementation details) from the individual components, so that new technology can be incorporated into the system without a complete software rewrite. As for graphical user interfaces (GUI), we chose MFC (Microsoft Foundation Class), because the Win32 API offered the greatest versatility in exploiting the features of Windows.

The user interface and functions of CAPPOIS parallels Simplant (Materialise, Belgium)[3,4,6,7], which is already commercially available, however, since the visualization and image processing algorithms involved in our software are developed using VTK and ITK, a plug-in evolutive software architecture is established, allowing for expandability, accessibility, and maintainability in our system. In addition, aiming to make the software simply accessible and fulfill the research requirements in academia, our future work is to make CAPPOIS a free, open source, and cross-platform (Windows, Linux and Mac Os X operating systems) software for preoperative planning in oral implantology.

2.2. Registration

In order to produce a bone-tooth-combined-supported surgical guide, a detailed visualization of dentition is a prerequisite. However, a 3D surface of the teeth created from CT images of the patient is not accurate enough; furthermore, for the cases involving amalgam fillings, the streak artifacts jeopardize the details of the occlusion [26]. In this study,

we presented a method of laser scanning combined with image registration technique to solve this problem.

At first, plaster casts of the patient were routinely made. These plaster casts were an accurate copy of the actual dentition of the patient [26]. Then, a commercially available 3D laser scanner was utilized to scan these plaster casts. The point cloud data acquired by the laser scanner were read and processed with 3DLaserRecon (a special software for laser-scanned data reconstruction developed by our institute). With the help of this software, data filtering and noise canceling were carried out, and then a 3D digitized model of the dentition (shown in Figure 2(a)) could be reconstructed through RBF (Radial Basis Functions) algorithm [30]. The regions of interest, i.e. the adjacent teeth surface in the edentulous region (shown in Figure 2), which matched the inner surface of surgical templates, were respectively cut from laser scanned model and 3D CT model, and then imported to MedRegCAD (a special software for image registration and surgical template design, developed by our institute) for registration.

Image registration refers to superimposing the 3D laser scanned dentition model onto the 3D skull model reconstructed from CT images. A two step method, respectively initial landmark registration and final surface registration, was used to accomplish this process, described as follows:

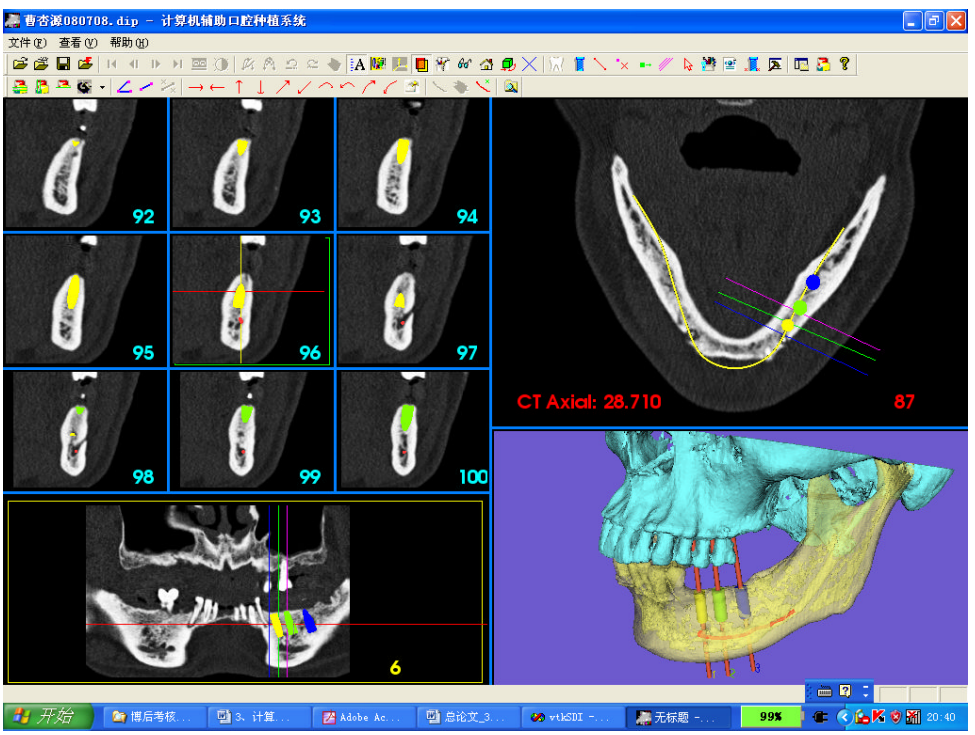
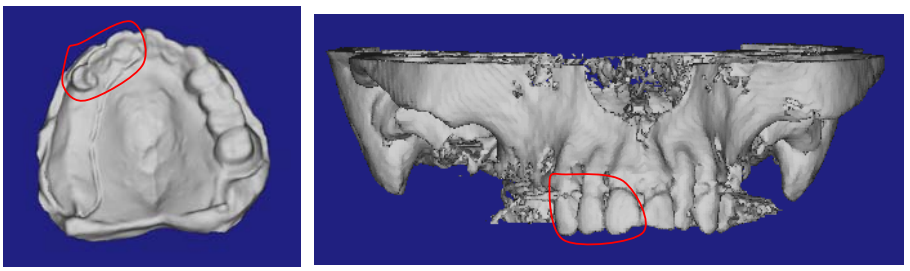


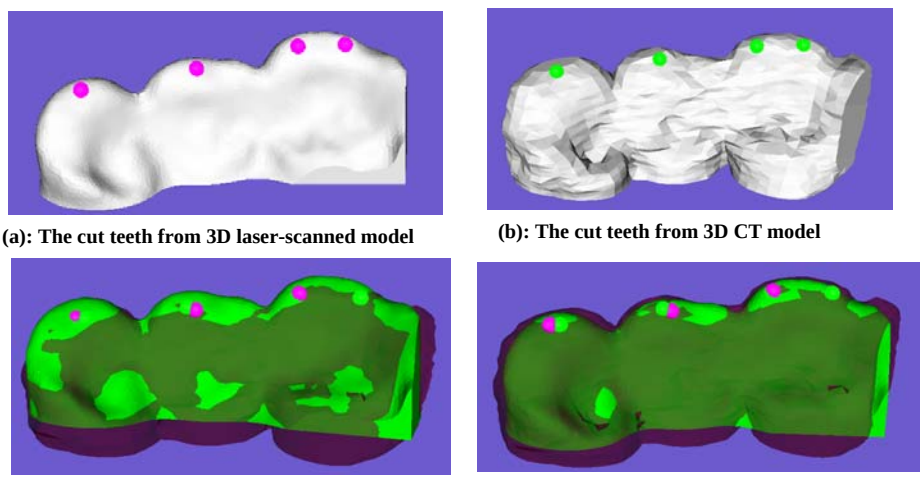
Figure 1. A preoperative plan is created for the ideal positioning of oral implants, in both 2D and 3D, while taking into account both clinical and esthetical considerations.



(a): 3D model from laser-scanned data **(b): 3D model from CT data**

Figure 2. 3D reconstructed models respectively from laser-scanned and CT data, the adjacent teeth (labeled in the enclosed red line area) in the edentulous region will be cut for registration

At first, at least three corresponding landmark pairs were indicated on the regions of interest: alternating between the 3D laser-scanned model and the 3D CT model (shown in Figure 3(a), (b)). Then, with the use of SVD (singular value decomposition) algorithm [27], landmark registration (shown in Figure 3(c)) obtained the best fit mapping one set of landmarks onto the other, in a least squares sense. After that, the second registration step was processed to match the two corresponding 3D surfaces using the iterative closest point (ICP) algorithm [28] (shown in Figure 3(d)). The core of the algorithm is to match each vertex in one surface with the closest surface point on the other, then apply the transformation that modify one surface to best match the other (in a least square sense), and the proper convergence of the surfaces is finally obtained by iterating the procedure. The point of this two step method is that the initial and final registration approaches are complementary. Landmark registration approximates the 3D laser scanned dentition model to the 3D CT space. It serves as the basis on which surface registration improves the overall registration accuracy.



(a): The cut teeth from 3D laser-scanned model **(b): The cut teeth from 3D CT model**

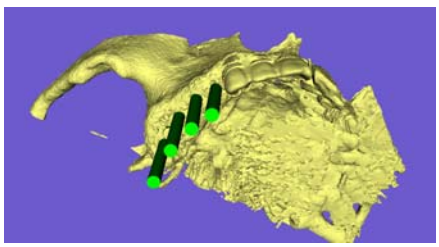
(c): The result from landmark registration (first step) **(d): The result from ICP registration (second step)**

Figure 3. Registration procedure. The red and green dots represent corresponding landmark pairs; for (c) and (d), the green and purple models respectively represent 3D laser-scanned and CT models.

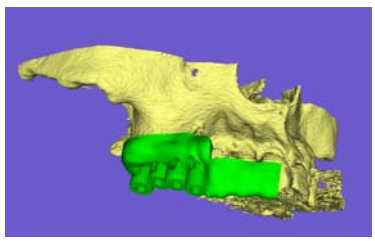
2.3. The Design and Manufacture of Surgical Templates

After registration, an "augmented" skull model with detailed dentition information was obtained (shown in Figure 4(a)). And then, with the use of MedRegCAD, the surface of alveolar bone and adjacent teeth in the edentulous region was determined by drawing a closed spline curve along with the region manually. This 2D surface was then extended to form a 3D solid model through an approach using the tangent vectors at the edges of the surface [29]. On the basis of preoperative planning, a 3D stl (stereolithography) model of the surgical template with cylindrical holes (shown in Figure 4(b)-(e)) was then generated through boolean operation, i.e., subtraction, between this solid model and the extended implants. Several windows with shape of hollow cylinder were designed on the buccal surface of the template to allow for irrigation with saline during the surgery (shown in Figure 4(d)). The implant surgery could be simulated in the software as well (shown in Figure 4(f)).

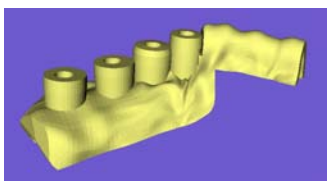
With respect to manufacturing, a rapid prototyping machine using the principle of stereolithography was employed to fabricate the resin surgical template (shown in Figure 4(g)). Finally, several surgical grade stainless steel tubes with suitable diameters were assembled to the cylindrical holes as metal sleeve guides in the template.



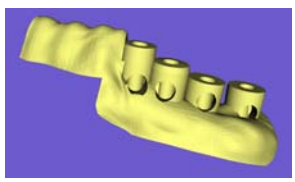
(a): The "augmented" skull model with detailed dentition information. The green cylinders represent the extended implants.



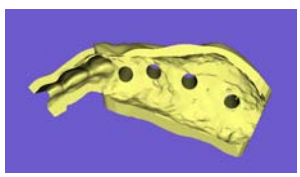
(b): 3D model of the surgical template, created through boolean operation



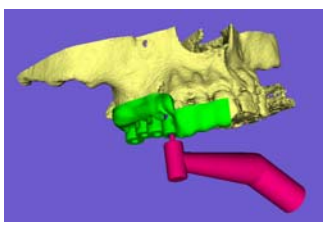
(c): 3D model of the surgical template, lingual side



(d): 3D model of the surgical template, buccal side



(e): 3D model of the surgical template, inner surface



(f): 3D simulation of the implant surgery procedure

(g): The resin surgical template fabricated through rapid prototyping technology, with several stainless steel tubes to be assembled

Figure 4. The CAD/CAM procedure of the surgical template.

Table 1. General information about the patient

Gender	Age	Indication	Prosthodontic rehabilitation method to adopt
Male	60	partially edentulous in the right posterior region of the maxilla	4 SCREW-LINE Promote® CAMLOG implants with diameter of 5 mm and length of 13 mm in tooth positions 2,3,4 and 5

3. Clinical Applications

The advantages of this sort of surgical templates are demonstrated through clinical applications. In the following context, a clinical case is reported in detail.

The general information about a severely resorbed edentulous patient is listed in Table 1.After the CT data of the patient were imported to CAPPOIS, the positions and orientations of the virtual implants were interactively designed to make optimal use of the bone volumes while protecting the critical anatomical structures including the maxillary sinus, the nasal cavity, the adjacent tooth roots, etc. (shown in Figure 5), and then, this plan and the laser-scanned data of the patient's plaster cast were transferred to MedRegCAD for image registration and template design. Finally, the bone-tooth-combined-supported surgical guide, as well as the stereolithographic model of the patient's maxilla, was manufactured via rapid prototyping technology (shown in Figure 6(a),(b)).

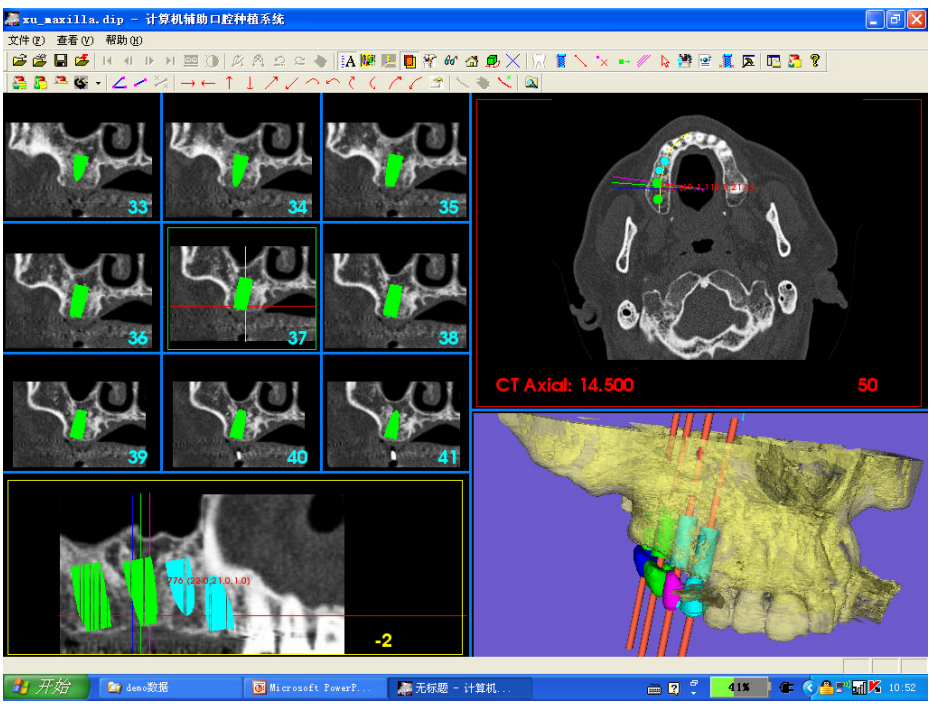
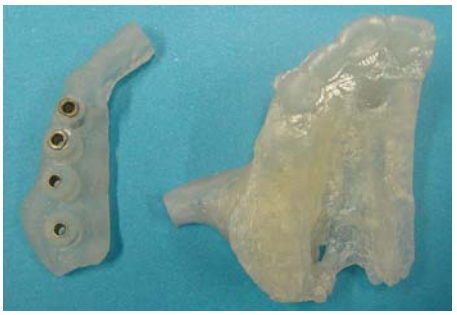
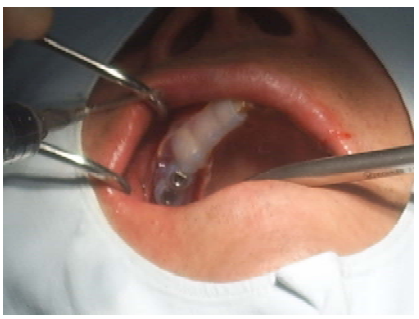


Figure 5. Preoperative surgical planning with the use of CAPPOIS.

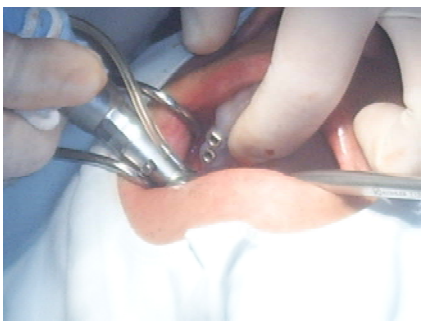


(a): The stereolithographic surgical template and maxillary phantom

(b): Matching of the surgical template with the maxillary phantom



(c): The template rested on the alveolar bone as well as the adjacent teeth



(d): The application of the template during the surgery

Figure 6. The application of the surgical template.

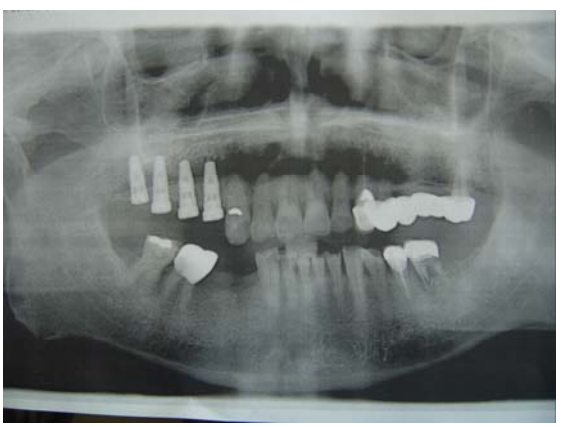


Figure 7. The post-operative panoramic radiographic image.

On the day of the surgery, a mucoperiosteal flap was carried out after anesthesia, and then the template was stably placed on the alveolar bone as well as the adjacent teeth, allowing the stainless steel tube to help guide the osteotomy procedure (shown in Figure 6(c), (d)). The post-operative panoramic radiographic image (shown in Figure7) demonstrated that all of the 4 implants were in ideal position and orientation, fulfilling anatomical and esthetic requirements.

Table 2. Resulting deviations

Implant No.	Distance deviation (mm) at the coronal point	Distance deviation (mm) at the apical point	Angle (°)
Patient 1			
1	1.1	1.2	2.6
2	0.6	1.0	2.1
3	0.4	0.6	1.9
4	0.3	0.7	1.2
Patient 2			
1	1.2	1.0	2.8
2	0.8	1.1	1.8
3	0.6	0.9	1.7
4	0.3	0.4	0.6
Mean	0.66	0.86	1.84
SD	0.34	0.27	0.71

Note: Implant No.1:The second molar area No.2:The first molar area
No.3:The second premolar area No.4:The first premolar area

After the placement of the implants, the patient was CT scanned, and then the postoperative images were aligned with the initial planning ones through an automatic image registration method using maximization of mutual information so that the overall accuracy could be calculated. Totally two cases with similar edentulous areas have been accomplished .Resulting deviations between the planned and the actual implants are shown in detail in Table 2. It reveals that the average distance deviations at the coronal and apical point of the implant were 0.66 mm (range: 0.3 to 1.2) and 0.86 mm (range: 0.4 to 1.2), and the average angle deviation was 1.84° (range 0.6°to 2.8°).

With respect to the conventional bone-supported surgical guides, there are a few papers published on the accuracy of the transfer to the surgical field. Sarment DP et al. performed cone beam CT scanning of epoxy edentulous mandibles, and then used stereolithographic guides to perform osteotomies. They reported average deviations of 0.9 mm at the entrance and 1.0 mm at the apex between planned and actual locations[31]. Di Giacomo GA et al. used six surgical guides for four patients with 21 implants inserted, and they reported average deviations of 1.45±1.42 mm and 2.99±1.77 mm[33]. Van Assche N et al. performed osteotomies for four cadavers and placed totally 12 implants with stereolithographic guides based on cone beam CT imaging and Procera® (Nobel Biocare AB, Go¨teborg, Sweden) software. They reported that placed implants (length: 10–15 mm) showed an average angular deviation of 2°(SD: 0.8, range: 0.7–4.0°) as compared with the planning, while the mean linear deviation was 1.1mm (SD: 0.7 mm, range 0.3–2.3 mm) at the entrance and 2.0mm (SD:0.7 mm, range 0.7–2.4 mm) at the apex[34]. Comparing the results presented above with the results achieved in this study, we concluded that the precision of the surgery was improved with the use of the novel bone-tooth-combined-supported surgical template. However, since only two cases have been accomplished currently, this is a pilot study and more clinical cases will be conducted to confirm the conclusions.

4. DISCUSSION AND CONCLUSION

As a prerequisite for CT-derived surgical templates, preoperative planning software is explicitly discussed in this study, including its various functions and image processing algorithms. In order to fulfill the increasing requirements of oral implantology, a plug-in evolutive software architecture is introduced. By separating the preoperative planning software into several DLL divisions, we enhance modularity and flexibility in our system.

To provide a link between the preoperative plan and the actual surgery, bone, mucosa, or tooth supported templates are commercially available, for example, SurgiGuide®, NobelGuide®, etc. Since the precision of the implant placement depends largely on the ability to position the drill guide accurately on the underlying tissue, it is crucial to ensure the unique match between the templates and receptor sites. For the conventional templates, because they are derived only from CT imaging, which was not as accurate as laser scanning (the resolution in x, y, z axis of laser scanning usually reaches 0.05mm, however the relatively poor z-axis resolution of CT imaging is often lower than 0.5mm), the match between the templates and the underlying bone ridge contour is not so perfect, and it also poses a significant challenge for prosthodontists to locate it at the optimal match position during the surgery. In addition, since an extensive flap needs to be raised in the edentulous situations, the templates are not so stable due to interference from the reflected flap.

In comparison, with regard to bone-tooth-combined-supported templates, the fixation are more stable because laser scanning technology enables detailed dentition information, which brings about the unique topography between the match surface of the templates and the adjacent teeth. Furthermore, an extended flap is not needed for the exposure of the surgical site since the adjacent teeth play a significant role to support the template. This approach takes advantage of both laser scanning and CT imaging, which are complementary: the former is more accurate, however can only reflect the outer surface information of an object; the latter, vice versa. With detailed dentition information obtained from registration, the fixation of this kind of template is unique, stable and reliable so that the accuracy of implant placement can be guaranteed.

Based on this principle, the same kind of mucosa-tooth-combined-supported template can also be fabricated. The advantage of mucosa-tooth-combined-supported template is that it allows flapless or minimally invasive surgery (MIS) with no incisions, no sutures and very little bleeding. Currently, the trend of MIS becomes a mainstream in oral implantology due to optimal esthetics with absence of scars produced by incisions and respect of papilla integrity [3-20]. From this perspective, the future of a combined-supported template is promising, and more clinical cases will be conducted to demonstrate its feasibility and reliability.

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Chapter 24

CONSIDERATIONS OF GRAFT MATERIALS IN MAXILLARY SINUS AUGMENTATION PROCEDURES

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ABSTRACT

After loss of teeth in the posterior maxilla, the alveolar ridge decreases by bone atrophy and pneumatization of the maxillary sinus cavity. Maxillary sinus augmentation is a well-established technique for functional rehabilitation of partially or completely edentulous patients with severe maxillary atrophy and the goal of sinus augmentation procedures is to create bone quantity and quality in order to ensure the placement of dental implants of sufficient length and satisfying initial stability. Various bone grafting materials have been used in sinus augmentation including autogenous graft, freeze-dried bone allograft, xenograft, and alloplastic material. Attempts have been made to increase the bone formation using growth factors, such as bone morphogenetic proteins (BMPs). Platelet-rich plasma (PRP) has been used in sinus augmentation procedures but conflicting results are being reported. Tissue engineering procedure using autogeous mesenchymal stem cells combined with scaffold has been applied in maxillary sinus augmentation procedure.

In this review, the effect and efficacy of biomaterials including growth factors and mesenchymal stem cells in sinus augmentation procedures will be addressed.

Keywords: sinus augmentation, maxillary sinus, bone, platelet-rich plasma, growth factor, mesenchymal stem cell.

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INTRODUCTION

After loss of teeth in the posterior maxilla, the alveolar ridge decreases by bone atrophy and pneumatization of the maxillary sinus cavity [1], and implant placement in the posterior maxilla frequently becomes a challenge because of the limited amount of residual bone height and the supposedly poor quality of bone [2].

Maxillary sinus augmentation is a well-established technique for functional rehabilitation of partially or completely edentulous patients with severe maxillary atrophy and the goal of sinus augmentation procedures is to create bone quantity and quality in order to ensure the placement of dental implants of sufficient length and satisfying initial stability [3]. Various bone grafting materials have been used in sinus augmentation including autogenous graft [4], freeze-dried bone allograft [5], xenograft [6], and alloplastic material [7].

The aim of this review is to describe the effect and efficacy of biomaterials including growth factors and mesenchymal stem cells in sinus augmentation procedures.

Considerations

There is threshold of osseous deficiency, vertical, horizontal, or both, at a site where a sinus augmentation is required for successful implant treatment regardless of residual bone quality [8]. The sinus augmentation should be strongly recommended to obtain adequate support for the placement of dental implants if there is vertical bone less than the certain criteria in the posterior maxilla [8].

An ideal bone graft material should have four characteristics: osteogeneisis, the formation of new bone by osteoblastic cells within the graft material; osteoinduction, the ability to induce differentiation of pluripotential stem cells from surrounding tissue to an osteoblastic phenotype; osteoconduction, the ability to support the growth of bone over its surface; and osteointegraftion, the ability to chemically bond to the surface of bone without an intervening layer of fibrous tissue [9].

It is reported that implant survival rates for sinus augmentation using lateral window technique showed similar survival rates as non-grafted posterior maxilla [10]. Similar outcomes were achieved in simultaneous and delayed procedures [11]. Implants placed in sinuses augmented with particulate grafts showed a higher survival rate than those placed in sinuses augmented with block grafts [10]. When implants are placed in grafted maxillary sinuses, the performance of textured or rough implants achieved better outcomes compared with machined surfaces [12]. Higher implant survival rates were seen when a membrane was placed over the lateral window [10].

Autogenous Graft

Autogenous bone has been considered to be the gold standard for reconstruction of the maxillofacial region, because of its osteoinductive and osteoconductive properties, the presence of growth factors and the lack of immunological responses [13]. Autogenous bone grafts obtained from the patient are one of the most widely used bone graft and these can be procured either intraorally or extraorally [2]. Autogenous bone graft showed high implant survival rates in sinus augmentation procedures [14].

However, there is some limitation in using autogenous bone. Harvesting bone from extra-oral sites has some major side-effects and is often performed under general anesthesia [15].

Main areas for harvesting autogenous bone grafts from intraoral regions are mandibular ramus and symphysis, extraction sites, and retromolar areas [16]. The intraoral sites provides a relatively small quantity of bone only suitable for the partially edentulous patient needing a unilateral sinus augmentation procedure [15]. In clinical cases with severe maxillary bone atrophy where a large amount of grafting material is required, other source of graft material is needed [17]. Devitalization of anterior mandibular teeth by involvement of tooth apices, damage to the mental or lower dental nerves, changes in the facial esthetics of the patient, and increased risk of mandibular ramus fracture also have to be taken in to considerations [2]. The incidences of grafted bone resorption have been problematic and the complete resorption of autogenous bone was observed in clinical cases [18].

Allograft

Allografts consist of bone tissue from a donor of the same species and they contain no viable cells [19]. They have been found to possess bone-stimulating proteins and possess osteoinductive properties, but these may not be recognized unless the grafts are utilized in either demineralized or morsellized forms [9]. Examples of allografts are fresh-frozen bone, freeze-dried bone and demineralized free-dried bone [19].

The use of homogeneous demineralized freeze-dried bone (DFDB) in one-stage sinus augmentation procedures results in a mechanical loading capacity of implants comparable to that achieved by autogenous cancellous bone from the iliac crest [20]. However, a chronic inflammatory reaction was seen in histolgical appearance when DFDB was used in sinus augmentation [21]. And it was suggested that DFDB homografts and heterografts cannot be recommended alone instead of cancellous autografts for augmenting the maxillary sinus in single-stage sinus augmentations [21].

Xenograft

Xenografts consist of bone mineral from animals or bone-like minerals (calcium carbonate) derived from corals or algae [19]. Deproteinized bovine bone (DBB) is the most researched grafting material and is widely used in maxillofacial region because of its similarity to human bone and proteins in DBB have been extracted to avoid immunologic rejection after implantation [19].

DBB graft promotes formation of new bone in a similar fashion to autogenous bone and DBB grafts have shown the very predictable results in the course of sinus augmentation procedures [22]. The use of DBB in combination with autogeous bone offers many additional advantages such as increase in the volume of the graft and longer space-maintaining effects due to prolonged resorption [2].

Alloplastic materials

Alloplastic materials represent a large group of chemically diverse synthetic calcium-based biomaterials, including calcium phosphate, calcium sulfate, bioactive glasses, and polymers [19], and these bone substitutes possess osteoconductive properties [9].

Beta tricalcium phosphate (β-TCP) is one of the earliest calcium phosphate compounds to be used as a bone graft substitute and structurally porous β-TCP has a compressive strength and tensile strength similar to cancelous bone [9]. β-TCP may show delayed resorption and

maintain the structural integrity [8]. Injectable β-TCP was developed and applied in sinus augmentation procedures with mesenchymal stem cells (MSCs) [8].

Calcium sulfate is thought to act as an osteoconductive matrix for the ingrowth of blood vessels and associated fibrogenic and osteogenic cells [9]. Successful osseointegration of implants were achieved with calcium sulfate [23].

Platelet-Rich Plasma (PRP)

Platelet-rich plasma (PRP) is platelet concentrate derived from blood, and platelet gel allows access to autogenous growth factors, which are capable of accelerating the normal process of bone regeneration, improving soft tissue healing and reducing postoperative mobidity [3, 24].

PRP is reported to be an effective regeneration adjunct when combined with autogenous bone in the reconstruction of mandibular defects [25]. The use of PRP for sinus augmentation was proposed as a way to reduce the time required for graft consolidation and maturation, and to improve trabecular bone density [26]. Sinus augmentation performed a composite graft of cortical autogenous bone, bovine bone and PRP showed that the graft mixture can be successfully used for sinus augmentation with greater bone maturation [2]. The adhesive capacity of PRP via its hemostatic capacity of fibrin rendered the additional advantage of easier handling of the graft material [2].

However, no significant differences in the production of vital bone or in the amount of implant-bone interface contact were seen between sinuses filled with PRP and those filled only with bovine bone [27]. Topical use of PRP also did not improve maxillary bone volume either clinically or statistically when compared to conventional treatments [28]. The factors that may contribute to the conflicting results are the variability in study designs, differing platelet yields, and differing methods of quantifying bone regeneration and wound healing [3]. Therefore, the use of PRP may not be recommended as a standard method to support bone regeneration for maxillary sinus augmentation [28].

Growth Factor, Bone Morphogenetic Protein (BMP)

Growth factors are present at low concentrations in bone matrix and plasma, but they execute important biologic functions [19]. The growth factors are believed to have an osseous regenerative effect on the MSCs and contribute to cellular proliferation, matrix formation, collagen synthesis and osteoid production [8].

Bone morphogenetic proteins are a group of growth factors which can induce a local immediate action, interact with extracellular matrix proteins and subsequently target cells [19]. BMPs have shown osteoinductive properties [29], and are able to induce MSCs to differentiate into osteoblasts and to produce new bone tissue [30]. Three BMPs, BMP-2, BMP-7 and BMP-14 have been applied in sinus augmentation procedures. Purified type I bone collagen has been used as a delivery material in a variety of animal studies and application of rhBMP-2/collagen sponge showed similar results to autogenous bone [31]. DBBM has excellent osteoconductive properties and it has been used for sinus augmentation clinically with rhBMP-7 [31]. In addition, β-TCP has been shown to be suitable as a biodegradable, highly biocompatible and osteoconductive carrier for BMPs in sinus augmentation procedures and it has been applied both experimentally and clinically [32].

Mesenchymal Stem Cell (MSC)

Various osteoconductive material have been used to augment the sinus floor, but these materials are cell-free and require more time for bone healing [26]. Therefore, researchers are very interested in a tissue-engineering procedure that uses autogenous MSCs combined with an osteoconductive scaffold as a live bone substitute [26]. MSCs are clonogenic, fibroblastic in shape, and can differentiate along multiple lineages such as osteoblasts, chondrocytes, adipocytes, and hematopoiesis-surpportive stroma [33].

Cells can be achieved from the maxillary tuberosity and they can be cultivated in vivo for certain periods, seeded on to the scaffold and then transplanted into the maxillary sinuses [22]. MSC can also be achieved from the periosteum of the mandible [13]. The osteogenic potential of periosteal mesenchymal cambial layer precursor cells were explored experimentally [13]. Cells of mandibular periosteum were cultured and then transferred onto the matrix and this *ex vivo* tissue-engineered combination was shown to be of value in sinus augmentation procedures [22]. Samples containing MSCs showed significantly higher differentiation of osteoblastic cells and bone formation than cell-free samples or fresh marrow [34].

CONCLUSION

Sinus augmentation procedures have been used clinically with very high success rate. Bone-substitute materials are reported to be as effective as autogenous bone when used alone or in combination with autogenous bone. It may be concluded that bone substitutes can be successfully used for sinus augmentation, reducing donor-site morbidity. Attempts have been made to accelerate bone formation with different scaffolds, growth factors and mesenchymal stem cells. Further studies are needed to find an optimal approach that can enhance bone formation in sinus augmentation procedures.

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Chapter 25

OVERDENTURE CONSTRUCTION OF IMPLANTS DIRECTIONALLY PLACED USING CT SCANNING TECHNIQUES

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ABSTRACT

CT scanning software is fast becoming a viable tool in the diagnosing of dental implant position and placement. Minimally invasive procedures may be requested by patients to reduce their anxiety and increase treatment acceptance rates. In areas where contours and width and height of bone are difficult to determine with conventional radiographic techniques, the CT scanning software allows diagnostic determination if bone quantity and quality exists and can be used to virtually place dental implants using the computer program prior to any surgical intervention. This is an outstanding tool in discussing the risks involved in surgical implant procedures and can help visualize the finished case before ever starting. Used in critical anatomic situations and for placing the implant in an ideal position in bone, CT scanning software eliminates possible manual placement errors and matches planning to prosthetic requirements. This innovative tool makes surgical placement of implants less invasive and more predictable. Prosthetic reconstruction is thus made simpler since the implants are appropriately positioned to allow for fabrication of the final prosthesis.

Keywords: Easyguide, Straumann Implant, Bredent attachment

Fabrication of a stable, comfortable maxillary removable complete denture using dental implants as the support mechanism begins with careful diagnosis and case planning. Simple two dimensional images created using conventional radiographic techniques may no longer

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be an adequate and predictable technique for proper implant placement. The surgeon's experience and manual placement techniques greatly influences the final functional and esthetic result. Any laboratory technician can tell you that often implants are placed in poor position or angulation making prosthetic fabrication difficult or retention comprosised.

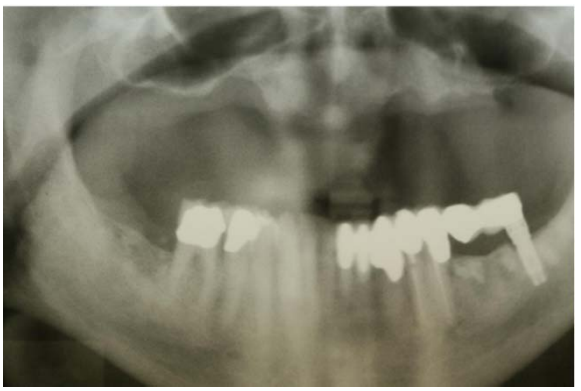


Figure 1.

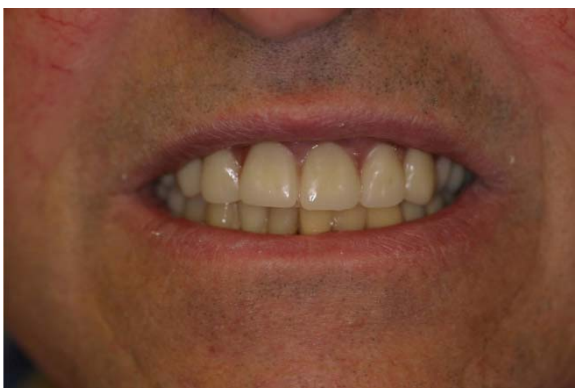


Figure 2.

Dental implants provide an outstanding treatment option demonstrating dramatic improvement in denture stability and increased chewing efficiency. There is an increase in quality of life that is rewarding to the dentist and gratifying for the patient. The use of endosseous implant designs, such as the Straumann dental implant system, has proven to have an outstanding prognosis and are reliable as retainers for overdentures.

This patient is a 68 year old white male who has worn a conventional maxillary complete denture opposing natural and implant retained dentition for many years. There are no medical contraindications to dental implant therapy. Following maxillary tooth removal several maxillary complete dentures were fabricated over the years which were not completely acceptable to the patient both functionally and esthetically. Form and function diminished over the years and the patient was anxious for a stable maxillary dentition.

Several options were discussed with the patient including fabrication of another new conventional case. The posterior vertical bone was minimal due to the large maxillary sinuses. The amount of anterior pre-maxillary bone was difficult to determine by radiographic

interpretation alone. The patient's main concern was that the existing maxillary case was not stable and his ability to chew and function had diminished. His quality of life had been compromised by the loss of his upper teeth. Discussion of the use of CT technology to determine the exact amount of bone available and the use of CT scanning software to determine precise position of potential implants helped motivate the patient to consider dental implant reconstruction.

There was significant facial resorption in the maxilla, so it was determined that an implant retained maxillary overdenture with proper lip support would best serve the patient. Struamann Dental Implants (Struamann Corp,, Boston) were chosen due to their long term successes. This system improves the dentist's and patient's access to superior and more effective treatment. Reliability and innovation are two strong qualities of the Straumann surgical and prosthetic techniques that made this the implant of choice in this situation. The implant provides an 8 degree Morse taper internal connection. The flared neck implant design used here is ideal for soft tissue contouring.

There are often concerns with any surgical procedures, especially in the sinus area or in bone where nerves are located. These concerns have popularized a newer concept in implant dentistry. We are now able to utilize our CAD/CAM computer software to visualize the patient's entire mouth anatomy in three dimensions, which takes all of ten minutes. The computer software allows us to simulate the placement of implants accurately before ever touching the patient. A surgical guide, created from the three dimensional images, helps us place the dental implants in the proper pre-determined positions, often without ever making a flap incision. This technique is proving to be a cost effective solution to assist the implant dentist in planning an esthetic and functional final result and minimizing any surgical challenges they may face.

The CAD/CAM technology is based on planning algorithms used clinically for more than 11 years. CT scans and 3D planning software can really improve our predictability and safety. The CAD/CAM techniques can be used for single tooth edentulous spaces, single tooth immediate extraction cases, partially edentulous spaces, fully edentulous maxillary and mandibular overdenture cases or fully edentulous maxillary or mandibular full arch permanent restorations. The surgical cases are, therefore, driven by the final esthetic and functional result. It is important to listen to your patients carefully to determine their goals and desires and design the implant reconstruction accordingly. It is critical today to make sure that the final tooth reconstruction is established before any surgical intervention. Placing the dental implants in the jaw before understanding tooth/implant position and the final result is a big mistake. [1,3,4]

The CAD/CAM planning and placement system provides a high level of comfort and safety for the patient by reducing surgical and restorative time. This is done by utilizing an accurate three dimensional plan prior to implant placement. There are obvious advantages including; easy visual understanding for clear case presentations, reduced surgical chair time, reduced restorative chair time in certain cases because of ideal implant positioning, reduced stress for the clinician and the patient, the avoidance of surprises during surgery, optimal implant placement for long term implant and prosthetic success and , most importantly, an improved esthetic result.

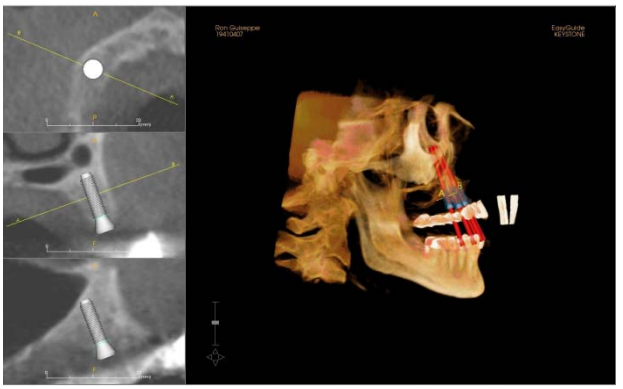
Prior to the CT scan a radiographic guide is fabricated by the dentist, which aids in visualization of the optimal prosthetic outcome. The teeth are positioned properly in wax and then a hard model to illustrate what the case will look like finished before ever starting. All

appropriate dental anatomy is included. The radiographic guide is placed in the mouth during the CT scan. This allows the clinician to see the ideal position of the teeth on a three dimensional model. The entire 3D image is analyzed and the implant planning and simulation of implant placement completed using the computer. The surgical placement of the implants can be done in a conventional manner using the newly created surgical guide to help direct the implants in the ideal position, but surgery can often be completed without making any incisional flap. The implants are placed in the desired depth using the computer software and the surgical guide.

It is imperative that the implants be placed as nearly parallel in all three dimensions, as possible, to the long axis of the bone and to each other. The implants in the right maxilla are parallel to each other as are the implants in the left maxilla. Due to the arch form it may be difficult to parallel all six implants sequentially. A clear surgical stent was fabricated using the information created using the CT scanning software. The guide is used to correctly position the implants in the first molar and cuspid areas to maximize stability of the final implant retained prosthesis No retraction of the soft tissue was needed, since the CT indicated in three dimensions the length and width and position of the implants to be used..

Figures 1 illustrates the loss of posterior maxillary bone and irregular shaped pattern of the pre maxillary bone making implant placement difficult to determine. Without CT diagnosis it may be speculated that adequate bone height and width exists, but this could not be exactly determined until a complete periodontal flap is made and surgical placement would be dictated by the experience of the implant dentist. Figure 2 illustrates the existing conventional maxillary complete denture with a closed vertical dimension of occlusion resulting in improper lip support and a poor frontal and lateral esthetic profile. The patients decrease in function may be due to the poor plane of occlusion and tooth position. Gagging was caused by the basic design of the conventional denture and poor fit. Quality of life was dramatically reduced. Figures 3 and 4 show how the CT scanning software is used to virtually place six anterior implants , their angulation and how the implants will be restored. In properly virtually placing the implants it was noted that the right and left arches would not allow parallelism of all six implants, so the three right side implants would be splinted together and the left side implants would be splinted separately. To achieve the best functional and esthetic result, as well as increase stability of any prosthesis, it was decided to parallel the implants as possible and place them in the pre-maxillary area and as posterior as the bone would allow. This is all determined virtually prior to any surgical intervention, a tremendous advantage to the less experienced implant surgeon.

Prior to the preparation of the osteotomies, the surgical directional guide is created with guiding openings in the stent. (Figure 5)This allows the pilot drill to penetrate the stent, through the soft tissue into the bone to a predetermined depth. Once the pilot drill makes an mark into the soft tissue and bone, a flap was made to visualize the precise contours of bone. The remaining osteotomies into the bone are made for the six implants chosen. The drills are used at approximately 800 RPM to the hub of the bur. 4.1mm X 10mm Straumann implants are threaded to place following the contours of the arch. (Figures 6,7) A radiograph is made of the implants in position (Figure 8) Remember depth into bone has been determined by the CT scanning softwsare. All angles are also predetermined with the virtual placement of the implants.



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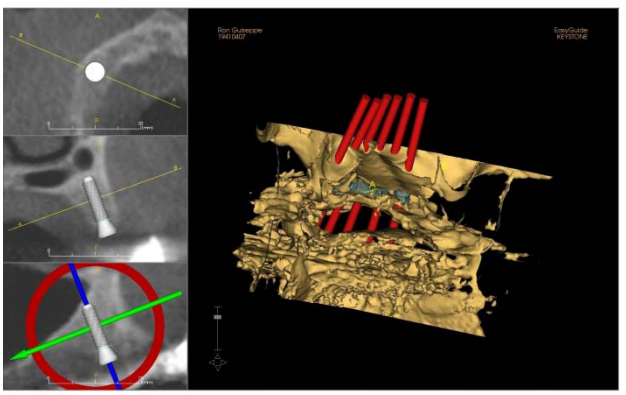

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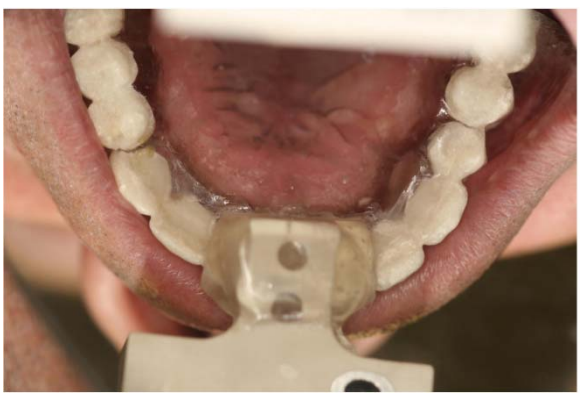

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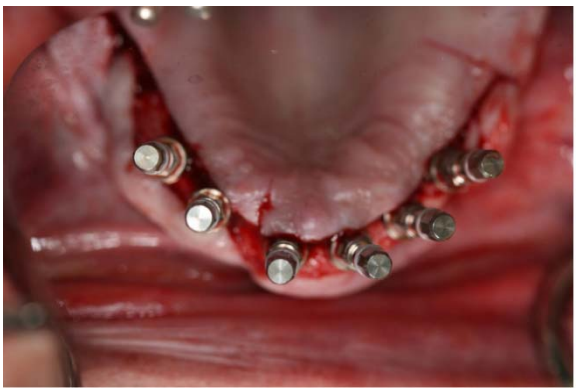

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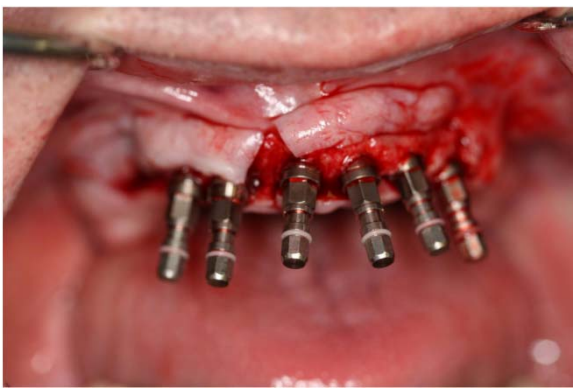

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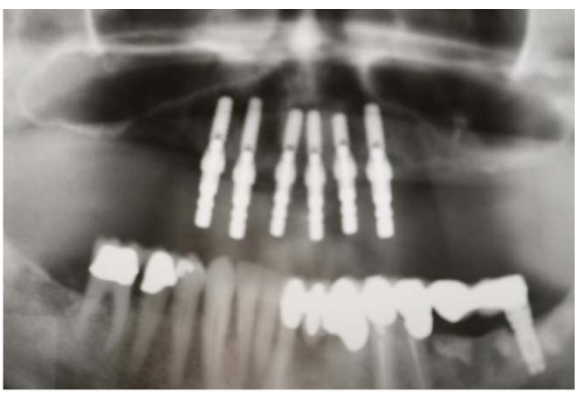

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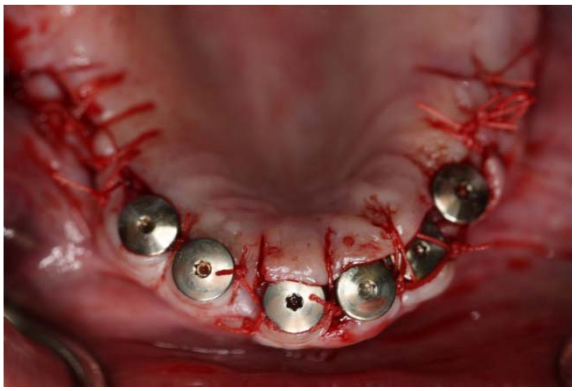

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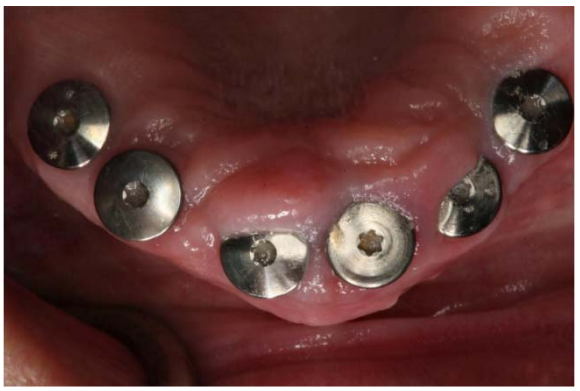
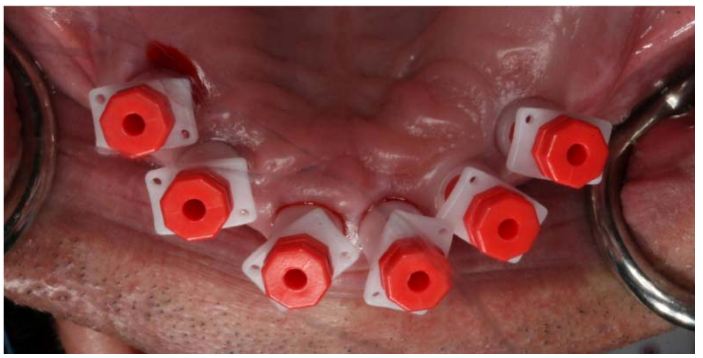


Figure 10.

The shoulder of the implants are left slightly coronal to the crestal bone to allow for easy access. 1.5mm tall closure screws were placed into each implant. (Figure 9) and the flap sutured closed using Vicryl sutures. Following three months of integration the tissue around the closure screws are pink and firm. (Figure 10) A closed custom tray impression of the implants was made using the Straumann impression cap and synOcta positioning cylinders.(Figure 11) A master impression was sent to the lab to have the proper analogs placed into the impression and placed into the impression for a master model pour up. The master cast is poured duplicating all peripheral borders. A bite registration was used to position the casts. Teeth are positioned to esthetics and function.

The Bredent (XPdent Corp.) attachments are intended to act as retentive devices for this overdenture case. Because of their design and incorporation into two screw retained bars the patient is able to easily align and seat the overdentures easily. Although not critical in bar fabrication, it is better that the implants be placed in a nearly parallel position to each other, to simplify the prosthetic construction. According to the manufacturer, the attachments are the most flexible and simplest attachment system on the market. There are three levels of retention to meet the needs of any individual and eventual changing of the attachments following wear is easily accomplished. [2]



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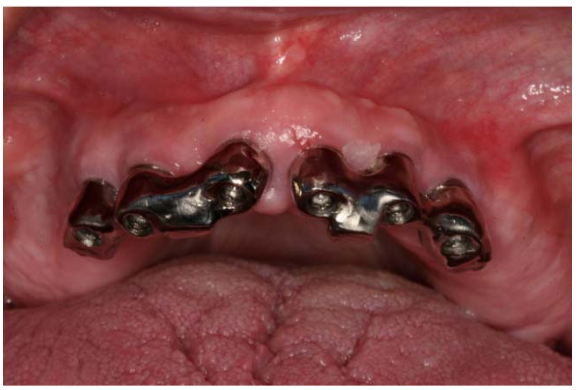

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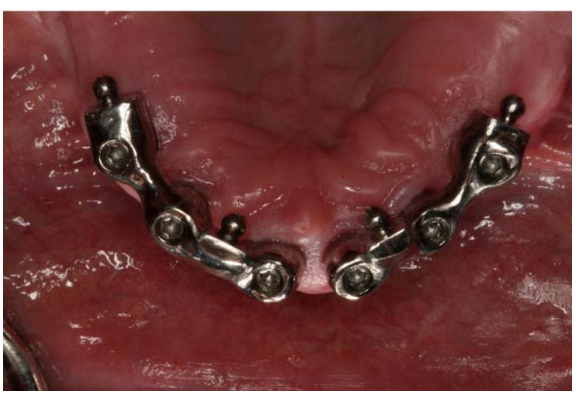


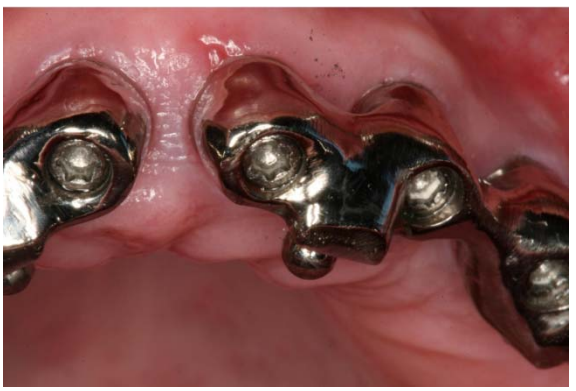
Figure 13.

When selecting an appropriate attachment for the overdenture, it is important to consider the amount of interocclusal space available. Retention requirements, ease of use and lifespan of attachment are also considered. One of the main benefits of the Bredent attachment is their reliability of retention and ease of use. The retentive mechanism is based on plastic female components that sit in a metal housing. The attachments come in three retentions, green is the

least retentive, yellow next and red the most retentive. Since four attachments were used in this case, yellow was chosen as retentive enough.

Conventional denture techniques are used to create the final esthetic contours. We create an outstanding functional and esthetic result, meet the patient's expectations and totally eliminate the gagging reflex caused by the old full palate conventional complete denture.

Figures 12 and 13 illustrate the facial and coronal position of the two screw retained bars, separated at the midline. Figure 14 is a close up of the male portion of the Bredent attachment. Figures 15 and 16 show the yellow attachment in metal housing in the palateless (horseshoe shaped) maxillary prosthesis. The shape of the denture completely eliminates any gagging reflex and tremendously imporoves taste sensation. A final panoramic radiograph (figure 16) shows the bars in place. Fortunately there was plenty of room for a bar design. If interocclusal clearance was a problem, a different attachment, such as the Locator type, would have been considered. Connecting the maxillary implants with bars improves the support and stablility of the implants placed in the relatively soft maxillary bone. It is this author's opinion that splinting the implants in the maxillary improves the long term prognosis of the implants themselves. Figure 17 is the final radiograph with the two bars in place. Note the smooth transition from metal framework to implant body.



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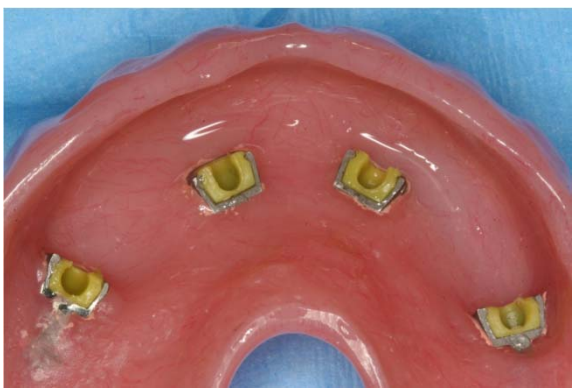

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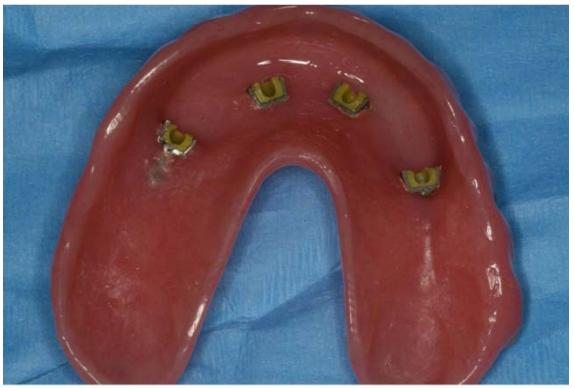

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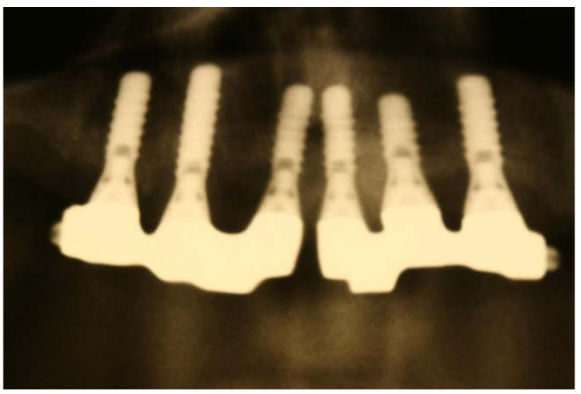

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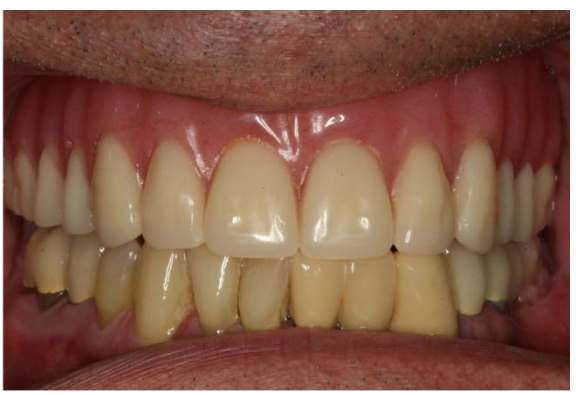


Figure 18.

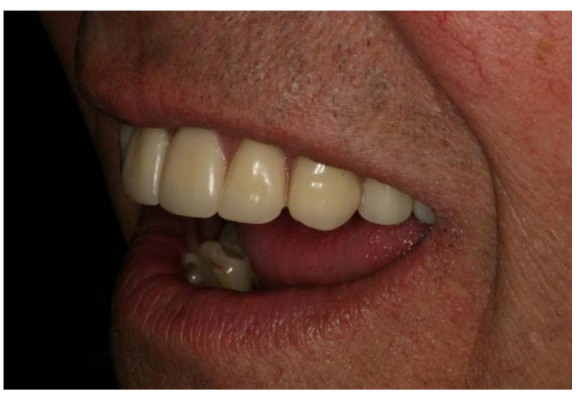
This type of prosthesis allows for excellent retention and stability for this patient. The Bredent attachments are positioned extracoronally on two bars separated at the midline. This allows for good palatal tissue adaptation and easy maintenance with a simple tooth brush or end-tuft brush. Follow up care includes clinical assessment for abutment stability, mobility of the implants and plaque accumulation. Since the perimucosal seal is vital to protect the underlying connective tissue and borne from migrating forces, probing a healthy implant is

not advised. Radiographs are taken yearly to determine bone position and contour. Metal scalers and ultrasonic instruments may scar and pit the titanium abutment surface, therefore, plastic, gold or graphite scalers should be used as necessary.

This patient exhibited a positive end result because of his understanding of the limiting factors involved in this case and the final prosthesis. He is, however, able to chew more efficiently and speak clearly without worry of the prosthesis loosening or any of the abutments decaying. Quality of life was dramatically improved using an implant retained overdenture. .Figures 18, 19,20 and 21 illustrate the final esthetic results.

The general dentist has an obligation to provide his/her patients with the most innovative, proven techniques available. CT scans and scanning software like the Keystone Easyguide program makes surgical placement of the dental implants rather routine. Anatomical anomalies are virtually determined prior to ever touching the patient. With better implant placement comes more routine and predictable prosthetic reconstruction. Since the GP is the professional the patient consults concerning their dental condition, he/she must educate himself with the treatment modalities. Many surgical therapies can be performed by the trained general dentist and certainly all general dentists should be able to restore these cases simply and easily. The predictable results only reinforces the modality. Maintenance is rather routine with a design of the bars that allows easy access with a proxy brush. As with any other dental appliance, professional evaluations and periodic radiographs are mandated.

Figure 19.



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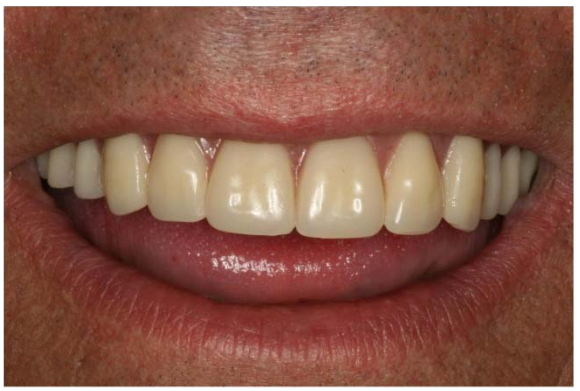

Figure 21.

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Chapter 26

ONLAY BLOCK BONE GRAFTING IN ALVEOLAR DISTRACTION OSTEOGENESIS TO INCREASE CALLUS VOLUME

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ABSTRACT

The functional and esthetical rehabilitation of the maxilla with dental implants when there is a severe alveolar bone deficiency can be achieved with the help of distraction osteogenesis. Although this method offers certain advantages over conventional augmentation methods, it sometimes requires secondary bone grafting because the bone of the transport segment may be too thin and semilunar excavations may form in the distraction zone. In this situation, the simultaneous application of distraction osteogenesis with a polytetrafluoroethylene membrane and autogenous onlay block bone grafting is an alternative solution to secondary bone grafting. In addition, this technique prevents semilunar excavations in the distraction zone and increases callus volume.

INTRODUCTION

Resorption of the anterior maxilla has several causes that range from soft and hard tissue pathologies to more complex injuries, including severe loss of the tissue complex of the nasal and sinus floors. Extensive loss of bone and teeth in the anterior maxilla presents a big problem for prosthetic rehabilitation with dental implants, especially in patients with severe bone loss (1). Implant-retained restorations require hard and soft tissues of adequate quality and quantity, or the final restoration will be compromised (2). Mild defects can be accommodated by a dental prosthesis, but severe bone defects in the anterior maxilla with deficient soft tissue and atrophic bone hinder prosthetic rehabilitation with dental implants (3). In this situation, onlay bone grafting, guided bone regeneration (GBR), or a split

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osteotomy are frequently used for alveolar bone augmentation. Since McCarthy *et al*. introduced the use of distraction osteogenesis to lengthen the human mandible in 1992, there have been many clinical studies on the vertical augmentation of alveolar bone deficiencies (4). Some of these studies noted that the distracted region in patients with severe alveolar hypoplasia did not have enough bone tissue bulk on the lateral surface of the distracted alveolar bone (Figure 1a, 1b). Horizontal bone deficiencies still require onlay bone grafting after distraction osteogenesis, and this involves another operation, prolonging the patient's rehabilitation.

This chapter discusses the simultaneous use of distraction osteogenesis and onlay bone grafting to increase the vertical and horizontal volume of the bone and soft tissue in one surgical procedure and the clinical outcomes with this technique.

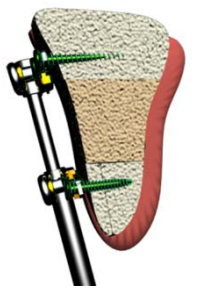

Figure 1a. Illustration of narrow alveolar bone distraction

Figure 1b. In narrow alveolar bone distracted bone still need horizontal bone augmentation for implant placement

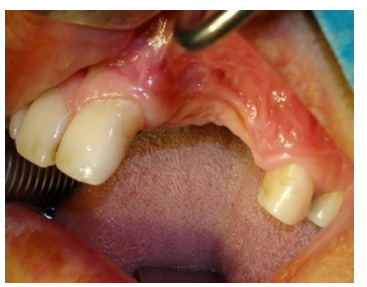

Figure 2a. Intraoral view of the defect site,

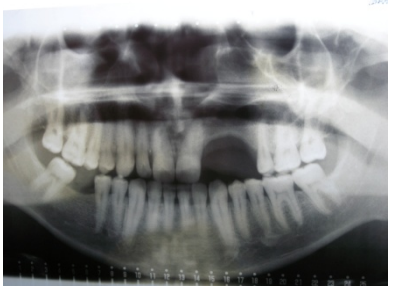

Figure 2b. Panaromic radiography of the patient

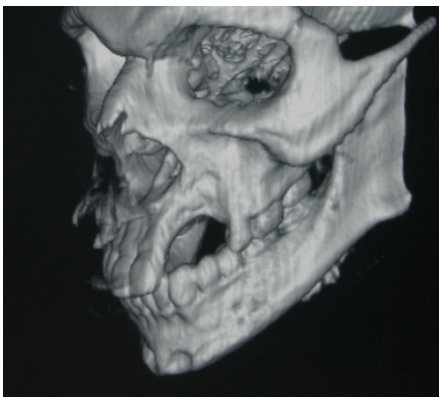

Figure 2c. 3D image of the defect

CLINICAL APLICATION AND METHOD

A 27-year-old male underwent multiple operations because of a recurrent keratocyst in the anterior maxilla. After a 2-year follow-up period without recurrence, the defect site was insufficient for implant rehabilitation in both the horizontal and vertical planes (Figure 2 a,b,c). For prosthetic rehabilitation with implant augmentation of a defect of the anterior maxilla, alveolar bone distraction osteogenesis was planned with simultaneous application of an onlay ramus graft in order to obtain a suitable bone height and width. Under general anesthesia, a flap was made, deep to the vestibular sulcus. The defect region was exposed (Figure 3a), and the bone segment for distraction was separated with one horizontal and two vertical osteotomies made with cutting disks and finished with osteotomes, without jeopardizing the palatinal soft tissue attachment of the osteotomized segment (Figure 3b). A 1×1.5-cm ramus graft was harvested and fixed with a screw at the recipient site (Figure 3c). Before positioning and fixing the membrane, the required distraction length should be determined intraoperatively, and the dimensions of the membrane should be adjusted accordingly. Polytetrafluoroethylene (PTFE) membranes are flexible and can tolerate the movements of distraction. The PTFE membrane was fixed to both segments. It was fixed to the transport segment through the onlay bone graft bicortically using miniplates from the Modulus distractor system (Medartis® AG, Switzerland) (Figure 4). The surgical site was sutured with 4/0 Polyglycan (Figure 5). The patient was instructed to distract the device by 0.5 mm twice daily for 12 days.

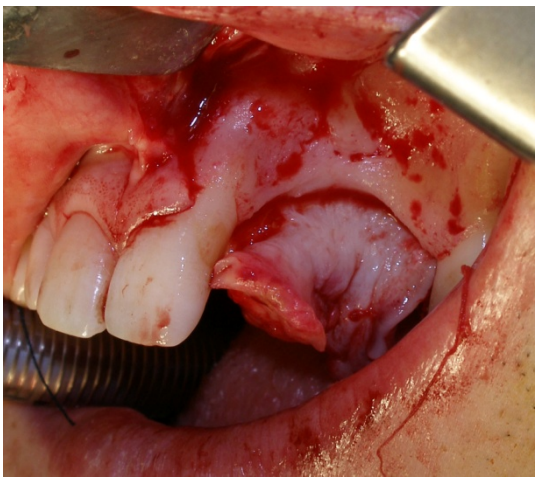

Figure 3a. intraoperative view of the horizontal and vertical insufficiency of the alveolar bone

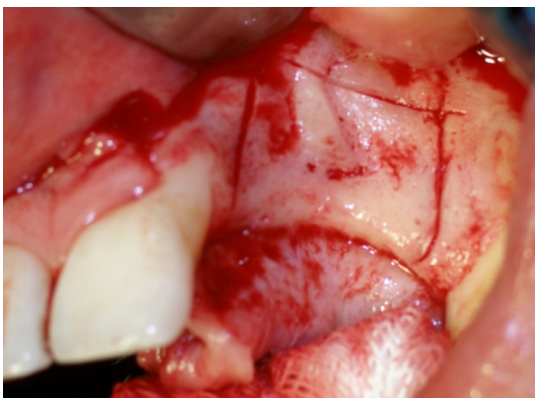

Figure 3b. horizontal and vertical osteotomies of the segment

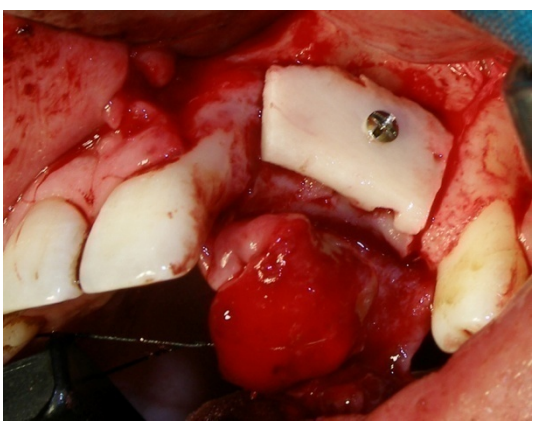

Figure 3c. application and fixation of the ramus bone graft for horizontal immediate augmentation

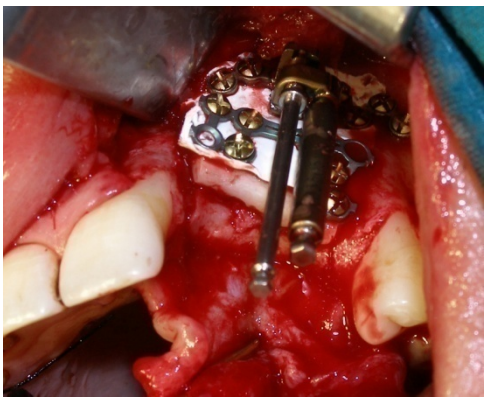

Figure 4. placement of PTFE membrane and its fixation to the transport and achorage bone segments by the distraction miniplates screws

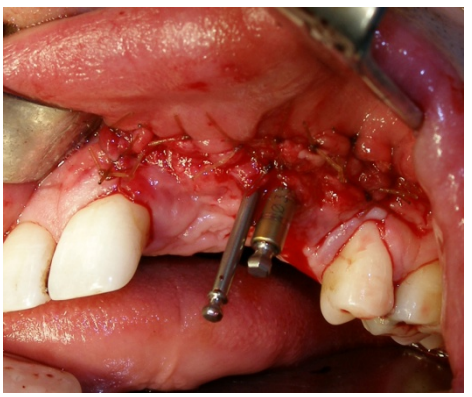

Figure 5. primary closure of the operation area

CLINICAL AUTCOMES

Four months later (Figure6), the distractor was removed after a radiological examination No complications developed, except slight edema and pain in first 2 weeks. No fibrous connective tissue or semilunar excavations were seen in the distraction zone (Figure 7a,b,c d). Callus volume was increased by the onlay bone graft (Figure 8a,b). After distraction, vertically by 10 mm and horizontally by 8 mm new bone was gained. For prosthetic rehabilitation, two dental implants (4.1 × 12 mm Straumann®, ITI, Switzerland) were placed in the distracted area (Figure 9a, b).

The use of alloplastic materials does not offer an ideal bed for rehabilitation with osseointegrated implants (5, 6). The vertical augmentation of alveolar bone with autogenic onlay bone grafts results in unpredictable bone resorption, especially before implant placement (7, 10). In addition, increased morbidity is expected because large amounts of bone must be harvested from intraoral or extraoral sites [7, 8, 10, 11]. The ability of GBR to increase the vertical bone dimension is very limited. In addition, there are risks of membrane exposure, infection, and limited vertical regeneration because of insufficient vascularization (9, 10, 12, 13).

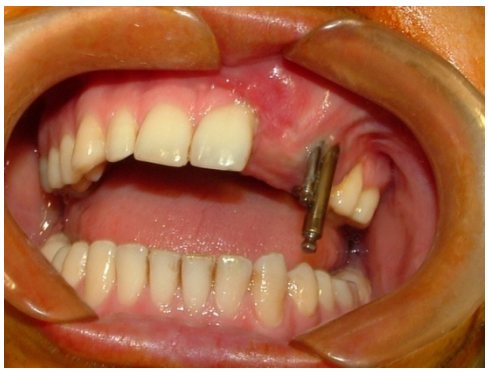

Figure 6. intraoral view before the removal of distractor

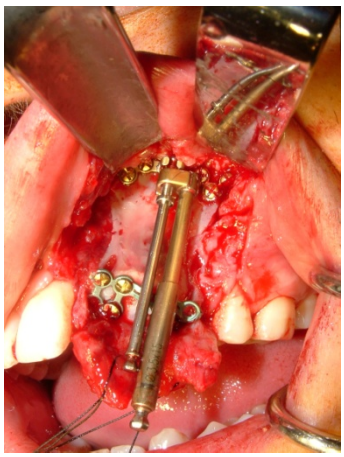

Figure 7a. intraoperative view of the distraction and the flexibility of the membrane with its adaptation to the distraction

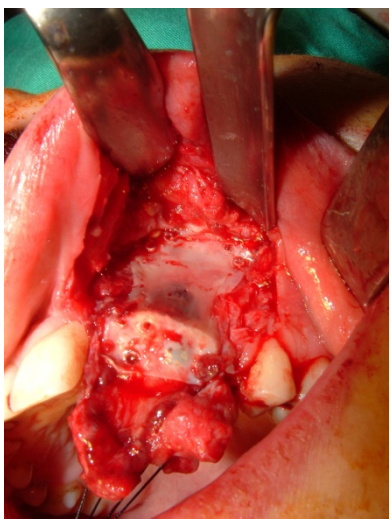

Figure 7b. after the removal of the distractor and see the adaptation of the PTFE membrane on bone

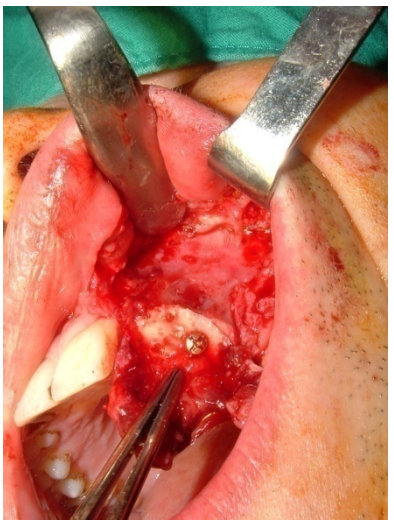

Figure 7c. smoothness of the newly generated bone under PTFE membrane

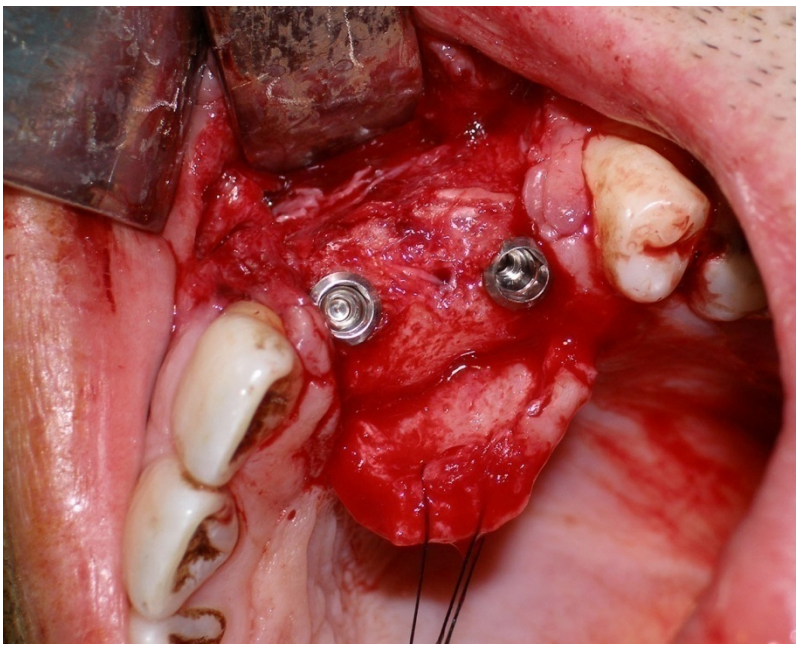

Figure 7d. 8mm thickness new bulky alveolar bone was seen. Sufficient bone bucaly and lingualy after implant placement.

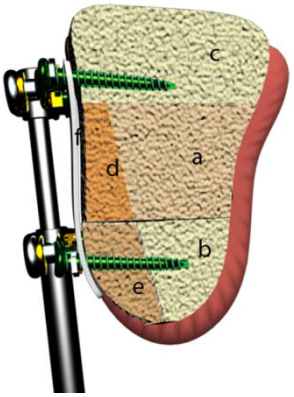

Figure 8a. a) gained bone after distraction, b) transport segment, c) maxillary alveolar bone, d) incrised callus wolume with distraction of onlay bone grafting, e) ramus onlay block bone graft

Figure 8b. After distraction of both transport alveolar bone and onlay bone graft sufficient horizontal and vertical bone volume was created for implant placement

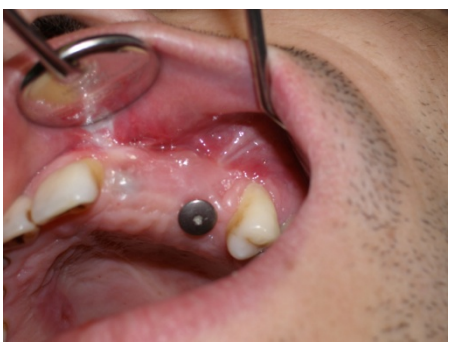

Figure 9a. after osteointegrarion period intraoral view of the dental implants

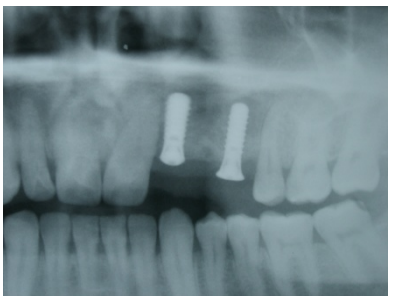

Figure 9b. radiographic view of dental implants

Distraction osteogenesis is an alternative augmentation technique that sufficiently increases the volume of bone and soft tissue for the placement of dental implants (11, 12, 14, 15). In this method GBR, onlay bone grafting, and distraction osteogenesis were combined in one operation in order to obtain the ideal augmentation of the anterior maxilla, while eliminating the disadvantages of each technique.

With distraction osteogenesis, there is a tendency for semilunar excavations to form at the distraction site due to the migration of fibrous tissue into distraction gap. This affects the quality of the bone and hinders implant placement (13, 14, 16, 17). Chiapasco *et al*. reported that one serious problem seen during the removal of the distractor is fibrous connective tissue migration through the distraction gap, causing a semilunar excavation on the buccal side of the regenerated bone (16, 18). These disadvantages were eliminated by using a PTFE membrane to prevent fibrous tissue migration to the distraction site, as reported by Dergin *et al*. (13). PTFE membranes have been used successfully for GBR for many years, although there is some risk of inflammation, exposure, and infection. Block *et al*. reported epithelialization up the rod of the distractor as a problem. The natural characteristic of the mucosal epithelium is to line the open tract in the oral cavity along the rod, which can lead to chronic infection and loss of both the distractor and regenerated bone (15, 16). The membrane serves as a barrier between the rod tract and the newly forming callus (17, 18). Moses *et al*. reported that PTFE membranes have a 41.2% risk of exposure in GBR at the alveolar crest, as compared with collagen membrane (Ossix) and collagen barrier (Bio-Guide) (18). However, the differences among the three groups were not significant, which suggests that premature membrane exposure is related more to a patient's healing capacity and the surgical procedure than to the type of membrane (18).

Block *et al*. reported that bone grafts in the distracted areas were frequently required after distraction for severe defects, because of the lack of horizontal bone formation when the distractors migrated toward the palate. They applied onlay grafts to the distraction site after an 8- to 12-week consolidation period and inserted dental implants after 6 months (15). Cortical ramus graft was used on the buccal side of the transport segment to increase the width of bone callus (fig 8a). It is hypothesized that increasing the volume of the transport segment also increases the volume of callus formed. One disadvantage of this method is resorption of the block bone graft during distraction. Cortical bone source should be selected to prevent bone graft resorption. However, fixing an onlay bone graft to a transport segment that has a rich blood supply from a palatal flap is not different from traditional onlay bone grafting.

Distraction osteogenesis of a membranous bone onlay graft to the alveolar bone may help shorten the treatment period in cases of severe mandibular hypoplasia that require both a bone graft and distraction. However, as in the present case, the extra operation can be eliminated with simultaneous onlay grafting and distraction, which can increase the callus formed between the segments and provide sufficient alveolar bone for implant placement.

The combination of distraction osteogenesis, onlay bone grafting, and membrane application is a promising method for preventing semilunar defects, which occur between the segments in distraction osteogenesis. In addition, applying an onlay bone graft to the transport segment can enhance the distracted callus volume, which may eliminate the need for secondary bone augmentation in severe combined horizontal and vertical bone deficiencies. Furthermore, the membrane can prevent infections via the distraction rod tract that opens in the oral cavity.

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Chapter 27

THE EMERGENCE AND INTEGRATION OF CAD/CAM DENTAL RESTORATIONS

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HISTORY

The CEREC system celebrated its 20th anniversary in 2006. This technology was envisioned and began its development with two people. Drs. Mörmann and Branestini were the ones to treat the first patients with this unique chairside system in 1985. The technology at the time was not what it is today, but the ideas were born and seeded into what was available. They knew that the hardware and software would eventually "catch-up" to their idea. So it has. The idea was that a dental restoration of solid porcelain could be manufactured chairside in one visit with the aid of computer design and milling. This would eliminate the need for impressions and laboratory fabrication. Additionally it would allow the clinician full control over the restoration from beginning to end.

TECHNOLOGY OF HARDWARE AND SOFTWARE CHALLENGED THE "IDEA"

The early years of CEREC offered only the ability to provide inlay restorations. The milling unit at that time used a diamond disc as the instrument to produce the restoration from the block of porcelain. In 1990, enhancements were made to the software to allow the machining of multiple surface inlays and onlays. In 1994 the software was upgraded and it made possible the production of a veneer. In that same time, the CEREC-2 was introduced but the production of crowns was not possible due to the limitations of the hardware. Later, in 1997, the milling unit changed from a disc/bur setup to a two bur style. This allowed the system to produce the restorations we can provide today. Today it is capable of making inlays, onlay, veneers, crowns. There have been other developments along the way that have given a wider field of technology to offer to the dentist. Some of these are the CEREC Scan

and the CEREC Link. These are products that help the practitioner acquire digital impressions and use the software for designing on laptop computers.

The software of CEREC has been bolstered by the boom of software in other industries and has allowed the practitioner to view their design process in three dimensional graphics. In the past, the graphics allowed only two dimensional viewing. This made the design process a little more difficult as the practitioner had to toggle between screens of the different tooth surfaces and develop a picture of the restoration in their mind. Now the process is intuitive and easy to learn.

With the constant advances in computer software and hardware, the original ideas of CEREC have been achieved. With the ongoing development of hardware and software industries this technology has had a phenomenal development. As time goes on the advances in the science of CAD/CAM and dentistry will provide even better, more sophisticated restorative possibilities. To date, there is now the ability to capture images continuously with the CEREC AC system.

The CEREC AC system uses a "Bluecam". The Bluecam captures more detail in less time with better precision than anything available at this time. A full arch digital impression can be made in two minutes and a half arch in as little as 40 seconds. The Bluecam is another step in the ongoing advancement of the technology of CAD/CAM dentistry today. With the success of the original CEREC system as evidenced over its 20 year history, another manufacturer has come to the market. Schein Company has recently been able to produce a similar CAD/CAM system called the E4D. Its hardware and software is similar to that of CEREC's. The difference in software is like comparing an Apple to a Microsoft program. They both produce the same result, but the graphics and design are different. In the future, there may be more companies coming to the market with systems of similar nature or better.

21 YEARS OF DEVELOPMENT

After 21 years of development and use in the dental profession, CAD/CAM technology has found a respected place in the field of restorative dentistry. To be sure, the level of care for the dental patient will continue to rise. To be able to provide restorations of high quality, incredible accuracy and respectable esthetics in a single visit without traditional methods and complete it in less than two hours is a monumental achievement. The future will bring even more astounding advances.

CEREC ONE, TWO, 3-D, AC

When the CEREC one was built, it was a large machine that was not well suited for use in a private practice. The machine housed both the computer and milling unit. The CEREC 2 tailored itself to be more office friendly. The machine was smaller and the milling unit was a separate station. The CEREC 3-D utilized the same hardware as the CEREC 2 but the breakthrough advancement was in the software. Having software that produced three dimensional real-time graphics greatly reduced the learning curve rate. Now the latest advancement is in software and hardware. The Bluecam light of the AC system allows the user to create restorations chairside and also send full arch digital impressions to laboratories

anywhere in the world. The new MCXL milling unit is capable of producing restorations faster. It also is a larger heavy duty design suited for a long service life.

RESTORATIONS AVAILABLE FOR EACH

The first CEREC system allowed for an inlay and onlay. When technology advanced in both software and hardware, the crown and veneer were able to be fabricated. Presently, with the advances in dental materials, coupled with that of the CEREC system, three unit fixed partial dentures can be made. Although at this time the making of three unit bridges is cost prohibitive to the general practitioner to keep in their office, it is easily made by emailing the digital acquisition file to the lab for fabrication. The cerec system will mill the framework for the fixed partial denture from a lithium disilicate block. The lab technician will then apply porcelain to the block in the conventional fashion.

Those in private practice though enjoy the ability to fabricate crowns, veneers, inlays, and onlays. These restorations are capable of being fabricated from a preformed block of material. The materials vary from composite to leucite reinforced porelain. All of these restorations can be made in just one visit.

The possibilities extend further. Some practitioners are doing anterior arch cosmetic restorations in one visit by using the quadrant function of the system. This enables the patient to receive an anterior reconstruction in one visit. An alternative approach is acquiring the images, temporizing the patient and making the restorations at a later time. This allows the practitioner to cut back porcelain, add on and fully customize the restorations. The result is an amazing transformation of smile with as little as a one day turnaround time.

OVERVIEW OF CAD/CAM SYSTEMS

There are two CAD/CAM systems on the market today. The Sirona Company has the CEREC and Schein Company has the E4D. Both systems employ the use of a computer, image acquisition unit (camera), and a milling unit. The word CAD/CAM means computer assisted design and computer assisted machining (or milling). The systems are capable of providing inlays, onlay, crowns, veneers, and bridges. The possibilities are only restricted by the dentists comfort level and experience.

The process starts with the acquisition of an image of a tooth preparation. The camera emits light that is reflected back into the camera. The light reflected is converted to an image. Each pixel of that image becomes a mathematical equation and represents a point in the three dimensional space of the information acquired. One cannot consider the camera as a device that takes a picture. Rather, it is an image with digital information. With this information, the software can run the program to enable the user to create a restoration. The software programs are unique in their appearance but similar in their methods. The user moves through a series of steps to create the restoration. Upon completing the design, the restoration is sent to a milling unit. The milling unit receives the information via wireless or cable connection to the acquisition unit. The information is then transferred to the machinery and the restoration is milled from a porcelain block. When the restoration is completed, the dentist then gives finishing, polishing, and delivery to the restoration to complete the process.

The cad/cam process is not without flaws. There are restorations that don't fit perfectly and those in which occlusion is considerably off. While the cause of such flaws can rarely be attributed to the computer aspect, the most likely reason lies with the operator of the system. The dentist is the human component to the fabrication of the restoration. He/she can only provide a restoration that is of a quality under their control. Meaning that the quality of the information that is given to the computer for making the restoration is exactly the quality of the restoration in its completed form. The old attage holds true, "Garbage in, garbage out". One cannot expect to receive a perfect restoration from a cad/cam machine if one hasn't provided perfect information to the cad/cam machine. With that, the expectation of a perfect restoration is entirely reasonable if the information provided is also perfect. In practice, perfect crowns are the norm, not the exception.

Sirona

Sirona is a German company that has been providing equipment and products to the dental industry for over 150 years. It has been the producer/manufacturer of the CEREC system since its inception. The Sirona system is the first on the market. Its technology has been constantly evolving and developing as it keeps pace the developments in the computer industry. While the software and hardware has evolved, its use in the dental practice has become more efficient, reliable, predictable and reproducible. The materials that are used to create the restorations have also been developing.

This past year, Sirona presented the marketplace with their own restorative block for milling with their system. It is the same porcelain as the most common restorative porcelain block, the Vitablock. Vita dental is the manufacturer of the all porcelain block. It comes in a variety of shades and sizes as most of the blocks on the market. The Sirona block is composed of the same material as the Vita block.

Schein

While second to the marketplace, the Schein product, the E4D, has opened the doors of competition. Their system is similar in design and nature, but the image acquisition is of a different method. Their design software is similar in the steps but the graphics are also different. It is similar enough to the Sirona system to produce a restoration of the same level of quality with the same materials.

The E4D has design principles similar to the CEREC but it does not use the same method to acquire the image. The image in the CEREC system uses a reflective powder and an infrared light. The E4D does not use a powder, and uses a red LED light. The E4D process requires many more images to gain the information necessary for designing a restoration than the CEREC. Research is needed to verify the light type

In comparing the software of both E4D and CEREC, while functionally similar in that many of the tools used in designing the restoration to achieve the same goal, they differ in their appearance and steps towards completion of design.

RESTORATION PROCESS

In making the various restorations, and describing the process, the CEREC technique will be used as the example because it is the most common and widely used technology to date. When first using this technology, it is advisable to work on a plastic typodont until the process is fully comprehended by the user. Then the first live patient attempts at restoration should be carefully chosen to ensure an efficient and successful operation.

Tooth selection for the restoration is fundamental to becoming proficient with this technology. After some experience, it will come naturally to treat virtually any tooth in the mouth under any situation. But for the novice, one should work on supragingival margins with good opposing occlusion. This will allow the practitioner to focus on the software design methods and not have to be concerned with the challenges of an oral environment that would make for a more difficult process.

Tooth Selection and Preparation

When first using the system it is wise to practice on a typodont or model. Both the CEREC and E4D have training education for the new user. The training is enough to gain familiarity with the systems but in no way should one consider themselves a master of the system with the basic training. There is nothing that will ever fully prepare the dentist for the first live patient. The first restoration performed on a patient will not be difficult in preparation or delivery. Preparation and delivery is the same as for any other restoration a practicing dentist is familiar with. The difficult task is to gain comfort and familiarity with the use of the camera and computer. As with any new endeavor, a learning curve exists. As long as one is first committed to quality, speed and efficiency will follow.

Tooth selection for the beginner should be in consideration of the following facts:

- A patient with good dental experience and interest in the technology.
- A tooth in the midsection of the arch such as a premolar or first molar
- An opposing occlusion that is of good form (i.e. no cross bite, no missing opposing tooth) and close to ideal occlusion.
- A tooth that has adjacent teeth both mesially and distally.
- A tooth in need of a crown as opposed to an inlay, onlay.

The rationale is that one would want to have as close to an ideal oral environment that would be similar to the typodont that was used in training. Also, having a patient with an interest in the technology produces an environment whereby both the practitioner and patient are comfortable and not stressed. This psychological benefit allows the practitioner to focus on the task at hand and not have to be concerned about managing the patients stress level.

The technology allows for the creation of a restoration regardless of the condition of the tooth to be restored. If a tooth is in good anatomical condition and the need for restoration is due to caries and a large restoration being present, then the crown can be modeled to replicate the existing anatomy. Alternatively, if the tooth is fractured or decayed to such an extent that the normal anatomy is no longer present, then a restoration can be produced from a library of files in the software which will create a tooth of size and form compatible with the existing dentition.

Preparation of a tooth for a crown is very much like any crown preparation for all ceramic. One distinguishing feature is that the occlusal table should be flat and not follow the contours of the cusps as in traditional gold and porcelain fused to metal crowns. The reasoning for this is twofold. One is the hardware. The milling of the restoration is better with the flat occlusal table than other forms. The other is for strength of the material.

When all-porcelain materials are used, a minimum thickness of 2mm is desirable for the occlusal table. Thickness' less than 2mm are not strong enough to tolerate the loading forces. Thickness' thinker than 2mm will cause the material to fracture as well. If the occlusal anatomy that is formed by the user during the design process is performed without regard for the milling process, the occlusion, and occlusal function then adjustments will need to be made upon delivery. So when adjustments are made, then the minimum thickness requirement will be violated. When this occurs (and it does so unintentionally) the crown will fail. When a preparation form of the kind in gold restorations is applied to the cad/cam systems, the likelihood of violating the minimum thickness requirement is very high. This is due to the fact that the internal anatomical surface of the restoration is limited by the diameter of the bur. So if a preparation surface has many facets, or slopes, the internal surface will have many stepwise bur marks to follow that surface. To minimize the stepping pattern of the internal surface, the preparation design should be as gradual or as flat as possible.

In a maxillary central incisor for instance, the incisal edge width of the preparation labiolingually can often be one millimeter. the diameter of the bur in the milling chamber is 1.6mm. so there will be more material removed in the ingot at the change of slope pattern than necessary for the preparation. The result is the creation of more space of the internal surface than is necessary. When the preparation surface has many slopes as in the contour of a molar, there will the "steps" in the internal surface of the restoration. If this is combined with an adjustment of the occlusion upon delivery, then the area will be too thin and a fracture will result.

It is important also to ensure that there are no sharp line angles. Sharp line angles in all - porcealain restorations have been associated with crack propagation and premature failure of the restoration. This is related to the direction and placement of occlusal forces on the restoration.

Image Acquisition

The image acquisition stage is the digital equivalent to taking an impression using conventional impression materials and then making a positive stone model. Where the conventional method involves many steps with many different materials over the course of hours, the digital impression involves only one step using using infrared light over the course of a few seconds. The result is a digital "stone" model on a monitor.

In acquiring the images necessary for restoration, a reflective powder must be placed in an even coat with the driest environment possible. This allows the image to be recorded in an even color tone to aid in the processing of the image to a digital model. There are a variety of powder coats available using different methods of placement. Some powder systems are attached to the delivery unit air lines, and others come in small pressurized cans.

The image should be taken quickly and accurately. A firm grasp of the camera with a finger rest on the teeth of the arch being treated must be done. Having an assistant is also ideal. The assistant will retract, suction and watch the operating field while the dentist places

the camera and watches the monitor to acquire the image. Between one and three images are commonly taken to acquire the needed information. When the image is acquired, it is saved in the computer in an image catalog file. It will then be used for the design process. When the image acquisition is completed, it is time to begin the design phase.

Restoration Design

At this time the assistant is rinsing the mouth to remove the reflective powder. The dentist is beginning the design of the restoration. The process begins by drawing the margin of the restoration on the simulated model of the preparation. Then contact points are viewed and adjusted. Occlusion is then viewed and adjusted if necessary. Contours, emergence profiles, anatomy are evaluated and can be edited if necessary. When the operator is satisfied with the restoration, it is saved and sent to the milling unit.

The process of designing a restoration uses basic computer skills. The understanding of proper design utilizes basic occlusion and anatomy knowledge. The design is prompted by a series of screens for the operator to enter the various factors in creating a restoration. The cavosurface margin area is drawn in first. Then a verification of the proximal contours is done. Finally, occlusal table, emergence profiles, cusp heights and occlusion are created.

Contact areas are created and placed precisely where the operator desires. The amount of proximal contact can also be placed. As in, a tight contact verses a light or no contact. The contact area is displayed with different colors onscreen to inform the user as to how much contact is applied to the adjacent teeth. The contact areas will be polished later, and the amount of porcelain removed in polishing can be accounted for in the design phase.

There are two modes of design in the present CEREC system, “simple” and “master”. Simple mode gives the computer more control over the restorations form and occlusion. The computer makes a restoration proposed restoration and the user can then refine it. In “master” mode, the user has complete control over all the restorations characteristics. This requires more steps in creating the restoration and a better understanding of the software system. In either mode of design, the outcome is the same. The restoration will be accurate, and predictable.

Once the restoration has been designed, it is ready for milling. The screen prompt will allow the user to pick a size of ingot block and the type of material for that block. There are some blocks which have layers of differing translucency. The user dictates the placement of these layers in the restoration to achieve the best esthetic result. Also of choice for the user is the ablitly to choose where the sprue will be placed on the restoration. The sprue can be placed on any surface except the occlusal.

Restoration Milling

The milling unit receives the information of the designed restoration and then begins the milling process. The milling process starts by prompting the user to choose which type and size ceramic block is desired. The computer then confirms that the size is acceptable and begins calibrating the block to be sure that the correct size has been placed. The milling begins and a message appears on the computer screen to indicate the amount of time before the milling is completed.

When the milling unit goes through the pre-mill sequence of checks, it is confirming that the information entered by the user matches what is detected in the milling chamber. Sometimes

human error will lead to the wrong material being placed in the chamber. This could be a milling bur that is not the correct size or shape or it could be the wrong milling block size.

The milling process can range from five minutes to 15 minutes, depending upon the block size, type of restoration, and the milling unit model. For instance, crowns on large molar teeth take longer to mill than an inlay on a lower premolar. The CEREC system has two milling units available, the newer MCXL and the standard unit. The newer MCXL is faster than the standard unit.

Restoration Finishing

When the restoration has completed milling, the next step is to finish and polish. All porcelain restorations are etched with a sandblaster of 50 micron particle size. Some practitioners also will etch the surface with Hydrofluoric acid. While the specifics and materials used in preparing the porcelain surface for cementation is highly debated, one fact is certain. Porcelain surfaces must be treated in order to increase the surface area for micromechanical retention.

After surface preparation is completed the restoration is finished with polishing points. Occlusal anatomy can be enhanced with a fine diamond point. Contact points are predictable and rarely need much adjustment. Occlusion is also very close and slight adjustments are performed with the fine diamond.

Alternatively, the surface can be easily stained and glazed in a glazing oven. This eliminates the hand polishing step. Staining and glazing will add 15-20 minutes more of treatment time. The restoration will have color and light characteristics tantamount to those made in the finest laboratories. Use of a stain and glaze oven is very rewarding and beneficial when providing anterior restorations. This especially so when making restorations in the anterior quadrants and with adjacent teeth that are polychromatic and have stains.

Restoration cementation

To complete the restoration, one can call on a variety of luting agents and cements available on the market. This area of dentistry is constantly changing and new materials are unveiled at the various trade shows across the country every year. The misconception is that because the tooth was produced with a CAD/CAM technology, then there must be a different way to cement the restorations. The truth is that these restorations are no different than any other all porcelain restoration received from a laboratory. The cementation process would be the same.

Some advocate the use of modified resin glass ionomers. These are the most popular cements on the market today. They provide adhesion, bonding to the tooth surface, and sealing of the margins. Also, they are easy to mix, place and clean up. Dual cure resins are also popular for these same reasons.

Other practitioners like to use resin cements, as there are claims of better bond strengths, less sensitivity, and ease of use. However, some resin materials need several steps to complete the bonding process. These steps are also highly technique sensitive. Thus it is also more time consuming.

Suffice it to say that regardless of the materials of choice for the cementation of the porcelain restorations, there are many available with a wide variety of materials science and they all are acceptable for use in the practice. The most important thing is to have the

knowledge of the steps involved and the characterisitics of the material to ensure the success of the restoration.

Some of the characteristics of the cements to be aware of are film thickness, bond strength, and thermal expansion. These characteristics vary among cements and can have an impact on post operative sensitivity and the success or failure of the restoration. The CEREC system gives the operator the options of adjusting the restoration for film thickness and space available between restoration and tooth.

ROLE OF CAD/CAM IN THE PRACTICE

Whereas any new technology offers an increase in the standard of care, it is often met with concern on how to implement the technology effectively. The success of implementation is directly dependent upon the commitment level of those trying to adopt it and the practicality of the item in providing care on a daily basis. Many dentists have shelves of retired items that have come and gone. Some things were great ideas and just not practical, while others were great ideas but difficult to adopt into daily care. CAD/CAM technology has been developed over the last twenty-two years and will continue to advance in the future. It is not the type of techonology that will dissipate and disappear.

CAD/CAM dentistry is a technology that is a great idea and can provide a higher standard of care on a daily basis. The success of its use though is the commitment level of the user in learning the technology. Once proficient in its use, the world of CAD/CAM will open doors to treatment that were never thought of.

For instance, a new user will be able to provide restorations in one visit. Crowns, onlays, inlays will become a rote one visit procedure. The issues of extended and multiple visits will be a thing of the past. The patients expectations for treatment will be raised and their increased interest in the technology and procedure will raise their level of interest in their home care and oral health.

The user will with advanced experience in CAD/CAM will find making veneers, quadrant dentistry, custom staining chairside and other advanced CAD/CAM procedures rewarding and productive.

The role of CAD/CAM dentistry today is to provide better restorations in a faster method. It is also to provide convenience, accuracy, esthetics and efficiency to the busy modern practice. In the growth and proliferation of this technology, new advances will be made and new treatment methods will emerge. By being able to provide a growing and constant improvement in care through CAD/CAM technology, the field of dentistry will advance.

CAD/CAM dentistry provides a greater ineraction of patient, doctor and the dental team. Using the system to its full potential, the dentist provides the restoration. The assistant aids in possibly the design. The assistant can finish, polish, stain and glaze the restorations. The hygienist can discuss and introduce the system to the patient. Explaining the benefits and advantages of needed restorations and how they can be delivered with the system. Because the system creates so much interest, it generates an increased morale and team approach to providing optimal care.

SUMMARY

The era of daily use of computers in dentistry is here. Most private offices employ the use of computers for scheduling and record keeping. Some offices have included the use of digital radiography and intraoral cameras. Some offices have incorporated the use of CAD/CAM systems like CEREC. The history and development of the CAD/CAM system is more than twenty years old. With the placement of restorations at that time that are still in service today, and years of independent research, it is clear that this technology is a lasting part to the modern dental practice.

The preparation of teeth for the CEREC system is very similar to conventional methods. The design and restoration process is predictable, accurate and efficient. The acquisition of the "model" for creating the restoration uses an optical scan verses impression materials. This is also a growing and changing technology. At this time, Sirona is presenting a new light source and camera that can more accurately scan not just a few teeth, but an entire arch in less than two minutes.

Milling the restoration in the office allows the practitioner to choose from many different materials and shades. Also, is possible for the provider to customize the restorations with staining and glazing techniques in the office. Delivering the restoration can be done with a variety of materials. Modified resin glass ionomer cements are the most common. Finally, the technology is available to the private practitioner in a system that is affordable, reliable and will be long lasting. Thus providing years of service of a technology that is constantly improving and developing.

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Chapter 28

IMMEDIATE IMPLANT LOADING FOLLOWING EXTRACTION OF A SPLIT CUSPID

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ABSTRACT

The therapeutic goal of implant dentistry is oral rehabilitation and tooth replacement. Considering dental implants as a treatment option provides patients with a positive, long term result. Implants have certainly developed into a viable alternative to conventional prosthetic reconstruction of edentulous areas. They provide outstanding support for single tooth replacements and are often less invasive than conventional crown and bridge techniques. However, implant dentistry has gone through many phases over the years. Modern technology and design allows us to predictably place our dental implants in immediate extraction sites and load the implants at the time of placement. Single too h reconstruction provides easy access for the patient to floss and clean the area compared with the relative difficulty in maintenance when crowns are splinted or bridges fabricated. This case demonstrates an immediate extraction of a fractured root and immediate placement of an OCO Biomedical ISI one piece implant and immediate function provided by a composite transitional crown.

Keywords: oco biomedical, isi, immpression pickup abutment, zirconia

Immediate loading of dental implants has been presented in many lectures and articles over the past years. However, predictability of immediately placing a tooth on an implant has often seemed compromised. Following an extraction of a tooth, the socket created is often larger or shaped elliptically which makes placement of any conventional implant tenuous. Often the practitioner elects to bury the implant following immediate placement of an implant following extraction, allowing integration to progress. Temporization is the most compromising concern for the patient. Fabrication of a removable "flipper" type appliance is often deemed the most probable solution to an edentulous space. Bonded transitional bridges can be used but in a functional area may be dislodged easily.

Dental implants have long provided an excellent treatment option to restore edentulous spaces. The advent of the endosseous implant provided relative surgical simplicity. Patient understanding of the benefits of dental implant therapy is a motivator to patient acceptance. Today, people often have a hard time psychologically accepting the idea of a removable appliance. The concept of placing dental implants immediately upon extraction of a tooth is relatively new, and loading the implants immediately following placement is often misunderstood. Smile design and emergence profile are key components to our current dental capabilities. The OCO Biocmedical ISI Complete One Piece implant is designed exclusively for immediate load, immediate function capability. The design a mini Cortic-O-Thread pattern at the top of the implant that locks into the cortical bone, and a bull nose, "auger" design at the apex that actually condenses bone around the tip and threads. This OCO Biomedical dental implant system is a minimally invasive, bone condensing implant system designed specifically for immediate function. Dual stabilization locks the implant in place to provide a true mechanical lock for immediate loading.(2)

The surgical techniques in the placement of the ISI implant is both user friendly and simple. Chair time is dramatically reduced due to immediate fabrication of a transitional crown. Final impressions can be made at the time of surgical placement. No special abutments are required and the final restoration is a cement on crown.

Figure 1 illustrates the periapical radiograph of a fractured root in the maxillary left cuspid area. Figure 2 shows the pre treatment mobile crown retained by a cast post in core in place. The patient presented at the end of the day in obvious concern. The decision was made to remove the fractured tooth (Figures 3 and 4). The root was removed in two distinct pieces with no trauma to the surrounding periodontal tissue and interdental papilla. The socket was curetted, but no granulation material was present since the tooth root just recently fractured. Typical surgical technique was utilized including the use of a pilot drill extending through the apex of the created socket. Since the socket site was rather large, no countersink drill was required. The final fluted 4.0mm x 12mm was used to create the osteotomy prior to implant placement. Since the implant is self tapping there was no need to use a thread former. The final drill is side cutting only and is used to form the osteotomy. The depth of the implant placement was determined by the pilot drill. There are no intermediate sized drills used in this technique.

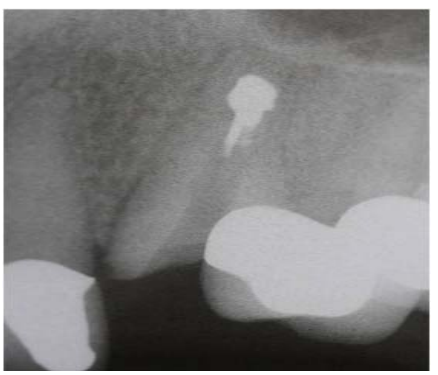
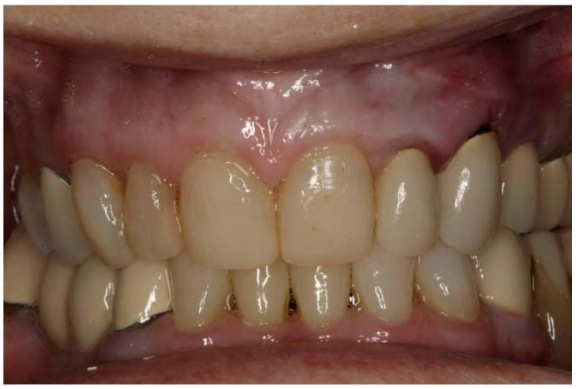


Figure 1



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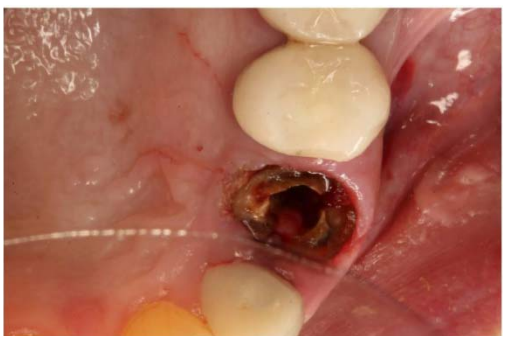

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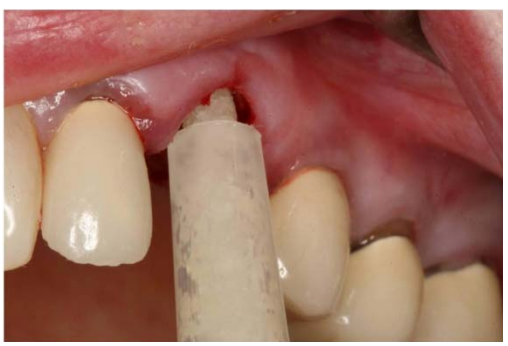
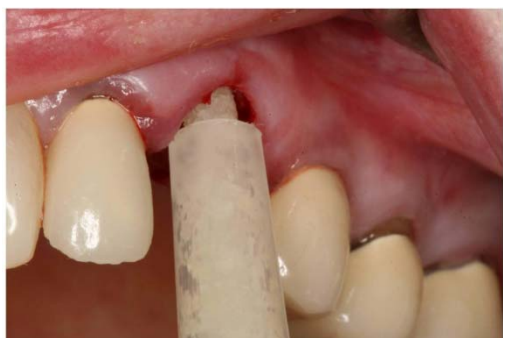


Figure 4.

Since the socket size was a bit larger than the final diameter of the implant, some Dynablast (Keystone Dental, Boston, MA) which is a mineralized/demineralized mixture of allograft material in a carrier, was injected into the prepared socket area.(Figure 5) (1) The ISI one piece implant was torque into the socket area and stopped when 75 Ncm of torque was achieved using a ratchet and the functional collar margin was subgingival by about 2 mm. (Figure 6). As the implant bottomed out, the bone is condensed at the apex and the cortical bone by the mini threads at the bottom of the tapered collar. Once the implant was placed an additional few turns were given to condense the bone at the tip and wedge the cortico thread into the cortical bone. A mechanical lock is achieved at the top and bottom of the implant. You can see that there is little or

no bleeding of the surgical site. (3,4) Figure7 illustrates the final implant position. Note that the implant obliterated the socket apex which allowed for immediate stabilization in bone. Since the interocclusal space was large there was no need to adjust the abutment head. The final crown would be fabricated over the one piece abutment. A simple machined acrylic coping is placed over the abutment (Figure 8) and a tooth colored composite crown fabricated using an indirect technique. This transitional crown has the appropriate margin adaptation and is cemented with a soft cement like Temp Bond. (Figure 9). The patient presented with an emergency situation and left the office in approximately 45 minutes with a transitional crown that was esthetically pleasing. Lateral interferences were removed from the transitional crown and the patient was maintained in a group function occlusion during the integration healing process. At the time of implant placement, it was determined that the final crown impression would also be made. A pick up impression abutment was snapped onto the abutment. It seats firmly into place (Figure 10) Polysiloxane impression materials are used to pick up the impression abutment and lock it into the final impression. A solid analog is simply snapped into the impression abutment (Figure 11) and a master cast fabricated. (Figure 12). Three months of healing was allowed prior to removing the transitional crown and evaluating the periodontal health. The tissue was pink and firm (Figure 13). Since we had made the final impression at the time of surgery, a zirconia coping was fabricate (Figure 14) and would be picked up in a final impression. This ensures proper final crown contours and creation of interdental papilla embrasures. Figure 15 illustrates the final crown created by the dental laboratory. The crown is cemented into place creating an esthetic free standing restoration which is easily maintained (Figures 16,17) The final periapical radiograph shows wonderful integration of the implant. (Figure 18)



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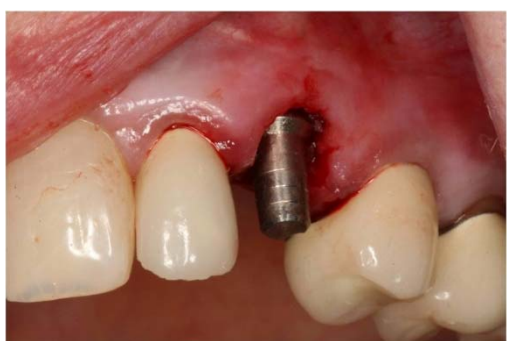

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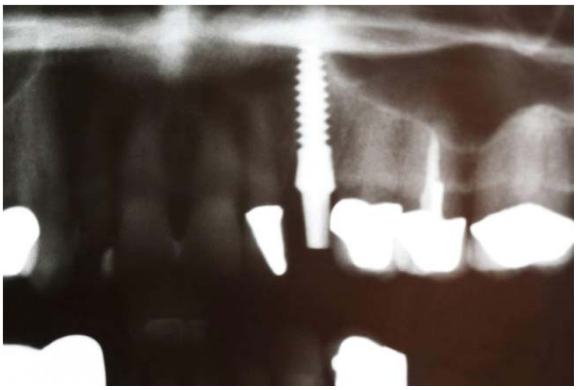

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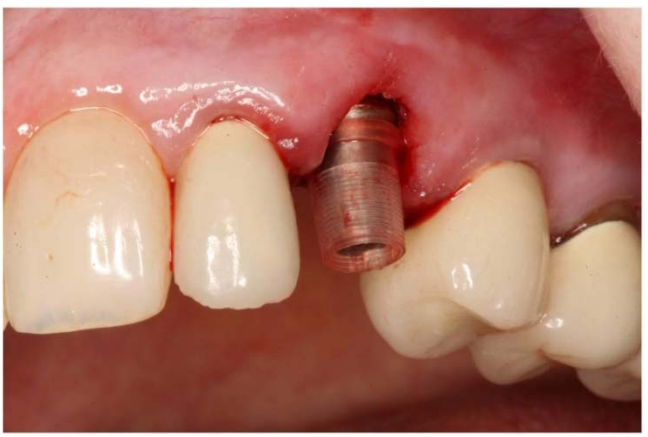

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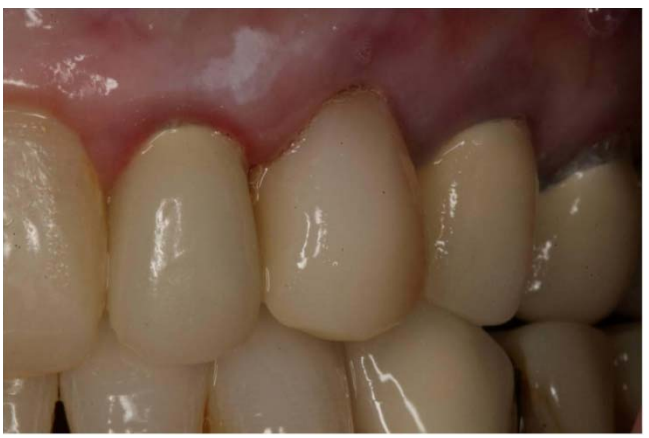

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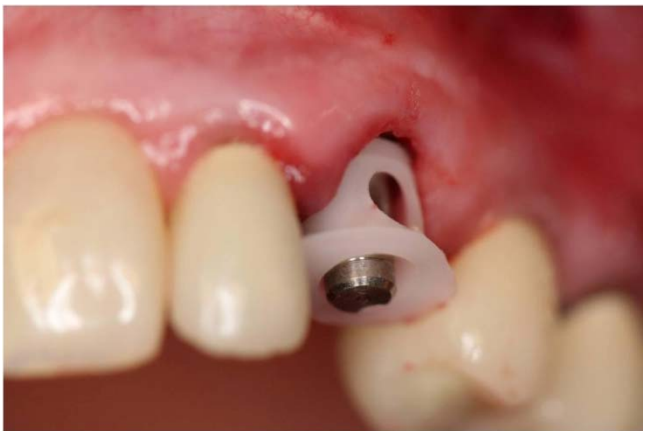


Figure 10.

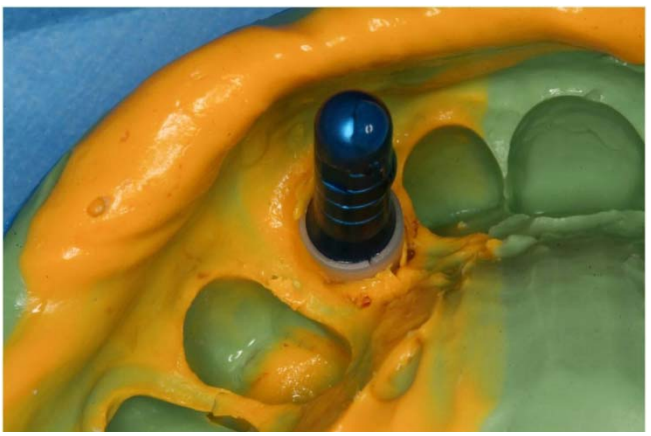
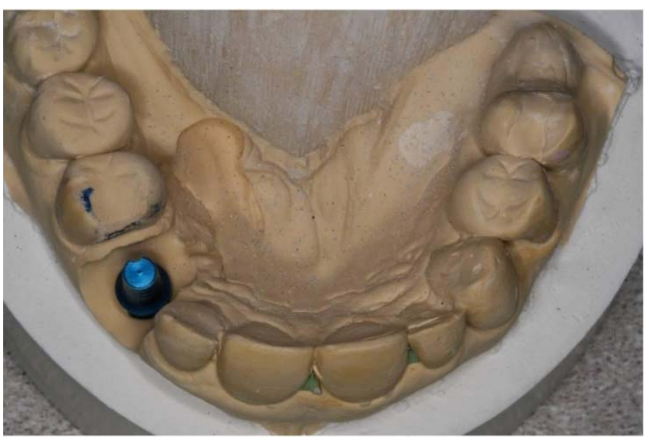


Figure 11.



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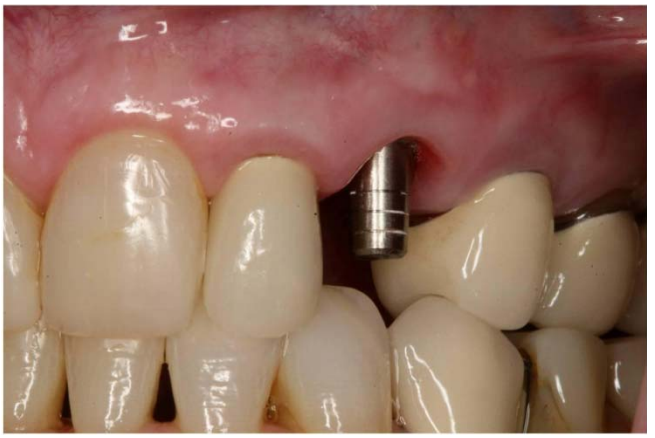

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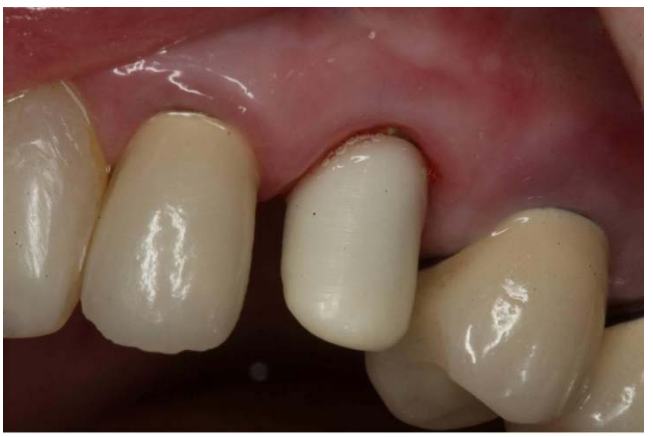

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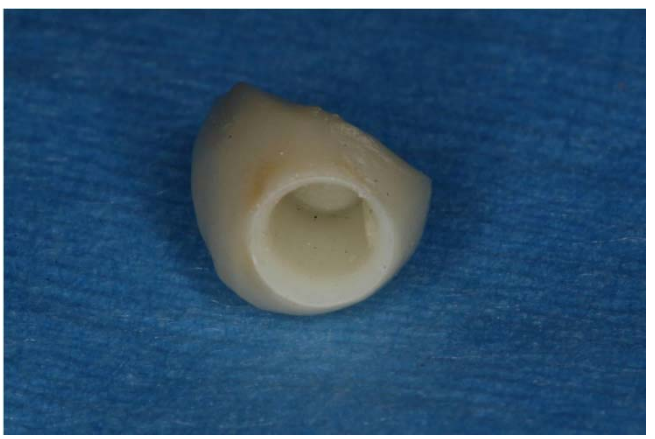

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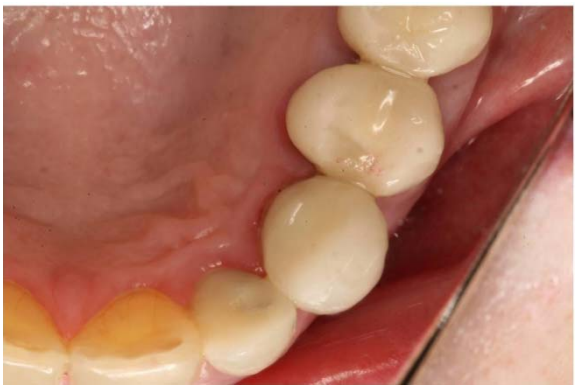

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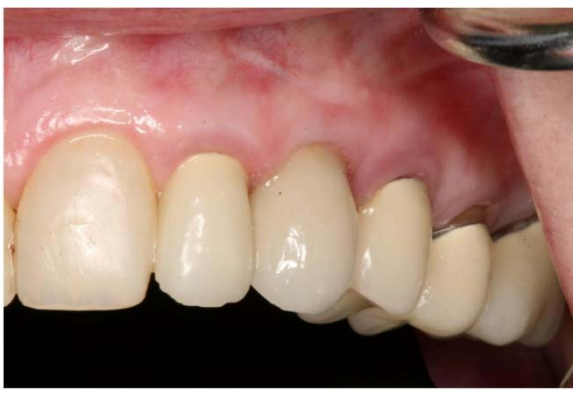

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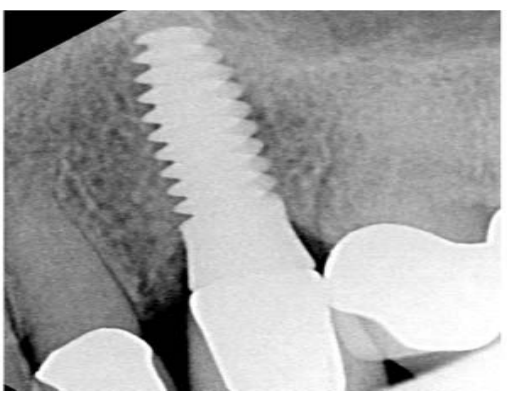

Figure 18.

RESULTS

The final esthetic zirconia crown restoration of the edentulous maxillary left cuspid was completed in a short clinical appointment. The non restorable root was extracted maintaining the gingival contours. Immediate placement of the implant insured maintenance of bone

height and contour. Immediate loading of the implant with a composite crown established triangular tissue in the interdental papilla area. This individual crown allowed for easy periodontal maintenance and patient compliance. Immediate placement of a transitional crown made the proposed implant therapy bearable and exciting to the patient.

DISCUSSION

The OCO Biomedical ISI one piece implant system allowed for surgical predictability, immediate stability and reliable osseointegreation. Simple prosthetic techniques made fabrication of the final implant retained crown as easy or easier than a conventional crown. No retraction was necessary and the technique was completed at the time of the initial surgery. Smile design and emergence profile considerations were addressed with proper planning and execution of the technique. This proved to be an outstanding treatment modality in an emotionally difficult circumstance. Giving patients their teeth immediately upon removal is a dynamic concept of implant dentistry today.

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Chapter 29

RISK FACTORS FOR CHRONIC PERIODONTAL DISEASES

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ABSTRACT

Chronic periodontal diseases include a group of inflammatory diseases that affect periodontal supporting tissues of the teeth and encompass destructive and nondestructive conditions. Periodontal diseases are multifactorial and the role of dental biofilm in their initiation is primary. However, whether dental biofilm affects a particular subject, what form the disease takes and how it progresses, are all dependent of a wide variety of factors. Therefore, the objective of this chapter is to outline the risk factors described for the most prevalent chronic periodontal diseases (plaque induced gingivitis and chronic periodontitis) and to explain some basic concepts related to the current understanding of the role of these risk factors based on *in vitro*, animal and human studies. The review will focus on the factors that may be associated with a direct increase in the likelihood of occurrence of disease or an increase in its severity. The following factors will be discussed: 1) host characteristics, such as age, gender and race; 2) social and behavioral factors (socioeconomic status, cigarette smoking and emotional stress); 3) systemic factors, e.g. diabetes mellitus and osteoporosis; 4) genetic factors; 5) tooth-level factors (root grooves, tooth position, caries, occlusal discrepancies, iatrogenic restorations, root abnormalities and periodontal parameters); and 6) the microbial composition of dental biofilm. Finally, this chapter will also present literature-based evidence on predictive factors associated with patients and tooth susceptibility for recurrence of periodontitis

after the end of the active periodontal therapy and will examine the use of some prognostic models which may be useful for clinicians in the identification high-risk groups of patients.

INTRODUCTION

Chronic periodontal diseases include a group of inflammatory diseases that affect the periodontal supporting tissues of teeth and encompass destructive and nondestructive conditions [12]. The term chronic periodontal diseases will refer, in this chapter, to both plaque-induced gingivitis and chronic periodontitis. Plaque induced gingivitis is the inflammation of the soft tissues without apical migration of the junctional epithelium [32]. In addition, chronic periodontitis, the most frequent form of periodontitis, results in inflammation of the supporting tissues of the teeth, progressive attachment and bone loss at a slow rate, characterized by pocket formation and/or gingival recession [34]. Cross-sectional epidemiologic studies from many countries have shown that gingivitis is highly prevalent in the primary and permanent dentitions of children [7] and affects many adults [5]. Further, chronic periodontitis is also a common entity worldwide [6]. Therefore, a knowledge of the factors that may influence the transition from health to disease and of the progression of the disease through various stages of severity are important in the development of effective strategies of prevention and treatment.

Gingivitis has already been established as a consequence of dental biofilm accumulation. It is produced as the result of a general increase in the number of microorganisms and a change in the composition of the flora associated with the increasing age of the dental biofilm [99]. Several studies show that periodontitis is preceded by gingivitis and, although the accumulation and duration of microbial dental plaque biofilm will predictably lead to the development of inflammation in the nearby gingival tissues, the duration of onset and the intensity of the inflammatory process vary considerably from person to person, as well as between teeth. Albandar et al. (1998) [4] studied a periodontally high-risk group comprising 156 young subjects that were examined twice during a period of six years to study the relationship between the presence of gingival inflammation (gingival bleeding) and the occurrence of attachment loss. They found that 9.3% of sites that had gingival bleeding and 0-2 mm of attachment loss at baseline showed a longitudinal attachment loss of ≥ 3 mm over 6 years, whereas only 4.8% of sites with no gingival bleeding at baseline showed a corresponding attachment loss. Hence, 90.7% of sites with gingival bleeding at baseline did not show any clinical attachment loss during the study period. This study showed that not all sites with gingival inflammation developed periodontitis during the study period. Thus, predisposition to periodontitis development varies significantly and may possibly be influenced by other factors. However, defining the factors involved in initiation and progression of chronic periodontitis is a more complex issue.

Chronic periodontitis is a multi-factorial disease. While the role of bacteria is primary, a number of host-related factors have been hypothesized as influencing its diverse clinical presentation and rate of progression [72]. Loe et al. (1986) [100], in a longitudinal study, evaluated a Sri Lanka population never exposed to any programs or incidents related to the prevention and treatment of dental diseases. This population did not practice any conventional oral hygiene measures. Three subpopulations were identified: 1) individuals with rapid

progression of periodontal disease (8%); 2) individuals with moderate progression (81%); and 3) a group who exhibited no progression (11%). When another longitudinal study was made comprising a sample of middle-class Norwegian men who had the benefit of a comprehensive health care program, a group that represented an extreme condition of periodontal maintenance when compared to the Sri Lanka population, two subpopulations (moderate disease and no disease) were found, despite the severity of attachment loss [164]. These studies illustrate significant differences in the pattern and rates of attachment loss among individuals, even when they receive regular and adequate professional and personal health care. Based on the evidence above, the identification of factors involved in the initiation and progression rate of chronic periodontal diseases has been the focus of considerable research in recent times.

Chronic inflammatory periodontal diseases have several etiological factors for which a plausible biological model of effect exists. The term risk factors is commonly used and it refers to an aspect of personal behaviors or lifestyle, an environmental exposure, or an inborn or inherited characteristic, which on the basis of epidemiological evidence is known to be associated with a health-related condition [99]. The presence of a risk factor implies a direct increase in the likelihood of a disease occurring [95]. Prospective longitudinal studies, and in particular clinical trials, provide the most powerful evidence for the existence, and the amount, of risk. However, in most cases these types of studies are not easily conducted. For this reason, most evidence for the existence of possible risk factors for periodontal diseases comes from cross-sectional studies. Although the identification of risk factors for disease is unfeasible using cross-sectional studies, when a proper study design is employed, these studies can provide valuable information on the presence or absence of an association between the variables under study and the occurrence of periodontal diseases. In order to make a distinction between the results of the different types of studies, it is customary to refer to significant effects assessed in cross-sectional studies as associations, whereas effects disclosed using case-control studies and prospective studies have been referred to as risk determinants, risk indicators, or risk markers [8].

Usually, the overview of factors associated with chronic periodontal diseases is systematically presented as host characteristics, social and behavioral factors, systemic factors, genetic factors, tooth-level factors and microbial factors [126]. In addition to the investigation of these factors at the onset of chronic periodontal diseases, longitudinal studies of patients treated for periodontitis try to determine the patient's susceptibility to disease recurrence [64, 96]. As a result, the prognostic factors (disease predictors), defined as characteristics related to the progression of preexisting disease [133], have been the subject of much discussion. The identification of groups and individuals at risk for disease progression during maintenance therapy still represents one of the greatest challenges in the management of periodontal patients. Thus, prognostic models aimed at identifying high-risk individuals or teeth in a clinical setting have been described [56, 91]. A question remains about the safety of these models routinely used to help clinicians in decision-making.

HOST CHARACTERISTICS

Age

Several epidemiological studies have clearly demonstrated an increase in the prevalence (percentage of persons), extent (percentage of teeth per person) and severity of periodontal attachment loss with increasing age [6, 9]. Papapanou et al. (1988) [132] examined full-mouth radiographs from 531 dentate individuals aged 25-75 years and found that bone loss increased with age. Moreover, two large epidemiological studies estimated the prevalence and extent of periodontal diseases in the United States using data from the National Health and Nutrition Examination Survey (NHANES) in the years 1985 to 1986 and 1988 to 1994 [6, 23]. It was demonstrated that 48.6% of persons 35 to 44 years old and 77.3% of those 55 to 64 years old had ≥ 3 mm attachment loss in the first survey. The same trend was observed in the second study, in which 48.5% for the 40 to 49 year old cohort and 74.8% for the 60 to 69 year old group had ≥ 3 mm attachment loss. Regarding the healing of periodontal tissues following periodontal therapy, Lindhe et al. (1985) [97] evaluated 62 patients and reported that, although age did not seem to have a significant effect on the results of periodontal treatment, there was a tendency for younger patients to have a shallower probing depth and gain more periodontal attachment than older patients.

With increasing age, people have to cope with a lifelong antigenic burden encompassing several decades of evolutionary unpredicted antigenic exposure, with a major impact on survival and frailty. In fact, there is a peculiar chronic inflammatory status characterizing aging, which has been denominated by Franceschi et al. (2000) [57] as inflamm-aging, and which is considered a random process detrimental for longevity, leading to long-term tissue damage, and related to a wide range of age-related diseases, including neurodegeneration, atherosclerosis, diabetes and osteoporosis among others, which share an inflammatory pathogenesis. It may therefore be speculated that this phenomenon may also affect the periodontium, in which after a lifetime's challenge by oral periodontopathogenic bacteria and their virulence factors, periodontal tissues may develop an intense subclinical inflammatory process, but also lead to healing/regeneration outcomes after periodontal therapy [15]. In vitro studies have clearly demonstrated an age-related decrease in the proliferation of periodontal ligament cells [15, 166]. Further, aging is able to modulate the expression of genes reported to participate in periodontal homeostasis (e.g. cytokines, metalloproteinases and their inhibitors and bone-related genes) by periodontal ligament cells [14, 15]. It is important to remember the role of periodontal ligament cells on periodontal health and disease because of their ability to proliferate, migrate and synthesize several components of the periodontium and also participate in the protective host mechanism that prevents periodontitis or impedes its progression [60]. Little information on the influence of aging on the periodontium is provided by animal studies. It has been documented that the periodontal ligament presented decreased cell density and collagen synthesis, and also a decreased number of cells in the osteogenic layer of the alveolar bone has also been reported [135, 165].

Despite the well-documented loss of attachment with increasing age and the rationale behind the association, the question as to what extent aging affects periodontal homeostasis is still a controversial issue in the periodontal literature. A number of arguments have been used against the presumed association. First, there are no marked increases in the probing depth with age. Furthermore, the prevalence of moderate and advanced periodontitis increased in

patients up to approximately 65 years of age, remaining steady until they were approximately 80 years of age, and decreasing thereafter [7]. There is also an indication that the effect of age may be reduced after adjusting for the effects of other confounders [1]. And finally, a diminished ability to perform daily oral hygiene activities has been blamed for the increased prevalence of periodontitis in older individuals [139].

Despite the questions that remain to be examined before consider aging as a risk factor for periodontal diseases, it may be reasonable to suggest that age is a good indicator of the degree of periodontal tissue loss that occurs due to periodontal diseases. However, more studies are needed to clarify the role of aging as a risk factor for the development and progression of periodontal tissue loss and in tissue regeneration following therapy [10].

Gender

Epidemiological surveys show an association between gender and attachment loss in adults, with men having a higher prevalence of and more severe periodontal destruction than women. In the NHANES I survey, a better periodontal status was reported for females than males in all age groups [9]. Subsequently, Hyman & Reid (2003) [76], in a study of risk factors for periodontal attachment loss among adults in the NHANES III survey, confirmed after adjustment for confounding variables, that males were at increased risk of attachment loss, deeper probing depths and a higher prevalence of periodontitis. Attachment loss thresholds of ≥ 3 mm, ≥ 4 mm and ≥ 5 mm were noted in 23%, 44% and 55% more males than females, respectively. This is attributed to a poorer standard of oral hygiene adopted by men and it is likely that hormonal and other physiological and behavioral differences between the two genders may also contribute to the higher risk for periodontal diseases in males than females [8]. Moreover, genetic predisposing factors have been related to the increased prevalence in males [10].

Race / Ethnicity

The level of attachment loss is influenced by race / ethnicity, although the exact role of this factor is not fully understood. Certain racial / ethnic groups, particularly subjects with an African or Latin American background have a higher risk of developing periodontal tissue loss than other groups [10]. In the United States, subjects of African and Mexican descent have a greater attachment loss than Caucasians [6]. However, the increased risk of periodontitis in certain racial/ethnic groups may be partly attributed to socioeconomic, behavioral and other disparities [143]. Moreover, there is evidence that increased risk may also be related to biologic/genetic predisposition [10]. A number of studies evaluating confounding variables have failed to find any differences in periodontitis prevalence and severity between different ethnic/racial groups [37, 76, 106, 107]. For example, Craig et al. (2001) [37] evaluated periodontitis progression rates among three ethnic / racial groups, Asian, African and Hispanic Americans, over a 2-month period. No significant differences in rate of attachment loss were observed between the three groups.

SOCIAL AND BEHAVIORAL FACTORS

Socioeconomic Status

Socioeconomic status is an important risk indicator of periodontal disease. Individuals with a low socioeconomic status have a higher occurrence of attachment loss and probing depth than those with a high socioeconomic status. Drury et al. (1999) [46] used an index comprising the individual's education attainment and family economic status and divided the United States population into four socioeconomic groups. They found that the prevalence of gingivitis and loss of attachment of ≥ 4 mm increased with the decrease in socioeconomic level. Furthermore, Dolan et al. (1997) [44] measured the attachment loss in 761 adult subjects and related these measurements to socioeconomic status and other potential risk indicators. They found that low income and residing in a rural area were significant risk indicators for attachment loss. Thus, it may be suggested that measurements of socioeconomic status, including income, education levels and urban status are fairly good risk indicators for periodontal diseases. Groups with a low socioeconomic status are at higher risk of having periodontal diseases than groups with a high socioeconomic status, and the higher level of risk in this group seems to be attributable to behavioral and environmental factors.

Smoking

It is now well established that tobacco use is among the most important, if not the most important, preventable risk factor in the incidence and progression of periodontal diseases. Cigarette smoking is associated with a two to eight-fold increased risk of periodontal attachment and/or bone loss, depending on the definition of disease severity and smoking dose [158]. For example, with the aim of examining the relationship between cigarette smoking and periodontitis and of estimating the proportion of periodontitis in the United States adult population that is attributable to cigarette smoking, Tomar & Asma (2000) [178] analyzed the data of 12,329 individuals from the NHANES III. In this study, current smokers were four times as likely to have periodontitis (the presence of ≥ 1 site with clinical periodontal attachment level ≥ 4 mm and probing depth ≥ 4 mm) compared to nonsmokers after adjusting for age, race or ethnicity, income, and educational level. Heavy smokers (≥31 cigarettes per day) had a greater risk than light smokers (≤9 cigarettes per day) with estimated odds ratios of 5.6 and 2.8, respectively. When a stricter definition of periodontitis was combined with heavy smoking in a Swedish population, the relative risk of disease ranged from 9.8 to 20.3 [19].

Summarizing the clinical findings in smokers, the gingival inflammatory response is dampened in smokers compared to non-smokers, as evidenced by a fibrotic appearance to the tissues and fewer sites that bleed upon probing smokers [18, 42]. Levels of supragingival calculus tend to be higher in smokers than in nonsmokers. This finding was independent of plaque levels. It is therefore possible to hypothesize that smoking may affect the mineralization rate of calculus [17]. Further, smokers have higher mean probing depths and more sites with deep probing [179, 182]. In addition, gingival recession is greater in smokers compared to nonsmokers [25]. Smokers have two to four times more teeth with furcation involvement [117] and demonstrate a greater loss of alveolar bone height [16]. Finally, smokers with periodontitis have a greater loss of teeth than patients with periodontitis and no history of smoking [52].

Smoking is also associated with periodontal attachment loss in individuals who are usually considered at lower risk because of their relatively young age. Rosa et al. (2008) [154], in a parallel-arm prospective study with eighty-one students considered not to have periodontitis, showed a greater clinical attachment loss and a lower mean alveolar bone height in the smokers compared to nonsmokers. Further data has revealed that even passive smoking, the exposure to environmental tobacco smoke in the home and/or workplace, has recently been associated with periodontitis. Persons exposed to tobacco had a 1.6 times greater chance of having periodontal disease compared to individuals not exposed to second-hand smoke [11]. Further, tobacco use has an adverse effect on the full spectrum of periodontal treatment approaches, ranging from mechanical debridement, local and systemic antimicrobial therapy to surgery and regenerative procedures [80, 90,144]. Interestingly, the deleterious effects of tobacco smoking may be suppressed by its cessation, despite the impossibility of reversing its past effects. In the study conducted by Tomar & Asma (2000) [178], the relative risk for developing periodontal disease was reported to be 3.97 for smokers and 1.68 for former smokers. In addition, among former smokers, the risk decreased with the number of years since quitting (3.22 after 2 years and 1.15 after 11 years).

Animal studies provide a basis of support for the evidence from human studies, since they permit the control of confounders such as behavioral and systemic factors that may also influence periodontal disease progression. Nociti et al. (2000) [122] showed, using a rat model, that nicotine administration associated with plaque infection increased the rate of periodontal loss. Subsequently, the authors, aiming to answer the question as to whether nicotine concentration could be critical in promoting a dose-dependent response, evaluated the effect of daily administration of high doses of nicotine on the bone loss rate in the furcation region of rats by histometric analysis [123]. Nicotine concentrations administered in this study were intended to reproduce the highest nicotine concentrations found in commercially available cigarette brands. The data suggested that nicotine was able to heighten the rate of bone loss in a dose-dependent manner in ligated and non-ligated teeth. However, nicotine is just one of the 2000-3000 potentially toxic substances in tobacco smoke, which presents a complex mixture of substances such as acrolein, acetaldehyde, carbon monoxide and hydrogen cyanide [158]. Therefore, in order to investigate the influence of cigarette smoke as a whole, the researchers used a cigarette smoke exposure chamber, an acrylic device where the animals were forced to breathe the cigarette smoke-contaminated air [26]. In this study, the authors first demonstrated that cigarette smoke inhalation significantly increased bone loss resulting from ligature-induced periodontitis. Furthermore, data analysis demonstrated that the cessation of cigarette smoke inhalation might positively affect the rate of bone loss resulting from periodontitis [27]. The results of these preclinical studies have thus reinforced previous clinical studies, minimizing possible confounding factors that may exist in human studies.

The mechanisms by which cigarette smoking influences the initiation and progression of periodontitis are not fully understood. It seems that tobacco smoke may affect both the composition of the microflora and the host response. Regarding microflora, while several investigators have reported no significant differences in the incidence and distribution of periodontal pathogens in the plaque biofilm of smokers [46], other studies have demonstrated significant differences in the recovery rates of periodontal pathogens in smokers [191]. Of particular interest are recent studies which demonstrate a high recovery rate of periodontal pathogens in shallow periodontal pockets and on oral mucous membrane [51, 69]. In addition,

a smaller reduction in periodontal pathogens was reported in smokers than in nonsmokers, following scaling and root planning [181]. These studies points to the role of smoking in altering the load environment of the shallower pockets, thereby promoting the growth of these microbial species, as well as possible alterations in the host response that would allow for the growth of these specific microorganisms.

The influence of tobacco smoke on host response may occur in two areas: the periodontal pocket and the tissue. The first host response events occur in the periodontal pocket; it appears that cigarette smoking may tip the balance even further away from the protective functions of neutrophils and antibodies in the periodontal pockets and towards greater destructive activity [158]. For example, several studies have demonstrated reduced imunoglobulin G and imunoglobulin A levels in smokers versus nonsmokers [58, 146]. Furthermore, the effects of smoking on neutrophil function have demonstrated impaired phagocytosis, chemotaxis in neutrophils exposed to acute levels of tobacco smoke [35, 105] and increases in the release of potentially destructive oxidative products, such as superoxide and hydrogen peroxide [156]. The next stage of pathogenesis occurs when the bacterial plaque biofilm has overwhelmed the host defenses in the periodontal pocket and the bacterial products penetrate the underlying soft tissues. Here, the balance between protection and destruction is mediated largely by the type of cytokine pattern secreted by monocyte cell population. The preponderance of evidence has suggested that smoking will tip the balance toward a more inflammatory/destructive profile. For example, in vitro studies have demonstrated high secreted levels of interleukin-1β in isolated mononuclear blood cells when exposed to in vitro smoke [157]. In addition, nicotine, whether or not in association with lipopolysaccharide from periodontopathogenic bacteria, has been shown to increase interleukin-6 and interleukin-8 production by human gingival fibroblasts [185]. In vivo, César-Neto et al. (2005) [27] indicated that smoking modulation of bone destruction in periodontal disease may involve reduced levels of anti-inflammatory and anti-resorptive factors, such as interleukin-10 and osteoprotegerin, respectively, and may also involve high levels of pro-inflammatory cytokines, such as interleukin-6. However, in clinical studies, the results of the effects of tobacco smoke on inflammatory components have been inconclusive.

Emotional Stress

Stress is a state of physiological and psychological strain caused by adverse physical, mental, or emotional, internal or external stimuli that tend to disturb the functioning of an organism and which the organism naturally desires to avoid [45]. Whether or not a subject exhibits a stress response depends on a myriad of factors, including coping behaviors, genetic predisposition, concomitant stressors, levels of social support, and other lifestyle factors. Stress is compatible with good health, which is necessary to cope with the challenges of everyday life. Problems start when the stress response is inappropriate to the size of the challenge, producing neuroendocrine and biochemical changes that result in significant adverse effects on the proper functioning of the immune system [38, 151]. Potential effects of the stress response that may be observed, or even measured, include anxiety, depression, impaired cognition and altered self-steem. Stressful stimuli can induce a set of reactions that produce effects on virtually all body systems [20]. Exposure to stress may affect the host immune response, making the individual more susceptible to the development of unhealthy conditions that damage periodontal health [137].

The most documented association between stress and periodontal disease is the one between acute forms of necrotizing gingivitis and periodontitis. An increased incidence of these conditions has been amply documented in military personnel during stressful activities and in students during examination periods [62, 67]. The association between stress and chronic periodontitis has been investigated. Wimmer et al. (2002) [188] conducted a retrospective case-control study of 89 patients with different forms of chronic periodontitis undergoing treatment. All participants completed a stress coping questionnaire, which served as a psychodiagnostic survey aimed at collecting data on stress coping strategies. The results showed that periodontitis patients with inadequate stress behavior strategies (defensive coping) are at greater risk for severe periodontitis. Later, the researchers discovered, by means of a 24-month prospective clinical trial, that passive coping strategies were more pronounced in advanced disease, as well as in cases of poor response to nonsurgical periodontal treatment. Patients with active coping modes had a milder form of the disease and a more favorable course of treatment [189]. A systematic review of case-control, cross-sectional and prospective studies examining psychologic factors, such as stress and depression and periodontal disease indicated that 57.1% of the studies reported a positive correlation between stress or other psychologic factors and periodontal disease, and that 14.2% did not [137]. In addition, a subsequent study confirmed the association between stress and depression and periodontal destruction [155]. The weight of evidence therefore seems to suggest an association between stress and periodontal health.

The biologic plausibility of such an association is not as yet completely elucidated. Stress can result in the dysregulation of the immune system, mediated primarily through the hypothalamic-pituitary-adrenal axis. Activation of hypothalamic-pituitary-adrenal axis by stress results in the release of an increased concentration of corticotrophin-releasing hormone from the hypothalamus. Corticotrophin-releasing hormone, in turn, acts on the anterior pituitary, resulting in the release of adrenocorticotrophic hormone (corticotrophin). The adrenocorticotrophic hormone then acts on the adrenal cortex and causes the production and release of glucocorticoid hormones (predominantly cortisol) into the circulation. The glucocorticoids then produce a response, modifying cytokine profiles, elevating blood glucose levels, and altering levels of certain growth factors [116]. The second major pathway to be activated is the sympathetic nervous system. Stress activates the nerve fibers of the autonomic nervous system, which innervate the tissues of the immune system. The nerve bodies secrete their products (cathecolamines) directly into the bloodstream. The release of catecholamines results in the hormonal secretion of norepinephrine and epinephrine from the adrenal medulla, which results in a range of effects that may act to modulate immune responses [116]. Eventually, the impact of stress on periodontal disease may be modulated by health-impairing behaviors that include neglecting oral hygiene practices, increased consumption of cigarettes and alcohol, disturbed sleeping patterns and bruxing [116].

Animal studies reinforce the hypothesis of a relationship between stress and periodontal disease by means of the influence of stress on the immune system via nervous and neuroendocrine because the model makes it possible to exclude the impact of various behavioral changes, such as smoking and less effective oral hygiene. Takada et al. (2004) [171] demonstrated that stress modulated the progression of periodontal inflammation and increased alveolar bone loss. More recently, Peruzzo et al. (2008) [138] showed, on the basis of the same rat model of restraint stress, that chronic stress increased bone loss resulting from

a ligature-induced periodontitis by a local increase in proinflammatory and proresorptive factors.

Based on the evidence described above, although further well-controlled prospective clinical trials are still required to definitively define stress as a risk factor for periodontitis, most studies point to the association between stress and periodontal disease. Stress management, therefore, may be a valuable component for current periodontal practice.

SYSTEMIC FACTORS

Diabetes Mellitus

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood [111]. It is a highly prevalent metabolic disorder; with 150 million cases estimated worldwide, which constitutes a global public health burden [142]. Diabetes is divided into two main forms: type 1 diabetes mellitus (formerly insulin-dependent diabetes mellitus) and type 2 diabetes mellitus (formerly non-insulin-dependent diabetes mellitus). Type 1 diabetes is caused by the immune-mediated destruction of the insulin-producing pancreatic β cells and accounts for 10% to 15% of all cases of diabetes. The more common form, type 2 diabetes, results from a combination of impaired insulin production and insulin resistance. Both forms of the disease are associated with a range of complications that increase the morbidity and mortality of affected individuals [142].

Periodontal disease has been called the sixth complication of diabetes, a view supported by several reviews which conclude that the bulk of evidence indicates there is a direct relationship between diabetes mellitus and periodontal diseases [101]. The presence of diabetes mellitus is often associated with increased gingival inflammation. Karjalainen & Knuuttila (1996) [83] observed that poorly controlled diabetes mellitus in children had higher levels of gingival inflammation than did well-controlled patients, regardless of plaque levels. Moreover, gingival bleeding significantly decreased after two weeks of insulin treatment of newly diagnosed type 1 diabetic children and adolescents. Recently, Dakovic & Pavlovic (2008) [40] confirmed that gingival inflammation is more evident in children and adolescents with type I diabetes mellitus than in healthy ones. An increased risk of periodontitis for individuals with diabetes has also been documented in several studies. The relation between diabetes and periodontal health status was first determined in a population of Pima Indians, where subjects with type 2 diabetes have an approximately three-fold increased risk of attachment loss [53]. Moreover, in a 2-year longitudinal study of the Pima Indian population, Taylor et al. (1998) [173] found that individuals with type 2 diabetes had an increased risk of progressive alveolar bone loss compared with non-diabetic subjects. The study also showed that the level of metabolic control had a significant effect on disease progression. Increased risk for progressive attachment and bone loss in poorly controlled diabetic patients have been confirmed in a meta-analysis of studies in various populations [134] and in more recent studies such as that conducted by Novak et al. (2008) [124].

Disease progression following periodontal treatment may also be related to metabolic control. Tervonen & Karjalainen (1997) [175] found that a group of type 1 diabetics with poor metabolic control had a significantly greater recurrence of deep probing depths 12

months after treatment than subjects with good or moderate diabetic control and non-diabetic controls. However, metabolically well-controlled diabetics responded to non-surgical and surgical periodontal therapy in a manner similar to that in which healthy controls responded [30, 186].

Many potential mechanisms have been studied by which diabetes could affect the periodontium. There are few differences in the subgingival microbiota between diabetic and nondiabetic patients with periodontitis [161, 162]. This suggests that alterations in the host immunoinflammatory response to potential pathogens may play a predominant role. Diabetes may result in impairment of neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate the persistence of bacteria in the periodontal pocket and significantly increase periodontal destruction [108, 110]. While neutrophils are often hypofunctional in diabetes, these patients may have a hyper-responsive monocyte/macrophage phenotype, resulting in a significantly increased production of pro-inflammatory cytokines and mediators [159, 160]. This hyperinflammatory response results in high levels of pro-inflammatory cytokines in the gingival crevice fluid. In addition, high glucose concentrations induce non-enzymatic glycation and oxidation proteins, such as collagen and lipids, resulting in the accumulation of advanced glycation end-products (AGEs) in diabetic tissues, including periodontal tissues. The AGEs, through their receptors (RAGEs), may also induce the expression of pro-inflammatory cytokines. The elevated pro-inflammatory cytokines in the periodontal environment may play a role in the increased periodontal destruction seen in many people with diabetes [111]. For example, Duarte et al. (2007) [50] showed an overexpression of interleukin-1β and interleukin-6, potent pro-inflammatory cytokines, in gingival tissues of diabetic patients diagnosed with chronic periodontitis [111].

In conclusion, studies indicate that diabetics with poor glycaemic control have an increased risk of periodontitis and disease progression.

Osteoporosis

Osteopenia and osteoporosis are systemic skeletal diseases characterized by low bone mass and micro-architectural deterioration with a consequent increase in bone fragility and susceptibility to fracture. According to the World Health Organization, osteoporosis is considered to be present when mineral density is 2.5 standard deviation (SD) or more below the mean for normal young Caucasian women. Further, osteopenia is defined as a bone density level between 1 and 2.5 SD below normal bone density [82]. Both osteopenia and osteoporosis are grave public health concerns, particularly associated with estrogen deficiency among postmenopausal women. The risk factors for osteoporosis include many risk factors associated with advanced periodontal disease [61]. Since both osteoporosis and periodontal diseases are bone resorptive diseases, it has been hypothesized that osteoporosis could be a risk factor for the progression of periodontal disease.

The effects of osteoporosis induced by an estrogen-deficient state have been widely studied in a rat model. Bilateral ovariectomies of female rats were able to induce this condition. Tanaka et al. (2002) [172] histomorphometrically investigated the alveolar bone following estrogen deficiency and showed osteoporotic changes and thin alveolar bone proper in the interradicular septum of the first molar of ovariectomized rats. Later, Duarte et al. (2006) [49] confirmed that an estrogen-deficient state may negatively affect the tooth-supporting alveolar bone, resulting in a lower density of alveolar bone than that observed in

estrogen-sufficient animals. It has also been shown that an estrogen-deficient state may significantly increase bone loss resulting from ligature-induced periodontitis and also at healthy sites [47, 48].

There have been a number of reports on the mechanisms involved in the estrogen regulation of bone metabolism. Since estrogen receptors in osteoblasts and osteoclasts were discovered [54, 128], it is believed that estrogen has a direct skeletal effect. It has also been shown that estrogen has an important role in controlling bone resorption through its action on osteoprotegerin (OPG) and the receptor activator of nuclear factor kB ligand (RANKL) mechanism [94, 189], as well as on bone-regulating factors such as interleukin-1, interleukin-6 and tumor necrosis factor [129, 63].

Animal studies have established a clear association between osteoporosis and oral bone density or osteoporosis and periodontitis-induced bone loss. In humans, the data gathered on the mostly cross-sectional studies appears to confirm a relationship between systemic and oral bone mineral density. For example, in a classic series of studies, Kribbs et al. (1983, 1989, 1990) [87, 88, 89] addressed this relationship in both normal and osteoporotic women. Although the technology used in those studies reflects the time at which the studies were carried out, they indicated an association between oral and systemic bone. More recent studies have included larger numbers of women with a wide range of bone mineral density in systemic bone. Wactawski-Wende et al. (1996) [183] showed positive correlations between alveolar bone loss and bone mineral density at the spine, trochanter, Ward's triangle or total femur. Further, cross-sectional data from 468 postmenopausal females enrolled in the oral ancillary portion of the Women's Health Initiative study revealed a significant correlation between basal bone density determined from intraoral radiographs and hip bone mineral density determined by DXA [79].

On the other hand, while some studies indicate osteoporosis as a risk indicator for periodontitis [153, 176], others have not detected a significant association [187]. Moreover, there is only a limited number of longitudinal studies evaluating the association of osteoporosis and periodontitis progression. Reinhardt et al. (1999) [147] prospectively analyzed the influence of serum estradiol levels and osteopenia / osteoporosis on common clinical measurements of periodontal disease over a 2-year period. No significant differences were found in attachment loss between osteoporotic and non-osteoporotic patients, although the authors reported a trend towards more attachment loss in non-smoking osteoporotic patients. In contrast, in a recent longitudinal study of 184 individuals aged 70 years [190], bone mineral density was associated with the number of progressive sites which had ≥ 3 mm additional attachment loss over 3 years, suggesting a significant relationship between periodontal disease and general bone mineral density.

It may therefore be concluded that the relationship between osteoporosis and periodontitis remains unclear. Confounding factors such as age, gender or smoking and the lack of precise methods for the assessment of osteoporosis in the jaws have been reported to affect the establishment of a clear interaction between osteoporosis and periodontitis. Larger prospective longitudinal studies are needed to further evaluate osteoporosis as a risk factor for progressive periodontitis.

GENETIC FACTORS

While microbial and other environmental factors are believed to initiate and modulate periodontal disease progression, there now exists strong supporting evidence that genes play a role in the predisposition to and progression of periodontal diseases [70, 73]. Support for this statement comes from studies in animals and humans which indicate that genetic factors influence the inflammatory and immune response in general, and periodontitis experience specifically. Different forms of genes (allelic variants) can produce variations in tissue structure (innate immunity), antibody responses (adaptative immunity) and inflammatory mediators (non-specific inflammation) [84]. Thus, complex diseases such as periodontitis may have multiple gene associations which individually have weak effects but which collectively combine with other influences, such as environmental factors, and result in various disease manifestations [120].

The hypothesis that genetic factors account for variation in phenotype expression of periodontal disease has been formally tested by comparing disease characteristics in monozygous and dizygous twins. It is assumed in these experiments that because a given set of adult twins grew up together in the same environment there is reason to believe that they should share most relevant habits and practices. Thus the similarity of such factors as personal habits, lifestyles and access to health care should not be different for members of twin pairs whether they are monozygous or dizygous. Michalowicz et al. (1991) [113, 114], in studies of both monozygous and dizygous twins reared together and apart, showed a significant genetic component for probing depth, attachment loss and radiographic alveolar bone height, supporting the role of genetics in periodontal disease. In a recent study, Michalowicz et al. (2000) [115] found that monozygous twins were found to be more similar than dizygous ones for clinical measurements such as probing depth, attachment loss, plaque and gingivitis. A statistically significant genetic variance was found for both severity and extent of the disease. Based on this study, chronic periodontitis was estimated to have approximately 50% heritability, which was unaltered following adjustments for behavioral variables including smoking. However, while monozygous twins were also more similar than dizygous twins for gingivitis scores, there was no evidence of heritability for gingivitis after behavioral covariates such as utilization of dental care and smoking were incorporated into the analyses. In short, these studies indicate that approximately half of the variance in chronic periodontitis in the population is attributed to genetic variation. Thus the basis of heritability of periodontitis seems to be biological and not behavioral.

Interest in identifying genetic risk factors for chronic periodontal diseases has been spurred by recent reports of associations with polymorphisms. Gene polymorphisms are locations within the genome that vary in sequence between individuals and are very prevalent, affecting at least 1% of the population. The rationale for studying single gene nucleotide polymorphisms is that they can be used to identify potential markers of susceptibility, severity and clinical outcome [84]. Various aspects of the host inflammatory response have attracted attention as potentially crucial variants influencing the host response in periodontitis.

Cytokines

Specific genotypes have been identified and linked to periodontal destruction. Polymorphisms of interleukin-1 (IL-1), IL-1β and IL-1RN genotypes have been identified as potential risk factors for periodontal destruction. In 1997, Kornman et al. [86] were the first to describe an association between polymorphisms and periodontal disease, creating a great interest in the topic. They found an association between polymorphisms in the gene encoding for IL-1α (-889) and IL-1β (+3953) and an increased severity of periodontitis. Functionally, IL-1 genotype is associated with high levels of IL-1 production [141]. A role has been suggested for IL-1 in the initiation and progression of periodontitis. IL-1 may activate the degradation of the extracellular matrix and bone of the periodontal tissues, and increased tissue or gingival fluid levels of IL-1β have been associated with periodontitis [84]. Moreover, it was found that the mean counts of specific bacteria species were higher in IL-1 genotype-positive individuals than in negative subjects [167]. Several studies have corroborated the association between IL-1 polymorphism and periodontal disease or tooth loss [39, 109, 184]. Furthermore, a recent systematic review and meta-analysis established a statistically significant association of IL-1A (-889) and IL-1B (+3953) polymorphisms with chronic periodontal disease [121]. However, Huynh-Ba et al. (2007) [75] in a previous systematic review suggested that there is insufficient evidence to establish whether a positive IL-1 genotype status contributes to the progression of periodontitis and/or treatment outcomes. The data thus remain inconclusive, and longitudinal studies are required to establish the extent to which this genetic factor plays a role in disease progression.

The polymorphism of tumor necrosis factor-α (TNF-α) has also been suggested as a possible risk factor for periodontitis. TNF-α is secreted as a response to bacterial stimulation by a variety of cell types [173]. It stimulates osteoclasts differentiation and together with IL-1 may result in bone resorption [103]. Furthermore, TNF-α promotes the release of collagenases (metalloproteinase) that destroy the extracellular matrix [21] and are produced in excessive amounts in inflamed periodontal tissues [66]. However, most studies have failed to link this polymorphism of the TNF-α gene to chronic periodontitis [36, 59, 121].

Human Leukocyte Antigens

Several investigations have studied populations of patients with different forms of periodontitis to determine the expression of polymorphisms of human leukocyte antigens (HLA). The HLA complex plays an important role in immune responsiveness and may be involved in antigen recognition of periodontal pathogens. Recognition of antigen peptides and their presentation to T cells is crucial for an effective antigen-specific immune response to periodontal pathogens and underlies genetic control. Because antigen presentation to and resultant activation of T cells is restricted by the major histocompatibility complex (MHC), the polymorphism of the human MHC molecules (human leukocyte antigens – HLA) may directly affect the binding capability of antigen peptides and thus the antigen-specific T-cell response [192]. A recent systematic review did not reveal any significant positive or negative associations [170]. However, few studies are available and those present significant limitations, such as the control group not always being healthy. On the other hand, when aggressive periodontitis was evaluated, an association with particular HLA polymorphisms was observed. Therefore, more studies are needed before definite conclusions can be drawn.

Immuno-Receptors

The association of immuno-receptors with periodontitis has been studied. Receptors for Fc domain of IgG FCγR) are categorized as a family of receptors, expressed on the cell surface of leukocytes, which bind IgG antibodies and immune complexes [102]. In humans, FCγRs are expressed on natural killer cells, macrophages, T lymphocytes, monocytes and mast cells [65]. The interaction between FCγRs and IgG triggers a variety of biological responses, including phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation [84]. Polymorphisms that influence the binding affinity between FCγR and IgG of different subclasses are considered important in susceptibility to periodontal diseases. The few existing studies of chronic periodontitis have investigated associations between FCγR polymorphisms and susceptibility to and severity of periodontitis. The majority of them indicate that polymorphisms of FCγR tend to be associated with the chronic form of peridontitis [31, 85, 112].

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are one of the most important groups of enzymes involved in periodontal connective tissue destruction [169]. Although few studies have suggested an association between MMP gene polymorphisms and chronic periodontitis [140, 169], there is strong controversial evidence for such an association. Itagaki et al. (2004) [140] reported that MMP-1 and / or MMP-3 single nucleotide polymorphisms were not associated with susceptibility to periodontitis in a Japanese population. More recently, polymorphisms in the gene for MMP-2 were studied and no definite correlation with periodontitis could be found [74]. Repeke et al. (2009) [150] observed a limited role for MMP-1 polymorphism in periodontitis. It seems that the extensive chronic antigenic challenge exposure overcomes the genetic control and plays a major role in the determination of MMP-1 expression. Therefore, due to the limited number of studies carried out to date, it is difficult to associate single nucleotide polymorphisms of MMP genes with chronic periodontitis.

Reports on the genetic polymorphisms associated with chronic periodontal diseases are increasing, encouraging the search for new specific markers by researchers, but the limitations of such studies have not been fully appreciated. For example, in nearly all the published studies, subjects have not been characterized as to behavioral risk factors such as smoking, stress or others. In addition, the heterogeneity of the diseases examined and the ethnic aspects of the distribution of the genetic markers may contribute to the disparity of the results [77]. In conclusion, some gene polymorphisms are associated with modest increases in the probability of periodontal disease developing. Further studies on the distribution and dynamics of genetic variation at many loci simultaneously might disclose the direct and epistatic (interaction among multiple alleles) genetic involvement in periodontitis.

MICROBIAL COMPOSITION

While periodontal disease is regarded as an opportunistic mixed microbial infection, specific periodontal pathogens have been proposed as predictors for further disease progression [72]. Although there are over 500 different intra-oral species and other that have not yet been identified, the majority of studies have focused on a subset of microorganisms including Agreggatibacter actinomycetencomitans (A.a.), Porphyromonas gingivalis (P.g.) and Tannerella forsythia (T.f.) [55, 126], presumably because they satisfy the criteria for Socransky's modifications of Koch's postulates:

- the organism must occur at higher numbers in disease-active sites than disease-inactive sites;
- elimination of the organism should arrest disease progression;
- the organism should elicit a humoral or cellular immune response;
- animal pathogenicity testing should infer disease potential;
- the organism should possess virulence factors relevant to the disease process;

Regarding the virulence factors, A.a., P.g. and T.f. share three common features that support their role as risk factors for initiation and progression of periodontitis. First, all are Gram-negative, and therefore produce lipopolyssacharide, which can modulate the local inflammatory response in host cells that express pattern recognition receptors. Moreover, all appear capable of invasion of the mucosal barrier to infection and possibly of being sequestered inside epithelial cells. And finally, all produce factors that enable them to evade the antibacterial functions of the innate immune response either passively (anti-phagocytic capsule) or actively (leukotoxin, gingipains, proteases, induction of apoptosis) [55].

However, evaluation of these three pathogens as risk factors for attachment loss over time has resulted in conflicting evidence. Some studies do not support the detection of these specific bacterial species for the identification of individuals at risk for periodontitis progression [98, 104]. On the other hand, a number of longitudinal studies have shown that the presence of high levels of these species at baseline is a prognostic indicator for disease progression [68, 106, 177]. Individually, A.a. has been implicated only in some cases of chronic periodontitis [24, 152, 177]. Its association has been most clearly demonstrated with localized aggressive periodontitis [71]. On the other hand, the importance of P.g. and T.f. in the initiation of chronic periodontitis as well as in its progression to advanced periodontitis is more clearly established in longitudinal studies [106, 180]. Further evidence suggests that B.f. and presumably P.g. are also associated with disease recurrence when patients are followed up after therapy [29].

Although this review has focused on the three bacterial species considered most likely to initiate the events resulting in chronic periodontitis, there are several other microorganisms that have been described as moderately associated with the disease. These species include Campylobacter rectus, Eubacterium nodatum, Fusobacterium nucleatum, Prevotella intermedia, Peptostreptococcus micros, Streptococcus intermedius-complex and Treponema denticola [127].

The evidence for the prognostic value of A.a., P.g. and T.f. remains inconclusive, and the role of the other pathogenic bacteria has likewise yet to be fully appreciated. Such evidences,

however, does lead us to believe that certain bacteria like P.g. and T.f. are indeed more important than others when it comes to considering risk indicators of chronic periodontitis.

Tooth-Level Factors

Individual variation in susceptibility to disease progression may be related to a number of a local clinical factors including tooth position [2], caries and defective restoration margins [3, 22], subgingival restoration margins [163], abutment tooth [145], presence of calculus [119], occlusal discrepancies [125], unsatisfactory root form [109] or root grooves [93].

A number of periodontal parameters have also been shown to influence periodontitis progression: gingivitis/bleeding on probing [43, 92], probing depth [13, 33], alveolar bone loss [145], tooth mobility [56], furcation involvement [41] and tooth type [118].

In particular, bleeding on probing, pocket depth and radiographic alveolar bone loss are considered to be of great importance by the clinicians for decision making [136]. But do these factors really predict future attachment loss?

Current theory holds that the gingival lesion is the precursor of periodontitis. Clearly, not all gingivitis lesions progress to periodontitis. It has been suggested that individuals are at lower risk for disease progression if the prevalence of bleeding on probing at a subject level is ≤ 25% [81]. However, the proportion of gingival lesions progressing to periodontitis and the factors causing this conversion have not yet been sufficiently clarified. Periodontitis and mean attachment loss have been positively associated with bleeding on probing [43]. Recently, a longitudinal study of a patient cohort of 565 males was performed over a 26-year period. Sites with consistent bleeding had 70% more attachment loss than sites that were consistently non-inflamed. Moreover, teeth with sites that were consistently non-inflamed had a 50-year survival rate of 99.5%, while teeth with consistently inflamed gingivae yielded a 50-year survival rate of 63.4% [92].

Regarding pocket depth, on a site basis, the presence of deep residual pockets has been associated with disease progression [13, 33]. A systematic review addressing the use of residual pocket depth, bleeding on probing and furcation status following initial periodontal therapy to predict further attachment and tooth loss found that, at the individual level, residual pocket depth was predictive of further disease progression [149].

Furthermore, longitudinal studies of periodontal disease have shown that the amount of alveolar bone loss present at baseline, which represents the patient's previous history of periodontitis, may be also used to predict further progression of untreated and treated periodontitis [56, 133, 145].

Despite the importance of clinical findings on the progression of periodontal disease, treatment planning based only on the assessment of disease severity rather than other documented risk factors such as environmental and systemic factors leaves much to be desired [136].

MULTIFACTORIAL RISK ASSESSMENT MODELS

The management of periodontal disease patients is used to be based on a "repair" model of care, in which clinician's goal was to diagnose the problem and resolve it via treatment. In recent years, however, an increasing understanding of the aetiology and risk factors for chronic periodontal diseases has developed. As a result, their management is undergoing a

transition from a repair model to the wellness model of patient care that guides the clinician toward a health care strategy based on risk reduction and disease prevention [130]. Rather than the mere application of the knowledge of the risk factors to maintain oral health and to prevent the onset of periodontal disease, attention has been drawn to the assessment of risk level for disease progression in individuals under supportive periodontal therapy, representing a population with a moderate to high level of tisk of periodontal breakdown has attracted attention. The assessment of risk level for disease progression in each individual patient would enable the practitioner to determine the frequency and extent of professional support necessary to maintain the attachment levels obtained following active therapy [91]. Moreover, the clinician often has to decide which teeth to retain, which treatment to prescribe, or how to maintain or restore a functional and aesthetically pleasing dentition. For decision making at a tooth level, it is of paramount importance to assess prognosis of each tooth in order to choose the treatment modality with the greatest probability of success [56].

Thus, as the study of prognostic factors has progressed, multi-factorial risk assessment models has been proposed using the combination of these factors to identify individuals and teeth at high risk for periodontitis progression [56, 91, 130, 136, 149].

Periodontal Risk Calculator (PRC) (Page et al., 2002)

Page et al. (2002) [130] developed a computer-based tool, the periodontal risk calculator (PRC), for assessing risk and predicting periodontal deterioration. The PRC is based on a mathematically derived algorithms that assign relative weights to various known risks that increase patients' susceptibility to develop periodontitis: patient age, smoking history, diabetes diagnosis, history of periodontal surgery, pocket depth, bleeding on probing, restorations below the gingival margin, root calculus, radiographic bone height, furcation involvements and vertical bone lesions. The aim of the PRC is to be user-friendly and to require only information that is gathered during a routine periodontal examination. The PRC determines the patient's level of risk on a scale from 1 (lowest) to 5 (highest). However, the details of the algorithm and weighting for the factors have not been published.

Page et al. (2003) [131] documented the extent of agreement between risk scores calculated using the PRC and information gathered during a baseline examination with the periodontal status 3, 9 and 15 years later. In a retrospective study, clinical records and radiographs of 523 men were used. Information from baseline examinations was entered into the risk calculator and a risk score on a scale of 1-5 for periodontal deterioration was calculated for each subject. Actual periodontal status in terms of alveolar bone loss determined using digital radiographs, and tooth loss determined from the clinical records, was assessed at 3, 9, and 15 years. The risk scores at baseline were found to be strong predictors of future periodontal status measured as worsening severity and extent of alveolar bone loss and tooth loss, especially loss of periodontally affected teeth. The authors concluded that risk scores calculated using the PRC and information gathered during a standard periodontal examination predict future periodontal status with a high level of accuracy and validity.

Periodontal Risk Assessment (PRA) (Lang & Tonetti, 2003)

Lang & Tonetti (2003) [91] constructed a functional diagram to assess patient's risk of recurrence of periodontitis based on a number of risk factors and risk indicators evaluated simultaneously. The PRA model consists of an assessment of the proportion of bleeding on

probing, the prevalence of residual pockets greater than 4 mm (≥ 5 mm), the tooth loss from a total of 28 teeth, the loss of periodontal support (proportion of sites with bleeding on probing) in relation to the patient's age, the systemic and genetic condition (e.g. diabetes mellitus and polymorphism of interleukin-1, respectively), and environmental factors, such as cigarette smoking. Each parameter has its own scale for minor, moderate and high risk profiles (Figure 1).

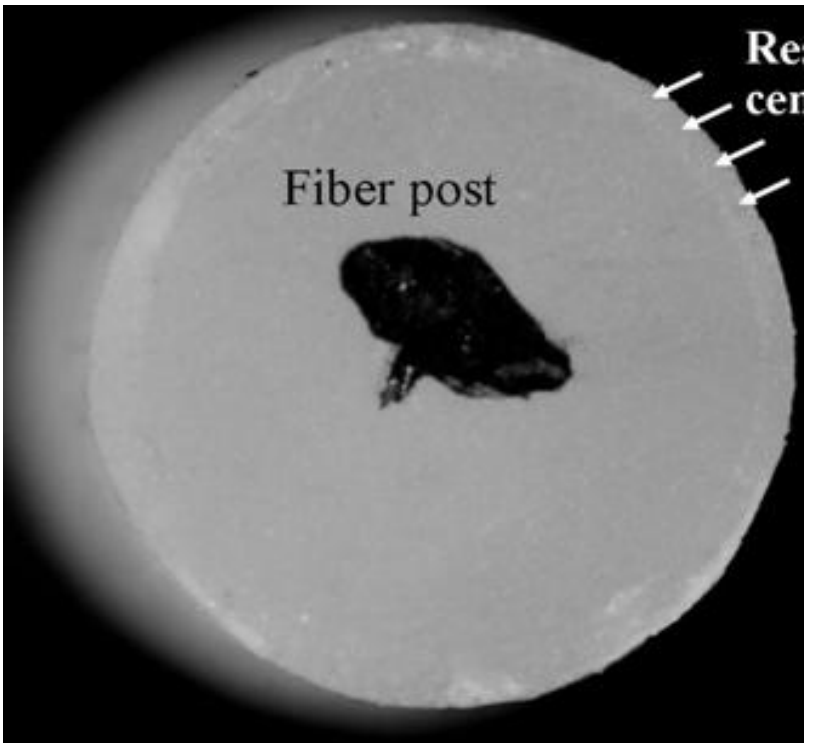

Figure 1. Schematic illustration representing a periodontal risk assessment functional diagram. Each vector represents a single risk factor or indicator. The area of low risk is found within the centre circle of the polygon, while the area of high risk lies outside the periphery of the second ring in bold. Between the two rings in bold is the area of moderate risk (Lang & Tonetti, 2003).

The authors provided evidence supporting the inclusion of each parameter within the diagram. The hexagonal risk diagram identified patients at low risk (all parameters within the low risk categories or, at the most, one parameter in the moderate) and those at moderate (at least two parameters in the moderate category, but at most one parameter in the high risk) and high risk (at least two parameters in the high risk category). Thus, a comprehensive evaluation of the functional diagram would provide an individualized total risk profile and determine the frequency and complexity of supportive periodontal therapy visits. However, this model was not validated and little evidence on its applicability is available. In a retrospective study including 100 patients who had received active treatment, Eickholz et al. (2008) [52] were the first to provide evidence that patients assigned to the high risk group according to the Lang & Tonetti risk assessment suffered from a higher rate of tooth loss after a 10-year follow-up than the other risk groups.

PRA / Multifactorial Risk Diagram (Renvert & Persson, 2004)

In this multifactorial risk diagram, a modification of the PRA model is described where the vector bone loss index (bone loss in relation to subject's age) is replaced by the proportion of sites with a distance ≥ 4 mm from the cementoenamel junction to the bone level [149]. The individuals were not more categorized as low, moderate or high risk. Here, the surface outlined between the various risk parameters was calculated to provide a numerical score of risk with the aid of a computer program (EXCEL XP for PC, Redmond, WA, USA). The authors suggested that in this way the risk scores can be monitored and compared over time, enabling the clinician to adjust the supportive therapy strategy as appropriate.

Prognostic Model for Tooth Survival (Faggion et al., 2007)

Faggion et al. (2007) [56] developed a prognostic model to estimate quantitatively survival rates for teeth in patients receiving treatment for periodontitis, in order to make evidence-based decisions about retaining or extracting teeth. With the aim of constructing the prognostic model, one hundred and ninety-eight patients were included in a retrospective study. At baseline, medical history (diabetes mellitus, coronary heart diseases, infectious diseases, allergies, coagulation disorders and radiation in the head and neck regions), clinical findings (teeth present, caries, dental restorations, probing depth, tooth mobility, approximal plaque index, sulcus bleeding test and tooth vitality) and full-mouth radiographs (alveolar bone level) were available. A logistic regression model revealed the following significant predictors for tooth loss during supportive periodontal therapy: a diagnosis of diabetes mellitus, the alveolar bone level, tooth mobility, root type and non-vital pulp at baseline examination. Based on these parameters, a prognostic model was constructed that provides estimates of tooth survival probability when periodontal therapy was performed (Figure 2). The authors showed that prognosis of tooth loss improved 14%, as compared with an alternative prognosis that did not consider any information provided by prognostic variables.

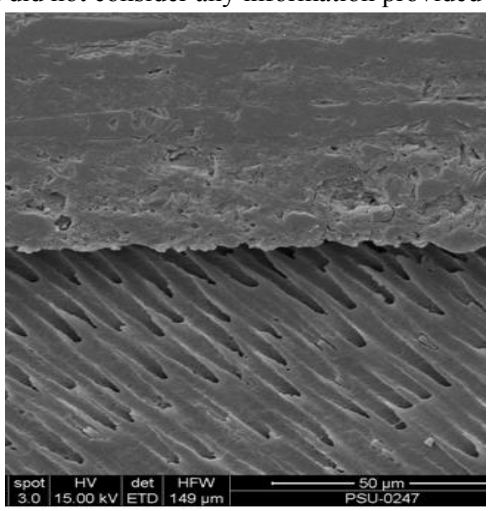

Figure 2. Schematic illustration representing a prognostic model for tooth survival. Each square represents a unique combination of predictors and the color coding on the bottom right indicates the likelihood of tooth survival probability (Faggion et al., 2007).

CONCLUSION

The above review clearly shows that chronic periodontal diseases are multifactorial disorders. Microbial dental plaque biofilm is the principal etiological factor, although several other local and systemic factors play an important modifying role in their pathogenesis. There is overwhelming evidence that both smoking and diabetes are important risk factors for periodontal tissue loss. In addition, the role of genetic factors and emotional stress has recently been highlighted. However, there is still a need for further studies to establish with great precision the contributions of other factors in the pathogenesis of these diseases.

Multifactorial risk models based on a knowledge of risk factors and risk indicators have been proposed to enhance the ability to predict risk for periodontal disease progression. However, prospective studies are virtually nonexistent to date. Moreover, few host-related factors are included in these models which may account for perhaps explain their limited improvement in predicting future disease events. Research in this field should be encouraged with the ultimate goal of helping the decision making during treatment planning and also to guide the clinician toward a strategy of risk reduction and disease prevention.

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Chapter 30

TREATMENT OF PERIODONTITIS

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RATIONALE OF PERIODONTAL TREATMENT

The goals of periodontal therapy according to the American Academy of Periodontology are to alter or get rid of the microbial etiology and causative risk factors for periodontitis, thus arresting the progression of disease and preserving the dentition in a state of health, comfort, and function with appropriate esthetics; and to prevent the recurrence of periodontitis. In addition, regeneration of the periodontal attachment apparatus, where indicated, may be attempted [1]. Mechanical debridement of the pocket has shown to significantly reduce the risk of tooth loss, slow down the rate of periodontal disease progression and improve gingival health [2,3].

After establishing a definite diagnosis of Periodontitis, a treatment plan is formulated initiated by Initial therapy. Also known as cause related therapy, initial therapy is aimed at controlling the etiologic agents for gingivitis and periodontitis and arresting further progression of periodontal tissue destruction. The Objectives of Initial therapy are: [4]

- Motivating the patient to understand and control dental disease.
- Instructions to the patient regarding self performed plaque control methods.
- Scaling and root planing.
- Removal of additional retention factors for plaque such as overhanging margins of restorations, ill fitting crowns, etc.

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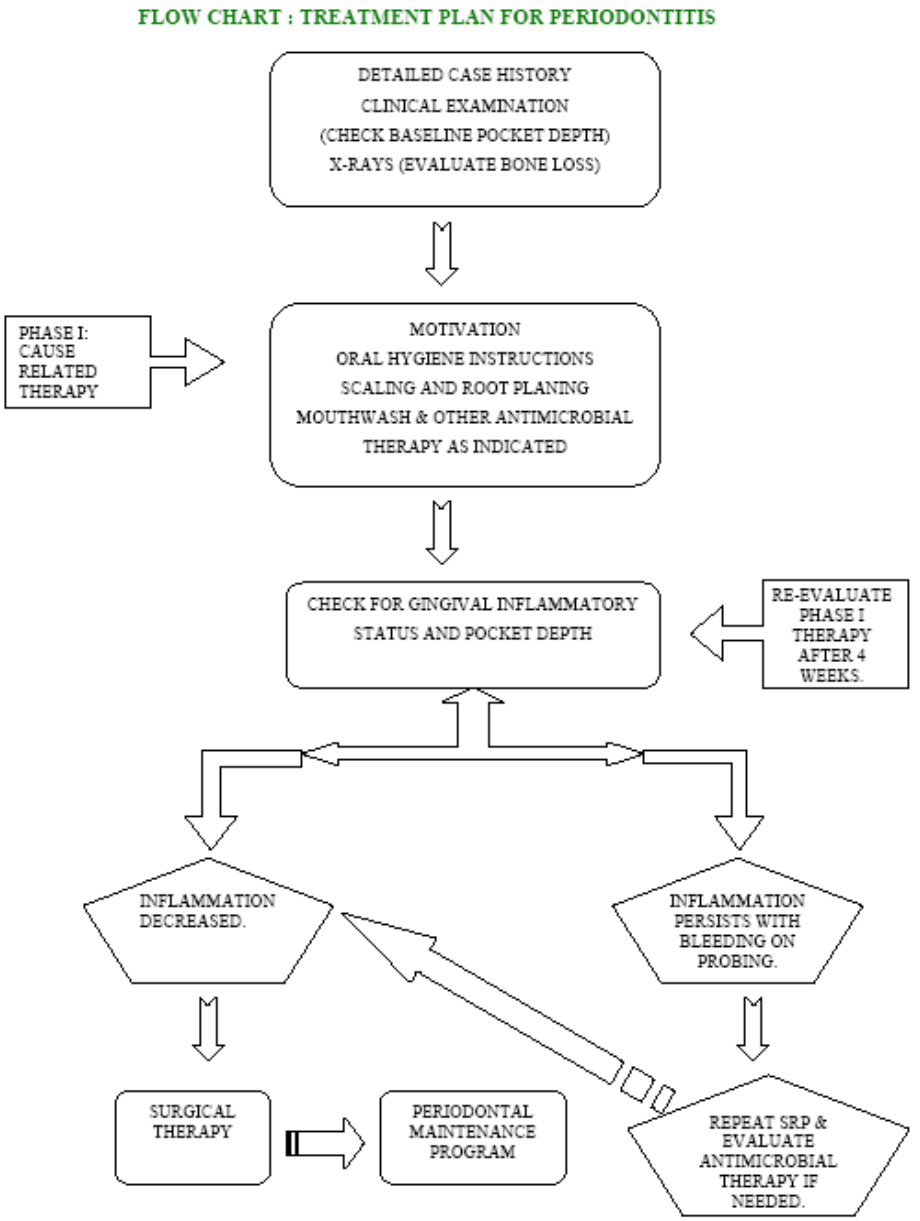
TREATMENT GUIDELINES

Certain treatment considerations have been laid down by American Academy of Periodontology (AAP) for treatment of chronic periodontitis with slight to moderate amount of bone loss. There are also certain factors that affect the decision of the treatment and the expected therapeutic result which includes age and systemic health of the patient, compliance, treatment preferences and patient's ability to control plaque. Other factors include the clinician's ability to remove subgingival deposits, restorative and prosthetic demands, and the presence and treatment of teeth with more advanced chronic periodontitis[1].



PATIENT MOTIVATION

It is the role of the dental health professional to assist patients to attain and maintain their oral health. Various effective ways are present to motivate people toward preventive dental care in general and toward preventive periodontics in particular. Each patient requires individually tailored oral health advice and information. One of the basic requirements in motivating a patient is communication between patient and the Periodontist. A well informed patient can be motivated easily and hence education and motivation goes hand in hand. Motivating patients for undergoing periodontal therapy is a task requiring considerable skills. This is because emergency care sells itself because the fear of pain and the need for self-preservation are active. The value of preventive measures is less substantial because time, effort and money must be expended to prevent possible future disease. Hence patients tend to neglect the detrimental effects caused by accumulation of plaque and calculus on the soft tissues as periodontal disease is quite painless in the initial, treatable stages and therefore, pain serves no great motivational purpose in causing people to act in a positive manner. Another factor is the lack of social pressure to have a plaque-free mouth[5]. Hence skilful communication with the patient educating him on periodontal maintenance and making him recognize the need for therapy is essential.

There are two procedures important for patient motivation [6,7]

1) Motivational interviewing
2) Stages of change model

Motivational interviewing involves a directive, patient centred counselling style that is compatible with the patient-centred clinical method. It encourages patients to speak and by doing so enables them to identify their oral health needs. The health professional acts as a medium only intervening when necessary thus allowing patients to recognise inner resistances reflected in lifestyle barriers. Although motivational interviewing was developed for use by addiction counsellors, some of its practical guidelines can be adapted for use in oral health settings. The Periodontist must take considerable time to explain to his patients regarding the importance of good periodontal health and bone support for survival of a sound tooth structure. Also the dentist should provide an atmosphere in which patients feel comfortable to speak, question and discuss the priority of their oral health needs. The dentist also has to evaluate the result of his counselling with the patient wherein the stages of change model can assist dental health professionals in their work with patients. It provides a framework by which they may evaluate their patients' progress from unawareness through motivation to compliance. The ‘stages of change model’ devised by Prochaska and DiClemente is divided into six different stages of behaviour change[8]. These are precontemplation, contemplation, preparation, action, maintenance and relapse. The stages reflect and hence provide a means of assessing progress from unawareness (precontemplation) through motivation (contemplation, preparation) to compliance (action, maintenance) [6]. Hence by using motivational interviewing and stages of change model, dentist can aid behavioural change in their patients and to achieve a long term goal of a stable dentition. Disclosing agents are used as motivational aids to educate patients to improve the efficiency of plaque control procedures. These agents are solutions or wafers which stain plaque on teeth and bacterial deposits on

tongue and gingiva. They are applied on to teeth using cotton swabs or solution is used as rinses. Hence disclosing agents are used as plaque control instruction in the dental office.

SCALING AND ROOTPLANING

SCALING: Is defined as the instrumentation of the crown and root surfaces of the teeth to remove plaque, calculus, and stains from these surfaces [9].

ROOTPLANING: A treatment procedure designed to remove cementum or surface dentin that is rough, impregnated with calculus, or contaminated with toxins or microorganisms[9].

Instruments used for Supra and Sub Gingival Scaling and Root Planning

Treatment process in a periodontitis patient is initiated by supragingival scaling. This removal of plaque and calculus is done using either hand instruments and or ultrasonic instruments. Hand instruments used for this purpose are Supragingival Scalers (Figure 1). Sickle scalers serve as an effective instrument to remove tenacious supragingival calculus from crowns of teeth and are used in a pull motion. The working end of it has unique design characteristics like a pointed tip with a triangular cross section and two cutting edges per working end. This shape makes the tip strong so that it will not break off during use. Both anterior and posterior sickle scalers are available [10,11]

Figure 1. Set of Supragingival Scalers. L to R: Sickle,bifid,Cumine,Surface & Posterior Interdental Scalers.

Instruments with slender working ends are needed for subgingival scaling like the curettes. Curettes are finer than sickle scalers and each working end has a cutting edge on both sides of the blade and a rounded toe without any sharp points or corners. They are used to remove deep subgingival calculus, root planing altered cementum and removing the soft tissue lining the periodontal pocket. Area specific curettes were developed by Dr.Clayton gracey and are popularly known as Gracey Curettes (developed by Hu-friedy manufacturing company) (Figure 2).

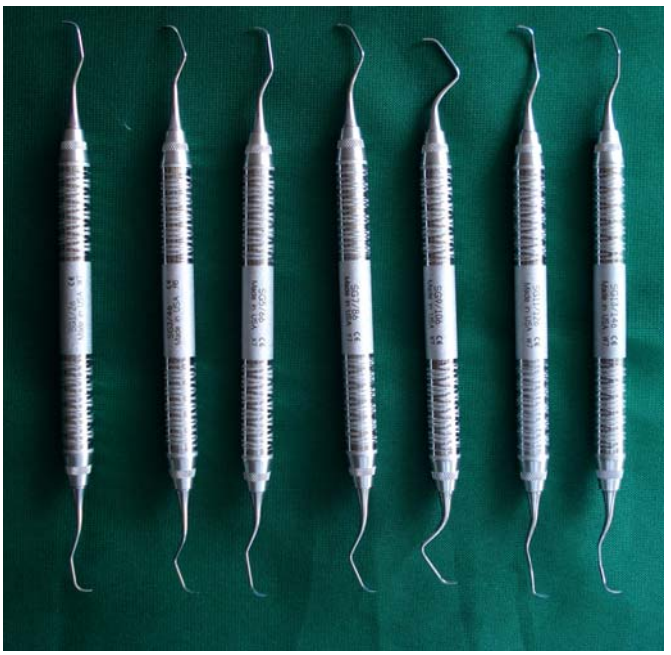


Figure 2. Set of Gracey Curettes. L to R: 1-2, 3-4, 5-6, 7-8, 9-10, 11-12 & 13-14.

Gracey Curettes are designed to adapt specific areas of dentition. Original Gracey series contains 14 single ended curettes, Gracey 1-14. Double ended Gracey curettes are paired as follows:

- Gracey 1-2 & 3-4: For Anterior teeth.
- Gracey 5-6 : For anterior teeth and Premolars.
- Gracey 7-8 & 9-10: For Posterior teeth (Facial and lingual).
- Gracey 11-12 : For Posterior teeth (Mesial).
- Gracey 13-14 : for Posterior teeth (Distal).
- Gracey 15-16 and 17-18 curettes are modifications developed to provide superior access to proximal surfaces of posterior teeth[12].

Ultrasonic and Sonic Instruments

Power driven scalers consists of Ultrasonic and sonic instruments. Sonic scalers operate at a low frequency of 3000 to 8000 cycles per second (cps) with a vibratory tip movement which is linear or elliptical in nature. Ultrasonic scalers are of two types namely Magnetostrictive and Piezoelectric working at a frequency range of 18000-45000 cps and 25000-50000 cps respectively. In magnetostrictive the vibration of the tip is elliptical, linear or circular depending on the type of unit. Here all the sides of the tip are active. In piezoelectric the vibration of the tip is linear or back and forth which allows only two sides of the tip to be active [13] (Figure 3). Removal of plaque and calculus by ultrasonic scalers is accomplished by the vibration of the tip of the instrument, acoustic streaming and cavitation effect. During operation, cooling water flows through the instrument handpiece onto the oscillating tip and the oscillating action of the tip within the water produces acoustic streaming. This causes a change in the streaming velocity to produce large hydrodynamic

shear stresses which can disrupt calculus. The water droplets of the spray directed at the tip forms tiny vacuum bubbles that collapse releasing energy in a process called as cavitation which serves to dislodge the calculus and debris [14,15]. One of the disadvantages of using ultrasonic scaler is the production of aerosols. Ultrasonic and sonic scaling is considered to produce the greatest source of aerosol contamination. This occurs due to interaction between the rapidly vibrating tip and the coolant liquid that comes in contact with the tip. The aerosols may contain infectious blood borne and air borne pathogens and is considered as a potential infection threat[16].

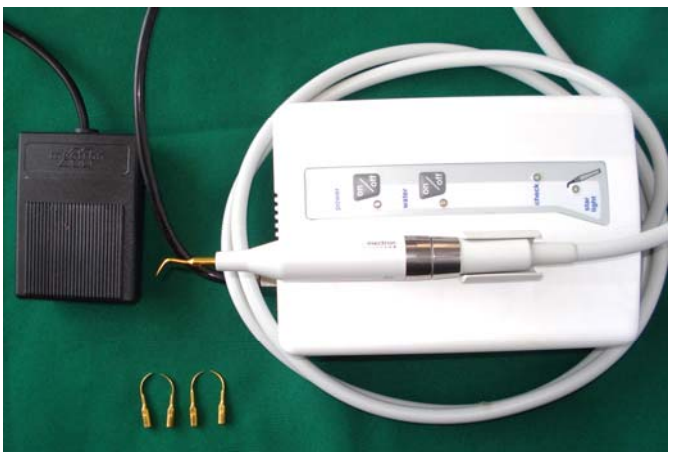


Figure 3. Ultrasonic Scaler with various tip designs.

Quadrant versus Full Mouth Scaling

Hand and power driven instruments are used for instrumentation of the root surfaces. Scaling and root planing can be performed quadrantwise or as a one stage full mouth scaling. Full mouth scaling is claimed by some researchers to be superior to standard scaling and rootplaning (SRP) quadrant wise. It was shown that a single course of SRP reduces the proportions of periodontopathic microorganisms which clearly correlates with improvement of the clinical periodontal parameters [17]. A single course of SRP unfortunately only temporarily reduces the proportions of subgingival pathogenic microorganisms [18] and hence the adjuctive use of antibiotics has been suggested [19]. However, a one stage full mouth disinfection with the use of an antimicrobial mouthrinse like chlorhexidine increases or prolongs the microbiological improvements of subgingival instrumentation without the need for antibiotics in the treatment of patients with Chronic Periodontitis [20,21,22]. Conceptually, this would reduce the microorganisms and diminish the amount of bacteria in the pockets and other intraoral habitats like the tongue, the mucosa, saliva etcs that may be responsible for reinfecting treated sites. Recent studies do not seem to prove that full mouth scaling and root planing is better than quadrantwise therapy. When short term comparison of microbiological changes following quadrantwise and full mouth SRP was done, both the treatment modalities showed similar microbiological outcomes and could not confirm that treated sites were at higher risk for bacterial reinfection in the presence of yet untreated periodontal lesions as in the case of quadrant wise root planing when compared with Full mouth root planing within 24 hours [23]. In another recent study both treatment modalities led to stabilised treatment outcomes over 6 and 12 months in pockets of 4-6mm wherein no

significant difference between the groups were present with respect to clinical attachment gain, Probing depth (PD) and Bleeding on probing (BOP) reduction [24].

Greenstein in his critical commentary after reviewing various clinical trials on the topic concluded that the concept of full mouth therapy provided additional benefits compared to partial disinfection [25]. Regardless of the type of modality adapted, a thorough removal of local factors is necessary which cause an improvement in the clinical parameters like BOP, PD and Clinical attachment gain.

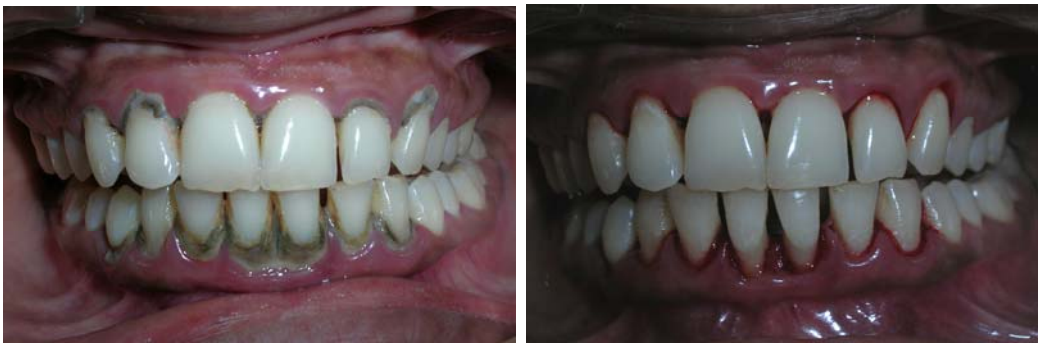
Hand versus Power Driven Instruments

Investigations concerning manual and power driven instrumentation of root surfaces have produced conflicting results. Certain studies have reported that hand instruments like curets produce either a smoother surface[26,27] or a rougher surface than ultrasonics[28,29]. A recent meta analysis on the topic clearly revealed that ultrasonic or sonic subgingival debridement can be completed in less time than subgingival debridement using hand instruments30. Although the time taken by power driven instruments is less, the clinical effects i.e gain in clinical attachment or decrease in pocket depth were similar for both hand or ultrasonics[30].

Healing after Scaling and Root Planing (SRP)

There is extensive evidence in support of Scaling and Root planing as an essential and effective component of therapy for inflammatory periodontal disease (Figure 4). In patients with Chronic periodontitis, subgingival debridement in conjunction with supragingival plaque control is an effective treatment in decreasing probing pocket depth and improving the clinical attachment level[31]. A review of nonsurgical mechanical pocket therapy by Cobb reveals mean probing depth reductions of 1.29mm and clinical attachment level gains of 0.55mm after mechanical therapy for initial probing depths of 4-6mm before treatment and probing depth reduction of 2.16mm and attachment level gain of 1.19mm after therapy for initial probing depths of > 6mm before treatment[32]. Lindhe et al determined the critical probing depth for scaling and root planing to be 2.9mm below which loss of attachment occurs following mechanical therapy[33].

Mechanical non surgical therapy has a profound effect on inflammatory components. The effect of scaling and rootplaning on the inflammatory cell subsets leads to a decrease in plasma cells, lymphocytes and immunoglobulin containing cells especially in periodontitis cases[34]. Marked effects are seen with the gingival tissue following mechanical therapy with distinct reduction in bleeding on probing. When collective analysis of the studies performed to evaluate the decrease in bleeding on probing and gingival inflammation was done, a 57% decrease in bleeding on probing was noted after mechanical non surgical therapy [32].



(A) (B)

Figure 4. A – Plaque and calculus covering gingival third of the teeth. B – Immediately after scaling and root planning.

Positive results of scaling and rootplaning on alveolar bone are well noted with an increase in alveolar bone density. Longitudinal studies with regard to the same were able to demonstrate a statistically significant increase in both superficial and deep average bone densities at 6months and 1 year post treatment which was analysed using standard radiographic technique and digital substraction radiography [35,36]. At the histologic level, scaling and root planing causes a re-establishment or reepithelialization in 2 weeks[37]. Though restoration of the junctional epithelium is complete within 2 weeks, granulation tissue still remains immature and not replaced by collagen fibers. Connective tissue repair continues for 4-8 weeks with specifically oriented collagen bundle fibers [38].

MECHANICAL AND CHEMICAL PLAQUE CONTROL

The responsibility of the dentist to treat a periodontitis patient not only lies in motivating and educating the patient about periodontal procedures and performing non surgical & surgical therapy, but also includes providing instructions to the patient in adequate home care measures like proper brushing, interdental cleaning and use of mouthwashes when needed. Self performed plaque control methods include proper brushing methods, use of interdental aids and mouth washes.

Mechanical Plaque Control

Good plaque control practices are particularly important for periodontal patients. It has been proved if dentogingival plaque is allowed to accumulate freely, subclinical symptoms of gingival inflammation in the form of an exudate from the gingival sulcus appears[39]. It has been noted that plaque growth occurs within a few hours and must be completely removed at least every 48 hours to prevent inflammation in subjects with sound periodontal health[40]. Periodontal patients should completely remove plaque from the teeth at least once every 24 hours because of their demonstrated susceptibility to disease[41]. The prevention and treatment of periodontal diseases are routinely approached by inhibiting plaque formation and instituting mechanical plaque removal measures.

Brushing and flossing is the first line approach to microbial reduction. The ADA recommends brushing for 2 minutes twice a day and flossing once a day[42]. Though tooth

brushing remains the bastion of oral health measures, majority of the population do not clean their teeth thoroughly enough to prevent plaque accumulation. Toothbrushes are available in myriad designs in the market. They were initially based on natural materials like hog bristles with a wooden or ivory handle. These natural materials were inherently unhygienic and hence were replaced by nylon filaments and plastic handles.

Tooth Brush Design Recommendations

Manual Toothbrushes

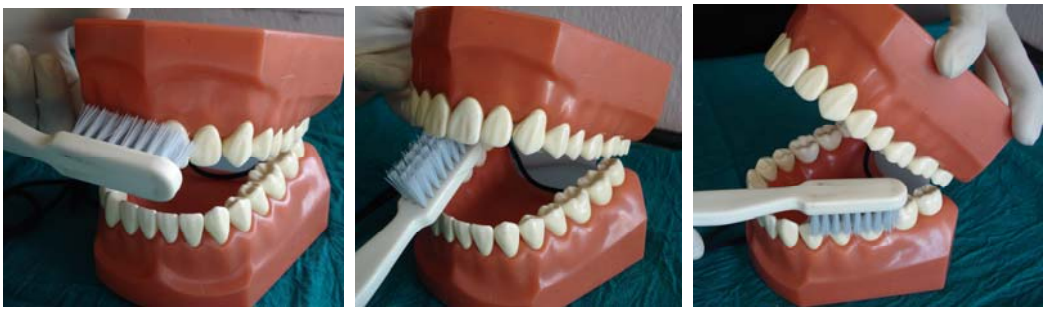
The European workshop on mechanical plaque control in 1998 has provided certain specifications with regard to the design of a toothbrush, the consensus of which is as follows:[43]

- Inclusion of a long contoured handle and the shape of the handle should be according to a particular style of toothbrush use.
- The head size should be according to the size of the user's mouth.
- Round ended Nylon or Polyester filaments to be used not larger than 0.23mm in diameter and soft filament configurations.

Bass recommended a straight handled brush with nylon bristles (0.2mm) in diameter and 10.3mm long with rounded ends, arranged in 3 rows of tufts with 6 evenly spaced tufts per row and 80-86 bristles per tuft. Nevertheless, if a patient perceives any benefit from a particular brush design characteristics, use of that brush should be encouraged[44].

Methods of Tooth Brushing

Various tooth brushing methods have been described in the literature. Among them the Modified Bass method is the most widely recommended brushing technique by the dentist. The other techniques include, the Charter's method for cleaning healing wounds after flap surgery and the modified Stillman method for patients with progressing gingival recession and root exposure to minimize abrasive tissue destruction[44]. The modified Bass method is designed to clean the cervical one third of the crowns of teeth and the area beneath the gingival margin. It can be recommended in subjects with a sound periodontal health or even with periodontal disease or during periodontal maintenance. This technique causes removal of plaque from the gingival sulcus and the interproximal area of teeth. The method consists of placing the brush bristles at an angle of 45 degrees to the long axis of the teeth directed apically (Figure 5). Gentle force is then applied to insert the bristles into the sulcus and is moved with short vibratory back and forth strokes without removing bristle ends from the sulcus with approximately 10 strokes to be completed covering 3-4 teeth at a time. The lingual surface of the anterior teeth is brushed using the heel of the brush placed vertically along the long axis of the teeth. The Occlusal surfaces should be cleaned by pressing the bristles firmly onto the Occlusal surface and a back and forth brushing stroke is activated[44].



(A) (B) (C)

Figure 5. Modified Bass Method of Toothbrushing. Demonstration of position of toothbrush on A, B – Maxillary anterior teeth: Facial & Lingual aspect respectively. C – Occlusal surface of mandibular teeth.

Powered Toothbrushes

Electric or Powered toothbrushes enhance cleaning of the teeth especially for people with poor manual dexterity (Figure 6). Plaque removal by powered toothbrushes is faster i.e takes 1minute compared to manual toothbrushes taking 6minutes to remove the same percentage of plaque[43]. Several types of powered toothbrushes are available. The basic patterns of head motion are reciprocating back and forth movement, arcuate or up and down movement and an elliptical movement[45]. Some of the available powered toothbrushes are as follows:

- Braun Oral-B (http://www.oralb.com/en)
- Philips Sonicare (http://www.sonicare.com/)
- Colgate Motion: (http://www.colgate.com/app/colgate/US/homepage.cvsp)
- Ultreo: (http://www.ultreo.com/)
- Crest Spinbrush: (http://www.spinbrush.com/)

Ultrasonic toothbrushes use filaments that vibrate at ultrasonic frequency (>20 Khz)[43].

Figure 6. Powered Tooth Brush.

Ionic toothbrushes use an electric current which is applied to the filaments during brushing that alters the charge polarity of the tooth resulting in the attraction of dental plaque towards the filaments and away from the tooth. No automated action is provided[43]. The polarity of tooth surfaces is changed from negative to positive. Plaque material is actively repelled by the teeth and drawn to the negatively charged bristles. Studies have been conducted comparing the efficacy of powered versus manual toothbrushes with no consistent significant results. One study found several types of electric brushes to be as efficient as manual brushing in plaque removal from facial surfaces[46]. Literature reviews have shown that powered brushes show superior benefit with regard to plaque removal and gingival

condition over manual brushes[47,48]. Nevertheless a recent systematic review on the topic has concluded the following. Limited evidence exists of the higher efficacy of powered brushes over manual in reducing dental plaque, gingival bleeding or inflammation in patients with gingivitis or periodontitis[49].

Interdental Cleaning Aids

Tough toothbrush is considered as an effective tool in plaque removal and reducing inflammation, the use of interdental aids in plaque removal is essential. It has been noted that toothbrushing is considered to be optimally capable of thoroughly cleaning the flat surfaces of the teeth while the proximal surfaces of teeth is not cleaned effectively. These areas have a high risk of developing periodontal lesions and caries. Hence interdental cleaning is crucial within the daily oral hygiene program for the treatment of periodontal diseases and the prevention of recurrence[50,51]. A wide range of interdental aids are available in the market from simple dental floss through woodensticks and brushes to mechanical or electrical devices (Figure 7). The choice of interdental cleaning aid depends on the size and shape of the interdental space. In general, embrasures with no gingival recession are adequately cleaned using dental floss while larger spaces with exposed root surfaces require the use of an interproximal brush. Interproximal spaces with no papillae covering requires single tufted brushes to remove plaque[44].

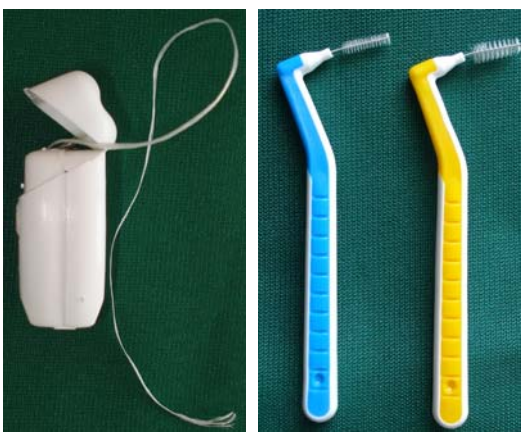


Figure 7. Interdental aids. L- R: Dental Floss and Interdental Brushes.

Chemical Plaque Control

Mechanical tooth cleaning through toothbrushing and the use of appropriate interdental aids is the most common form or oral hygiene practiced by people. Unfortunately, many individuals remove only around half of the plaque from their teeth even when brushing for 2 minutes[52]. Hence an adjunctive use of chemical plaque inhibitory mouthwash may have a major effect on improving the oral health of the individual. The most commonly used chemical plaque control agent is the Mouthwash. Other vehicles which carry the chemicals are chewing gums, varnishes, sprays, irrigators. Some of the chemicals used are Bisbigauanide antiseptics (Chlorhexidine, alexidine, Octenidine), Quaternary ammonium compounds (Cetylpyridinium chloride, banzalconium chloride), Phenols and essential oils

(Thymol, ecalyptol, triclosan etc), Fluorides, Oxygenating agents (Hydrogen perioxide). The most widely used chemical agent is chlorhexidine mouthwash.

Chlorhexidine Mouthrinse

Chlorhexidine (CHX) molecule is a bisbiugnide compound, cationic agent having a desirable property of effectively inhibiting plaque and thus preventing the onset of gingivitis. Its dicationic nature makes it extremely interactive with anion which is relevant to its efficacy, safety and local side effects. Clinical efficacy of chlorhexidine as a mouthwash is achieved in two concentrations 0.2% and 0.12%. 10ml solution of 0.2% delivers 20mg and 15ml of 0.12% delivers 18mg[53].

Mechanism of Action and Unwanted Effects of Chlorhexidine

The cationic Chlorhexidine molecule binds to the negatively charged phosphate and carboxyl groups on bacterial cell surface facilitated by electrostatic forces. This alters the integrity of the bacterial cell membrane and chlorhexidine molecule is attracted towards the inner cell membrane. Further CHX binds to the phospholipids in the inner membrane leading to increased permeability of the inner membrane and leakage of low molecular weight components such as potassium ions. This occurs at low concentrations of the solution leading to bacterostatic action. At higher concentrations, coagulation and precipitation of the cytoplasm occurs by the formation of phosphated complexes leading to cell death[54,55]. Chlorhexidine's prolonged substantivity i.e ability to adsorb onto and bind to hard and soft tissues explains the long-standing bacteriostatic effect of the drug in the mouth. The main adverse effect of Chlorhexidine is extrinsic brown staining of teeth. This may be due to the precipitation of chromogenic dietary factors on to the teeth. It can alter taste sensation.

Clinical Usage

Chlorhexidine is used as an adjunct to mechanical oral hygiene and in physically and mentally challenged individuals with decreased manual dexterity to maintain an effective oral hygiene. It is also indicated in medically compromised patients predisposing to oral infections. Chlorhexidine is prescribed after periodontal surgeries as it offers the advantage of decreasing the bacterial load in the oral cavity and preventing plaque formation. There is a lack of supporting evidence that using mouthrinses on a regular basis has any therapeutic value at retarding progression of chronic periodontitis in untreated patients suffering from the disease[56]. Chlorhexidine is useful for short periods of up to 2 weeks following periodontal surgery when oral hygiene maintenance may be difficult[53]. Several studies conducted on Chlorhexidine, Listerine etc have shown that these antiseptics retard the acculumation of dental plaque and decrease the severity of gingivitis when used as a supplement. A study demonstrated that Listerine was effective in decreasing existing plaque and gingivitis scores at 1,3 and 6 months when used as an adjunct to normal oral hygiene[57.]

CHEMOTHERAPEUTIC AGENTS IN THE TREATMENT OF PERIODONTITIS

Antibiotics and other chemotherapeutic agents are a powerful group of compounds used for management of dental infections either locally or systemically administered. Microorganisms in periodontal infections are heterogeneous in nature. They vary significantly from one patient to another and is site specific even in one individual. Hence, it is difficult to recognize the need of antibiotics in periodontitis cases as an adjunctive therapy. Nevertheless local and systemic antibiotics have been used to treat periodontal diseases and earlier studies have even reported the use of these agents as a monotherapy including tetracycline hydrochloride, minocycline, metronidazole, doxycycline etc58.

Systemic Administration

There is a consensus that use of systemic antibiotics as an adjunctive therapy in the treatment of Aggressive forms of periodontitis and refractory periodontal disease along with conventional periodontal therapy and in situations that cannot be managed with mechanical therapy alone[59].

Table 1. Review of commonly used antimicrobial agents to treat periodontal diseases

Group	Agent	Action	Suggested Dosage & duration to treat Periodontal Diseases[62,63,64].
Semisynthetic Penicillins (Extended Spectrum)	Amoxicillin	Bactericidal-inhibiting cell wall synthesis.	500 mg tid for 8-10 days
Semisynthetic Penicillins + β lactamase inhibitors.	Amoxicillin + Clavulanic acid	Clavulanic acid permeates the outer layer of cell wall of bacteria & inhibits β lactamase enzyme.	250 or 500 mg tid for 10 days.
Tetracyclines (Broad Spectrum Antibiotics)	Tetracycline Hydrochloride	Bacteriostatic- inhibits protein synthesis by binding to 30S ribosomes in susceptible organisms.	250mg QID for 14-21 days.
	Doxycycline Hyclate		100mg bid first day followed by 100mg OD for 10-14 days.
Antiamoebic agent (Nitroimidazoles)	Metronidazole	Cidal activity against protozoa & certain anaerobic bacteria by disrupting bacterial DNA synthesis in conditions with a low reduction potential.	250-500 mg tid for 10 days.
Quinolones	Ciprofloxacin	Bactericidal – inihibits enzyme bacterial DNA gyrase & digests DNA by exonucleases.	500mg bid for 8 days.
Combination therapy.	Amoxicillin + Metronidazole		250 mg of each drug tid for 8 days.
	Metronidazole + Ciprofloxacin		500 mg of each drug bd for 8 days.

Also systemic therapy has been reserved for advanced cases of periodontitis for sites that have not responded well to debridement and in progressive tissue destruction and for certain medically compromised patients[60,61]. For successful management of infections, bacterial

species should be isolated, cultured and tested for antibiotic sensitivity rather than blind prescribing of drugs. When culture and sensitivity testing are not feasible, one has to choose antibiotic based on patient presentation and history. A recent review suggests the following approach to choose the appropriate antibiotic when culture and sensitivity testing is not feasible. Patients without previous history of antibiotic therapy may respond well to tetracycline group. Alternatively for patients not allergic to penicillins, amoxicillin and clavulic acid may be effective[62].

Clinical use of Systemic Antimicrobial Agents in Treatment of Periodontitis

Among the various antimicrobial agents listed, Tetracyclines are widely used in the treatment of Periodontitis. They have the ability to concentrate in the periodontal tissues and is known to inhibit the growth of microorganisms like Actinobacillus actinomy cetem comitans. These anaerobic bacteria can be cultivated from Chronic periodontitis and Aggressive Periodontitis cases. However, in mixed infections these antibiotics may not provide sufficient suppression of subgingival pathogens to arrest disease progression[65]. Tetracyclines have been investigated as adjuncts in the treatment of Aggressive periodontitis where Actinobacillus actinomycetemcomitans which is a tissue invasive bacteria is the causative microorganism. Systemic tetracycline along with mechanical removal of calculus and plaque can eliminate tissue bacteria, arrest bone loss and has even shown an increase in the post treatment bone levels[63]. A 11 month follow up study using tetracyclines in periodontitis cases revealed decreased probing depth and motile organisms[66]. Similar results were obtained in an 18 month follow up study using Doxycycline 100mg/day for 14 days in recurrent periodontal disease case[67]. Long term Tetracyclines prescription has led to infecting organism's resistance to the drug. A long term study of patients taking 250mg of tetracycline per day for 2-7 years showed persistence of deep pockets with high proportions of tetracycline resistant gram negative rods such as fusobacterium nucleatum[63]. Metronidazole has been used in a few instances as an adjunct to Scaling and Root Planing. In a 6 week follow up study, when metronidazole was administered 250mg TDS for a week, a significant reduction in probing depth and apparent gain in attachment level was found relative to patients in the positive control group. Also this was associated with a significant reduction in the need for periodontal surgery in the metronidazole treated patients[68]. Amoxicillin and Clavulanate was tested in the treatment of refractory cases with a history of periodontal surgery, tetracycline therapy and regular periodontal maintainance. 250mg of Augmentin TDS was systemically admistered for 14 days along with full mouth scaling and root planing performed under local anesthesia. Clinical evaluation after 3 months post therapy showed a gain in attachment which remained stable throughout the 1 year recall study. Probing depth decreased over 6 months with a decrease in frequency of bleeding. Hence the results proved that non surgical periodontal treatment with adjunctive use of selected antibiotic decreases the incidence of attachment loss in individuals who had been previously refractory to treatment[69]. A recent metaanalysis on the efficacy of systemically administered antimicrobial agents in the treatment of periodontal infections suggested that subjects with Aggressive periodontitis received greater benefits from these agents than Chronic Periodontitis patients but significant benefit was achieved with Chronic Periodontitis subjects. Though authors of the systematic review suggested that antimicrobial agents are useful additions in the treatment of periodontal infections, certain aspects regarding dosage,

choice of drug, patient selection, duration of treatment in relation to mechanical debridement and nature of hazards such as antibiotic resistance need to be explored better in the treatment of periodontitis patients[70].

Local Delivery of Antibiotics

Antimicrobial agents must reach adequate concentration to have a therapeutic effect to kill or inhibit the growth of target microorganisms. Though systemic administration of drugs are beneficial on periodontal tissues by providing a ready exposure of all periodontal sites to the antimicrobial agent, it poses a risk of adverse reactions to non oral body sites including nausea, vomiting, headache, urticaria, GI upset, abdominal discomfort etc. Also certain drugs like penicillins may induce allergic reactions and bacterial resistance in patients. Another aspect which needs to be considered is the drug has to reach the site where the organisms exist sustaining its localized concentration at effective levels for a sufficient time and evoking minimum or no side effects. Though systemic administration of doxycycline or tetracycline was highly concentrated in the Gingival Crevicular Fluid (GCF) at levels 5-10 times more than found in serum, even this hyperconcentration of the drug in the GCF resulted in a level of antibiotic to which many organisms were notsusceptible[71,72]. Hence considering these factors local delivery of antimicrobials was developed.

Local drug delivery (LDD) agents have been classified as professional applied and home applied agents. One of the main advantages of LDD is the high concentration of the drug released in gingival crevicular fluid. For example, placement of tetracycline hydrochloride fibers subgingivally results in substantially higher dose of the drug in the pocket (1590 µg/ml in GCF & 43 µg/ml in the tissue) than in systemic dosing (2-8 µ g/ml)[61]. It has been suggested that a local concentration of 30µg/ml eliminates most pathogenic bacteria associated with periodontal disease. Though the concentration of drug in GCF is high, it's serum concentration do not exceed 0.1 µg/ml.

Table 2. Local Drug Delivery Agents

AGENT	TRADE NAME	AVAILABLE AS
Tetracycline	Actisite	Non resorbable fibers of ethyl vinyl acetate, 25% saturated with Tetracycline Hydrochloride.
Doxycycline	Atridox	10% Doxycycline gel in a syringe.
Minocycline	Dentamycin, Periocline	2% minocycline hydrochloride gel in a syringe.
	Arestin	2% minocycline encapsulated into bioresorbable microspheres in a gel carrier.
Metronidazole	Elyzol	25% gel, a biodegradable mixture in a syringe.
Chlorhexidine	Periochip Biodegradable chip	

The other advantages being, LDD decreases potential problems with patient compliance, adverse drug reactions are eliminated, reduces the development of drug resistant microbial population at non oral body sites[73]. One of the disadvantages of LDD is difficulty in

placement of the drug in deeper pockets. Placement of the drug in various sites in periodontitis patients is time consuming. Sometimes the need for a second appointment for fiber (non resorbable) removal is required. Also these agents do not markedly affect the periodontal pathogens present in the adjacent gingival connective tissue, tongue, tonsils etc which increases the risk of later reinfection[73].

Studies demonstrated that LDD agents applied with scaling and root planing improved periodontal clinical parameters. In a study comparing the efficacy of tetracycline fibers placed subgingivally in localized recurrent periodontal sites in maintainance patients with scaling and rootplaning alone revealed that at 1,3 and 6 months postoperatively, adjunctive fiber therapy was significantly better in reducing probing depth and bleeding on probing than scaling and rootplaning alone[74]. Also at 6 months, fiber therapy was significantly better in promoting clinical attachment gain. In a double blind, randomized trial, patients with pockets at least 5mm deep were selected and either minocycline 2% gel or vehicle were applied once every 2 weeks for four applications after initial scaling and root planing. Microbilogical assessment of the subgingival flora done using DNA probes at 2, 4, 6 and 12 weeks revealed statistically significant reduction of P.gingivalis, P.intermedia and Aa. Also reduction in probing depth was significantly greater with minocycline gel. Sites with 7mm pockets displayed statistically significant better results than with 5mm pockets[75]. The combination of Scaling and Root planing with metronidazole gel was proved superior to the conventional treatment of scaling and root planing alone in Chronic adult Periodontitis patients. Significantly greater reduction in pocket depths of ≥ 5mm at baseline was seen compared to scaling and root planing alone and the difference was maintained for a period of 9 months[76]. Studies have also shown no significant difference between groups receiving scaling and root planing alone versus scaling and root planing and LDD agent. In a study conducted to evaluate the effectiveness of a controlled release Chlorhexidine chip in the treatment of chronic periodontitis proved no statistically significant difference between the two groups for any of the clinical or microbiological parameters. Also both groups presented a significant improvement in papillary bleeding score, probing depths and relative attachment level but no significant difference was seen between the two groups. Also for both treatments, there was a significant reduction in the percentage of BANA positive sites and the improvements in the BANA test were similar for both groups after 3 and 9 months[77]. Another interesting finding in the study was the adverse reaction seen in patients treated with Chlorhexidine chip. The most common reactions were gingival pain, discomfort, local irritation and gingival edema. Gingival abscesses were found in three sites; however, this side-effect was minor and transient, with resolution usually complete within a few days and requiring no intervention or medication.

Metanalysis on local drugs like tetracycline, minocycline, metronidazole and Chlorhexidine revealed some interesting results. Adjunctive use of local antibiotics though appeared to have an impact on probing depth improvements or gain in attachment level, it was only in the range of about 0.25 – 0.5mm and 0.1 – 0.5mm respectively. According to the results obtained from the analyses of numerous studies with respect to probing depth and clinical attachment level, local minocycline might be the most promising adjunctive therapy followed by tetracycline. Side effects from these adjunctive therapies are relatively minor. Adjunctive therapies may be used routinely as treatment alternatives when isolated sites do not respond adequately to scaling and root planing. The difference between the added effects of the adjunctive treatment and scaling and root planing alone narrows with time.

Nevertheless at all time periods, Scaling and root planing with adjunctive therapy seems to be more effective than scaling and root planing alone[78].

HOST MODULATION

In periodontitis which is initiated by bacteria, the "host" harbors these pathogens. Though the presence of these pathogens especially gram negative bacteria is required, it is not sufficient to induce periodontal disease[79]. Ultimately it is the host's reaction to the presence of bacteria that mediates tissue destruction which is also influenced by certain risk factors like environmental, acquired and genetic factors[80]. Hence modulating the host in periodontal management strategies has a significant potential for improving treatment outcomes. Host modulation is a new concept introduced in dentistry and in the periodontal context host modulation means modifying or modulating destructive or damaging aspects of the inflammatory host response that develops in the periodontal tissues as a result of the chronic challenge presented by the subgingival bacterial plaque[81]. Bacterial challenge to the host leads to an upregulation of inflammatory mediators and destructive enzymes such as IL-1α, IL-1β and IL-6. In response there is an increase in the anti inflammatory mediators such as IL-1ra (receptor antagonist) and tissue inhibitors of matrix metalloproteinases (TIMPs)[82,83]. An imbalance with an excessive level of the proinflammatory or destructive mediators present in the host tissues will lead to tissue destruction. Hence host modulatory therapy is to restore balance between, on the one hand, pro-inflammatory mediators and destructive enzymes, and, on the other hand, anti-inflammatory mediators and enzyme inhibitors.

Host modulatory therapy (HMT) can be included as one of the available adjunctive treatment. This is achieved by downregulating or modifying destructive aspects and / or upregulating protective or regenerative components of the host response. HMT consists of systemically or locally delivered pharmaceutical agents that are prescribed as part of periodontal therapy and are used as adjuncts to conventional periodontal treatment such as scaling-root planing and surgery. Numerous agents have been evaluated as host response modulators, including the nonsteroidal anti-inflammatory drugs, bisphosphonates, and tetracyclines.

Non Steroidal Anti-Inflammatory Drugs (NSAID)

They inhibit the formation of inflammatory mediators like PGE_2 produced by variety of cells like neutrophils, macrophages, fibroblasts etc in response to bacterial lipopolysaccharides (LPS). PGE_2 , a potent inflammatory mediator elevated in periodontitis patients is known to upregulate bone resorption by osteoclastic activity[84]. Studies have shown that NSAIDs like Flurbiprofen, indomethacin and others administered daily for 3 years significantly decreased the rate of alveolar bone loss[85,86]. NSAIDs need to be administered for an extended period of time (years) for the periodontal benefits to become apparent. However, these agents have some disadvantages like gastro intestinal problems, hemorrhage, renal and heparic impairment. Also a patient may experience a 'rebound effect' once he stops taking the drug leading to an increase in the rate of bone loss[81]. Selective clyclo-

oxygenase-2 inhibitors were investigated as HMT agents. However serious adverse effects of these agents were identified and were withdrawn from the market. Hence NSAIDs due to their unwanted effects, their use as adjuncts to periodontal treatment is not justified.

Tetracyclines

Tetracyclines molecules have been modified by removing all the antibiotic properties but retaining the host modulatory, anticollagenolytic effects. Such agents are known as Chemically Modified Tetracyclines (CMT). CMT-3 and CMT-8 have shown to inhibit osteoblastic bone resorption and promote bone formation, enhance wound healing and inhibit proteinases produced by periodontal pathogens[87].

Subantimicrobial Dose Doxycycline (SDD)

Are an effective HM agent indicated in the treatment of Periodontitis. It is marketed as "Periostat" and has the ability to downregulate certain enzymes like Matrix Metallo Proteinases (MMP) which degrades a variety of extracellular matrix molecules including collagen[81]. Doxycycline has several benefits when used as an adjunctive treatment. It inhibits connective tissue breakdown by a multiple non-antimicrobial mechanisms. They may have a direct inhibition of the MMPs, promote excessive proteolysis of pro-matrix metalloproteinases into enzymatically inactive fragments, decrease cytokine levels and also may have a pro anabolic effect such as increase in collagen production and osteoblastic activity[88].

Bisphosphonates

Are bone sparing agents which have been used in the management of osteoporosis. They are absorbed by the bones and locally released during acidification with osteoclastic activity[89]. Bisphosphonates at the tissue level decrease bone turnover by decreasing bone resorption and by reducing the number of new bone multicellular units. At the cellular level they decrease osteoclast and osteoblast recruitment, decrease osteoclast adhesion and also decrease the release of cytokines by macrophages[90]. Clinical trials have been performed to examine the role of bisphosphonates in the management of periodontal bone loss. A study was conducted on type II diabetic subjects with established periodontitis. Patients treated with scaling and root planing and alendronate- 10 mg/day for 6 months induced improvement in alveolar bone crest height than control therapy. Alendronate induced a significant decrease in urine N-telopeptide which was used as a biochemical marker of bone resorption[91]. Some bisphosphonates have undesirable effects such as inhibiting bone calcification and inducing changes in white blood cell count[87].

Host response modulation has emerged as a convincing treatment concept for the management of periodontal disease. To date, only subantimicrobial dose doxycycline has been approved specifically as a host response modulator for the treatment of periodontitis[81] and the majority of clinical trials of this drug have clearly demonstrated a benefit. The prevention of bone loss associated with periodontal disease progression may be enhanced by

modulating the host response which in turn may be an auxillary to the management of Periodontitis.

SURGICAL PHASE

Rationale for Periodontal Surgery

The aim of effective treatment of periodontal disease is to arrest the inflammatory diseases process and establish an environment compatible with periodontal health. The success of periodontal therapy is measured in terms of improvement in clinical attachment levels, decrease in probing pocket depths, reduction in bleeding on probing and maintenance. With regard to the ecological environment, periodontal therapy results in a microbiota more representative of health. Presumptive periodontopathogens like P.gingivalis, F.nucleatum and C.rectus are decreased and gram positive facultative organisms are increased[92]. Numerous studies have utilized probing depth measurements to assess the need for therapy and to evaluate the response to treatment. Deep sites experience greater disease progression and further when the risk/ratio of developing disease progression in deep sites as compared to shallow sites evaluated over 5-36 months was usually found around 3 times greater at deep sites[92]. Also there is a direct relationship between the type of pathogens and increased probing depths. Deep periodontal pockets are associated with increased levels of spirochetes and motile forms[92].

A thorough mechanical therapy brings about improvements in both clinical and microbiological parameters of the gingival tissue. Clinician needs to evaluate their ability to instrument deep pockets and decide if non surgical therapy can achieve the preferred outcomes of periodontal therapy i.e resolution of clinical signs of inflammation, attaining shallow probing depths, stabilization and gain of clinical attachment, radiographic resolution of osseous defects, occlusal stability and decreased plaque to a level associated with health[92]. With respect to these objectives, non surgical therapy should be used as long as it attains favourable results. However, numerous investigations have shown that the difficulty of this task increases as the pocket becomes deeper [92,93]. As the pocket deepens, the surface to be scaled increases, more irregularities appear on the root surface and accessibility is impaired especially in the furcation involved sites[94]. Hence the need for surgical access therapy is needed by displacing the soft tissue wall of the pocket which increases the visibility and accessibility of the root surface[95].Therefore surgical therapy for access and pocket reduction should be considered when non surgical treatment is unsuccessful or the desired result cannot be achieved[92]. A classic study by Lindhe et al determining the critical probing depths stated an improvement in attachment level occurs when surgery is performed in pocket depths measuring >4.2mm[33]. However, A final decision on the need for periodontal surgery should be made only after a thorough evaluation of Phase I therapy. The assessment is made not less than 1-3months and sometimes as much as 9 months after completion of phase I therapy[96]. Clinicians have to evaluate every case and determine what type of treatment will best preserve the dentition in a state of health.

Evaluation of Patients after Phase I Therapy

The purposes of re-evaluation of the tissue are to determine the need for further therapy and to determine the effectiveness of scaling and root planing and to review the proficiency of home care[97]. Re-evaluation consists of examination of the gingival tissues, bleeding on probing, probing depth and occlusal factors. A study performed on a group of teenagers with gingivitis, showed a decrease in plaque index and bleeding on probing from baseline to 15 and 30 days after being treated with ultrasonic scaling[98]. The American Academy of Periodontology world workshop agreed that a 4-6 week interval was usually adequate to assess the initial response to phase I therapy[99]. As stated earlier, reepithelialization of attachment (junctional epithelium) occurs in 1-2 weeks. Hence revaluation of the soft tissue response should not be done earlier than 2 weeks after instrumentation[100]. Repopulation of periodontal pockets by microbes after instrumentation occurs within 2 months in the absence of improved plaque control. The ideal time for reevaluation is between 4-8 weeks[101].

Objectives of Periodontal Surgery

The surgical phase consists of techniques performed for pocket therapy and for correction of certain mucogingival defects. The objectives are to improve the prognosis of teeth and improve esthetics. The purposes of periodontal surgery as proposed by Barrington 1981[102] are as follows:

- To eliminate pockets by removing and or recontouring soft tissues or bone.
- To remove diseased periodontal tissues by creating a favourable environment for new attachment and/or readaptation of soft tissues or bone.
- To correct mucogingival defects.
- To establish tissue contours to facilitate oral hygiene.
- To establish esthetics by reducing soft tissue in cases of gingival enlargement.
- To establish a favourable restorative environment.
- To establish drainage (periodontal abscess).
- To facilitate regeneration of bone and soft tissue.

Indications for Periodontal Surgery

Carranza and Takei proposed the following indications[96] :

- Sites with irregular bony contours, deep craters and other defects.
- When complete removal of root irritants is not considered clinically possible especially in pockets of posterior teeth.
- Grade II or grade III furcation involved teeth wherein surgery ensures the removal of irritants.
- Root resection or hemisection requires surgical intervention.
- Intrabony pockets on distal areas of last molars, frequently complicated by mucogingival problems, are usually unresponsive to nonsurgical methods.

- Persistant inflammation in areas with moderate to deep pockets may require surgical approach.

Surgical Methods for Periodontal Pocket Therapy

The most common method is the removal of the periodontal pocket wall which can be accomplished by non surgical and surgical methods. Scaling and root planing procedures resolve tissue inflammation thereby causing shrinkage of the gingiva and hence reduction in the pocket depth. Surgical therapy consists of tissue resection performed by gingivectomy technique or by means of flap procedure (undisplaced flap). Apically displaced flap technique leads to an apical displacement of the flap thereby reducing the pocket wall. Elimination of pocket depth is carried out by New Attachment technique. New attachment is defined as the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion or connective tissue adaptation or attachment and may include new cementum[103].

Treatment Guidelines for Periodontal Pocket Problems

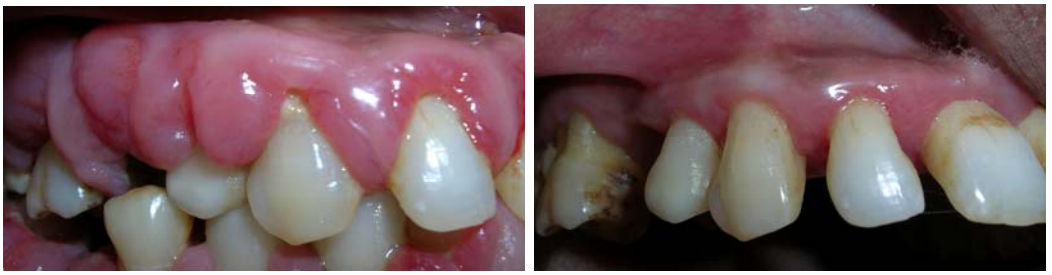
In sites with gingival or pseudo pockets, gingivectomy (excision of the gingiva) is the treatment of choice for pockets with a fibrotic wall[96]. In cases involving larger areas of the dentition flap technique is needed. A conservative approach and satisfactory oral hygiene is sufficient to control the disease in cases with slight periodontitis with shallow to moderate pockets. Sites with deeper pockets as in case of moderate periodontitis, surgical procedure of choice in the anterior teeth with wide interproximal spaces, the papilla preservation flap technique is considered as the first choice[96]. This technique offers less recession and reduced soft tissue crater formation interproximally[104]. In teeth very close interproximally, the sulcular incision flap is the next choice and Modified Widman flap is chosen when esthetics are not the primary consideration. Access to posterior teeth for periodontal therapy is difficult due to their root morphology, furcation involvement etc.. Accessibility can be obtained by either the undisplaced flap or the apically displaced flap. The flap of choice for regeneration of osseous defects in the posterior teeth is the papilla preservation flap and followed by sulcular flap and the modified widman flap[96].

Periodontal Surgical Procedures

Gingivectomy

Excision of the gingiva is performed to eliminate suprabony pockets which are fibrotic and to eliminate gingival enlargements. The technique is performed by means of scalpel, electrosurgery or lasers. The pockets are marked with pocket marker after careful exploration of the pockets using a periodontal probe. Periodontal knives (Kirkland and orban) are used for making the incisions other than Bard-Parker knives. Incision must be placed at a level more apical to the level of the points marked and should be beveled at approximately 45 degrees to the tooth surface[105,106]. As far as possible, the normal festooned pattern of the gingiva is created. The excised pocket wall is removed and granulation tissue is curetted. Plaque and

calculus is removed and the wound areas are covered with a periodontal pack. The pack is removed after a week (Figure 8).



(A) (B)

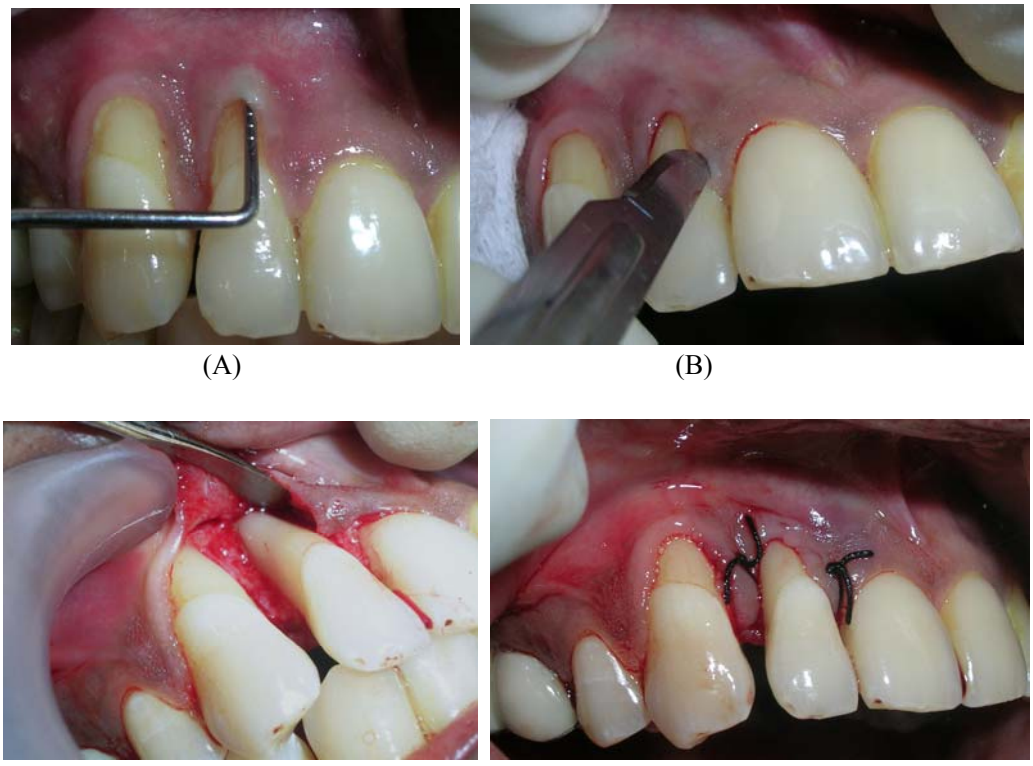
Figure 8. Gingivectomy A – Gingival enlargement in relation to the first quadrant. B – Post operative view after Gingivectomy.

Modified Widman Flap

Is defined as a scalloped, replaced, mucoperiosteal flap, accomplished with an internal bevel incision that provides access for root planning[107]. This flap procedure was described by Ramfjord and Nissle in 1974[108]. The initial incision is the internal bevel incision given 1.0 mm away from the gingival margin following the contour of the gingival margin. This may be performed using a bard parker blade (no.11) which should be parallel to the long axis of the tooth. A similar incision technique is used on the palatal aspect. The incisions should be placed as far as possible between the teeth such that sufficient amounts of tissue can be included in the palatal flap to allow for proper coverage of the interproximal bone when the flap is sutured. Next a periosteal elevator is used to reflect the flap such that a few millimetres of the alveolar crest is exposed. A second crevicular incision is made around the teeth to facilitate the separation of the collar of pocket epithelium and granulation tissue from the root surfaces. After the flap is reflected, a third incision is made in the interdental space close to the surface of the alveolar bone crest to remove the gingival collar. Granulation tissue is removed using curettes. The exposed root surfaces are scaled and planed. The flaps are then trimmed and the facial and lingual interproximal tissue is adapted such that there is complete coverage of the interproximal bone. Recontouring of bone from the outer aspect of the alveolar process may be needed if it prevents good tissue adaptation. The flaps are sutured together with interrupted direct sutures. Finally surgical dressing (periodontal pack) may be placed. The sutures and dressing is removed after 7 days. Advantages of modified widman flap are the ability to coapt the tissues to the root surfaces, access to the root surfaces, less likelihood of root sensitivity and caries and a positive environment for oral hygiene maintenance. The disadvantage being the presence of a flat or concave interproximal soft tissue contours may be seen after the surgery[107].

Modified Flap Operation

Kirkland described this technique which is an access flap for proper root debridement. It makes use of only intracrevicular incisions through the bottom of the pocket. Both buccal and lingual flaps are reflected and debrided. The flaps are then replaced to the original position and sutured interproximally (Figure 9 and Figure 10).



(A) (B)

(C) (D)

Figure 9. Flap Surgery A – Deep Pocket on facial aspect of maxillary right lateral incisor. B – Incision given, C – Flap reflected and debridement performed, D – Sutures placed

Coronally Advanced Flap

The periosteum is considered as having a regenerative potential due to the presence of osteoprogenitor cells. The barrier type effect by the repositioned periosteum and the cellular activity of the periosteum is considered as the reason for its regenerative potential[103].

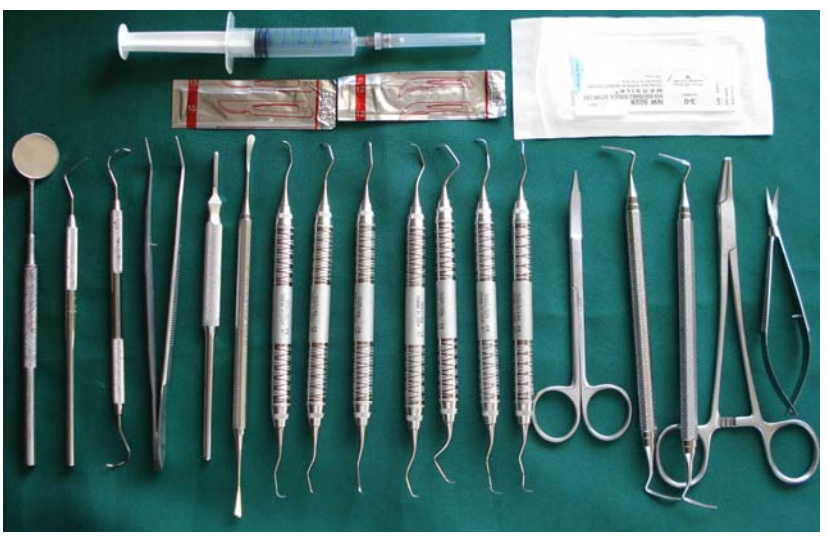


Figure 10. Instruments used for Periodontal flap Surgery.

Reconstructive Periodontal Surgery

Periodontal regeneration is defined as the restoration of lost periodontium or supporting tissues and includes formation of new alveolar bone, new cementum and new periodontal ligament[103]. New attachment is defined as 'the reunion of connective tissue with a root surface which has been deprived of its periodontal ligament. The reunion occurs by the formation of new cementum with inserting collagen fibers'[109]. Though new attachment is the ideal treatment outcome, achieving this end point requires repopulation of a detached root surface by cells from the periodontal ligament which is a prerequisite for new attachment formation. Other therapeutic results may be seen such as formation of a long junctional epithelium, root resorption and ankylosis, recession or reccurrence of periodontal pockets[110].

A study was undertaken to evaluate four periodontal regenerative procedures on the connective tissue attachment level. Tissue sections analyzed 12 months after surgery revealed that healing following the 4 different regenerative procedures resulted in the formation of a long junctional epithelium along the treated root surface with no connective tissue attachment[111]. It has been reported that granulation tissue derived from gingival connective tissue produced root resorption and granulation tissue derived from bone produced ankylosis of roots deprived of their PDL and root cementum[109]. Despite the various treatment outcomes seen, regeneration of the periodontal tissues with new attachment seems to be the ultimate goal.

Root Surface Conditioning

This method is perhaps the oldest and most frequently attempted type of regeneration. Periodontal disease leads to structural and biochemical damage of the root surface including decreased insertion of collagen fibers, alterations in mineral density and surface composition and root surface contamination by bacteria and their endotoxins[112]. Use of certain chemical modifying agents on the altered root surfaces serve for cell attachment and fiber insertion. Agents which are commonly used for root conditioning are citric acid, ethylenediaminetetra acetic acid (EDTA), tetracyclines and fibronectin.

Citric acid (pH 1 for 2min) when used on denuded root surfaces in dogs led to formation of cementum pins extending into dentin tubules to facilitate regeneration[113]. Citric acid treated root surfaces produced wide zones of demineralization dominated by exposed collagen fibrils[114]. Removal of smear layer from root planed surfaces was seen with citric acid resulting in depressions corresponding to open dentinal tubules[115]. It also initiated wound healing by clot stabilization which may result in new connective tissue attachment[116]. Citric acid is also known to reduce aerobic and anaerobic bacteria[117] and endotoxins from root surfaces. Effect of tetracycline root conditioning on cell adhesion, migration and proliferation of fibroblasts was seen in a study and was concluded that tetracyclines increased attachment of fibroblastic cells[118]. Though studies on animals show the beneficial effects of root conditioning agents, human studies have shown contradictory results[119].

A recent systematic review concluded that the use of citric acid, tetracyclines or EDTA to modify the root surfaces provides no clinical benefit to the patient with respect to decrease of probing depth or gain in clinical attachment level in chronic periodontitis patients[112].

Bone Grafts

Bone replacement grafts remain among the most widely used therapeutic approaches for the correction periodontal osseous defects. Many investigators have focused upon bone regeneration as the prerequisite for new attachment formation and hypothesizede that this will also lead to induction of new cementum[109].The rationale behind the clinical use of grafting procedures is that the complete regeneration of the attachment apparatus (including new bone formation and new connective tissue attachment) would be improved by various biomaterials due to their osteogenetic (Any tissue or substance with the potential to induce growth or repair of bone is said to be osteogenic[9]) potential if the graft contained viable bone-forming cells, osteoinductive capacities (exerted by the release of boneinducing substances), or osteoconductive properties (i.e. the possibility to create a scaffold to support bone formation)[120].

Bone grafts are classified as Autogenous bone grafts, Allografts, Xenografts Alloplasts.

Autografts are tissue transferred from one position to another in the same person[9]. Iliac crest of the pelvis is the most common extraoral site for procuring graft. Common intraoral sites are healing extraction sockets, edentulous sites, tuberosity region distal to the last molar and mental symphisis below the teeth. Trephines, saws or drills are used to procure intraoral autogenous bone with profuse faline irrigation to prevent overheating and also to maintain viability of the bone cells. Techniques like osseous coagulum (a mixture of bone dust with patient's blood) and bone blend (a triturated mixture of cortical or cancellous bone with saline) have been used in periodontal regeneration with successful results of new bone, cementum and new attachment seen at the interdental osseous defect site[121]. A technique also know as bone swaging has been proposed which requires the existence of an edentulous area adjacent to the defect from which the bone is pushed into contact with the root surface without fracturing the bone [122].

The main advantage of Autogenous bone grafts is that they contain viable cells which may go on to actively form new bone. The disadvantages are creation of a second surgical site for harvesting the bone which may lead to increased risk of morbidity due to post operative complications, availability of limited quantity of bone and difficulty in procuring the same from intraoral sites and chances of root resorption when fresh ilac grafts are used[123].

Allografts are grafts between genetically dissimilar members of the same species; a processed human bone graft obtained from a tissue bank[9]. Allografts are available from commercial tissue banks. Grafts are obtained from cadaver bone, freeze dried and treated to prevent transmission of disease to get Freeze dried bone allografts (FDBA) or may be demineralised to obtain demineralised freeze dried bone allografts (DFDBA). The antigenic properties of allografts are reduced by radiation, freezing or chemical treatment[109].

FDBA is regarded as an osteoconductive material wherein it acts as a scaffold for natural bone to grow into but does not activate bone growth. Eventually the graft is resorbed and replaced by new bone. A recent academy report on periodontal regeneration revealed bone fill ranging from 1.3-2.6 mm when FDBA was used in controlled clinical trials to treat periodontal defects[124]. DFDBA stimulates bone formation due to the influence of bone inductive proteins called as Bone Morphogenetic Proteins (BMP) a group of polypeptides belonging to the transforming growth factor- β (TGF-β) family which gets exposed during the demineralization process. They stimulate bone formation through osteoinduction by inducing pleuripotential stem cells to differentiate into osteoblasts. Hence DFDBA elicits

mesenchymal cell migration, attachment and osteogenesis when implanted in well vascularized bone[125]. It has been reported that DFDBA have demonstrated bone fill similar to that achieved with FDBA ranging from 1.7-2.9 mm[103]. Variability has been reported in the ability of DFDBA to induce new bone. In a study conducted to compare the ability of DFDBA, Guided tissue regeneration (GTR) membrane and growth factors, it was seen that DFDBA were least effective in promoting bone growth[126]. Variability in results could be attributed to insufficient quantity of BMPs present especially in adult cortical bone[127] or bone inductive components of the graft may be in an inactive form. Further natural variation in human donors may exist which may explain the variation in the osteoinductive capacity of DFDBA[125].

Human mineralized bone has been developed recently. It contains human mineralized component, organic matrix and collage. This mineralized bone allograft (MBA) which is solvent preserved by tutoplast process and low dose gamma irradiation is more osteoconductive material than FDBA[103]. This treatment has been claimed to preserve the bony trabecular pattern and is shown to exhibit increased porosity than FDBA[128]. MBA with or without membrane when compared with open flap debridement in the regenerative ability of class II furcation defects in molars revealed significantly improved bone fill. Hence MBA has been introduced to periodontal therapy and recently been evaluated for its use in regenerative and bone augmentation [129,130].

Alloplast is a synthetic graft or inert foreign body implanted into tissue[9]. These are synthetic, inorganic bone graft materials which are osteoconductive in nature. Various alloplasts are available namely Hydroxyapatite (HA), β-tricalcium phosphate, Polymethyl methacrylate/hydroxyethylmethacrylate (PMMA/HEMA), calcium layered polymer and bioactive glass. Alloplastic materials must possess certain properties which will make it ideal as a regenerative material. The material should be biocompatible with host tissues, non allergenic, non carcinogenic and non inflammatory. They should possess sufficient porosity to allow bone conduction and have the ability to stimulate bone induction, resorbability with replacement of bone and be radiopaque[107]. The bioactive glass showed a greater increase in bone fill and significantly greater probing depth reduction than open flap debridement procedure[120].

Xenograft is a graft taken from a donor of another species and is referred to anorganic bone[9]. There are minimal clinical data supporting the use of xenografts in periodontal defects. An anorganic bovine derived graft marketed as bio-oss has been used to treat osseous defects in periodontitis with successful new attachment and bone regeneration. They contain porous matrix containing minerals from cancellous or cortical bone which is bovine derived. Though the organic components of the bone are excluded, the bony trabecular architecture is still retained[131].

Guided Tissue Regeneration

It provides a barrier to epithelial downgrowth and excludes gingival connective tissue cells thereby allowing cells with regenerative potential i.e periodontal ligament and bone cells to enter the wound first. A biocompatible membrane is used to isolate the defect from the gingival epithelium and connective tissue. Resorbable and non resorbable membranes are available. Increase in gain of clinical attachment and probing depth reduction in the treatment of furcation defects and intrabony defects has been noted[132].

Biologic Modifiers

Certain naturally occurring molecules with matrix proteins known as growth factors regulate the biologic events necessary for regeneration namely mitogenesis, migration and metabolism[133]. Numerous growth factors have been identified and characterized. Studies in non human primate model showed Platelet derived growth factors (PDGF) has the capacity to stimulate bone formation and periodontal regeneration[133]. It has also shown to be an important stimulator of cellular chemotaxis, proliferation and matrix synthesis enhancing influx of fibroblasts into the wound site and increases extracellular matrix production[133]. Combination of recombinant PDGF and Insulin like growth factors (IGF) has shown promising results in the treatment of intrabony defects and furcation involvement[134]. Bone Morphogenetic Proteins (BMP) are natural proteins which play important roles during embryogenesis and mediate in specific aspects of skeletal growth and development. They are osteoinductive in nature. BMP-2 & BMP-7 have shown improved regenerative results when used for treatment of periodontal defects[103]. Certain proteins are secreted by hertwig's epithelial root sheath during development of tooth and induces acellular cementum formation. These proteins which are known as enamel matrix proteins are believed to favour periodontal regeneration[135]. One of the enamel matrix derivative (EMD) has been approved by the U.S Food and drug administration for use in achieving periodontal regeneration in osseous defects. They are marketed under the trade name of Emdogain. It consists of a viscous gel of enamel derived proteins from tooth buds in a polypropylene liquid and is delivered by a syringe to the defect site[110]. Amelogenin is the major protein present in the mixture[136]. Emdogain has promoted increased gain of radiographic bone and clinical attachment onto diseased root surfaces associated with intrabony defects in periodontitis subjects compared to control group who received a placebo application[137]. A recent systematic review has shown the beneficial effects of EMD in periodontal regeneration and decreasing probing depth[136] and also there is a strong evidence that EMD favours wound healing and new periodontal tissue formation[138].

Resective Osseous Surgery

Osseous Surgeries are procedures to modify bone support altered by periodontal disease, either by reshaping the alveolar process to achieve physiologic form without the removal of alveolar supporting bone, or by the removal of some alveolar bone, thus changing the position of the crestal bone relative to the tooth root[9]. The rationale of osseous resective surgery is that the discrepancies in level and shapes of the bone and gingiva may predispose patients to the recurrence of pocket depth post surgically. Thus resective osseous surgery leads to reshaping the marginal bone to resemble that of alveolar process undamaged by periodontal disease[139]. Osteoplasty (reshaping of the alveolar process to achieve a more physiologic form without removal of alveolar bone proper[9]) and Ostectomy (the excision of a bone or portion of a bone[9]) procedures are utilised in osseous surgery. Osteoplasty is used to reduce buccal and lingual bony ledges, shallow intrabony defects and incipient furcation involvements that do not necessitate removal of supporting bone[140]. Ostectomy is used to treat shallow (1-2mm) to medium (3-4mm) intrabony and hemiseptal osseous defects and to correct reverse osseous architecture. The apically displaced flap design is utilized with this technique to provide minimum probing depth and gingival tissue morphology that enhances good self performed oral hygiene and periodontal health.

Healing after Flap Surgery

Following flap surgery, the area between the flap and tooth / bone surface is established by blood clot consisting of fibrin reticulum with numerous neutrophils, erythrocytes, debris of injured cells and blood capillaries[141]. 1-3 days post surgery the epithelial cells migrate over the border of the flap and by one week and epithelial attachment to the root is established. The clot is replaced by granulation tissue derived from gingival connective tissue, bone marrow and periodontal ligament. Collagen fibers appear parallel to the tooth surface 2 weeks after surgery and a fully epithelialized gingival crevice with a well defined epithelial attachment is seen one month after surgery[141]. Full thickness flaps results in necrosis of bone which peaks at 4-6 days following surgery[141]. Repair of the osseous lesion may be seen when flap surgery is carried out in an area with a deep infrabony lesion. Various factors influence the amount of bone fill such as the anatomy of the defect, crestal bone resorption and extent of inflammation[106]. The presence of retained cementum on the root surface is beneficial during the healing process. It was seen that resorption occurred prior to new cementum formation and connective tissue attachment in those areas where cementum had been planed from the root[142].

PERIODONTAL MAINTENANCE (SUPPORTIVE PERIODONTAL THERAPY)

Upon completion of active periodontal therapy, a routine periodontal maintainance (PM) visits should be formulated for patients which is also known as Supportive periodontal therapy. The importance of regular maintenance visits should be made aware to patients for a long term control of the disease. This will include the following:

- To revise medical and dental histories of the patients.
- To assess oral hygiene status.
- To Evaluate periodontal soft tissues and dental hard tissues.
- To perform mechanical scaling to remove plaque, stains and calculus with adjunctive chemotherapeutic agents when indicated.
- To identify any new risk factors and formulating an appropriate treatment for the same.

Achieving a stable attachment level with absence of clinical signs of inflammation after periodontal therapy is important. Hence prevention of the progression and recurrence of periodontal disease in patients previously treated for gingivitis and periodontitis is one of the objectives of periodontal maintenance[143]. Another objective is to decrease the incidence of tooth loss by monitoring patients' dentition. Also concurrent diseases within the oral cavity can be recognized and treated in periodic maintenance visits. It has been reported that there was decreased probing depth and tooth loss in patients who had received periodic periodontal maintenance compared to subjects who had not received the same for 10 years following completion of periodontal therapy[144]. It was also seen in a study that subjects who adhered to strict periodontal maintenance lost fewer teeth. Also patients were assessed for percentage

of compliance (total number of visits the patient should have made divided by the number of visits actually made) wherein it was seen that patients whose percentage of compliance was higher maintained their teeth longer than those less compliant[145].

Bacteria present within the tissues in Chronic and Aggressive Periodontitis patients[146,147] may not be eliminated completely in certain areas[148] and these bacteria may recolonize the pocket and may be the cause for recurrent periodontal disease. Debridement of pockets in periodontitis patients suppresses the components of the subgingival microflora but may return to their baseline levels in approximately less than 3 months[144]. Hence constant monitoring and mechanical debridement is needed for good maintenance results. Healing after periodontal surgery often leads to the formation of a weak long junctional epithelium and inflammation may lead to the separation of this epithelium from the tooth leading to recurrence of disease activity. Hence treated periodontal patients may be at a risk of developing recurrent periodontal disease if regular maintenance care is not optimal which is essential for a long term preservation of the dentition. The frequency and quality of such recall visits is important and has been reported that an average periodontal maintenance visit should last for an hour and should be scheduled every 3 months[149].

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Chapter 31

THE ROLE OF ANTIMICROBIAL PEPTIDES IN PERIODONTAL DISEASE

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ABSTRACT

The oral cavity is a warm, moist environment, in which a number of microorganisms colonize and live in harmony as a community, a so-called biofilm. In this environment, antimicrobial peptides may play a critical role in maintaining normal oral health and controlling innate and acquired immune systems in response to continuous microbial challenges in periodontal disease. Two major families of antimicrobial peptides, found in the oral cavity, are defensin and cathelicidin. Members of the defensin family are cysteine-rich peptides, synthesized by plants, insects, and mammals. These peptides vary in length and in the number of disulfide bonds, and have a beta-sheet structure. In the oral cavity, four alpha-defensins are synthesized and stored in neutrophil granules, which are converted into active peptides by proteolytic processing, while three human beta-defensins (hBDs), hBD-1, hBD-2, and hBD-3, are predominantly produced by oral epithelial cells. The only member of the cathelicidin family found in humans is LL-37, an alpha-helical peptide that contains 37 amino acids and begins with two leucines at its NH_3-terminus. LL-37 is derived from enzymatic cleavage of a precursor peptide, namely, human cationic antimicrobial peptide-18. Clinically, differential expression of antimicrobial peptides has been reported in specific types of periodontal disease, and their presence has been shown in saliva and gingival crevicular fluid. Current evidence suggests that alpha-defensins, beta-defensins, and LL-37 have distinct, but overlapping, roles in antimicrobial and pro-inflammatory activities. Several studies have shown antimicrobial activities of hBD-2, hBD-3, and LL-37 against several periodontal pathogens, suggesting their potential role as antimicrobial agents for periodontal disease. The clinical significance of antimicrobial peptides in periodontal disease has recently been demonstrated in morbus Kostmann syndrome, a severe congenital neutropenia, in which chronic periodontal infection in young patients, resulting from a deficiency of neutrophil-derived antimicrobial peptides, causes early tooth loss. Although researchers

initially focused their attention on antimicrobial activities, it is now becoming evident that defensins and LL-37 are multifunctional molecules that mediate various host immune responses, and may thus represent essential molecules of innate immunity in periodontal disease. In this chapter, basic knowledge and the clinical importance of antimicrobial peptides in periodontal disease will be discussed in detail.

INTRODUCTION

The warm and moist environment in the oral cavity is a unique niche suitable for a number of microorganisms to colonize, proliferate, and live in harmony as a community, a so-called biofilm. Oral epithelium plays a main role as a physical barrier between the microbial biofilm in the external environment and underlying connective tissue and blood vessels. Naturally, this barrier can be disrupted, since the oral epithelium is the only site in the body normally penetrated by a hard tissue, namely, a tooth. The junction between oral epithelium and the tooth is, therefore, considered a site that is readily susceptible to infection from various microorganisms living in dental plaque. Previously, the role of oral epithelium was viewed as that of an innocent bystander. However, it is now apparent that oral epithelial cells can respond to continuous microbial challenges from the dental plaque by production of cytokines, chemokines, and antimicrobial peptides, which enhance inflammation and immune response in periodontal tissues. Uncontrolled inflammation and immune response from excessive production of these pro-inflammatory molecules is considered one of the etiological factors in the pathogenesis of periodontal disease.

In the oral cavity, antimicrobial peptides may play a critical role in maintaining balance between periodontal health and disease. Therefore, their biological and clinical significances, particularly the ones that are pertinent to periodontal disease, will be emphasized in this chapter. These include the differential expression of antimicrobial peptides in healthy and diseased periodontal tissues and in gingival crevicular fluid (GCF), their antimicrobial effects against a variety of periodontal microorganisms, and their novel functions, related to host immune responses in periodontal disease. Furthermore, some recent studies have demonstrated a connection between the deficiencies in antimicrobial peptide production or function and patients affected with some types of periodontitis, highlighting the clinical importance of these antimicrobial peptides.

Two well-characterized families of antimicrobial peptides, including defensin and cathelicidin, are present in saliva and GCF, and localized in the oral mucosa (Dale and Fredericks, 2005). These peptides include β-defensins that are expressed in the oral epithelial cells, α-defensins that are secreted from neutrophil granules, and LL-37, the only human antimicrobial peptide in the cathelicidin family, which mainly derives from neutrophil granules and to a lesser extent from oral epithelial cells (Dale et al, 2001). The synthesis of some of these antimicrobial peptides can be considerably up-regulated upon exposure to oral microorganisms; thus, these peptides are regarded as essential effector molecules in innate immunity. In this chapter, basic knowledge, regarding expression and regulation of defensins and LL-37, as well as their antimicrobial activities and other functions, will be extensively reviewed. However, a review of other antimicrobial peptides present in the oral cavity, such as calprotectin, adrenomedullin, histatins, etc., is beyond the scope of this chapter and will not be discussed.

GENERAL INFORMATION ON HUMAN CATHELICIDIN AND DEFENSIN

Cathelicidin is a family of antimicrobial peptides that contain a cathelin domain at their NH_3-terminus and an antimicrobial domain at their COOH-terminus (Zanetti et al, 1995). Whereas the amino acid sequence of the cathelin domain is conserved throughout animal species tested to date, the sequence of the antimicrobial domain exhibits considerable variations, accounting for various molecular structures, such as α-helix, β-sheet, etc., possibly reflecting the nature of microbial diversity. The cathelin domain, categorized as a member of the cystatin family (Ritonja et al, 1989), primarily functions as a cathepsin L inhibitor, from which the name of this domain is derived (Kopitar et al, 1989). However, it was later demonstrated that this domain also possesses an antimicrobial function against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (Zaiou et al, 2003), yet its antimicrobial mechanism is still largely unknown. The first cathelicidin antimicrobial peptide was isolated from bovine neutrophils (Romeo et al, 1988). Subsequently, several cathelicidin peptides were identified in various mammals, particularly humans. The only cathelicidin in humans, LL-37, is derived from proteolytic processing of a precursor peptide, human cationic antimicrobial protein-18 (hCAP-18), and contains two leucines at its NH_3-terminus (Agerberth et al, 1995; Cowland et al, 1995).

Defensin is a family of small cationic antimicrobial peptides, containing six unique cysteine amino acids that form three disulfide bonds, functioning in stabilization of their β-sheet structure (Zasloff, 2002; Ganz, 2003). Moreover, these peptides, comprising several positively charged amino acids that favorably interact with negatively charged microbial membranes, can form a complex structure, such as a dimeric structure (Hill et al, 1991). In addition, the defensin peptides contain both hydrophobic and hydrophilic domains in their molecules, a so-called amphipathic structure. All of these properties, thus, make the defensins suitable for membrane integration that eventually leads to a pore formation in the membrane. The pore-forming mechanism of the defensins is then believed to be a crucial process in their antimicrobial function. Therefore, it has been shown by a number of studies that the defensins exert their broad spectrum of antimicrobial activities against gram-negative and gram-positive bacteria, fungi, and some enveloped viruses (Ganz, 2003).

The human defensin family can be further divided into two subfamilies, i.e., α-defensin and β-defensin subfamilies. In the α-defensin subfamily, four of the six α-defensins, human neutrophil peptide-1, -2, -3, and -4 (HNP-1, -2, -3, and -4), are synthesized and stored in neutrophil granules (Ganz et al, 1985; Wilde et al, 1989), while the other two α-defensins, human defensin-5 and -6 (HD-5 and -6), are synthesized and stored in the granules of Paneth cells, specialized epithelial cells located at the crypts of Lieberkühn of the small intestine (Jones and Bevins, 1992; 1993). Being encoded by the same gene, the pro-peptide of HNP-1, -2, and -3 comprises 94 amino acids, which is successively cleaved by putative proteolytic enzymes, yielding different sizes of the mature peptides that are stored in azurophilic granules (Valore and Ganz, 1992). The number of amino acids in the mature peptides of HNP-1 to HNP-3 varies from 29 to 30 amino acids. On the other hand, HD-5 and HD-6 are stored in Paneth cell granules as pro-peptides, and are subsequently activated by trypsin digestion upon release into the intestinal lumen (Ghosh et al, 2002). HNP-4 is encoded by another gene, and its amino acid sequence completely differs from that of HNP-1, HNP-2, and HNP-3, leaving only the identical characteristic cysteines and some arginines (Wilde et al, 1989).

In the β-defensin subfamily, four human β-defensins, human β-defensin-1, -2, -3, and -4 (hBD-1, -2, -3, and -4), are principally expressed in epithelial cells that cover several tissues and organs, particularly skin and the mucosal surfaces of gastrointestinal, respiratory, and urogenital tracts, whereas hBD-5 and hBD-6 are expressed only in epididymis (Semple et al, 2003). However, only hBD-1, -2, and -3 are found in the oral cavity (Abiko et al, 2007). HBD-1 and hBD-2 peptides are localized in differentiated epithelial cells within the suprabasal layers of normal gingival epithelium (Dale et al, 2001), whereas hBD-3 peptide is expressed in undifferentiated epithelial cells within the basal layer (Lu et al, 2005), suggesting a potential role for hBD-3 as a mediator to signal the underlying connective tissue cells. HBD-1 is constitutively expressed in several epithelial cell types studied to date, especially gingival epithelial cells (Krisanaprakornkit et al, 1998), whereas expression of hBD-2, hBD-3, and hBD-4 is inducible upon stimulation with pro-inflammatory cytokines or contact with microorganisms. The regulation of human β-defensins will be discussed below.

EXPRESSION AND REGULATION OF HUMAN CATHELICIDIN AND DEFENSINS

Human cathelicidin is mainly isolated from neutrophil granules in the amount of 0.627 micrograms per one million neutrophils (Sørensen et al, 1997). After synthesis, human cathelicidin is stored in granules distinct from those that store proteolytic enzymes, such as neutrophil elastase, proteinase-3, etc., to prevent premature activation of the cathelicidin peptide inside the neutrophils. Upon being released into neutrophil phagosomes after bacterial phagocytosis or being released into extracellular environment, the neutrophil cathelicidin is proteolytically cleaved into a mature LL-37 peptide by the proteinase-3 (Sørensen et al, 2001). In addition to regulation of cathelicidin activation by enzymatic cleavage in human neutrophils, cathelicidin expression in other cell types is controlled by exposure to microorganisms, growth factors, and differentiating agents. For instance, LL-37 expression in skin keratinocytes and gastric epithelial cells is induced by *Staphylococcus aureus* and *Helicobacter pyroli*, respectively (Midorikawa et al, 2003; Hase et al, 2003). Furthermore, LL-37 expression in skin keratinocytes is up-regulated by insulin-like growth factor-I and vitamin D, known to promote wound healing and differentiation, respectively (Sørensen et al, 2003; Weber et al, 2005). In addition, LL-37 expression in gastric and small intestinal epithelial cells is induced by short chain fatty acids, including butyrate, via mitogen activated protein (MAP) kinases (Schauber et al, 2003).

Expression of LL-37 can also be found in natural killer cells, monocytes, B- and T-lympocytes (Agerberth et al, 2000), mast cells (Di Nardo et al, 2003), epithelial cells lining respiratory (Bals et al, 1998) and gastrointestinal tracts (Tollin et al, 2003), reproductive organs (Agerberth et al, 1995; Frohm Nilsson et al, 1999; Malm et al, 2000), salivary glands (Murakami et al, 2002a), sweat glands (Murakami et al, 2002b), and in inflammatory skin disorders (Frohm et al, 1997). In the oral cavity, LL-37 is expressed in buccal and tongue mucosa (Frohm Nilsson et al, 1999), and its expression is up-regulated in the inflamed gingival tissues (Hosokawa et al, 2006). Correspondingly, the concentrations of LL-37 in the gingival tissue, whether derived from neutrophils or from gingival epithelium, correlate positively with the depth of the gingival crevice, suggesting that the LL-37 levels may be

used as one diagnostic tool in inflammatory periodontal disorders (Hosokawa et al, 2006). In addition, LL-37 peptide is detected in saliva (Murakami et al, 2002a) and GCF (Puklo et al, 2008), and LL-37 levels in GCF are significantly elevated in patients with chronic periodontitis compared to those in patients with gingivitis or to those in healthy volunteers (Türkoğlu et al, 2009). However, it is likely that LL-37 present in saliva and GCF originates mostly from neutrophil granules (Dale and Fredericks, 2005).

The neutrophil α-defensin gene (*DEFA1*) is located on chromosome 8 (8p23) (Sparkes et al, 1989), and the number of such genes in different individuals varies from two to three genes per diploid cell (Mars et al, 1995). HNP-1, HNP-2, and HNP-3 mRNAs are mainly expressed in neutrophils, and their respective proteins were first characterized from azurophilic granules (Ganz et al, 1985) that also comprise other antimicrobial peptides, such as myeloperoxidase, cathelicidin, etc. Moreover, expression of HNP-1, HNP-2, and HNP-3 can be detected in lymphocytes (Blomqvist et al, 1999; Agerberth et al, 2000) and Langerhans cells in the vicinity of epithelial dysplasia adjacent to precancerous lesions and oral squamous cell carcinoma (Mizukawa et al, 1999), but their expression is not found in normal oral mucosa. They are also present in ductal cells of submandibular salivary glands from patients with oral cancer (Mizukawa et al, 2000).

With respect to periodontal tissue, the detectable amounts of HNP-1, HNP-2, and HNP-3 in GCF can vary from 270 to 2000 nanogram per site (or approximately equivalent to mg/ml) (McKay et al, 1999), which is sufficient for their antimicrobial function in periodontium. By virtue of matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), it has been demonstrated that HNP-1 is most abundant in GCF, whereas HNP-3 is least abundant (Lundy et al, 2005). Moreover, the concentrations of HNP-1 to HNP-3, as well as those of LL-37 and hBD-3, have been quantified in saliva. These concentrations (up to twelve μg/ml) are variable in the human population (Tao et al, 2005). In addition, the median levels of HNP-1 to HNP-3 in saliva are significantly higher in children without dental caries than in those with dental caries experience, whereas the median levels of LL-37 and hBD-3 do not correlate with caries experience (Tao et al, 2005), suggesting the protective role of neutrophil α-defensins against dental caries.

Enteric α-defensin genes are located on chromosome 8 in the same vicinity as *DEFA1*, suggesting the duplication of α-defensin genes during evolution (Bevins et al, 1996). Up to now, only very weak HD-5 expression has been identified in a few oral tissue samples, whereas HD-6 expression is not detectable at all, indicating that enteric α-defensins do not play any role in the innate immunity of the oral cavity (Dunsche et al, 2001).

β-defensins are somewhat larger than α-defensins. Although 28 β-defensin genes have been discovered by computer searching of the human genome (Schutte et al, 2002), expression of only six human β-defensins, hBD-1 to hBD-6, has been characterized to date in human tissues and organs. HBD-1 is the first human β-defensin, isolated from hemofiltrate passing through the kidney at the nanomolar levels (Bensch et al, 1995). The gene encoding hBD-1, *DEFB1*, is on chromosome 8, in close proximity to *DEFA1*, around 100-150 kilobases apart (Liu et al, 1997). However, both the amino acid sequence and the pairing between two cysteine amino acids that form the disulfide bond in hBD-1 greatly differ from both the sequence and pairing in HNP-1; thus, creating a new β-defensin subfamily. *DEFB1* contains two exons with one large 6962 base pair (bp) intron (Liu et al, 1997). The two exons encode a 362 bp complementary DNA (cDNA) that is translated into an hBD-1 pro-peptide

(Liu et al, 1997). The hBD-1 pro-peptide is subsequently cleaved to yield several hBD-1 mature peptides, ranging from 36 to 47 amino acids long. Widespread and low expression of hBD-1 has been detected in various epithelia lining several organs, including trachea, bronchus, prostate gland, mammary gland, placenta, thymus, testis, skin, small intestine (Zhao et al, 1996), pancreas and kidney – especially, the collecting duct, distal tubule, and loop of Henle – (Schnapp et al, 1998), vagina, endometrium, Fallopian tube (Valore et al, 1998), and salivary glands (Bonass et al, 1999; Sahasrabudhe et al, 2000).

In the oral mucosa, hBD-1 expression is found in gingival epithelium, but is not associated with the amount of IL-8 expression in the gingival tissue (Krisanaprakornkit et al, 1998). In other words, the amount of hBD-1 expression in gingival tissue does not correlate with the degree of tissue inflammation, but varies among different individuals (Krisanaprakornkit et al, 1998). Moreover, confluent cultured gingival epithelial cells constitutively express hBD-1 mRNA at baseline levels; however, its expression is up-regulated in a post-confluent culture, representing the state of cellular differentiation *in vitro* (Dale et al, 2001). In this study, the state of differentiation is shown by increased mRNA expression of profilaggrin, a late marker for differentiation. Consistent with the increased hBD-1 mRNA expression in the post-confluent culture, hBD-1 mRNA and peptide are localized in the suprabasal layers of oral epithelium *in vivo* (Dale et al, 2001). On the other hand, it has been demonstrated that increased hBD-1 expression can, in turn, induce differentiation in skin keratinocytes (Frye et al, 2001).

By using a protein chip array together with surface enhanced laser desorption/ionization (SELDI) and time-of-flight mass spectrometry, hBD-1 peptide at a molecular mass of about 4.7 kilodalton (kDa) is detected in culture medium of gingival epithelial cells (Diamond et al, 2001). Unlike known concentrations of neutrophil α-defensins in GCF, the precise levels of hBD-1 present in GCF have not yet been accurately quantified. Highly variable amounts of hBD-1 peptide have been found in saliva and GCF, collected from different normal individuals (Diamond et al, 2001). It is possible that salivary ductal cells may also contribute some hBD-1 peptide detected in saliva in addition to hBD-1 peptide synthesis by oral epithelial cells (Sahasrabudhe et al, 2000). It is noteworthy that hBD-1 and hBD-2 are neither expressed in cultured gingival fibroblasts (Krisanaprakornkit et al, 1998; 2000) nor found in the underlying connective tissue of the oral mucosa (Dale et al, 2001).

The second human β-defensin, hBD-2, was first isolated in large amounts from psoriatic skin keratinocytes (Harder et al, 1997a). The gene encoding hBD-2 is *DEFB4*, which is located on chromosome 8, region 8p22-p23.1, in close proximity to *DEFA1* and *DEFB1* (Harder et al, 1997b). *DEFB4* contains one 1639 bp intron (Liu et al, 1998), and two small exons that encode a signal peptide domain and a mature peptide, whose sizes are 23 and 41 amino acids long, respectively (Harder et al, 1997b). Expression of both hBD-1 and hBD-2 is localized in the suprabasal layers of normal epidermis (Ali et al, 2001), identical with their expression in normal oral mucosa (Dale et al, 2001). HBD-2 peptide is stored in lamellar granules in the spinous layer of epidermis, and later released into the extracellular environment with other lipids in the granular layer, suggesting that lipids covering the skin function as a natural barrier against water permeability and microbial invasion due to the presence of antimicrobial peptides (Oren et al, 2003).

As with the inducible expression of hBD-2 by microorganisms and pro-inflammatory cytokines in other cell types, hBD-2 mRNA is up-regulated in cultured gingival epithelial

cells in response to stimulation with IL-1β, TNF-α, phorbol ester, a potent epithelial activator, and Gram-negative periodontal bacteria, including *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* (Mathews et al, 1999; Krisanaprakornkit et al, 2000; Noguchi et al, 2003; Chung et al, 2004; Taguchi and Imai, 2006; Laube et al, 2008). Nevertheless, unlike the critical role of CD14, a lipopolysaccharide (LPS) co-receptor, and nuclear factor-kappa B (NF-κB) in hBD-2 induction in respiratory epithelial cells and mononuclear phagocytes (Becker et al, 2000; Harder et al, 2000; Tsutsumi-Ishii and Nagaoka, 2002), CD14 and NF-κB are neither critical nor essential for hBD-2 up-regulation in gingival epithelial cells (Krisanaprakornkit et al, 2002). In fact, a purified LPS fraction of either *Fusobacterium nucleatum* or *Aggregatibacter actinomycetemcomitans* is a poor hBD-2 activator in gingival epithelial cells (Krisanaprakornkit et al, 2000; Laube et al, 2008, respectively). Furthermore, p38 MAP kinase and c-Jun N-terminal MAP kinase (JNK) control hBD-2 mRNA up-regulation in response to *Fusobacterium nucleatum* in gingival epithelial cells (Krisanaprakornkit et al, 2002). Likewise, the MAP kinase pathways, but not the NF-κB transcription factor, are critical for hBD-2 up-regulation by the outer membrane protein 100 (Omp100; named after its molecular mass) of *Aggregatibacter actinomycetemcomitans* (Ouhara et al, 2006). Taken together, these findings suggest different cellular receptors and intracellular signaling mechanisms to control hBD-2 up-regulation by different stimulants in distinct cell types.

In addition to the involvement of p38 MAP kinase and JNK in hBD-2 up-regulation by *Fusobacterium nucleatum*, it is shown that an increase in intracellular calcium ion and phosphorylated phospholipase D, two important molecules in regulating epithelial cell differentiation (Exton, 1999; Bollag et al, 2005), are involved in hBD-2 up-regulation by *Fusobacterium nucleatum* (Krisanaprakornkit et al, 2003; 2008). It is noteworthy that treatment of gingival epithelial cells with either exogenously added calcium ions or thapsigargin, an inhibitor of the sarcoendoplasmic reticulum calcium (SERCA) pump, an inhibitor that leads to continuous calcium ion release from its intracellular storage, induces hBD-2 mRNA, whereas BAPTA-AM, a cell permeable calcium chelator, blocks hBD-2 mRNA up-regulation by *Fusobacterium nucleatum* and thapsigargin in a dose-dependent manner (Krisanaprakornkit et al, 2003). In summary, the regulation of hBD-2 expression can be controlled by both inflammation from bacteria and epithelial differentiation.

Consistent with this conclusion, the strongest hBD-2 expression in gingival tissue is found at the gingival margin, adjacent to microbial plaque accumulation, and hBD-2 expression is localized in differentiated epithelial cells within the suprabasal layers of gingival epithelium (Dale et al, 2001). Moreover, the localization of hBD-2 peptide is found not only in cultured gingival epithelial cells that express involucrin, another marker for differentiation, but also in stimulated cells with infectious and pro-inflammatory stimulants (Dale et al, 2001). In contrast, neither hBD-1 nor hBD-2 is expressed in junctional epithelium (Dale et al, 2001), which consists of relatively undifferentiated epithelial cells, implying that the junctional epithelium may be more susceptible to infection than other areas of gingival epithelium because of the lack of some antimicrobial peptides. However, it is probable that other antimicrobial peptides, such as α-defensins, LL-37, etc., released from neutrophils that transmigrate from blood vessels into the junctional epithelium and gingival crevice, may perform this antimicrobial function instead (Dale and Fredericks, 2005).

Using biochemical and molecular biology techniques, the gene encoding hBD-3 (*DEFB103*) has been cloned from human skin keratinocytes and alveolar epithelial cells, and the amino acid composition of hBD-3 has been sequenced and classified as a novel peptide in the β-defensin subfamily (Harder et al, 2001). *DEFB103*, containing two small exons, is located 13 kb upstream from *DEFB4* that encodes hBD-2 on chromosome 8 (Jia et al, 2001). HBD-3 cDNA is translated into an hBD-3 pro-peptide that comprises a signal peptide domain (22 amino acids long) and a mature peptide (45 amino acids long). The amino acid sequence of hBD-3 is 43% identical to that of hBD-2 (Jia et al, 2001).

In addition to skin keratinocytes, hBD-3 is expressed in various epithelia lining several tissues, including gingiva (Jia et al, 2001; Dunsche et al, 2002), tonsils (Harder et al, 2001), esophagus, trachea, placenta, and fetal thymus glands (Jia et al, 2001). In the oral cavity, hBD-3 mRNA and peptide are localized in the basal layer of normal gingival epithelium (Lu et al, 2005), whereas hBD-1 and hBD-2 are expressed in the suprabasal layers (Dale et al, 2001). Furthermore, hBD-3 mRNA is expressed in both inflamed and non-inflamed epithelium and salivary glands (Dunsche et al, 2001), and its expression is up-regulated in leukoplakia and oral lichen planus (Nishimura et al, 2003). *In vitro*, hBD-3 mRNA expression is induced in cultured epithelial cells that are stimulated with IFN-γ, TNF-α, and IL-1β (García et al, 2001; Harder et al, 2001; Jia et al, 2001), although IFN-γ does not up-regulate hBD-2 mRNA (García et al, 2001). Consistent with the findings obtained from these studies, it was later demonstrated that IFN-γ is a primary inducer for hBD-3 expression, whereas IL-1β and TNF-α are major stimulants for hBD-2 expression (Joly et al, 2005).

With respect to up-regulation of hBD-3 by oral microorganisms, hBD-3 mRNA expression is induced by live nonperiodontopathic bacteria (Ji et al, 2007a), including *Streptococcus sanguinis* and *Streptococcus gordonii*, and some periodontopathic bacteria, including *Aggregatibacter actinomycetemcomitans* (Feucht et al, 2003), *Prevotella intermedia*, and *Fusobacterium nucleatum* (Ji et al, 2007a). In contrast, three well known causative pathogens in chronic periodontitis, including *Porphyromonas gingivalis*, *Tanerella forsythia*, and *Treponema denticola*, down-regulate hBD-3 mRNA expression, as well as IL-8 production and secretion in an oral epithelial cell line (Ji et al, 2007a). This indicates that these so-called "red-complex" periodontal pathogens may suppress innate immune responses of oral epithelial cells by an immune-evading mechanism, known as "chemokine paralysis" (Darveau et al, 1998). Furthermore, the red-complex bacteria can tolerate the host immune response by being more resistant to LL-37 and phagocytosis by neutrophils (Ji et al, 2007b), indicating their strong implication with chronic periodontal infection.

ANTIMICROBIAL ACTIVITY

Up to the present, there have been an enormous number of *in vitro* studies, showing the antimicrobial activity of LL-37 and human defensins against various pathogens associated with a variety of human diseases. All of these studies cannot be completely mentioned in this chapter due to the space limitation. Therefore, the scope of this topic will be restricted to the antimicrobial effects on oral pathogens, especially the ones associated with periodontal disease. In the oral cavity, the warm temperature and moistened mucosal and tooth surfaces are suitable for microbial colonization and then the formation of biofilm, so-called dental

plaque. The dental plaque is essential for some specific oral microorganisms to survive and thrive in this complex community. It is conceivable that the exopolysaccharide-producing plaque can protect oral microorganisms from exposure to antibiotics, or antimicrobial peptides in the context of this discussion. As with antibiotics, it is, therefore, likely that plaque microorganisms are more resistant to destruction by antimicrobial peptides than are planktonic microrganisms present in the saliva. Consequently, antimicrobial peptides can be regarded as one of the selective pressures that oral microorganisms must overcome in order to establish colonies in the dental plaque.

Furthermore, it should be emphasized that the results obtained from most studies that examine the susceptibility of one or more microbial species to individual antimicrobial peptides *in vitro* may not represent the real effectiveness of antimicrobial peptides due to the complexity of interactions between host and microorganisms or between two different types of microorganisms in the dental plaque. However, it is rather difficult to evaluate the effectiveness of antimicrobial peptides in such a complicated situation *in vivo*. Fortunately, some recent *in vivo* studies have shed light into the clinical significance of antimicrobial peptides for periodontal homeostasis. In this regard, it has been shown that genetic and acquired deficiencies of some antimicrobial peptides are associated with the pathogenesis of some types of periodontitis (Pütsep et al, 2002; Puklo et al, 2008), and this will be discussed under the next heading.

Other factors that influence the antimicrobial effects of some antimicrobial peptides are high salt concentrations that are shown to inhibit antimicrobial functions in other parts of the body (Goldman et al, 1997; Midorikawa et al, 2003) and the presence of inhibitors in serum (Tanaka et al, 2000). However, in the oral cavity, antimicrobial peptides may not be affected by these factors, since the peptides function at the mucosal surface, where the concentrations of salt or inhibitors, diluted with saliva, are too low to exert any significant inhibitory action.

At the outset of the study of the antimicrobial effects on oral bacteria, the bactericidal activity of LL-37 was tested against different strains of *Aggregatibacter actinomycetemcomitans* and *Capnocytophaga* spp., which are implicated in the pathogenesis of juvenile periodontitis and gingivitis, respectively (Tanaka et al, 2000). It was found that the concentrations of LL-37 (below 12 μg/ml) already killed all strains of these two bacteria by 99%. Subsequently, under a more detailed investigation into the antimicrobial effects of LL-37 against different kinds of periodontal bacteria, involved with various stages of dental plaque formation, it was demonstrated that the early colonizing yellow-complex bacteria, such as oral *Streptococci*, *Actinomyces*, etc., and the bridging orange-complex bacterium, i.e., *Fusobacterium nucleatum*, are susceptible to the bactericidal activity of LL-37 with low minimum inhibitory concentrations (MICs) in μg/ml (Ji et al, 2007b). Similar results have also been obtained from another study (Ouhara et al, 2005), which shows the antimicrobial effects of LL-37 against various gram-positive oral *Streptococci*. In contrast, the red-complex periodontopathic bacteria, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola*, are more resistant to LL-37 than are other bacteria (Ji et al, 2007b), suggesting their strong involvement with periodontitis.

Furthermore, LL-37 exerts its candidacidal activity by disrupting the yeast cell membrane, leading to membrane fragmentation and a release of intracellular contents, such as adenosine triphosphate (den Hertog et al, 2005). With respect to the antimicrobial activity of neutrophil α-defensins, oral microorganisms are usually resistant to HNP-1 to HNP-3, even

though a synergistic antimicrobial effect is revealed between HNP-1 and LL-37 against *Escherichia coli* and *Staphylococcus aureus* (Nagaoka et al, 2000).

The antimicrobial activities of hBD-1, hBD-2, and hBD-3 peptides have been tested against different strains of gram-negative and gram-positive oral bacteria and fungi in several *in vitro* studies. In brief, it is found that, among these three human β-defensins, hBD-3 has the strongest antibacterial activity against oral *Streptococci* and some periodontal bacteria, especially all strains of *Fusobacterium nucleatum*, while hBD-1 and hBD-2 are less effective against both oral gram-positive and gram-negative bacteria (Ouhara et al, 2005). This may be owing to the strong basic property of hBD-3 due to several positively charged amino acids in its molecule (Schibli et al, 2002). However, hBD-2 exerts its antimicrobial activity well with cariogenic bacteria, including *Streptococcus mutans* and *Streptococcus sobrinus* (Nishimura et al, 2004). Generally, aerobic bacteria are more susceptible to hBD-2 and hBD-3 peptides than are anaerobic bacteria (Joly et al, 2004). Although the antimicrobial activity of β-defensins is normally inhibited by high salt concentrations, as shown in other studies (Goldman et al, 1997; Midorikawa et al, 2003), the antimicrobial activity of hBD-3 against periodontal and cariogenic bacteria is not much influenced by high salt concentrations (Ouhara et al, 2005). It can be concluded that, among the antimicrobial peptides of the defensin and cathelicidin families, hBD-3 and LL-37 exhibit the greatest degrees of antimicrobial effects against various oral bacteria, especially most aerobic bacteria and some periodontal bacteria. Although the red-complex periodontopathic bacteria are more resistant to hBD-3 and LL-37, it is likely that hBD-3 and LL-37 may still play a role in the pathogenesis of periodontal disease by reducing the number of early colonizing and bridging bacteria so that the late colonizers, including the red-complex periodontopathic bacteria, cannot colonize and thrive in dental plaque.

Interestingly, some pathogenic bacteria have evolved other virulence mechanisms that enable them to resist the activity of antimicrobial peptides. For example, antimicrobial peptides can be degraded by distinct enzymes secreted from bacterial pathogens, including SufA, a novel subtilisin-like serine protease of *Finegoldia magna* (Karlsson et al, 2007), streptopain of *Streptococcus pyogens*, elastase of *Pseudomonas aeruginosa*, gelatinase of *Enterococcus faecalis* (Schmidtchen et al, 2002), and the 50 kDa metalloprotease (ZapA) of *Proteus mirabilis* (Belas et al, 2004). By analogy, *Porphyromonas gingivalis*, one of the red-complex bacterial triad, can also be resistant to the bactericidal activity of antimicrobial peptides due to its ability to synthesize a group of enzymes, called gingipains. In fact, it has been recently demonstrated that the gingipains efficiently degrade several different antimicrobial peptides, including HNP-1, hBD-1, hBD-2, and hBD-3 (Carlisle et al, 2009). However, it was formerly shown that the degradation of antimicrobial peptides by gingipains does not appear to contribute to the resistance of *Porphyromonas gingivalis* to the antimicrobial action (Bachrach et al, 2008).

The possible alternative mechanisms for the resistance of *Porphyromonas gingivalis* may be due to the possibility that gingipains secreted from *Porphyromonas gingivalis* may prevent destruction of its commensal bacteria, i.e., *Fusobacterium nucleatum*, which is easily destroyed by antimicrobial peptides. Otherwise, gingipains and proteases released from *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively, may inactivate cystatins, inhibitors that function against endogenously-derived proteases, such as host cathepsins, etc. This ultimately releases cathepsins from their tight control by cystatins. The active cathepsins,

including cathepsin B, L, and S in the cysteine protease family, may then proteolytically degrade antimicrobial peptides, resulting in depletion of antimicrobial activity (Taggart et al, 2003). The gingipains and other virulence factors make *Porphyromonas gingivalis* one of the critical periodontal pathogens, and antimicrobial peptides may then be regarded as an important determinant for the "normal" and "diseased" states of periodontium.

As with *Porphyromonas gingivalis*, *Treponema denticola*, another red-complex periodontal pathogen, is resistant to the antimicrobial activity of human β-defensins, but by other distinct mechanisms, since *Treponema denticola* does not produce degrading enzymes. These mechanisms include an efflux pump of defensin peptides that enter the cytoplasm (Brissette and Lukehart, 2007) and reduction of defensin binding to the microbial surface due to the lack of LPS (Brissette and Lukehart, 2002). Furthermore, *Treponema denticola* cannot induce the host innate immune response, i.e., expression of hBD-2 and IL-8, in gingival epithelial cells (Brissette et al, 2008). The immune tolerant mechanisms of *Treponema denticola*, including resistance to the antimicrobial effect of antimicrobial peptides and silencing host innate immunity, may, therefore, partly explain the strong association of *Treponema denticola* with chronic periodontitis.

IMMUNOREGULATORY EFFECTS

In addition to its antimicrobial activities, LL-37 can elicit host innate and acquired immune responses. For example, LL-37 inhibits the binding of endotoxin LPS to its receptor complex, comprising Toll-like receptors (TLRs) and CD14, which results in prevention of sepsis (Fukumoto et al, 2005; Mookherjee et al, 2006) and suppression of the synthesis of nitric oxide (Ciornei et al, 2003), TNF-α, prostaglandin E_2 (PGE_2), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-2 (MIP-2) (Ohgami et al, 2003). Moreover, LL-37 can block macrophage stimulation with lipoteichoic acid and lipoarabinomannan, indicating that LL-37 can bind to various molecules on bacterial cell membranes (Scott et al, 2002).

LL-37 chemoattracts monocytes, neutrophils, CD4 T lymphocytes, and eosinophils along its concentration gradient via a G-protein coupled receptor, namely, formyl peptide receptor-like 1 (FPRL1), on these cells (De Yang et al, 2000; Tjabringa et al, 2006). However, the appropriate LL-37 concentrations fall within the range between 10^{-7} and 10^{-5} molar, which are far greater than those of chemokines used in chemotaxis. In this regard, it is possible that LL-37 can play a role as a chemoattractant at inflamed periodontal sites only when elevated concentrations of LL-37 derived from inflamed gingival epithelial cells and granules of neutrophils, which are abundant in diseased tissues, are sufficient to exert the chemotactic effect. Moreover, LL-37 attracts migration of mast cells in rats (Niyonsaba et al, 2002a) and induces histamine release from mast cell granules via intracellular calcium mobilization (Niyonsaba et al, 2001), leading to enhanced phagocytosis of opsonized microorganisms. LL-37 can also induce dendritic cell differentiation, which then activates cell-mediated acquired immunity through a Th1 profile (Davidson et al, 2004).

Several studies have also shown the inducible effect of LL-37 on the expression of several immune-related genes. For instance, LL-37 can induce expression of chemokines and chemokine receptors (Scott et al, 2002) via MAP kinase pathways (Bowdish et al, 2004). It can also induce expression of intercellular adhesion molecule-1 (Edfeldt et al, 2006),

implying an indirect role for LL-37 in chemotaxis in addition to its direct role, as indicated above. LL-37 transactivates an epidermal growth factor receptor (EGFR) through induction of matrix metalloproteinase activity, resulting in interleukin-8 (IL-8) up-regulation and increased cell proliferation in human bronchial epithelial cells (Tjabringa et al, 2003). Similarly, LL-37 enhances IL-8 expression and release by human airway smooth muscle cells, albeit through purinergic receptors (Zuyderduyn et al, 2006). In addition, in the presence of IL-1β, lower LL-37 concentrations can synergistically induce IL-8 synthesis in both human keratinocytes and bronchial epithelial cells (Filewod et al, 2009), and up-regulate expression of IL-6, IL-10, MCP-1, and MCP-3 in peripheral blood mononuclear cells (Yu et al, 2007).

With respect to periodontal cells, we have recently found similar IL-8 mRNA up-regulation by LL-37 in both gingival epithelial cells and gingival fibroblasts in dose- and time-dependent manners (Figure 1). Interestingly, the kinetics of IL-8 up-regulation between these two cell types shows distinct profiles, indicating different signaling pathways controlling IL-8 expression (Figure 1). Therefore, it is possible that LL-37 may be responsible for controlling neutrophil transmigration from blood vessels into diseased periodontal tissue in chronic periodontitis.

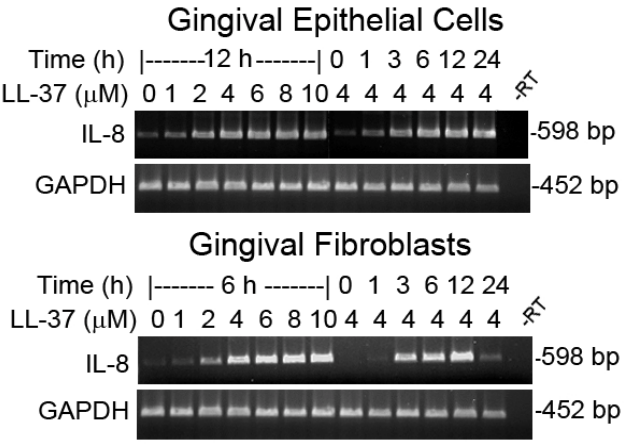

Figure 1. Up-regulation of IL-8 mRNA expression by treatment with various doses (0-10 μM) of LL-37 for indicated times (0-24 hours) in gingival epithelial cells and gingival fibroblasts. Note a dose-dependent increase in IL-8 expression. While IL-8 mRNA was transiently induced by LL-37 in gingival fibroblasts, up-regulation of IL-8 mRNA in gingival epithelial cells accumulated from 0 to 24 hours. Expression of glyceraldehyde phosphate dehydrogenase (GAPDH) was equivalent among all samples, indicating the equal mRNA loadings in this experiment. –RT represents a negative control sample where a reverse transcriptase enzyme was omitted from the reaction.

As with LL-37, neutrophil α-defensins also exert their immunomodulating effects on various types of immune cells. For example, HNP-1 and HNP-2 can induce chemotaxis of T-lymphocytes (Chertov et al, 1996), dendritic cells (Yang et al, 2000), macrophages, and mast cells (Grigat et al, 2007). Neutrophil α-defensins enhance cytokine expression in T-lymphocytes and immunoglobulin G production in B-lymphocytes (Tani et al, 2000), induce IL-8 expression in lung epithelial cells (van Wetering et al, 1997), and promote IL-1β release through posttranslational processing (Perregaux et al, 2002).

With regard to the immunoregulatory effects of human β-defensins, hBD-1 and hBD-2 chemoattract immature dendritic cells and memory T-lymphocytes through a G-protein

coupled chemokine receptor, i.e., CCR6, indicating the ability of these two β-defensins to bridge innate and acquired immunity (Yang et al, 1999). HBD-1 activates monocyte-derived dendritic cells and promotes the synthesis of several cytokines (Presicce et al, 2009). Moreover, hBD-1 up-regulates expression of CD91, a scavenger receptor that recognizes defensins, on the dendritic cell surface, indicating a positive feedback of dendritic cell activation (Presicce et al, 2009). However, hBD-2, but not hBD-1, enhances chemotaxis of mast cells (Niyonsaba et al, 2002b) and neutrophils treated with TNF-α (Niyonsaba et al, 2004), possibly via a CCR6 that mediates the signal through activation of phospholipase C. Furthermore, hBD-2 induces histamine release from mast cells and prostaglandin D synthesis (Niyonsaba et al, 2001). As with the dissociation of antimicrobial activities from the host immunostimulatory activities of LL-37 (Braff et al, 2005), it has recently been demonstrated that the chemoattractant and antimicrobial activities of β-defensins are exerted by distinct domains, and both of these activities do not rely on the intramolecular disulfide bridges of β-defensins (Taylor et al, 2008).

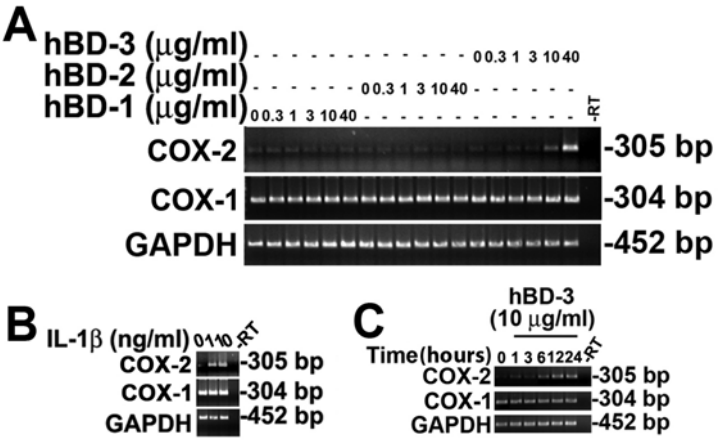

Figure 2. COX-2 mRNA up-regulation by hBD-3. Human gingival fibroblasts were treated for 18 hours with (A) various doses (0-40 μg/ml) of hBD-1, hBD-2, hBD-3, (B) IL-1β as a positive control, (C) 10 μg/ml of hBD-3 for various times (0-24 hours), or left untreated as a negative control. Total RNA was harvested and RT-PCR was conducted to analyze mRNA expression for cyclooxygenase-1 (COX-1), COX-2, and GAPDH. Note constitutive COX-1 mRNA expression, while COX-2 mRNA was up-regulated by hBD-3 treatment in dose- and time-dependent manners. This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

Regarding a potential role for human β-defensins in modulating host immune responses in periodontal disease, we have very recently shown that only hBD-3, but not hBD-1 or hBD-2, induces mRNA and protein expression of cyclooxygenase-2 (COX-2) in gingival fibroblasts in dose- and time-dependent fashions (Figures 2 and 3, respectively).

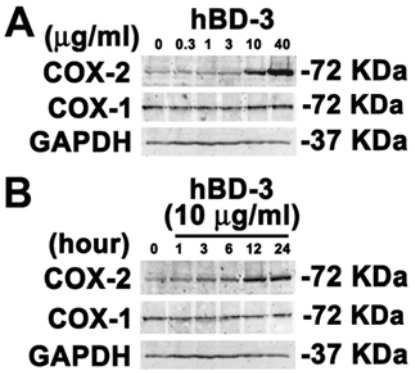

Figure 3. Up-regulation of COX-2 protein by hBD-3 in human gingival fibroblasts. Consistent with COX-2 mRNA up-regulation, COX-2 protein expression was up-regulated by hBD-3 treatment in (A) dose- and (B) time-dependent manners. Note constitutive COX-1 protein expression. This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

In comparison to up-regulation of COX-2 mRNA by 1-10 ng/ml of IL-1β, up-regulation by hBD-3 requires much higher concentrations (Figure 2), suggesting that epithelial-derived hBD-3 may act as a local immunomodulator on fibroblasts in adjacent connective tissue, where its concentration is sufficient to reach the low range of μg/ml. This concentration can probably be achieved by persistent inflammation in chronic periodontitis. Furthermore, up-regulated COX-2 expression by hBD-3 results in raised PGE_2 levels in cell-free culture supernatants (Table 1), which is confirmed by an experiment using a specific inhibitor of COX-2 activity, i.e., NS-398 (Figure 4). In summary, all of these findings suggest the potential role and ability of hBD-3 in initiating localized inflammation within periodontal tissues.

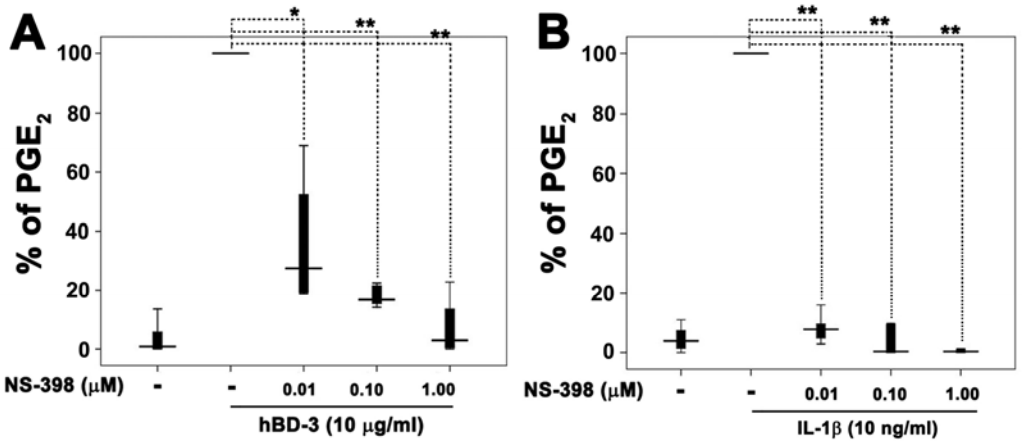

Figure 4. Elevated PGE_2 levels result from induced COX-2 expression. Human gingival fibroblasts were pretreated with indicated doses of NS-398, a specific COX-2 inhibitor, for 30 minutes prior to treatment with either (A) 10 μg/ml of hBD-3 or (B) 10 ng/ml of IL-1β for 18 hours. Cell-free culture supernatants were collected and analyzed for the PGE_2 levels by ELISA. Note a significant inhibition of elevated PGE_2 levels by NS-398 (*, $P < 0.05$; **, $P < 0.01$). This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

Table 1. HBD-3 treatment results in elevated PGE_2 levels in cell-free culture supernatants in a dose-dependent fashion. The cell-free culture supernatants from Figure 2 were collected and analyzed for PGE_2 concentrations (pg/ml) by ELISA. This table is modified from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell

Concentration (μg/ml)		Median PGE_2 concentration (range)
Control		36.40 (34.49-38.32)
hBD-1	0.3	35.91 (33.74-38.08)
hBD-1	1.0	39.22 (36.28-42.16)
hBD-1	3.0	37.42 (34.99-39.85)
hBD-1	10.0	37.35 (34.96-39.73)
hBD-1	40.0	39.61 (35.43-43.78)
hBD-2	0.3	38.30 (35.70-40.89)
hBD-2	1.0	37.58 (35.00-40.16)
hBD-2	3.0	39.64 (34.86-44.42)
hBD-2	10.0	40.51 (38.37-42.66)
hBD-2	40.0	36.82 (32.94-40.69)
hBD-3	0.3	21.33 (20.00-23.00)
hBD-3	1.0	35.55 (22.64-48.31)
hBD-3	3.0	53.16 (48.31-58.00)
hBD-3	10.0	260.59* (260.56-266.03)
hBD-3	40.0	1934.00* (1824.20-2048.10)

* denotes statistically significant difference from untreated control cells at $P < 0.05$.

OTHER BIOLOGICAL ACTIVITIES

Besides the immunomodulation, LL-37 plays a role in tissue repair by stimulating airway epithelial cell proliferation and wound closure (Shaykhiev et al, 2005) and by activating keratinocyte proliferation and migration in the process of re-epithelialization (Heilborn et al, 2003) via transactivation of EGFR and phosphorylation of the signal transducers and activator of transcription 3 (STAT3) (Tokumaru et al, 2005). Consistent with these *in vitro* studies, the levels of LL-37 decrease in chronic ulcer epithelium (Heilborn et al, 2003), whereas adenoviral transfer of LL-37 to the wound in mice results in a significant improvement of wound healing by enhanced re-epithelialization and granulation tissue formation (Carretero et al, 2008). Furthermore, it has recently been shown that LL-37 can suppress keratinocyte apoptosis via a COX-2-dependent mechanism (Chamorro et al, 2009), which is in agreement with the function of LL-37 in promoting cell proliferation and tissue repair, as indicated above. Therefore, it is interesting to determine whether LL-37 plays any role in tissue repair and/or regeneration after periodontal surgery.

Interestingly, exogenously added LL-37 into the wound induces angiogenesis that corresponds to an *in vitro* study (Koczulla et al, 2003), which demonstrates endothelial cell

proliferation and increased numbers of new blood vessel formation through FPRL1 on cultured endothelial cell membrane in response to LL-37 treatment. As in keratinocyte migration, LL-37 also induces migration of human corneal epithelial cells, as well as expression of IL-1β, IL-6, IL-8, and TNF-α (Huang et al, 2006). Moreover, it has been shown that LL-37 can internalize into human lung epithelial cells through endocytosis, and subsequently accumulates in the perinuclear region (Lau et al, 2005).

There are a number of reports that show other biological effects of neutrophil α-defensins and human β-defensins on various cell types. For instance, α-defensins enhance mitosis in some cell types (Murphy et al, 1993), promote tissue repair in airway epithelial cells via MAP kinase pathways (Aarbiou et al, 2002), regulate expression for adhesion molecules on endothelial cells (Chaly et al, 2000), control smooth muscle cell contraction via an α2-macroglobulin receptor (Nassar et al, 2002), induce proliferation of lung fibroblasts and collagen synthesis (Han et al, 2009), and induce expression of some mucin genes, i.e., *MUC5B* and *MUC5AC* (Aarbiou et al, 2004). As with the induction of mucin genes by neutropil α-defensins, it has lately been demonstrated that LL-37 also up-regulates *MUC2* and *MUC3* expression in intestinal epithelial cell lines (Otte et al, 2009). Furthermore, α-defensins affect histamine release from mast cell granules through a G-protein coupled receptor, suggesting their indirect role in vasodilatation (Befus et al, 1999).

Among human β-defensins, hBD-2 activates the differentiation of dental pulp mesenchymal cells into odontoblast-like cells, confirmed by up-regulation of dentin sialophosphoprotein (*DSPP*) gene expression (Shiba et al, 2003). In addition, stimulation of odontoblast-like cells with recombinant hBD-2 leads to increased mRNA expression of several inflammatory genes, including IL-6, IL-8, and cytosolic phospholipase A_2 (Dommisch et al, 2007). Consequently, it is probable that hBD-2 plays a role in reparative dentin formation, as well as immune regulation, in addition to its antimicrobial effect. Furthermore, like LL-37, hBD-2, hBD-3, and hBD-4 stimulate cell migration and proliferation, and production of cytokines and chemokines in skin keratinocytes (Niyonsaba et al, 2007).

DISEASE IMPLICATIONS

Several studies have shown the association between altered expression of epithelial-derived antimicrobial peptides, including LL-37 and human β-defensins, and various skin and epithelial diseases, e.g., acne vulgaris (Chronnell et al, 2001), oral lichen planus, leukoplakia (Nishimura et al, 2003), oral candidiasis (Abiko et al, 2002), condyloma acuminatum, verruca vulgaris (Conner et al, 2002), cholesteatoma (Jung et al, 2003), chronic nasal inflammatory disease (Kim et al, 2003), etc.

Due to space limitations, only one classic example of alteration in antimicrobial peptide expression is presented here to demonstrate the clinical significance of these antimicrobial peptides in the pathogenesis of inflammatory skin diseases. This example is described in some studies related to two well-characterized skin diseases, psoriasis and atopic dermatitis. In psoriatic lesions, expression of LL-37, hBD-2, and hBD-3 is up-regulated (Frohm et al, 1997; Harder et al, 1997a; Harder et al, 2001), whereas expression of these three peptides is significantly reduced in atopic dermatitis lesions (Ong et al, 2002). The difference in the levels of antimicrobial peptide expression between psoriasis and atopic dermatitis can be

elaborated by different cytokine milieus between these two skin diseases, a Th1 versus a Th2 profile, respectively (Nomura et al, 2003). It has been demonstrated that enhanced production of IL-4 and IL-13, two cytokines categorized as a Th2 profile, in atopic dermatitis, can block expression of some antimicrobial peptides in skin keratinocytes (Nomura et al, 2003), which may then account for the reduction of antimicrobial peptide expression in this lesion.

It is known that one basic function of human skin is to form a natural barrier against microbial colonization and invasion, which leads to tissue homeostasis. To further enhance this function, the skin can also produce several antimicrobial peptides, which help control the number and types of microorganisms on the skin. If the production of antimicrobial peptides is impaired by dysfunction of the host immune system as a result of the pathogenesis of skin diseases, an increased risk of opportunistic infections from bacteria or viruses in the skin lesion ensues. Consequently, the deficiency of antimicrobial peptides, particularly LL-37, in atopic dermatitis lesions causes frequent infections from vaccinia virus (Howell et al, 2004). Similarly, a drastic reduction of LL-37 protein expression that results in increased susceptibility to infections has also been observed in patients with acute myeloid leukemia (An et al, 2005).

With respect to periodontal disease, data regarding the expression of β-defensin antimicrobial peptides in different types of periodontal diseases compared to healthy periodontal tissue are still contradictory and inconclusive. For example, the findings from one study (Dommisch et al, 2005) showed no significant differences in β-defensin mRNA expression in different clinical stages of periodontal disease as compared to that in normal tissue. Nevertheless, in the same study, hBD-2 expression was found to be significantly higher than hBD-1 expression in both gingivitis and periodontitis groups (Dommisch et al, 2005). In contrast, it was later shown in another study (Vardar-Sengul et al, 2007) that the levels of hBD-1 expression did not significantly differ from those of hBD-2 expression in patients with gingivitis. However, in patients with periodontitis, hBD-1 expression was significantly higher than hBD-2 expression in chronic periodontitis, whereas hBD-2 expression was significantly higher than hBD-1 expression in aggressive periodontitis (Vardar-Sengul et al, 2007). The reason behind these discrepancies may be due to a small number of patients and healthy volunteers, recruited in each study. Consequently, before any conclusions can be drawn for the relationship between β-defensin expression and periodontal disease, a larger study is required for assessing more accurate levels of β-defensin expression in both healthy and diseased tissues, obtained from different types of periodontal diseases.

It is noteworthy that significant up-regulation of both hBD-1 and hBD-2 expression is found in periodontal pocket epithelium as compared to the adjacent healthy epithelium from the same patient (Lu et al, 2004). In contrast, higher levels of hBD-3 expression are found in periodontally healthy tissues as compared to diseased tissues (Bissell et al, 2004). These may suggest differential functions between hBD-1/hBD-2 and hBD-3 in periodontal disease, and also a more protective role for hBD-3 in regulating host immune responses to microbial assaults, as mentioned under the previous headings.

To the best of our knowledge, there has been no report that shows the relationship between the deficiency of β-defensin expression in periodontal tissues and periodontal diseases. On the contrary, both LL-37, which is mainly derived from neutrophils, and neutrophil α-defensins show a direct link to the pathogenesis of a certain type of periodontitis. This is revealed by one study (Pütsep et al, 2002) that shows the deficiency in

LL-37 and the reduction of neutrophil α-defensins in patients with morbus Kostmann syndrome, a severe congenital neutropenia. These patients suffer from recurrent gingivitis and even severe periodontitis during early childhood that result from the lack of neutrophil-derived antimicrobial peptides. Furthermore, it has been demonstrated *in vitro* that several periodontal pathogens, e.g., *Aggregatibacter actinomycetemcomitans*, are sensitive to the bactericidal effects of LL-37 (Tanaka et al, 2000; Isogai et al, 2003), so it is likely that the defective antimicrobial function of neutrophils from patients with morbus Kostmann syndrome, who are deficient in LL-37, cannot eliminate *Aggregatibacter actinomycetemcomitams*, which is highly associated with early-onset periodontitis.

In this regard, it is interesting to further investigate whether the deficiency of these antimicrobial peptides is also implicated with other forms of periodontitis associated with a syndrome, for instance, juvenile periodontitis in Papillon-Lefèvre syndrome, whose abnormalities result from cathepsin C mutations (Hart et al, 1999; Toomes et al, 1999). Is it probable that some antimicrobial peptides are substrates for cathepsin C enzyme, and these peptides may become more active after enzymatic degradation? If the answer is positive, one can assume that impaired cathepsin C function may not yield sufficient amounts of active antimicrobial peptides to exert their antimicrobial effects on periodontal pathogens. The deficiency in active antimicrobial peptides finally leads to repeated periodontal infections.

CONCLUSIONS AND INTERESTING RESEARCH TOPICS

Substantial variations in expression of small cationic antimicrobial peptides, including LL-37 and defensins, in periodontal tissues, GCF, and saliva, exist and may be correlated with the pathogenesis of periodontal disease, as well as that of other oral inflammatory and infectious diseases. Therefore, the association between altered expression of antimicrobial peptides and some types of periodontitis, specifically the ones that are associated with syndromes, should be further explored in detail. Moreover, expression of some antimicrobial peptides and their clinical significance in other oral diseases should be further studied. Perhaps, it is possible that some peptides could be further developed as biomarkers for diagnosis and/or prognosis of oral diseases in the future.

Up to now, accumulated data gathered from *in vitro* and *in vivo* studies have exhibited a broad range of antimicrobial activities against oral microorganisms, especially some periodontal pathogens in a planktonic state. With respect to these data, it is interesting to further examine the microbicidal effects of antimicrobial peptides on dental plaque microorganisms. In addition, it is now becoming increasingly evident that the functions of antimicrobial peptides are not restricted to their antimicrobial activities, as was initially thought. It is, therefore, likely that other novel, but undiscovered, functions of these peptides will be unraveled in the near future. Consequently, additional studies into new biological activities of antimicrobial peptides are needed and will be beneficial for us to better understand and gain deep insight into the importance of these multifunctional molecules, particularly their essential roles in maintaining tissue homeostasis during the healthy and diseased states of the periodontium.

Furthermore, it is still necessary to continue regulation studies, involving cellular receptors and intracellular signaling pathways that mediate up-regulation of some inducible antimicrobial peptides, in order to understand the mechanisms used to enhance the expression

of these peptides. In quest of new adjunctive treatment modalities against periodontitis, it is possible that enhancement of antimicrobial peptide expression by putative components of commensal periodontal bacteria that are not harmful to the human body, or by non-toxic agents, similar to vaccination, may be of significant interest in controlling the number of periodontopathic bacteria. Finally, we, as members of the health professions, should be constantly aware of the clinical significance of these antimicrobial peptides in the pathogenesis of oral infectious and inflammatory diseases, especially periodontal disease.

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Chapter 32

PROGNOSIS: PREDICTABILITY REDEFINED

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ABSTRACT

This comprehensive review highlights a detailed overview related to devising a periodontal prognosis. A precise predictability of the results of a disease is profound and crucial for proper treatment planning. Since the understanding of periodontal disease has progressed to include the influence of risk factors, assigning a prognosis has become more perplexing to the clinician. Various factors that influence the overall and individual tooth prognosis have been enumerated. The classification systems required to assign a prognosis has also been included. The potential adverse influences of both local and systemic factors have also been discussed. An experienced clinician should analyze all these factors, along with the patients attitude towards dental therapy, prior to arriving at a judgment for a single tooth or teeth. With newer trends in treatment modalities, patients can seek better options for treatment, thus improving the long term prognosis.

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INTRODUCTION

Prognosis simply means “forecast”. The term derives its origin from the Greek words “pro” which means “before” and “gignoskein” which means “know”. It is the art of foretelling the duration, course, result and termination of disease [1]. A precise predictability of the results of a disease is both essential and profound in the treatment plan.

In earlier days, when only fewer periodontal diseases had been identified both diagnosis and prognosis were easier. But with discovery of complex forms of the disease, prognosis became more complex. To further complicate matters, periodontal diseases have predilection for individual teeth rather than the complete dentition or even a segment of the entire dentition. The ability to prognosticate accurately for the entire dentition or an individual tooth is important for many reasons. The patient uses this information to determine whether the treatment seems worthwhile. It would benefit the patient for insurance purposes and lastly, the clinician uses these factors to determine which treatment modality would be most effective to develop restorative recommendations.

Periodontal literature have considered many factors which judge whether a tooth should be retained or not, but since the understanding of periodontal diseases has progressed to include the influences of risk factors such as genetics, smoking, stress etc in the occurrence and severity of periodontal diseases, assigning a prognosis has become even more perplexing to the clinician.

This compilation provides a detailed overview on the various factors that determine prognosis and highlights its relevance in predicting the newer treatment modalities.

VARIOUS TERMINOLOGIES RELATED TO PERIODONTAL PROGNOSIS

Provisional prognosis: a provisional or tentative prognosis is that which allows the clinician to initiate treatment of teeth that have a doubtful outlook in the hope that a favorable response may tip the balance and allow teeth to be retained.[1]

Guarded prognosis : The prognosis is graded as Guarded, if plaque or calculus control is poor or if resolution of inflammation is inadequate. If increasing attachment loss, radiographic evidence of increasing bone loss, increasing mobility, or persistent third-degree mobility is found 1 to 3 months after anti-infective and initial occlusal therapy, the prognosis may be assigned as guarded or poor.[2]

Diagnostic prognosis: It is an evaluation of the course of disease without treatment. It answers the queries, on the current status of the teeth and the anticipated future of these teeth. [3]

Therapeutic prognosis: refers to the effect of periodontal treatment on the course of the disease. Information of a prognosis, the clinician may be dealt with various circumstances which require skillful therapeutic judgment. For example: the clinician may find situations in which teeth have ample support but are mobile and teeth that have little support but are firm or incases with two walled defects that are narrow and deep, that can be successfully treated with techniques that aim at new attachment and bone regeneration than deep horizontal defects which lack regenerative capacity.[3]

Prosthetic prognosis: Once the anticipated result of periodontal treatment and periodontal health status is obtained for the patient, the following queries arise regarding the forecast for success of prosthetic restoration, whether the prosthesis is therapeutic or detrimental and dictating the necessity of the prosthesis. [3]

PROGNOSIS AND RISK

Prognosis and risk are terms used interchangeably and often creates a certain degree of confusion. Risk is the probability that an individual will get a specific disease at a given period or an unwanted outcome may occur in the future [4]. Risk factors are objective findings that indicate a strong probability of developing unwanted outcomes in people who have not been subjected to the given disease.

Many times, the factors associated with a poor prognosis are the same as those associated with increased risk. Specifically, if factors that increase the risk of acquiring the disease are present while the patient has the disease, they can worsen its prognosis. Knowledge about certain measurable factors may provide information about future disease onset and / or disease progression. Genetic susceptibility to disease in patients with juvenile periodontitis is an example of a factor that predisposes to both disease onset and progression.

In contrast, prognostic factors are characteristics or factors that predict the outcome of a disease once a disease is present. The process of using prognostic factors to predict a course of a disease is called Prognosis Assessment [5]. In some cases risk factors and prognostic factors are the same. For example some factors such as smoking may be both risk factor and prognostic factor .Thus once a person has a disease, two processes may be considered that is, reducing the risk in healthy sites and increasing the risk of a positive prognosis in the sites with the disease.

Prognostic Indicators

These are factors which are found to be associated with further disease progression. The mere co-existence of the indicators in subjects or sites exhibiting an ongoing periodontal destruction does not necessarily suggest a cause and effect relationship. In order to determine if a prognostic indicator is a true prognostic factor, it is necessary to follow untreated periodontitis patients longitudinally, and determine an association between the disease progression and the presence / absence of the marker in question. [4]

Individual tooth prognostic factors such as type of bone loss, probing depth, mobility including whether or not, one or more sites are undergoing an episode of active periodontal disease are important. Some patients may have active disease at multiple sites, and other patients may be in remission. [6]

Machtei EE et al [7] in a longitudinal study examined the clinical, microbiological and immunological indicators to determine whether the presence or combination of these parameters would correlate positively with true prognostic factors. The results showed that the overall mean attachment loss and bone loss were almost identical. They concluded that past periodontal destruction, smoking habits, Bacteriodes forsythus, Prevotella intermedia and Porphyromonas gingivalis at baseline are prognostic factors for further periodontal breakdown.

Factors to be Considered when Determining a Prognosis

The assignment of prognosis is one of the most important functions involved in clinical practice. It involves an examiner identifying one or more commonly taught parameters as they uniquely apply to the tooth.

Glickman (1973) considered two aspects to the determination of prognosis in patients with periodontal disease: The *overall prognosis* and the *prognosis of individual teeth*.

Carranza [4] considered some factors that may be more important in determining a prognosis [refer Table 1]. Consideration of each factor may be beneficial to the clinician. In most cases, analysis of these factors allows the clinician to assign a prognosis.

Table 1. Factors to be considered for determining prognosis [4]

Overall Clinical Factors	Systemic / Environmental Factors	Local Factors	Prosthetic/ Restorative Factors
Patient age	Smoking	Plaque / calculus	Abutment selection
Disease severity	Systemic disease / condition	Subgingival restorations	Caries
Plaque control	Genetic factors	Anatomic factors:	Nonvital teeth
Patient compliance	Stress	Short, tapered roots Cervical enamel projections Enamel pearls Bifurcation ridges Root concavities Developmental grooves Root proximity Furcation involvement Tooth mobility	Root resorption

Prognosis for Patients with Gingival Diseases

Gingival diseases are diverse group of complex and distinct pathological entities found within the gingiva resulting from a variety of etiologies. A classification system consisting of four main types were developed. The common form is plaque induced gingivitis resulting from dental plaque only [refer Table II] The other three types of plaque-associated gingival diseases are those modified by: 1) systemic factors 2) medications 3) malnutrition [refer Table III]

Prognosis for Patients with Periodontal Diseases

Two main aspects are considered for determination of prognosis in patients with Periodontitis:

1) Overall prognosis: refers to the dentition as a whole. Many specific dental conditions can affect the overall prognosis of the dentition. [Table 1]

Table 2. Classification for dental plaque-induced gingival diseases and their prognosis [4]

Plaque induced gingival diseases	Prognosis
1. Gingivitis associated with dental plaque only a) Without other local contributing factors b) With local contributing factors	Prognosis of gingivitis associated with dental plaque is considered as good, provided 1. all local irritants are eliminated, 2. other local factors contributing to plaque retention are eliminated 3. gingival contours conducive to the preservation of health are attained, 4. the patient cooperates by maintaining good oral hygiene.
2. Gingival disease modified by systemic factors a) Associated with the endocrine system 1) Puberty-associated gingivitis 2) Menstrual cycle-associated gingivitis 3) Pregnancy-associated a) Gingivitis b) Pyogenic granuloma 4) Diabetes mellitus- associated gingivitis b) Associated with blood dyscrasias 1) Leukemia- associated gingivitis 2) Other	The long-term prognosis for these patients depends not only on control of bacterial plaque, but also on control or correction of the systemic factor(s).
3) Gingival diseases modified by medications a) drug-induced gingival diseases 1) drug-induced gingival enlargements 2) drug-induced gingivitis b) Oral contraceptive-associated gingivitis	Reductions in dental plaque can limit the severity of the lesions. The long-term prognosis is dependent on whether the patient's systemic problem can be treated with an alternative medication that does not have gingival enlargement as a side effect. Long-term prognosis in these patients is dependent not only on the control of bacterial plaque, but also on the likelihood of continued use of the oral contraceptives.
4. Gingival diseases modified by malnutrition a) Ascorbic acid-deficiency gingivitis b) Others	The prognosis in these cases may be dependent on the severity and duration of deficiency and on the likelihood of reversing the deficiency through dietary supplementation.

Table 3. Classification for Non-plaque induced gingival lesions and their prognosis[4]

1. Gingival diseases of specific bacterial origin a) Neisseria Gonorrhea – associated lesions b) Treponema Pallidum-associated lesions c) Streptococcal species-associated lesions d) Other	Prognosis is dependent on elimination of the source of the infectious agent.
2. Gingival diseases of viral origin a) Herpesvirus infections i) herpetic primary gingivostomatitis ii) recurrent oral herpes iii)varicella-zoster infection b) Others	Prognosis is dependent on elimination of the source of the infectious agent
3. Gingival diseases of fungal origin a) Candida- species infections b) Linear gingival erythema c) Histoplasmosis d) Others	Prognosis is dependent on elimination of the source of the infectious agent
4. Gingival manifestations of systemic conditions a) Mucocutaneous disorders 1) Lichen planus 2) Pemphigoid 3) Pemphigus vulgaris 4) Erythema multiforme 5) Lupus erythematosis 6) Drug- induced 7) others b) Allergic reactions 1) Dental restorative materials A) Mercury b) Nickel c) Acrylic d) others 2) Reactions attributable to a) toothpastes/dentifrices b) mouthrinses/mouthwashes c) chewing gum additives d) food and additives 3) Others	Prognosis for these patients is linked to management of the associated dermatologic disorder Prognosis for these cases is dependent on avoidance of causative agent
5.Traumatic lesions (factitious, iatrogenic, accidental) a) Chemical injury b) Physical injury c) Thermal injury	Prognosis for these cases is dependent on avoidance of causative agent
6. Foreign body reactions	Prognosis for these cases is dependent on elimination of the causative agent.

Factors Influencing Overall Prognosis in Periodontitis Patients

1) Types of Periodontitis

a) Chronic periodontitis

It is the most common form of periodontitis,that can present in a localized or generalized form. It is a slow progressive disease associated with well-known local environmental factors that generally influences the normal host bacterial interaction. In cases of slight to moderate periodontitis, the prognosis is generally good; provided good oral hygiene is maintained and local plaque retentive factors are eliminated. In patients with severe form of chronic periodontitis, as evidenced by furcation involvement and increasing clinical mobility or non-compliant patients, the prognosis may be fair to questionable[4]. In cases, where the etiology is as a result of smoking or stress, elimination of these factors can lead to favourable prognosis.

b) Aggressive Periodontitis

Aggressive periodontitis comprises a group of rare, often severe, rapidly progressive forms of periodontitis, characterized by an early age of clinical manifestation and a distinctive susceptibility for cases to aggregate in families[8]. Since this disease usually occurs at early age, it implies that etiologic agents have been able to cause clinically detectable levels of disease over a relatively short time. Diagnosis of aggressive periodontitis requires exclusion of the presence of systemic diseases that may severely impair host defenses and lead to premature tooth loss.

The prognosis for patients with aggressive periodontitis depends on whether the disease is generalized or localized and on the degree of destruction present at the time of examination. In general, the treatment of patients with generalized forms of aggressive periodontitis should be very similar to that of patients with refractory forms of the disease. The generalized forms, which are usually associated with some systemic disease, have a worse prognosis than the localized forms. The rate of disease progression may be faster in these younger individuals, and therefore the clinician should monitor such patients more often.

Flare-ups of proliferative gingival inflammation can be observed early when the patient is on a frequent monitoring cycle. Currently, monitoring every 3 weeks or less is suggested while the disease is in an active phase. Aggressive periodontitis rarely undergoes spontaneous remission. It is important to obtain earlier radiographs to assess the stage of the disease. In case of localized aggressive periodontitis, a number of treatment modalities have been attempted in the past with varying degrees of success. However, the response has been unpredictable[4].

Patients who are diagnosed as having an early form of aggressive periodontitis may respond to standard periodontal therapy. In general, the earlier the disease is diagnosed (as determined by less destruction), the more conservative the therapy may be and the more predictable the outcome.

c) Periodontitis as a manifestation of systemic diseases

Periodontitis as a manifestation of systemic diseases can be divided into two categories. (1) Those associated with hematological disorders such as leukemia and acquired neutropenias and (2) those associated with genetic disorders such as familial and cyclic neutropenia, Down syndrome, Papillon-Lefevre syndrome and hypophosphastasia. Although the primary etiologic factor in periodontal diseases is bacterial plaque, systemic diseases alter the prognosis. For example, decreased numbers of circulating neutrophils (as in acquired neutropenias) may contribute to widespread destruction of the periodontium. Unless the neutropenia can be corrected, these patients present with a fair-to-poor prognosis. Similarly, genetic disorders that alter the way the host responds to bacterial plaque (as in leukocyte adhesion deficiency syndrome) also can contribute to the development of periodontitis. Because these disorders generally manifest early in life, the impact on the periodontium may be clinically similar to generalized aggressive periodontitis. The prognosis in these cases will be fair to poor. [4]

d) Necrotizing Periodontal Diseases

Necrotizing periodontal diseases can be divided into necrotic diseases that affect the gingival tissues exclusively that is, necrotizing ulcerative gingivitis (NUG) and necrotic ulcerative periodontitis (NUP) that affect deeper tissues of the periodontium. In necrotizing ulcerative gingivitis the main predisposing factor is bacterial plaque. However, this disease is usually complicated by the presence of secondary factors such as acute psychological stress, smoking and poor nutrition, all of which can contribute to immune suppression.Therefore superimposition of these secondary factors on a preexisting gingivitis can result in painful, necrotic lesions characteristic of necrotizing ulcerative gingivitis. With control of both the bacterial plaque and the secondary factors, prognosis for such patients is good.

In systemically healthy patients, this progression may have resulted from multiple episodes of necrotizing ulcerative gingivitis or the necrotizing disease may occur at a site previously affected with periodontitis. In these cases the prognosis is dependent on alleviating the plaque and secondary factors associated with necrotizing ulcerative gingivitis. However, many patients presenting with necrotic ulcerative periodontitis are immune compromised through systemic conditions, such as HIV infection. In these cases, the prognosis is dependent on not only reducing local and secondary factors, but also on dealing with the systemic problem. Prognosis in these cases is unfavourable. [4]

2) Overall Clinical Factors

(i) Age

Many of the studies confirm that older age groups have consistently more destruction compared to the younger [9,10].This is attributed to the chronicity of the disease process. However aging, per se is not likely to be a predisposing factor for periodontal disease.

The classical work carried out by Loe et al, on experimental gingivitis was studied in the young (20-24 year old) and the old (65-78 year old) age groups, gingival inflammation was considerably more rapid and more severe in the elderly groups [11].

The strong association between age and periodontal destruction reported in cross-sectional studies is mostly due to effect of age as surrogate for the length of exposure to etiologic factors.

Limited information suggests that increased age may be associated with slower bone healing after extractions, placement of intraosseous implants and bone grafting [12].

Although aging does not appear to affect the outcome of periodontal therapy, it is a very important factor that should always be considered when assessing patient susceptibility.

(ii) Disease severity

Knowledge of past dental health status is a good predictor for future oral health status of the dentition. Steady progression implies a poorer prognosis that does arrest disease, even at an advanced stage. Rapid progression about many teeth, simultaneously or sequentially, carries a poorer prognosis than does a rapid destruction of the periodontal tissues of a single tooth. The severity of the disease might be slight, moderate or severe.It can be determined by clinical or radiographic examination. Severity depends on pocket depth, level of attachment,degree of bone loss, tooth mobility, and crown-root ratio. The determination of the level of clinical attachment reveals the approximate extent of root surface that is devoid of periodontal ligament.

Loe et al [13] conducted a study on the attachment loss in 480 male labourers at two tea plantations in Sri Lanka. Based on the inter-proximal loss of attachment and tooth mortality rates, three sub populations were identified: 1) individuals with rapid progression of periodontal disease (8%), 2) individuals with moderate progression (81%), 3) individuals who exhibited no progression of periodontal disease beyond gingivitis (11%). It was noted that at the age of 35 years, the mean loss of attachment in the rapid progression group was around 9mm, the moderate periodontitis group showed around 4mm attachment loss and no progression group had less than 1mm loss of attachment. Ten years later, the mean loss of attachment in the rapid progression group was 13mm, moderate group was around 7mm. the annual rate of destruction in the rapid progression group varied between 0.1-1mm, in the moderate progression group between 0.05-0.5mm and in the no progression group between 0.05-0.09mm. Since this population was caries free, essentially all missing teeth were lost due to periodontal disease. In moderate progression group, the tooth mortality started after 30 years of age and increased throughout the decade. At 45 years of age the mean loss of teeth in this group was 7 teeth. The no progression group did not show any tooth loss.

(iii) Patient Compliance / Cooperation

The most important determinant of treatment outcomes in clinical practice is patient compliance. Regardless of the type of initial periodontal therapy rendered, patients with poor oral hygiene who fail to comply with recommended recall schedules are more likely to have less favorable results. The prognosis for patients with gingival and periodontal disease is critically dependent on the patient's attitude; desire to retain the natural teeth, and willingness to maintain good oral hygiene [14]. Patients should be clearly informed of the important role they must play for treatment to succeed.

Dental literature covers two principal areas: compliance with oral hygiene regimens and utilization of dental care by the public. The reasons for non-compliance are highly variable but include lack of pertinent information, fear, economics and the patient's perception of lack of compassion on the part of the dental therapist. In general, it has been found that patients comply better when they are positively reinforced and when barriers to treatment are reduced.[15]

Chace [16] retrospectively studied data from 166 fairly compliant patients who were seen for periodontal maintenance therapy every 3 months for 40 years. Only 12% of teeth initially classified as having a questionable prognosis were extracted during the follow-up period. The average survival rate for the teeth extracted was 8.8 years. These data suggest that when patients comply with recommended program of periodontal maintenance care, they have an excellent chance of retaining most of their teeth.

(iv) Plaque control

Bacterial plaque is the prime etiologic factor associated with periodontal disease. Therefore effective removal of plaque on a regular basis by the patient is critical to the success of periodontal therapy and to the prognosis. The patient who does not have the motivation, dexterity, and discipline to keep the plaque score at baseline levels will definitely have a poorer prognosis. Some patients have more viscous saliva than others, which provides for a more rapid plaque accumulation. Others accumulate plaque slowly and in scanty amounts,hence, they have a better prognosis. Persons with high caries rates may exhibit a relatively poor prognosis.

3) Systemic / Environmental Factors

(i) Smoking

Smoking is related to periodontal disease in a dose related manner and appears to exert its most hazardous effects on areas in direct contact with smoke, such as lingual aspect of maxillary teeth. This deleterious relationship between smoking and periodontal disease is seen in smokers regardless of their overall levels of plaque accumulation. However, the specific microbial flora in smokers may shift to a more pathogenic profile[17]. These findings suggest that tobacco smoking may itself promote the development of local environments that favor growth of such pathogenic species, in addition; substances in smoke such as cotinine may also promote the pathogenic activites of periodontal flora.

A series of epidemiological studies[18,19] was done to compare the periodontal destruction in current smokers, former smokers and non smokers. The results from these studies indicate: (i) level of periodontal destruction in former smoker's lies somewhere between the level of periodontal destruction of current smokers and nonsmokers. (ii) The progressive damage seen in smokers can be retarded or halted with smoking cessation. (iii) The clinical response to flap surgery is poorer in smokers when compared to non smokers; the clinical response of former smokers is similar to nonsmokers as long as the former smokers do not resume their smoking habit. These types of studies demonstrate that smoking cessation can markedly improve the prognosis and outcomes of periodontal treatment [20].Therefore

the prognosis in patients who smoke and have slight-to-moderate periodontitis is generally fair to poor. In patients with severe periodontitis, the prognosis may be poor to hopeless.

However, it should be emphasized that smoking cessation can affect the treatment outcome and therefore the prognosis. Patient's with slight to moderate periodontitis who stop smoking can often be upgraded to a good prognosis, whereas those with severe periodontitis who stop smoking may be upgraded to a fair prognosis.

(ii) Systemic background

Although the relationship of general health status and systemic disorders to periodontal disease has been studied extensively, there is no conclusive evidence that they are primary etiologic factors in periodontal disease. It is more accurate to consider systemic diseases as contributing factors in the pathogenesis of periodontal disease. The patient's systemic background affects overall prognosis in several ways. In patients with known systemic disorders that could affect the periodontium (for example, nutritional deficiency, hyperthyroidism, and hyperparathyroidism) prognosis improves with the correction of systemic problem.

Diabetes: Of all the systemic diseases that are relatively common, diabetes has emerged in recent years as the one with the strongest potential influence on periodontal diseases. In well-controlled diabetics, clinical responses to both surgical and nonsurgical periodontal therapy produced similar results to those observed in non-diabetics [21]. In general, poorly controlled diabetes appears to be associated with an increased risk of loss of attachment by gingival inflammation ranging from marginal gingivitis to periodontitis. With control of the diabetes, this group of symptoms may be expected to decrease in severity and occasionally subside [22]. The controlled diabetic exhibits a more favorable prognosis.

Immunodeficiency states: HIV-infected patients may also present with common forms of periodontal disease such as chronic periodontitis. Because of their severely compromised immune system, AIDS patient generally have poor prognosis. An Epidemiologic survey have shown a higher prevalence of bone loss and attachment loss in HIV-infected patients accompanied by a greater degree of gingival recession and shallower probing depths compared to control populations[23]. However, the effects of HIV infection on the long-term prognosis of the dentition in chronic periodontitis remain unresolved. On the one hand, a more rapid progression of bone loss and attachment loss in a HIV-infected periodontal patient may imply a poorer prognosis for the dentition when compared to the HIV-negative patient. With the advent of highly active retroviral drugs and proteinase inhibitor, long term prognosis turns out to be good.

The prognosis is guarded, when surgical periodontal treatment is required but cannot be provided because of patient's health incapacitating conditions that limit patient's performance of oral hygiene procedures (Parkinson's disease) also adversely affects the prognosis. Newer automated oral hygiene devices such as electric tooth brushes may be helpful for these patients and improve the prognosis.

(iii) Assessment of the past bone response

The past response of the alveolar bone to local factors is a useful guide for predicting the bone response to treatment and the likelihood of arresting the bone destructive process. This

entails consideration of severity and distribution of the periodontal bone loss in terms of the following: the patient's age; the distribution, severity, and duration of local irritants such as plaque, calculus, food impaction, occlusal abnormalities and habits[24].

If the amount of bone loss can be accounted for by the local factors, conventionall treatment can be expected to arrest the bone destruction; then the overall prognosis for the dentition is good.

If the bone loss is more severe than one would ordinarily expect at the patient's age in the presence of local factors of comparable severity and duration, factors other than those in the oral cavity are contributing to the bone destruction. The overall prognosis is then poor, because of the difficulty generally encountered in determining the responsible systemic factors. The prognosis is not necessarily hopeless without systemic therapy, provided the disease is detected early and sufficient bone remains to support the teeth. In such cases, local treatment often can retain the dentition in useful function for many years by eliminating local destructive factors and limiting the bone destruction to that caused by the systemic conditions.

(iv) Genetic Factors

An important problem related to research in the hereditary of periodontitis is that, whatever the etiology of the disease, the symptoms are same, such as deepening of pocket, loss of attachment, bone loss. In most of cases, the development of periodontitis at an individual level depends probably on the collective presence of a number of environmental risk factors in conjunction with number of susceptibility factors at a given time during life. Literature search suggests genetic polymorphisms in certain genes involved in immune response(eg. IL-1, IL-10, Fc-gamma receptors) may be associated with susceptibility to severe periodontitis in some populations.

Familial aggregation studies support the idea that both aggressive and chronic periodontitis tends to cluster within families and single/major locus autosomal dominant gene is involved in the disease [25]. This supports the use of family background of periodontitis as a risk factor for the development of future periodontal disease. Tumor necrosis factor-α polymorphisms, HLA antigens, and FcγR genotypes have been evaluated for their association with chronic periodontitis. But results have been equivocal [4]. The genetic susceptibility test for severe chronic periodontitis that is commercially available is the Periodontal Susceptibility Test (PST). This test evaluates the simultaneous occurrence of allele 2 at the IL-1A +4845 and IL-1B +3954 loci.A patient with allele 2 at both of these loci is considered "genotype- positive" and therefore more susceptible to developing chronic periodontitis [26]. The rationale for this association is that persons with this combination of alleles tend to produce more 1L-1 in response to bacterial challenge and therefore will be predisposed to have more inflammation and tissue-damage. The influence of genetic factors on prognosis is not simple. Although microbial and environmental factors can be altered through conventional periodontal therapy and patient education, genetic factors currently cannot be altered. However, detection of genetic variation that are linked with periodontal disease can potentially influence the prognosis in several ways. First, early detection of patients at risk due to genetic factors can lead to early implementation of preventive and treatment measures. Second, identification of genetic risk factors later in the disease and / or during the course of treatment can influence treatment recommendations, such as the use of adjunctive antibiotic

therapy or increased frequency of maintenance visits. Finally, identification of young individuals who have not been evaluated for periodontitis, but who are recognized as being at risk can lead to the development of early intervention strategies. In each of these cases, early diagnosis, intervention and / or alterations in the treatment regimen may lead to an improved prognosis for the patient.

(v) Stress

Physical and emotional stress as well as substance abuse may alter the patient's ability to respond to the periodontal therapy performed. Stress, distress and coping behavior are regarded as important risk indicators for periodontal disease[27].

A deleterious effect of stress and psychosocial factors on health was extensively elucidated by Selye (1946) [28]. He established the term "General Adaptation Syndrome", describing the sum of all non-specific, systemic reactions of the body that ensue upon long, continued exposure to stress. The type of tissue response to irritation and infection influences prognosis.

High stress / low coping patients have more susceptibility to periodontal breakdown as elevated systemic levels of cortisol can suppress several host response mechanisms such as T-helper cell function, antibody production and neutrophil function. Reducing psychological depression and improving coping strategies for stressful life events may improve periodontal prognosis and treatment outcomes [29]. There are also studies which do not agree to the above results [30].

4) Economic considerations

Periodontal disease is more severe in individuals of lower socio-economic status and poorer education. However, when periodontal status is adjusted for oral hygiene and smoking, the associations between low socio-economic and educational status and severe periodontal disease are not seen. Thus socio economic and educational status does not appear to directly affect disease progression.

Gamonal JA, Lopez NJ, Aranda W [31] conducted a survey involving 1150 Chileans aged 35-44 and 65-74 years. Prevalence of chronic inflammatory periodontal disease was 90% in subjects aged 35 – 44 years and 100% in subjects aged 65-74 years. A total prevalence for both age cohorts were 92%. A significant association between socio-economic status and periodontal health was found. Prevalence was 56% in subjects of high status, 98% in subjects of low-socio economic status. An association between educational level and periodontal health was apparent. The only subjects who were periodontally healthy were in the group with university education. There was also a significant association between educational level and loss of teeth. Thus their study shows that periodontal health is better in the educated and also people belonging to higher socio-economic status.

5) Oral habits and compulsions

Habit is an important factor in the initiation and progression of periodontal disease. Habits of significance in the etiology of periodontal disease include:

1) Neuroses
2) Occupational habits
3) Miscellaneous

Recognition and elimination of a habits detrimental to periodontal health (like tongue thrusting, mouth-breathing, bruxism) are of utmost importance in the treatment of the periodontal manifestation. Unless the dentist understands the damage that can or does occur due to a deleterious habit and the need for eliminating it, he will find himself hindered in periodontal therapy. Therefore, the prognosis depends on the degree of destruction caused by the habits on the periodontium. If the underlying etiology is corrected, then the prognosis will be favorable[32].

Chewing habits: betel quid and tobacco chewing may result in increase in prevalence of chronic gingivitis, acute necrotizing ulcerative gingivitis as well as periodontitis. Increased accumulation of plaque and calculus formation has been observed in smokers. Gingivitis toxica, characterized by the destruction of gingival and the underlying bone, has been attributed to the chewing of tobacco.The magnitude of the occlusal forces placed on teeth can vary from patient to patient and affect the prognosis. If the patient has a habit of clenching or grinding the teeth, the bone support for the teeth will have to be greater than for a person who does not have these parafunctional habits. If the occlusal forces are excessive, the prognosis is often limited [32].

6) Malocclusion

Irregularly aligned teeth, malformations of the jaws and abnormal occlusal relationships may be important factors in the etiology of periodontal disease, as they may interfere with plaque control or produce occlusal interferences. A frequently encountered situation is, when tooth migrates or tips mesially, that surface of the teeth can become inaccessible for self performed oral hygiene. This can lead to clinical attachment loss and bone loss at the mesial tooth sites, which might pose some risk for development of periodontal inflammation that could lead to loss of support.

The overall prognosis is poor, for occlusal discrepancies that cannot be corrected. The distribution of teeth is also important. Having teeth on both sides of a mouth is more favourable than having all the teeth on one side of the mouth. It is usually favourable to have all the remaining teeth either in the posterior part of the mouth or in both the anterior and posterior parts of the mouth. If all the remaining teeth are in the anterior part of the mouth, prognosis is usually less favourable.

7) Prosthetic / Restorative Factors

The overall prognosis requires a general consideration of bone levels (evaluated radiographically) and attachment levels (determined clinically) to establish whether enough teeth can be saved either to provide a functional and aesthetic dentition or to serve as abutments for a useful prosthetic replacement of the missing teeth. When a tooth is lost, the structural integrity of the dental arch is disrupted, and there is subsequent realignment of teeth as a new state of equilibrium is achieved. Teeth adjacent to or opposing the edentulous space frequently moves into it. At this point, the overall prognosis and the prognosis for individual

teeth overlap because the prognosis for key individual teeth may affect the overall prognosis for prosthetic rehabilitation. When few teeth remain, the prosthodontic needs become more important, and sometimes periodontally treatable teeth may have to be extracted if they are not compatible with the design of the prosthesis. [4]

Selection of the Type of Prosthesis

Missing teeth may be replaced by one of three prosthesis types, a removable partial denture, a tooth supported fixed partial denture, or an implant supported fixed partial denture. Several factors must be weighed when choosing the type of prosthesis to be used in any given situation.

Abutment Evaluation

Teeth that serve as abutments are subjected to increased functional demands. This is of particular significance when designing and fabricating a fixed partial denture, since the forces that would normally be absorbed by the missing tooth are transmitted, through the pontic, connectors, and retainers to the abutment teeth. More rigid standards are required when evaluating the prognosis of teeth adjacent to edentulous areas [33]. A tooth that has undergone endodontic treatment that has a post is more likely to fracture when serving as a distal abutment supporting a distal removable partial denture. However, the tooth should have some sound, surviving coronal tooth structure to ensure longevity. Even then some compensation must be made for the coronal tooth structure that has been lost.

This is accomplished through the use of dowel core or pin-retained amalgam or composite resin core. Teeth that have been pulp capped should not be used as fixed partial denture abutments unless they are endodontically treated. There is a risk that they will require endodontic treatment later, with the resultant destruction of retentive tooth structure and of the retainer itself.

The supporting tissues surrounding the abutment teeth must be healthy and free from inflammation before any prosthesis can be contemplated. Normally, abutment teeth should not exhibit mobility, since they will be carrying an extra load. The roots and their supporting tissues should be evaluated for three factors:

(i) Crown-root ratio

The ratio is a measure of the length occlusal to the alveolar crest of the bone compared with the length of root embedded in the bone. As the level of the alveolar bone moves apically, the lever arm of the portion of bone increases and the chances for harmful lateral forces is increased. The optimum crown-root ratio for a tooth to be utilized as a fixed partial denture abutment is 2:3. A ratio of 1:1 is the minimum ratio that is acceptable for a prospective abutment under normal circumstances [33].

(ii) Root configuration

This is an important point in the assessment of an abutment's suitability from a periodontal standpoint. Roots that are broader labiolingually than they are mesiodistally are preferable to roots that are round in cross-section. Multirooted posterior teeth with widely

separated roots will offer better periodontal support than roots that converge, fuse, or generally present a conical configuration. The tooth with conical roots can be used as an abutment for a short span fixed partial denture if all other factors are optimal. A single rooted tooth with evidence of irregular configuration or with some curvature in the apical third of the root is preferable to the tooth that has nearly perfect taper [33].

(iii) Periodontal ligament area

Larger teeth have a greater surface area and are better able to bear added stresses. When supporting bone has been lost because of periodontal disease, the involved teeth have a lessened capacity to serve as abutments [33] The length of the pontic span can be successfully restored, in part, by the abutment teeth and their ability to accept the additional load. In a statement designated as "Ante's law" by Johnson et al [34] the root surface area of the abutment teeth had to equal or surpass that of the teeth being replaced by pontics. As a clinical guideline, there is some validity in this concept .Fixed partial dentures with short pontic spans have a better prognosis than do those with excessively long spans. It would be an oversimplification to attribute this merely to the overstressing of the periodontal ligament. Failures from abnormal stresses have been attributed to leverage and torque rather than overload.

FACTORS INFLUENCING PROGNOSIS OF INDIVIDUAL TEETH

Individual Tooth Prognosis

The prognosis for individual teeth is determined after the overall prognosis and is often affected by it. [35] In periodontal procedures, the main goal is the preservation of the entire dentition as a functioning unit. This means that individual components are not as vital as the overall function of the entire unit. The loss of single tooth or several teeth does not destroy the dentition, if the teeth can be restored to function and esthetics. For example, in a patient with a poor overall prognosis the dentist likely would not attempt to retain a tooth that has a questionable prognosis because of local conditions. Many of the factors listed under local factors and prosthetic / restorative factors have a direct effect on the prognosis for individual teeth in addition to any overall systemic or environmental factors that may be present [11].

1) Bone topography

The single most important factor in the prognosis of an individual tooth affected by periodontal disease is the topography of the bone surrounding it. Other factors that are considered are morphology of the bony deformity, surgical accessibility for correction of the defect, anatomy of the root/s, and the functional demands to which the tooth is subjected. If the tooth is supported adequately with bone and the osseous defect can be corrected by surgical intervention, then the pocket can be eliminated,resulting in a favourable prognosis [1]. All pockets are not amenable to surgical correction; the regional anatomy frequently makes pocket elimination impossible and the long term prognosis unfavourable.

2) Percentage of bone loss

Greater the bone loss, poorer the prognosis. The depth of a pocket is relatively equivalent to the amount of bone loss seen in radiographs. Gingival (pseudo) pockets do not generally have accompanying bone destruction, and hence can be treated and restored to health, for the prognosis to be graded fair to good. When greater bone loss has occurred on one surface of a tooth, the bone height on the less involved surfaces should be taken into consideration. Because of the greater height of bone in relation to other surfaces, the center of rotation of the tooth will be nearer the crown, resulting in a more favourable distribution of forces to the periodontium and less tooth mobility. If the bone loss occurs as a result of acute infectious disease like a periodontal abscess, prognosis is generally favorable as the resultant angular defects are more amenable to regenerative procedures. A similar bony deformity caused by a chronic process induces horizontal bone loss making prognosis less favourable. Progressive bone resorption may also lower the prognosis but the deciding factor should be the etiology of the progressive bone loss and the probability of its correction [3].

Janson L et al [36] in his 10 year follow up study found the annual marginal bone loss to be approximately 0.09 mm. Prognosis was graded favourable as the bone level remained in relatively stationary position after initial bone loss.

3) Probing depth and pocket formation

Evaluation of pocket probing depth has been the most critical marker for determining prognosis. It remains the most reliable indicator of past periodontal destruction [37]. Longitudinal clinical observations of probing depths that increase over time are associated with future loss of attachment. The pocket depth, level of attachment, and type of pocket are most important for determination of prognosis. These are determined by probing and radiographic evaluation. Prognosis is adversely affected, if the base of the pocket (level of attachment) is close to the root apex. The injurious bacterial products may reach the pulp through the apical foramina. Root canal therapy is necessary in such cases to obtain optimal results from periodontal treatment. When the periodontal pocket extends to involve the apex, the prognosis is generally poor. The presence of apical disease as a result of endodontic involvement also worsens the prognosis. However, good apical and lateral bone repair can sometimes be obtained by combining endodontic and periodontal procedures.

The location of the pocket is important. Some deep infrabony pockets on the proximal surfaces respond by fill and reattachment, others do not: a deep infrabony proximal pocket that does not respond will result in reverse architecture and will have a poor prognosis. Shallow defects can be treated by either fill or elimination. Pockets made deeper by inflammatory hyperplasia (pseudopockets) have a good prognosis[1].

4) Presence and severity of furcation

The anatomical location of the molar, makes the tooth vulnerable to periodontal destruction and eventual loss. The concavities and convexities of the furcal aspects of molar roots and of the maxillary first bicuspid are potential sites for plaque retention[1].

The presence of furcation involvement does not indicate hopeless prognosis. However, when the lesion reaches the furcation, it causes two additional problems; it causes difficulty of access to the areas both for scaling, root planing and performing surgery. The second is the inaccessibility of the area to plaque removal by the patient. If both these problems can be solved, then prognosis is similar to or even better than that of single rooted teeth with similar degree of bone loss.

Prognosis for teeth with furcation invasions depends on: [1]

1) Extent of bone destruction horizontally and vertically in the interradicular space.
2) Number of roots and their morphology.
3) Morphology of the inter-radicular space
   a) Width
   b) Depth
4) Condition of the periodontal attachment as determined by clinical mobility tests and percussion.
5) Access for surgical correction of the deformity.
6) Patient's access for oral hygiene after therapy.

Mandibular molars: These molars with furca involvement usually have a more favorable prognosis than maxillary molars with furca because of better access for oral hygiene. Mandibular first molars with bone loss in the furca have a favourable prognosis if the roots are reasonably long and interrradicular space is wide. Root caries is often a greater threat to the longevity of mandibular first molars with furcation invasion than periodontal disease.

Mandibular second molars: These teeth with furcation invasions usually have a less favorable prognosis than first molars since their roots are shorter and the interradicular space is constricted. The proximity of the ascending ramus of the mandible on the distal aspect and the position of the external oblique ridge and muscle attachments on the buccal aspect of the second molar often makes it impossible, even with modern techniques, to obtain an adequate zone of attached gingival for a favorable long term prognosis. These anatomic restrictions often make it impossible to create a gingival papilla in the furca because a selective recession cannot be secured that will place the gingival margin apical to the furca. In this event, the pocket will be eliminated and the long range prognosis is poor.

Maxillary bicuspids: Maxillary first bicuspids often have two roots-buccal and palatal. Joseph I et al [38] examined the furcation anatomy of 100 of these teeth. In 62% of the bifurcated teeth, a furcal concavity was seen on the palatal aspect of the buccal root. The mean furcation width was 0.71mm; less than the diameter of a curette. Concavities were found on the proximal surfaces of all teeth with deeper concavity on the mesial than the distal aspect. Maxillary bicuspids may also display a V-shaped groove on the proximal surfaces. These often persist towards the apical region and are associated with greater loss of attachment than that found around non-grooved teeth.

Maxillary molars: Maxillary first molar with furcation invasion usually have a favourable prognosis if the septal bone is still present in the interfurca. The position of the furcation entrance, particularly in maxillary molars is important [39]. Furcation involvement on the mesial aspect or buccal aspect has a favourable prognosis since they are accessible for surgical reconstruction that makes oral hygiene possible. Furca invasion on distal aspect of these teeth are generally hopeless because of inaccessibility. Prognosis of the maxillary

second molar with a furca invasion is less favourable than for a similar invasion of the first molar because of the second molar's smaller root structure, restricted inter-radicular space, and more distal position in the arch.

As seen from the classic observations of Hirschfeld and Wasserman, the teeth with minimal(class I) or no furcation invasions generally have good prognosis. The greater the amount of attachment loss in the furcation, the worse the long term prognosis.The teeth with complete loss of bone in the coronal aspect of the furcation(class III) generally have a poor prognosis[40].

5) Mobility and clinical crown-to-clinical root ratio

Tooth mobility and clinical crown-to-clinical root ratio, influence prognosis and are important factors in determining the number of teeth that must be used as abutments if missing teeth are to be replaced [41].

The prognosis for reducing tooth mobility through periodontal therapy is related to the etiology of mobility. Increased tooth movement may be related to inflammation in the gingival and / or periodontal ligament, the presence of occlusal trauma, loss of periodontal support or a combination of any of these factors.

If extreme mobility is detectable but pocket depth is not, it can be presumed that the loosening is due to trauma from occlusion or from oral habits and compulsions. This can be determined by the clinical examination and dental history. It can be presumed that such looseness will be co-related with widening of periodontal ligament space around the teeth as seen in radiographs. If these findings are confirmed, the prognosis can be considered as favourable and the mobility can be resolved by selective tooth grinding, use of night guards and/or fixed splinting [42]. However, if mobility is related to occlusal trauma and / or loss of attachment, occlusal adjustment and/or splinting may be indicated as part of the therapy and the long-range results are less predictable.

The root form and length should be compared with the degree of mobility and the amount of bone loss, pocket depth. Teeth with bulky, long rooted teeth with extreme mobility in which only the apical third of the bone is present will have a better chance of survival following periodontal therapy and splinting, thus favouring prognosis. Teeth with spindly roots and advanced mobility in which only the apical third of the bone remains have very poor prognosis.

6) Root Anatomy

The amount of cemental surface available for periodontal ligament attachment varies with the length, shape, and circumference of each root. Root circumference is usually greatest at the cervix. As it tapers toward the apex, the circumference decreases and less surface area are available for attachment. Root contour must be considered as well as the clinical-crown-to-clinical-root ratio. Prognosis for a tooth with a rectangular-shaped root is more favorable than for a tooth of the same length with a cone-shaped root. Scaling and root planing of root surfaces are fundamental, if successful treatment is to be attained and anything that decreases the efficiency of this procedure, such as bizarre root morphology can decrease the prognosis.[14]

Good oral hygiene is also essential for maintaining the healthy state obtained after therapy. This too can be made difficult by various root morphologies. Determination of prognosis and proper treatment planning is based on recognition of these diverse root forms.

7) Local Factors

(i) Plaque / calculus

A unifying concept emerged in 1965, when the cause and effect relationship between plaque and gingival inflammation was demonstrated in a classic study by Loe et al [11]

The principal concept was that plaque was the primary and essential disease initiator from health to gingivitis and if the gingivitis were untreated it might progress to adult periodontitis.

Plaque retentive factors are important in the development and progression of chronic periodontitis because they retain plaque microorganisms in close proximity to the periodontal tissues, providing an ecologic niche for plaque growth and maturation. Calculus is considered the most important plaque retentive factor because of its ability to harbor plaque bacteria on its rough surface. As a result, calculus removal is essential for the maintenance of a healthy periodontium.

(ii) Subgingival Restoratons

Subgingival margins, in addition to influencing the progression of periodontitis, can have other effects on the attachment apparatus.

a) Effects of subgingival margins

The area between the depth of a healthy gingival sulcus and the alveolar crest is described as the biologic width. This constitutes the junctional epithelium and the supracrestal connective tissue.

Schatzle et al [43] in a 26 year prospective cohort study analysed the gingival indices and attachment level and compared between those who did and those who did not have restorative margins greater than 1 mm from the gingival margin. After 10 years, the cumulative mean loss of attachment was 0.5 mm more for the group with sub gingival margins, which was statistically significant.

b) Overhanging margins

Overhanging margins of dental restorations contribute to the development of periodontal disease by changing the ecologic equilibrium of the gingival sulcus to an area that favours the growth of disease – associated organisms (predominantly gram negative anaerobic species) at the expense of health – associated organisms (gram positive facultative species) and by inhibiting the patients access to remove accumulated plaque. A highly significant statistical relationship has been reported between marginal defects and reduced bone height [44]. Removal of overhangs permits more effective plaque control, resulting in reduction of

gingivitis and a small increase in radiographic alveolar bone support [45]. Overhangs can also impinge on the interproximal embrasure space, making cleansing with floss difficult and cause displacement of the gingiva. Violation of the biologic width by overhanging restorations is another possible mechanism by which they may damage the periodontium [46].

The sub gingival zone is composed of the margin of the restoration, the luting material, and prepared as well as the unprepared tooth surface. Sources of marginal roughness include (1) grooves and scratches in the surface of carefully polished acrylic resin, porcelain restoration (2) separation of the restoration margin and luting material from the cervical finish line, thereby exposing the rough surface of the prepared tooth. (3) dissolution and disintegration of the luting material between the preparation and the restoration, leaving a space (4) inadequate marginal fit of the restoration.

c) Contour

The facial and lingual contours of restorations are also important in the preservation of gingival health. The most common error in recreating the contours of tooth in dental restorations is overcontouring of the facial and lingual surfaces. It generally occurs in the gingival third of the crown and results in an area in which oral hygiene procedures are unable to control plaque [47]. Consequently, leading to plaque accumulation and gingival inflammation. Apparently, undercontoured preparation is not nearly as damaging to the gingiva as over-contouring.In patients in whom periodontal disease causes the gingival margin to be in a more apical position than it was in health, the facial and lingual contours become even more significant. In these cases, the bulge on the facial contour of the crown, which normally would be subgingival appears supragingival. If the furcation has been exposed by periodontal surgical procedure or by gingival recession, it is important that the restoration be contoured in such a way as to facilitate access for oral hygiene. Occlusal surfaces should be designed to direct masticatory forces along the long axis of the teeth. The anatomy of the occlusal surface should provide well formed marginal ridges and occlusal sluiceways to prevent interproximal food impaction. [48]

d) Restorative materials

Surface textures of restorative materials differ in their capacity to retain plaque. All can be adequately cleaned if they are polished and accessible to oral hygiene measures. Damage to the periodontal tissue might occur during the preparation and fabrication of the restoration; the materials used might contain components that irritate tissue; and the physical or chemical properties of the restorations may cause retention of bacterial plaque in the long term, thus affecting prognosis[48].

e) The effect of surface finish of restorative materials on the periodontium

The surface of restorations should be as smooth as possible to limit plaque accumulation. Roughened tooth and restoration surfaces in the subgingival region result in increased plaque accumulation and increased gingival inflammation. There is evidence that the amount of plaque that accumulates in patients with relatively poor oral hygiene is not affected to a significant degree by minor changes in root surface configuration. All restorative materials

placed in the gingival environment should have the highest possible polish to avoid plaque accumulation and to give a favourable prognosis[49].

(iii) Anatomic Factors

Anatomic factors that may predispose the periodontium to disease and therefore affect the prognosis include short, tapered roots with large crowns, cervical enamel projections (CEPs) and enamel pearls, intermediate bifurcation ridges, root concavities, and developmental grooves. The clinician must also consider root proximity and the location and anatomy of furcations when developing a prognosis[4].

a) Short tapered roots: Prognosis is poor for teeth with short, tapered roots and relatively large crowns. Because of the disproportionate crown-to-root ratio and the reduced root surface available for periodontal support, the periodontium may be more susceptible to injury by occlusal forces.
b) Cervical enamel projections (CEP), Enamel pearls: CEP's are flat, ectopic extensions of enamel that extend beyond the normal contours of the cementoenamel junction. They extend into the furcation of 28.6% of mandibular molars and 17% of maxillary molars[50]. They are most likely to be found on buccal surfaces of maxillary second molars. Enamel pearls are larger, round deposits of enamel that can be located in furcations or other areas on the root surface.These projections can affect plaque removal and should be removed to facilitate maintenance.
c) Furcation Ridge: The anatomy of the furcation is complex. The presence of bifurcational ridges, a concavity in the dome, and possible accessory canals complicates not only conventional and surgical therapy, but also periodontal maintenance.These ridges run from one root to the other, and in some maxillary molars continue apically. In mandibular molars there may be a central bifurcational ridge that forms distinct pits in the roof of the furcation. Hou and Tsai [51] determined that these ridges are strongly associated with attachment loss in furcations. These reports emphasize the complexity of the furcation topography of molars that has to be taken into consideration when debriding teeth with furcal attachment loss.
d) Root Concavities / Proximities: Root concavities exposed through loss of attachment can vary from shallow flutings to deep depressions. They appear more marked on maxillary first premolars, the mesiobuccal root of the maxillary first molar, both roots of mandibular first molars, and the mandibular incisors. Any tooth, however, can have a proximal concavity. Although these concavities increase the attachment area and produce a root shape that may be more resistant to torquing forces, they also create areas that can be difficult for both the dentist and the patient to clean. Access to the furcation area is usually difficult to obtain. Maxillary first premolars offer the greatest difficulties, and therefore their prognosis is usually poor when the lesion reaches the mesial-distal furcation. Maxillary molars also offer some degree of difficulty because of their furcation width. Sometimes

their prognosis can be improved by resecting one of the buccal roots, thereby improving access to the area. When mandibular first molars or buccal furcations of maxillary molars offer good access to the furcation area, their prognosis is usually fair [4].

e) Developmental Grooves: Other anatomic considerations that present accessibility problems are developmental grooves. Developmental grooves, which sometimes appear in the maxillary lateral incisors (palatogingival groove) or in the lower incisors, create an accessibility problem. They initiate on enamel and can extend a significant distance on the root surface, providing a plaque-retentive area that is difficult to instrument. These palatogingival grooves are found on 5.6% of maxillary lateral incisors and 3.4% of maxillary central incisors. Treatment consists of odontoplasty of the groove, placing bone substitutes, and surgical management of the soft tissue and underlying bone. Radicular grooves can result in 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 needs to be extracted due to a poor prognosis.[4]

Leknes KN, Lie T, Selvig KA [52] did a retrospective study on 103 extracted teeth with grooves, to evaluate the effect of proximal root grooves as a risk factor in periodontal attachment loss. Following staining, the teeth were examined under light microscopy. On each tooth, loss of attachment was measured along the long axis of the root from the cemento-enamel junction to the most coronal level of the stained periodontal ligament remnants on mesial as well as on distal surfaces. For both groups, a statistically significant greater loss of attachment was present on grooved than on non-grooved surfaces. Generally, there was a direct relationship between groove location and maximum loss of attachment. The results indicate that proximal root grooves should be considered in periodontal diagnosis, prognosis, and treatment planning.

8) Caries, Nonvital Teeth, and Root Resorption

For teeth mutilated by extensive caries, the feasibility of adequate restoration and endodontic therapy should be considered before undertaking periodontal treatment. Extensive idiopathic root resorption or root resorption that has occurred as a result of orthodontic therapy, jeopardizes the stability of teeth and adversely affects the response to periodontal treatment. The periodontal prognosis of treated nonvital teeth is not different from that of vital teeth. Caries destroys tooth structure, creating open contacts, poor embrasure form and plunger cusps, all of which encourage food impaction, plaque formation and periodontal disease. In the presence of debris and decay, the adjacent gingival soft tissue can become more inflamed and caries can extend deep into periodontal pockets, especially around defective restorations that suffer from recurrent caries. The removal of dental caries and the restorations of sound tooth structure are necessary components of early treatment of a patient with periodontal disease. The re-establishment of marginal integrity with normal interproximal contacts and proper embrasure space will facilitate oral hygiene, prevent plaque

accumulation, and create a local environment conducive to health. Restoration of dental caries should be as conservative as possible to maintain natural tooth structure and provide for gingival margins that are able to be kept plaque free by the patient, which will improve prognosis[4].

9) Pulpal involvement

The relationship that exists between the pulp of a tooth and the surrounding periodontium is undisputed. Healthy periodontal tissue provides nourishment and support for the roots. Unhealthy pulpal tissue or an infected pulpal space can contribute to loss of the periodontal attachment. Direct communication exists between the pulp and periodontal ligament by way of dentinal tubules, lateral and / or accessory canals and the apical foramina[2]. While pulp vitality may not often be affected by periodontal disease, evidence exists that periodontal disease can affect the health of the pulp. For example, in the presence of long-standing periodontal disease, the pulp may exhibit degenerative changes such as internal resorptions, calcifications and infarctions. Lesions affecting the periodontium may be the result of inadequate or incomplete root canal treatment, perforations, fractures, resorptions, or coronal leakage, in addition to an unhealthy or necrotic pulp. Treatment may involve nonsurgical treatment and/or surgical management. When the etiology is removed, the potential for healing exists. The greater the periodontal involvement, the poorer the prognosis. The healing potential should dictate the course of treatment [53].

10) Strategic value

Ultimately, one of the first decisions that must be made is the strategic value of a tooth. This will have a bearing on whether the tooth is retained or extracted. For example, a third molar in an arch with many missing teeth may need to be saved so that it can be used as an abutment for a partial denture.

This same decision process can be made for other strategic abutment teeth. A full denture is a poor substitute for natural teeth; however, an average patient is usually satisfied with a maxillary complete denture after some period of adjustment. The same cannot be said for many mandibular complete dentures. A mandibular complete denture is much more difficult to adapt to and every effort should be made to maintain strategic abutment teeth as long as possible to provide retention for a partial denture. This is especially true if the patient is a poor candidate for a dental implant.

A few strategically placed teeth can serve as abutments for a fixed prosthesis and can have a favorable prognosis than an implant. Drifting of teeth often occurs following failure to replace missing teeth. It often creates conditions that lead to initial periodontal disease. The pattern of changes that may follow due to failure to replace missing first molar is characteristic[32]

(i) The drifting of second and third molars often result in a decrease of the vertical dimension.

(ii) The premolars drift distally and the mandibular incisors tilt or drift lingually. The mandibular premolars while moving distally lose their intercuspating relationship with the maxillary teeth and may tilt distally.
(iii) Anterior overbite is increased, the mandibular incisors strike the maxillary incisors near the gingiva or traumatize the gingiva.
(iv) Diastemata are created by the separation of anterior teeth.

The disturbed proximal contact relationships lead to gingival inflammation, food impaction and pocket formation followed by tooth loss and mobility. Occlusal disharmonies created by altered tooth positions traumatize the supporting tissues of the periodontium and aggravate the destruction caused by inflammation [54]. Reduction in periodontal support leads to migration of teeth and occlusal problems.

(i) Teeth adjacent to edentulous area

Teeth that serve as abutments are subjected to increased functional demands. More rigid standards are required in evaluating the prognosis of teeth adjacent to edentulous areas [1].

(ii) Relation to adjacent teeth

In dealing with a tooth with questionable prognosis, the chances of successful treatment should be weighed against the benefits that would accrue to the adjacent teeth, if the tooth under consideration were extracted. An attempt to retain hopelessly involved teeth jeopardizes the adjacent teeth. Extraction of the questionable tooth is followed by partial restoration of the bone support of the adjacent teeth [48].

(iii) Location of remaining bone in relation to the individual tooth surfaces

When greater bone loss has occurred on one surface of a tooth, the bone height on the less involved surfaces should be taken into consideration, when determining a prognosis. Because of greater height of bone in relation to other surfaces, the center of rotation of the tooth will be nearer the crown. This will result in a more favourable distribution of forces to the periodontium and less tooth mobility [48].

11) Therapist's knowledge and skill

Although precise and well directed treatment planning is important, skillful and efficient performance is just as important. Needless trauma to flaps, dehydration of the tissues and imprecise closure of the wound are all crucial contributions to the therapeutic failures. Such errors make the difference between success and failure of the therapy. Restorative skill is every bit as important as periodontal skills. Good therapy is ineffective in the face of poor restorative sequelae. [1]

12) Trauma from Occlusion

Occlusal trauma has been associated with periodontal disease for many years. Various animal studies[55,56] were conducted to demonstrate this relationship and controversial results were obtained. Some investigators did not support the concept that excessive occlusal forces were causative agent of periodontal destruction. Further, Glickman and co-workers summarized all their work and concluded that excessive occlusal forces were a co-destructive force in the presence of gingival inflammation and could lead to vertical osseous defects. Only a few studies have evaluated the effects of excessive occlusal forces on the periodontium. These studies have indicated that treating occlusal discrepancies may lead to better results following periodontal treatment[55].

The outlook for retaining teeth that are affected in periodontal occlusal trauma depends on the degree of control the dentist has over the etiological factors and the severity of the periodontal tissue loss.If the occlusal trauma is related to a restoration that was placed in supraocclusion, the prognosis is favorable because the dentist has control over the occlusal contours of restorations. Also, if the occlusal trauma is related to reduced periodontal support, splinting and replacement of missing teeth can often control the occlusal trauma for long periods.

13) Mucogingival deformities

Assessment of the mucogingival status of a patient is an essential part of an oral examination, particularly if there are major restorative or orthodontic work planned. Mucogingival status refers to the quality and quantity of keratinized gingival tissue, the amount of gingival recession, the presence of aberrant frena and the depth of the vestibule.

Gingival recession can be a problem for patients for esthetic reasons, dentinal hypersensitivity or interference with normal hygiene procedures. A number of factors have been implicated in the etiology of gingival recession. An extreme buccal or lingual positioning of the tooth in the dental arch, whether natural or due to orthodontic movement, can lead to thinning of the alveolar plate and associated gingival tissues. This makes the area more susceptible to recession, either from trauma or inflammation. Trauma is usually the result of vigorous tooth brushing. Plaque-induced disease can also cause recession, particularly in patients with a thin periodontium. [57]

The outlook for preventing further loss of tissue in areas of gingival recession depends primarily on maintaining the tissues free of inflammation along with the severity and etiology of the recession. In general, if the recession is not too extensive and the etiological factors can be identified and corrected,then the prognosis for preventing further recession is favourable.

The restoration of gingiva on root surfaces previously denuded by recession is possible and is indicated when gingival health cannot be established because of a mucogingival defect or for esthetic reasons. If an adequate zone of attached gingiva is present on an adjacent tooth, a pedicle flap can be positioned over the exposed root, and the outlook for covering, it is quite good. If a suitable donor site is not located on an adjacent tooth, a free autogenous soft-tissue graft must be used. While the coverage of denuded root surfaces with this method is possible, it is not as favourable as with the pedicle graft.

When a high frenum or muscle attachment is involved, a frenectomy or detachment of the muscle is done in conjunction with the procedures just mentioned.

Prognosis may improve when the following treatment is instituted

- Root demineralization with citric acid
- Orthodontic movement or recontouring of the tooth to place it within the alveolar housing.
- Replacement of overcontoured restorations, improper prosthetic appliances.

PROGNOSIS AND RADIOGRAPHS

The radiograph is a valuable aid in the diagnosis of periodontal disease, determination of patient prognosis, and the evaluation of the outcome of the treatment. However it is an adjunct to the clinical examination, not a substitute for it. [1]

Radiographic evaluation of bone changes in periodontal disease is based mainly on the appearance of the inter-dental septa because the relatively dense root structure obscures the facial and lingual bony plates. The width and the shape of the interdental septum and the angle of the crest normally vary according to the convexity of the proximal surfaces of the teeth at the level of the cemento-enamel junctions of the approximating teeth. The angulations of the crest of the interdental septum are generally parallel to a line between the cemento-enamel junctions of the approximating teeth. When there is a difference in the levels of the cemento-enamel junctions, the crest of the inter-dental bone appears angulated rather than horizontal.

- The alveolar bone, the alveolar process and the periodontal space on the mesial, distal, apical aspects of the root are recorded on the x-ray in a single plane.
- The clinical crown to root ratio is recorded
- Dense deposits of calculus and margins of metallic restoration may be observed on the proximal surfaces of the teeth.

Radiographic Appearance of Bone Destruction in Periodontal Disease

The radiograph does not reveal minor destructive changes in bone, therefore slight radiographic changes in the periodontal tissues mean that the disease has progressed beyond its earlier stages. Atleast 40% of bone reduction should be present to be seen in the radiograph. The radiographic image tends to show less severe bone loss than is actually present. The difference between the actual alveolar crest height and the height as it appears on the radiograph ranges from 0 to 1.6mm, most of which can be accounted for x-ray angulations. It is an indirect method for determining the amount, distribution and pattern of bone loss. [1]

PROGNOSIS OF IMPLANTS

The replacement of missing teeth in partially or fully edentulous patients has conventionally involved fixed or removable and partial or full prostheses supported by natural teeth, soft tissues or both. Prosthetic reconstructions were often limited by the number or distribution of abutment teeth, the morphology of the alveolar ridges, the periodontal health

or the remaining hard tissue structures of the abutment teeth. The possibility of adding abutments by the insertion of oral implants has been a treatment goal desired by practitioners. However, until the early 1980s, dental implants lacked sufficient long-term evaluation [58]

Among the earlier implant designs, only the mandibular transosteal staples demonstrate sufficient long-term efficacy. Although subperiosteal and blade implants improved the functional and aesthetic comfort of patients, the overall long-term assessment of such implants did not fulfill the requirements for long-term success. Long-term success rates from several studies of blade implants healing by fibro-osseous integration range from 42% to 83% over 5 years. Although improvements with biomaterials and designs have been claimed, the installation of such implants is still accompanied by a high number of long-term complications.The type of dental implants widely used today as a result of their high predictability is endosseous root form implants healing with direct bone-to-implant contact (osseointegration). This high level of predictability could finally be achieved by applying new basic knowledge of biomaterials and the study of the reactions by the adjacent tissues to the design of a new generation of implants. Additionally, important surgical and restorative concepts necessary for implant survival were recognized.

Based on the predictability in fully edentulous jaws, implants have more and more been used in partially edentulous patients to another single crowns or fixed partial dentures. Data in the literature indicate that the implant survival rates may be expected to be as high as in the treatment of fully edentulous ridges. However, not all of these studies have been designed in a prospective format, as described. A prospective trial with non-submerged implants including a 5-year life-table analysis demonstrates that implants do not have to be submerged under the mucosa to achieve osseointegration and survival rates comparable to submerged implants.

- For implants with a relatively smooth surface, lower success rates have to be expected in bone of lesser quality (density) and quantity.
- For this type of implant, shorter fixtures (<8 mm) have had higher failure rates in fully edentulous arches than longer implants (> 7mm). This difference was, however, not found when implants were used to support fixed partial dentures.
- Implants with a rough or porous surface do not show that difference between mandibular and maxillary survival rates.

A controversy exists regarding the use of hydroxyapatite coating. Despite the lack of adequate long-term clinical data in the literature, coated implants are very popular in clinical practice. The enthusiasm about hydroxyapatite-coated implants is the results of their osteoinductive capacity, leading to a higher number of bone-to-implant contacts, which may influence implant survival in spongy bone positively. However, a number of long-term problems have been reported with hydroxyapatite-coated implants[58]. The long-term prognosis of acid-etched implant strongly depends on the degree of tissue integration during the first year following implantation[59, 60].

CASE SELECTION FOR IMPLANT SURGERY

1) Primary judgement (prosthetic level)

When patients are referred seeking implant treatment, initial oral prophylaxis and any specific oral problems must first be identified by the clinician, after which different treatment alternatives can be presented. If patient has any oral problems and if healing is delayed, this can lead unfavourable prognosis [61].

2) Secondary assessment (surgical level)

In this stage, the medical condition of the patient as well as the local health and bone morphology of the future implant sites is analyzed. The medical state of the patient should be taken into consideration prior to any surgical treatment. So far, no specific condition has been identified which would exclusively prevent implant surgery. Factors like gender, age do not seem to have much influence on the outcome. Still, it should be remembered that elderly patients are more susceptible to infections and/or slow healing, and therefore may constitute potential risks for problems pre-operatively and post-operatively. Patients with history of any disease should be thoroughly examined prior to posting for implant surgery, thus favouring prognosis.

Intraoral health and bone morphology: It is also important to examine the intaoral health status of the soft and hard tissues as well as of the bone morphology in future implant areas. This is mainly done using both clinical and radiographic parameters. Any defects should thereby be identified and treated prior to implant procedure. Furthermore, the clinical examination should include a judgement of interarch and interdental spaces to see if that there is accessibility for the instruments as well as for the future prosthetic construction. It is also important to study the jaw relation, as that will have influence on the implant direction [61].

3) Treatment planning (combined surgical-prosthetic level)

Based on clinical and radiographic data collected, a final treatment planning is carried out by the clinician. As the placement of the implants is an important part of the strategy, the clinician should take part in detailed planning regarding implant location, that is participate in team approach. In order to create acceptable function and esthetics, the best position and direction of the implants must be identified together with the number and type of implants that can be inserted.

Implant placement: The main purpose of the implant surgery is to establish the anchorage for the future fixed prosthetic construction. In order to create a favorable and lasting result, it is first of all important to understand that the jawbone is living tissue that cannot be violated during surgery. Other factors are which should be considered during placement are flap design, bone drilling, implant position, implant direction, abutment selection and implant selection [62]. All these factors can influence the long term prognosis.

ASSIGNING PROGNOSIS

According to McGuire and Nunn [63] following the initial phase of periodontal therapy and prior to placing the patient on maintenance recall, each tooth is assigned a prognosis. [refer Table IV]. Excellent, good and hopeless are the only prognoses that can be established

with a reasonable degree of accuracy. Fair, poor and even questionable prognoses depend on a large number of factors that can interact in an unpredictable number of ways. In many of these cases, it is advisable to establish a provisional prognosis until phase I therapy is completed and evaluated [4].

Table 4. Assigning prognosis according to Mcguire and Nunn[63]

Excellent prognosis: No bone loss, excellent gingival condition, good patient cooperation, no systematic environmental factors.
Good prognosis (one or more of the following): Control of the etiologic factors and adequate periodontal support as measured clinically and radiographically to assure the tooth would be relatively easy to maintain by the patient and clinician, assuming proper maintenance.
Fair prognosis (one or more of the following): Approximately 25% attachment loss as measured clinically and radiographically and / or class I furcation involvement. The location and depth of the furcation would allow proper maintenance with good patient compliance.
Poor prognosis (one or more of the following): 50% attachment loss with class II furcations. The location and depth of the furcations would allow proper maintenance, but with difficulty.
Questionable prognosis (one or more of the following): Greater than 50% attachment loss resulting in poor crown-to-root ratio. Poor root form Class II furcations not easily accessible to maintenance care or class III furcations. 2 + mobility or greater. Significant root proximity.
Hopeless prognosis: Inadequate attachment to maintain the tooth. Extraction performed and suggested.

Recently, Kwok and Caton [64] designed a periodontal prognostication system based on the probability of disease progression. The individual tooth prognosis relies on the prediction of future stability of the periodontal supporting tissues. Basically comprises of four classifications [refer Table V].The classification system comprises primarily of favorable, questionable, unfavorable and hopeless prognosis.

Table 5. A Periodontal Prognostication System by Kwok and Caton [64]

Favorable Prognosis: local and/or systemic factors can be controlled and the periodontal status of the tooth can be obtained with comprehensive periodontal therapy and maintenance.
Questionable Prognosis: Local and/or systemic factors may or may not be controlled. However, periodontal stability is achieved through comprehensive periodontal therapy and maintenance, provided, these factors are controlled. Otherwise, future breakdown may occur.
Unfavorable Prognosis: Local and/or systemic factors cannot be controlled. Periodontal breakdown is likely to occur even with comprehensive periodontal therapy and maintenance.
Hopeless Prognosis: Extraction is the only therapy

Prognosis of Dentition

All branches of dentistry strive at the ultimate goal, that is preservation of teeth/tooth. In periodontal procedures, the prime consideration is the functioning unit. This means that the individual components are not as vital as overall function of the entire organ. In most cases, one should consider removing the tooth with poor prognosis, especially in patients with systemic conditions that compromise the overall prognosis. Teeth with good periodontal

prognosis should be maintained, provided the patient is capable of maintaining oral hygiene and follows the recall appointments [65].

CONCLUSION

Prognostication is a skill not easily acquired and is highly dependent upon the experience and skill of the clinician. The ability to predict the response of the dentition to periodontal therapy is essential in developing a definitive periodontal maintenance and restorative treatment plan. It is generally agreed that a tooth with a hopeless prognosis is one that despite the patients and clinicians best efforts is not going to improve. In such situations, it is difficult to arrest the disease process and restore periodontal health.

In this review, the potential adverse influences of a variety of factors (local and systemic) on prognosis have been discussed. While each of these factors alone may have a detrimental effect on prognosis and treatment outcomes, it should be kept in mind that the presence of any of these factors alone or in combination does not imply poorer outcomes.

In order to reduce the likelihood of developing the disease and improving prognosis, patients are encouraged to control as many of these factors which include smoking cessation, stress reduction, microbiological testing etc. With recent advances in microbiological and radiographical diagnostic methods, the clinician can utilize these aids to monitor the recurrence of on going periodontal disease and predict the outcome. Also considerable amount of work has to be done on possible genetic disposition of certain individuals to periodontal diseases.

All in all, prognosis is dependent on the experience of the operator, his ability to examine and interpret findings, his judgement concerning the healing capacity of the patient and his technical ability all combined with the patients co-operation.

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Chapter 33

BIOLUMINESCENT *LUX* GENE BIOSENSORS IN ORAL STREPTOCOCCI: DETERMINATION OF COMPLEMENTARY ANTIMICROBIAL ACTIVITY OF MINOCYCLINE HYDROCHLORIDE WITH THE ANESTHETIC LIDOCAINE/PRILOCAINE OR THE ANTISEPTIC CHLORHEXIDINE

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ABSTRACT

Background: Plaque-induced periodontitis is gingival inflammation at sites undergoing loss of connective tissue, apical migration of junctional epithelium and loss of alveolar bone. Non-surgical treatment of plaque-induced periodontitis typically involves removal of biofilm conducted through mechanical scaling and root planing (SRP) procedures. The antibiotic minocycline hydrochloride, delivered as a sustained-

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release product[1] used for professional subgingival administration into periodontal pockets, has been shown to be beneficial as an adjunct to conventional SRP. Use of chlorhexidine rinse is also a typical adjunct therapy to SRP procedures for chemical control of supragingival plaque. Lidocaine (2.5%) and prilocaine (2.5%)[2] provides localized anesthesia for SRP. The objective of this study is to develop and use bioluminescent recombinants of oral streptococci in determining the potential antibacterial activity of minocycline hydrochloride used either alone or in combination with the anesthetic lidocaine/prilocaine, or with the antiseptic chlorhexidine.

Methods: Recombinant plasmids containing the bioluminescence-generating *lux* gene from *Photorhabdus luminescens* were transformed into the oral bacterium *Streptococcus mutans*, strains UA159 and ATCC 25175. Transformants were verified as *S. mutans* derivatives by selection and growth on mitis salivarius agar supplemented with bacitracin, in addition to an antibody test directed specifically against *S. mutans* cell wall proteins and polymerase chain reaction experiments targeting sequences in the *S. mutans* glucosyltransferase (*gtf*) gene. *S. mutans* transformants were then subjected to growth analysis for comparison of absorbance and bioluminescence activity. Minocycline hydrochloride and lidocaine/prilocaine, or minocycline hydrochloride and chlorhexidine, were used in combination to determine the potential interactive effects of these agents on the antibacterial activity of minocycline hydrochloride.

Results: Using two distinct *S. mutans* transformants representing both strains UA159 and ATCC 25175, we observed rapid and pronounced bacteriostatic activity when using high doses of minocycline hydrochloride ($\geq$1 μg/ml), which were statistically distinct from untreated cultures (p=0.000058) when measured at the peak of metabolic activity. Reduced bacteriostatic activity was seen using lower doses. When lidocaine/prilocaine at doses >100 μg/ml is used in conjunction with minocycline hydrochloride, we observed an additive antibacterial effect. The *S. mutans* transformant strain UA159, when treated with chlorhexidine (0.01%) in conjunction with either high (1 μg/ml) or low (0.1 μg/ml) doses of minocycline hydrochloride, displayed reduced levels of cell mass accumulation, as measured by absorbance, that were additive when both antimicrobial agents were deployed. Bioluminescence determinations, which are a direct measure of metabolic activity and an indirect measure of cell number when cells are in logarithmic stage of growth, displayed similar reductions when cultures were treated with minocycline hydrochloride and chlorhexidine used singularly or in combination.

Conclusions: The *S. mutans* lux transformants serve as sensitive biosensors in the determination of antimicrobial activity, and can rapidly monitor inhibition of bacterial metabolism. We conclude that the anesthetic lidocaine/prilocaine does not interfere with the potent bacteriostatic activity of minocycline hydrochloride, and actually has an additive antibacterial effect. The potent bacteriostatic activity of minocycline hydrochloride can also be complemented with the addition of chlorhexidine. The application of the *lux* biosensor system in the assessment of minocycline hydrochloride and lidocaine/prilocaine, or minocycline hydrochloride and chlorhexidine, represents its first use in examining antimicrobial drug interactions in periodontology.

Keywords: *lux* biosensors, Minocycline hydrochloride-Lidocaine/prilocaine interactions, *Streptococcus mutans*, minocycline hydrochloride, lidocaine/prilocaine, chlorhexidine

[1]Brand name for minocycline hydrochloride used as a sustained release product is Arestin

[2]Brand name for the lidocaine (2.5%) and prilocaine (2.5%) anesthetic is Oraqix.

INTRODUCTION

Plaque-induced periodontitis is gingival inflammation at sites where there has been loss of connective tissue, apical migration of junctional epithelium and loss of alveolar bone [1]. The subgingival plaque linked with chronic periodontitis is usually associated with Gram-negative anaerobes that coexist with hundreds of other species of bacteria in a highly organized biofilm [2,3]. Biofilms are natural communal aggregations of microbes that form on a wide range of surfaces, including teeth [4]. Plaque is a highly organized biofilm [5] that possesses several features that help to protect and increase the nutritional advantages for bacteria [6] and provide competitive advantages over free-floating bacteria. Bacteria in biofilms produce a matrix, or glycocalyx, that encloses and shelters the microbial community from harmful effects of the surrounding environment. These bacterial matrixes form slimy, insoluble coatings that promote retention of bacteria, and inhibit removal by surrounding fluids, such as saliva and crevicular fluid [6]. In active periodontitis, biofilm removal and resultant healing of periodontal tissues can often only be achieved by clinical means, such as scaling and root planing (SRP) procedures [7,8].

SRP becomes substantially more difficult as pocket depths increase [9], with periodontal pockets > 4 mm retaining up to 66% of the plaque and calculus on root surfaces following non-surgical therapy [10-12]. Despite an improvement in periodontal status, periodontal pathogens remain subgingivally within the residual biofilm, in the remaining calculus, and within the exposed dentin tubules [7,8,13,14,15]. With the probability of pocket re-infection under these circumstances being high, additional means to decrease microbial load should be considered with non-surgical therapy [8].

USE OF MINOCYCLINE HYDROCHLORIDE ANTIBIOTIC AS ADJUNCT THERAPY FOR SRP

Minocycline hydrochloride (Arestin, OraPharma, Inc., Warminster, PA) is a sustained-release product for professional subgingival administration into periodontal pockets, and has been shown to be beneficial as an adjunct to conventional SRP in the treatment of periodontal disease [16-19]. Each unit-dose cartridge delivers product equivalent to 1 mg of minocycline free-base that is transported via microspheres. The minocycline free-base is slowly hydrolyzed and liberated over a 2-3 week period upon contact with moisture, with concentrations > 300 micrograms per ml being released into the gingival crevicular fluid [16]. Minocycline hydrochloride is a member of the tetracycline class of antibiotics and has a broad spectrum of activity. It is bacteriostatic and exerts its antimicrobial activity by inhibiting protein synthesis. *In vitro* susceptibility testing has shown that many putative periodontal pathogens, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*, are susceptible to minocycline hydrochloride at concentrations ≤ 8 micrograms per ml [20].

The typical indication for use of minocycline hydrochloride is in a pocket of >5 mm with bleeding on probing. Minocycline hydrochloride has been demonstrated to more effectively decrease the red complex periodontal pathogens as compared to SRP alone, and also act to block collagenases, which are involved in host tissue breakdown [21]. Increased benefits have

been shown in patients that have advanced periodontal disease and in smokers [22,23]. Likely explanations for these observations are that there are more susceptible pathogens in these sites since SRP is not as effective in the deeper periodontal pockets, and that smokers may exhibit higher proportions of periodontal pathogens. Additionally, minocycline hydrochloride retains anti-metalloproteinase properties that may counteract the increased protease activity exhibited in smokers [24].

In addition to the beneficial effects of minocycline hydrochloride with SRP [21,25-27], recent studies have suggested that this antibiotic may also be used as an adjunct for surgical therapy [28] and for treatment of peri-implantitis [29-31].

USE OF CHLORHEXIDINE ANTISEPSIS AS AN ADJUNCT THERAPY FOR SRP

Chlorhexidine diglucanate (0.12%) has been used in the treatment of periodontitis for over 40 years and has well-documented success as an anti-plaque and anti-gingivitis mouthrinse [32,33]. Chlorhexidine is a cationic bisbiguanide that has a broad-spectrum antimicrobial effect due to its ability to bind to the negative charge of the cell walls of Gram-positive and Gram-negative bacteria, causing rupture of the membrane through alteration of the osmotic equilibrium [34,35]. This antiseptic agent has demonstrated effectiveness due to its ability to absorb onto cationic substrates, such as pellicle and salivary glycoproteins, and subsequent release over an 8-12 hour period [34,36]. Combined, these properties have demonstrated chlorhexidine to be highly effective for the treatment and prevention of gingivitis [37,38]. However, in the case of periodontal disease, chlorhexidine has decreased effectiveness due to difficulty in delivery of the drug to the depth of deep pockets [39] as well as difficulty in penetrating structured biofilm. Thus chlorhexidine has been shown to be more effective in conjunction with mechanical removal of the biofilm [40]. The clinician has several choices for mode of delivery, as chlorhexidine can be used as a mouth rinse, a supra- or subgingival irrigant, or as a locally delivered antimicrobial in the form of a bioabsorbable chip.

As a mouth rinse, typical dosing with chlorhexidine is to rinse with one-half fluid ounce for 30 seconds after brushing and flossing, with no eating or drinking for the following 30 minutes. Because chlorhexidine is poorly absorbed in the gastrointestinal tract, it has a high margin of safety [34]. Additionally, antibiotic resistance to chlorhexidine has not been demonstrated as with systemic antibiotics [41]. Although chlorhexidine rinse provides many benefits, it also has several reversible side effects. Chlorhexidine use may cause extrinsic staining of teeth, restorations, and tongue, as well as an altered or decreased taste perception [42,43]. Patients may also exhibit increased calculus formation [44-46]. In rare instances, gingival desquamation and painful mucosa have been also reported [33,42].

Due to its side effects, chlorhexidine should not be considered for long-term use in every periodontal patient. Indications for use may include treatment of gingivitis or peri-implantitis [47], or post-surgically to prevent infection [48,49] or to enhance healing [50]. Chlorhexidine can also be used to prevent infections in patients who are immunocompromised, as in the case of cancer or transplantation [49]. Some clinicians use local chlorhexidine application as part

of a daily oral hygiene regimen for implants. It has even been advocated for improved fixed prosthetic restoration impressions [51].

In 1998, a locally delivered, sustained-released chlorhexidine chip was developed for use as an adjunct therapy for SRP. The advantages to the use of the chlorhexidine chip include its reliable effect on subgingival sites, continued release, and lack of visible staining of teeth [26]. The PerioChip (Perio Products Ltd.) is supplied in the form of a bioabsorbable 5 x 4 x 0.35 mm hydrolyzed gelatin chip which contains 2.5 mg of chlorhexidine gluconate. In the process of dissolving over the 7-10 days following placement, chlorhexidine is released at an average drug concentration >125 micrograms per ml in the gingival crevicular fluid [52], which is higher than the minimal inhibitory concentration (MIC) for more than 99% of the subgingival microorganisms from periodontal pockets [53].

USE OF LIDOCAINE/PRILOCAINE PERIODONTAL GEL AS ANESTHETIC DURING SRP

Lidocaine/prilocaine, a composite of 2.5% lidocaine and 2.5% prilocaine (Oraqix, DENTSPLY Pharmaceutical, York, PA), is applied directly to the periodontal pocket to provide effective, non-invasive localized anesthesia during SRP procedures [54,55]. The anesthetic mixture contains poloxamers that allows reversible temperature-dependent gelation. Therefore, lidocaine/prilocaine exists as a low-viscosity fluid at room temperature and as an elastic gel in the periodontal pocket. Once gelation occurs in the periodontal pocket, the anesthetics, lidocaine and prilocaine, are released to provide sufficient anesthesia for SRP.

Potential Interactions between Minocycline Hydrochloride and Lidocaine/ Prilocaine and Between Minocycline Hydrochloride and Chlorhexidine

Because both minocycline hydrochloride and lidocaine/prilocaine may be applied subgingivally in the same periodontal pocket, with the anesthetic applied prior to SRP and the antibiotic applied following SRP, an interaction seems plausible. The purpose of this study is to test the efficacy of minocycline hydrochloride, when used in conjunction with the anesthetic lidocaine/prilocaine periodontal gel. We will also test the interactions of minocycline hydrochloride and chlorhexidine, which is commonly used for treatment of periodontal patients. To begin developing an understanding of these interactions, we transformed oral mutans streptococci with the luminescence (*lux*) gene from *Photorhabdus luminescens* for use as biosensors for antibiotic or drug sensitivity tests. Streptococci were selected as biosensors since they are among the most common colonizers in plaque biofilm, which will reform following SRP. These bacterial species will influence the ultimate biofilm composition, including the numbers and types of periodontopathogens present [56]. Mutans streptococci have also been found in the subgingival plaque of patients with periodontitis, and may be important in the development of root caries [57]. The *lux* biosensors will allow rapid and uniform determination of *in vitro* drug sensitivity experiments, either singular or in combination with test agents, and provide measurements of inhibition. Thus, these biosensors will assess potential effects of the lidocaine/prilocaine anesthetic, or chlorhexidine antiseptic, on the bactericidal activity of the minocycline hydrochloride antibiotic.

MATERIALS AND METHODS

Lux Recombinant and Oral Streptococci Strains

The *lux* recombinant contains the *lux* operon (*A-E*) from *Photorhabdus luminescens* modified for expression in *Streptococcus pneumoniae* [58,59]. The recombinant *lux A-E* operon reconstitutes an aldehyde-recycling pathway [60], and allows for continual build-up of substrate (long chain aliphatic aldehyde) to drive the generation of measureable light. The *lux* recombinant also contains an *Escherichia coli* origin of replication and erythromycin-resistance gene, used for recombinant plasmid generation in *E. coli* and selection of transformants, respectively. *Streptococcus mutans* strains UA159 (also known as ATCC 700610), ATCC 25175 and ATCC 35668, in addition to *Streptococcus sobrinus* (ATCC 33478) and *Streptococcus salivarius* (ATCC 25975), were obtained from the American Type Culture Collection (ATCC; Manassas, VA).

Transformation of Oral Bacteria with *Lux* Recombinants and Validation of *Streptococcus Mutans* Strains

Cultures of oral streptococci were prepared by inoculating 10 ml of THYE broth (Todd Hewitt broth supplemented with 0.3% yeast extract) and incubating overnight at 37°C with 5% CO_2. Cultures were then diluted 1:20 in pre-warmed THYE broth and incubated until reaching an A_{600} of 0.1. *Lux* recombinant plasmid DNA (200 ng) was added to 0.5 ml of cells, and incubated for an additional 2.5 hours. Transformation mixes were then plated on THYE agar plates, containing either 250 μg/ml or 500 μg/ml of erythromycin, for selection of transformants. Mutans streptococci transformed with the *lux* recombinant include *Streptococcus mutans* UA159 and *Streptococcus mutans* ATCC 25175. These transformants were validated as being *S. mutans* derivatives with the subsequent growth and selection on mitis salivarius agar (MSA) supplemented with bacitracin (10 U /ml) and affirmed positive reaction on the Saliva-Check Mutans antibody test (GC America; Alsip, IL), which uses monoclonal antibodies against *S. mutans* cell wall components.

Polymerase Chain Reaction (PCR) and Acidification Reactions

Additional validation of the transformants as *S. mutans* derivatives was obtained with PCR using specific primers directed against *S. mutans* or *S. sobrinus* gene targets. We developed primers recognizing sequences from the glucosyltransferase genes in *S. mutans* UA159 (Genbank accession numbers AE014133 [complete genome] and NC 004350 [*gtfB* gene], position of *gtfB* gene in *S. mutans* genome [951112 – 955542]) and in *S. sobrinus* (Genbank accession number D63570, *gtfI* gene [position 1 – 6368]). Specific primer sets include: 1) mutans *gtfB* forward primer 793 (position in *gtfB* gene; 85 -> 106) and mutans *gtfB* reverse primer 1309 (position in *gtfB* gene; 601 -> 580) and 2) sobrinus *gtfI* forward primer 871 (position in *gtfI* gene; 1723 -> 1744) and sobrinus *gtfI* reverse primer 1582 (position in *gtfI* gene; 2434 -> 2413). PCR was conducted with an initial denaturation step at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds, with final extension at 72°C for 5

minutes followed by 4°C soak. GoTaq Green Master Mix (Promega, Madison, WI) was used in all amplifications.

Acidification reactions (D-ribose, L-arabinose, D-mannitol, D-sorbitol, D-lactose, D-trehalose, inulin, D-raffinose, amidon, and glycogen; derived from API-20 Strep kit, Biomurieux SA) using the two transformants were compared against *S. mutans* strains UA159, ATCC 25175 and ATCC 35668, and *S. salivarius*. Acidification reactions discriminating *S. mutans* strains include the use of D-mannitol, D-sorbitol and D-raffinose. Validated transformants were grown in TYYE broth supplemented with erythromycin and tested for bioluminescent activity.

Minocycline Hydrochloride and Lidocaine/Prilocaine and other Chemicals

Minocycline hydrochloride (microspheres, 1 mg/cartridge) was obtained from OraPharma, Inc. (Langhome, PA). Lidocaine/prilocaine was obtained from Dentsply Pharmaceutical (York, PA). Chlorhexidine gluconate oral rinse (0.12%) was obtained from Patterson Dental (St. Paul, MN). Mitis salivarius agar, Todd Hewitt broth, and yeast extract were obtained from Becton, Dickinson and Company (Sparks, MD). Bacitracin (0.6 U/mg) was obtained from Acros Organics USA (Morris Plains, NJ) or from Sigma Chemical (St. Louis, MO).

Treatment of Transformants with Minocycline Hydrochloride, Lidocaine/ Prilocaine, or Chlorhexidine

Lux transformants UA 159 or ATCC 25175, or corresponding non-transformed *S. mutans* strains, were treated with minocycline hydrochloride (0 – 100 μg/ml) for up to 6 hours, and assayed every 30 minutes for bioluminescence using luminometry and for cell mass accumulation by measuring absorbance at 600 nm. Parallel cultures were also treated with minocycline hydrochloride and concurrent application using lidocaine/prilocaine (0 – 200 μg/ml), and also assayed every 30 minutes for bioluminescence and cell mass accumulation. In independent experiments, *lux* transformant UA 159 was treated with minocycline hydrochloride (0.1 μg/ml or 1.0 μg/ml) in the presence of absence of chlorhexidine (0.01%) for up to 5.5 hours, and assayed every 30 minutes for bioluminescence and cell mass accumulation.

Luminometry and Absorbance Assays and Culture Medium

Bioluminescence was measured as relative light units (RLUs) with the Turner Biosystems Veritas Luminometer. Absorbance at 600 nm wavelength was measured using a Novaspec II visible spectrophotometer. Streptococci were plated on mitis salivarius agar (MSA, Difco™; Becton, Dickinson and Company, Sparks, MD), which utilizes high saccharose and vital dyes (ie: crystal violet and bromophenol blue) as selective agents. MSA was supplemented with potassium tellurite (Difco™; Becton, Dickinson and Company, Sparks, MD); 1 ml of 1% aqueous potassium tellurite was added to 1 liter of MSA. To

validate the sub-group of mutans streptococci, MSA including potassium tellurite, was supplemented with bacitracin (10 Units/ml).

Statistical Analysis

All bioluminescence determinations were conducted in quadruplicate. Descriptive statistics, including mean values and standard error of the mean for each data point, were conducted. Standard error bars, even those nearly indistinguishable from corresponding mean values, are displayed in graphs. In selected cases where multiple enumerations were conducted, as in the chlorhexidine experiments, we conducted pair-wise comparison using one-way between-subjects Analysis of Variance (ANOVA) modified for multiple comparisons with the use of the Bonferroni correction.

RESULTS

Validation of Streptococcus Mutans *Lux* Transformants

The two transformants used in these studies have been validated as *S. mutans* derivatives, consistent with the specific identification of *S. mutans* strains UA159 and ATCC 25175. This is based on selection and growth on mitis salivarius agar supplemented with bacitracin, in addition to an antibody test directed specifically against *S. mutans* cell wall proteins (Saliva Check Mutans test) and PCR experiments targeting sequences in the *S. mutans gtf* gene. Using the Saliva Check Mutans kit, positive immunoreactivity lines were formed using *S. mutans* strains UA159 and ATCC 25175, in addition to the two transformants, but not with *S. sobrinus* or *S. salivarius*. In addition, PCR using *S. mutans*-specific primers amplified the correct size fragment from the two transformants, and from stock cultures of *S. mutans* UA159 and ATCC 25175, but not from *S. sobrinus* or *S. salivarius* (unpublished observations). We conclude that both transformants are confirmed as *S. mutans* strains.

In addition, acidification reactions were conducted using a panel of metabolic substrates in the API 20 Strep kit (Table 1). Each ampule was inoculated with aliquots of *S. mutans* ATCC 25175 (two independent sources), UA159 or ATCC 35668, or the two transformants, or *S. sobrinus* or *S. salivarius*. All inocula were calibrated to the equivalent of 4 McFarland Units, as recommended by the manufacturer. As can be observed (Table 1), and as previously described [61-63], *S. mutans* and *S. sobrinus* can be distinguished by differential raffinose acidification (*S. mutans*: raffinose positive acidification; *S. sobrinus*: raffinose negative acidification). *S. mutans* strains UA159 and ATCC 25175 can be distinguished by differential sorbitol acidification (ATCC 25175: sorbitol positive acidification; UA159: sorbitol negative acidification). The combination of negative inulin acidification and positive raffinose acidification distinguish *S. salivarius* from *S. mutans* (either strain) or *S. sobrinus*. We therefore conclude that the two *S. mutans* transformants are consistent with the identification of derivatives of UA159 and ATCC 25175.

Table 1.

Bacterium	Sugars Metabolism									
	RIB	ARA	MAN	SOR	LAC	TRE	INU	RAF	AMD	GLYG
S. mutans, ATCC 25175, source A	-	-	+	+	+	+	+	+	-	-
S. mutans, ATCC 25175, source B	-	-	+	+	+	+	+	+	-	-
S. mutans, UA159	-	-	+	-	+	+	+	+	-	-
S. mutans, ATCC 35668	-	-	+	+	+	+	+	+	-	-
Lux Transformant A	-	-	+	-	+	+	+	+	-	-
Lux Transformant B	-	-	+	+	+	+	+	+	-	-
S. sobrinus	-	-	+	+	+	+	-	-	-	-
S. salivarius	-	-	+	-	+	+	-	+	-	-

The metabolic substrates include D-ribose, L-arabinose, D-mannitol, D-sorbitol, D-lactose, D-trehalose, inulin, D-raffinose, amidon, and glycogen. Manufacturer states that yellow color reactions are positive for acidification using varied substrates, and that orange/red colors are negative for acidification. *Lux* transformants A and B have been subsequently confirmed as derivatives of the UA159 and ATCC 25175 strains, respectively.

Growth Curve Analysis of UA159 Transformant Demonstrates Increase in Bioluminescence Activity, Reflective of Cell Mass Accumulation during Log Phase

Cultures of the UA159 transformant were inoculated for overnight incubation, and then re-initiated the following morning at reduced concentration in fresh medium to allow regenerated growth. Aliquots from the regenerated culture were measured for both absorbance (A=600 nm) and for bioluminescence (Figure 1, Panels A and B, respectively). We observe that bioluminescence increases as cell mass accumulates during logarithmic phase of growth, and as the culture enters stationary phase, bioluminescence drops, reflective of the decrease in the metabolic activity of the culture (Figure 1, Panel B). Similar results were also obtained using the ATCC 25175 transformant (unpublished observations).

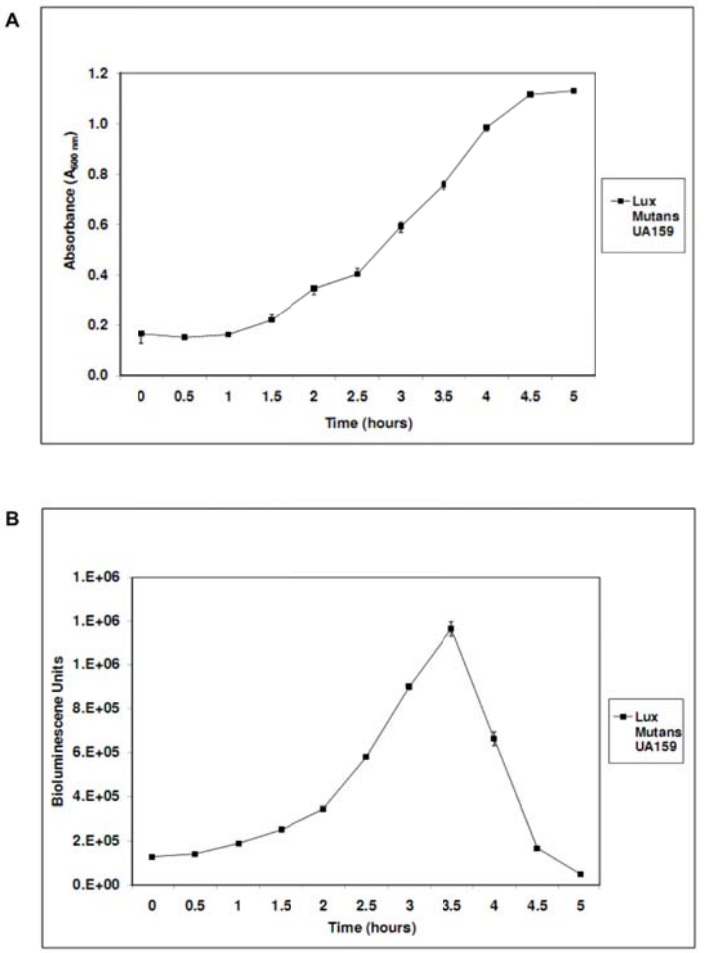

Figure 1. Panels A and B: Growth curve analysis of *lux* UA159 transformant examining absorbance and bioluminescence, respectively. Absorbances were measured at 600 nm. Note that measurements in Panels A and B were conducted with 4 replicate determinations, and that data points represent mean values. Standard error bars have been placed on each data point; in many cases, the standard error bars are indistinguishable from the data point itself.

S. Mutans UA159 Strain and *S. Mutans Lux* Transformants have Near-Equivalent Sensitivity to the Antibiotic Minocycline Hydrochloride

Cultures of non-transformed *S. mutans* UA159 were treated with varying doses of minocycline hydrochloride (0.01 – 100 μg/ml), and absorbance (A_{600} nm) was measured at specific time intervals (Figure 2, Panel A).

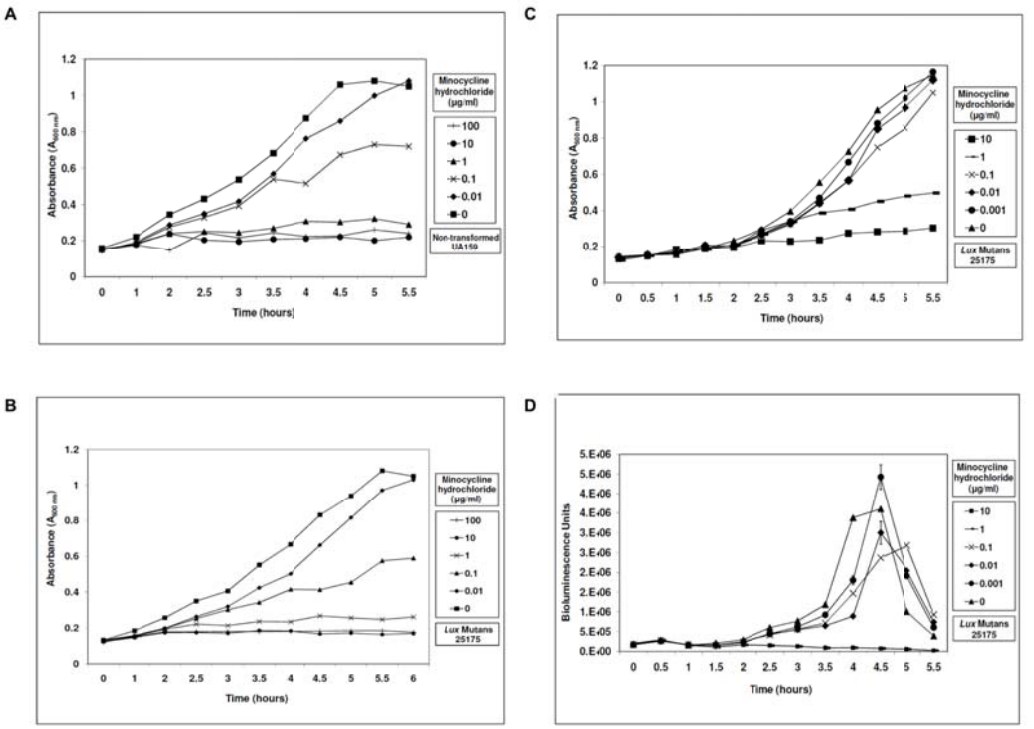

Figure 2. Panel A: Absorbance measurements of non-transformed *S. mutans* UA159 treated with varying doses of minocycline hydrochloride (0.01 – 100 μg/ml). Panel B: Absorbance measurements of *lux* ATCC 25175 transformant treated with varying doses of minocycline hydrochloride (0.1 – 100 μg/ml). Panel C and D: Matched experiment examining absorbance (Panel C) and bioluminescence (Panel D) measurements of *lux* ATCC 25175 transformant treated with intermediate range doses of minocycline hydrochloride (0.001 – 10 μg/ml). Absorbances were measured at 600 nm. Replicate determinations (n = 4) were conducted for all experiments displayed in Panel D. Standard error bars have been placed over all data points, with some standard error bars indistinguishable from the data point itself.

Non-treated cultures (0 μg/ml minocycline hydrochloride) served as controls for growth. Repeated experiments demonstrated that 1 μg/ml minocycline hydrochloride was the minimum effective dose for immediate and sustained reduction of accumulated cell mass as measured by absorbance (Figure 2, Panel A). Similar profiles were observed for both *S. mutans* transformants, including the ATCC 25175 transformant (Figure 2, Panel B), which also demonstrated considerable inhibition of cell mass accumulation using 1 μg/ml minocycline hydrochloride. This was reproduced when using an intermediate range of

minocycline hydrochloride (0.001–10 μg/ml; Figure 2, Panel C). When bioluminescence was simultaneously tracked in the ATCC 25175 transformant during the same matched experiment, we observe rapid and marked reduction of emitted light at the two highest minocycline hydrochloride doses (1 μg/ml and 10 μg/ml; line graphs are similar), with progressively less effect at the lower doses compared to the non-treated controls (Figure 2, Panel D). Interestingly, bioluminescence for the non-treated control peaked at approximately 4.5 hours and decreased with subsequent time points, indicative of the metabolic activity of the culture, which becomes slowed as the cultures become saturated and enter stationary phase (compare Panels C and D in Figure 2; also illustrated in Figure 1, Panels A and B).
When comparing bioluminescence values obtained at 4.5 hours, or the peak of metabolic activity, we find that the bioluminescence values for cultures treated with 1.0 or 10 μg/ml minocycline hydrochloride were statistically different from bioluminescence values for untreated cultures ($p=0.000058$ in both cases; p values have been factored with the Bonferroni correction for multiple comparisons). All other ATCC 25175 transformant cultures treated in this same experiment with sub-optimal doses of minocycline hydrochloride (0.001–0.1 μg/ml) also appeared to drop in bioluminescence activity at 4.5 hours or later, prior to saturation of growth (Figure 2, Panel D).

Lidocaine/Prilocaine has Antimicrobial Activity at Higher Concentrations in the UA159 Transformant and is not Contraindicative to the Antimicrobial Activity of Minocycline Hydrochloride

Cultures of the UA159 transformant were treated with varying doses of lidocaine/prilocaine (0 - 200 μg/ml) and absorbance was measured at specific time intervals (Figure 3, Panel A). The majority of doses of lidocaine/prilocaine (0.02–20 μg/ml) tested in this series had minimal effect on the growth of the UA159 transformant, with the exception of the highest dose (200 μg/ml), which resulted in immediate and sustained reduction of cell mass accumulation as measured by absorbance (Figure 3, Panel A).

We tested the hypothesis that lidocaine/prilocaine may have a contraindicative effect on the antimicrobial activity of hydrochloride by treating the UA159 transformant with combinations of minocycline hydrochloride (at 1 μg/ml) and varying doses of lidocaine/prilocaine (0–200 μg/ml), and determined that lidocaine/prilocaine has no contraindicative effects on the antimicrobial potency of minocycline hydrochloride (Figure 3, Panel B). In additional experiments, bioluminescence determinations (in relative light units or RLUs), which represent assessments of the metabolic activity of the cultures, were conducted with the UA159 transformant. Bioluminescence values in RLUs were depressed using the combination of minocycline hydrochloride at 1 μg/ml and all doses of lidocaine/prilocaine tested (compare Figure 3, Panel C to absorbance determinations in the absence of minocycline hydrochloride in Figure 3, Panel A).

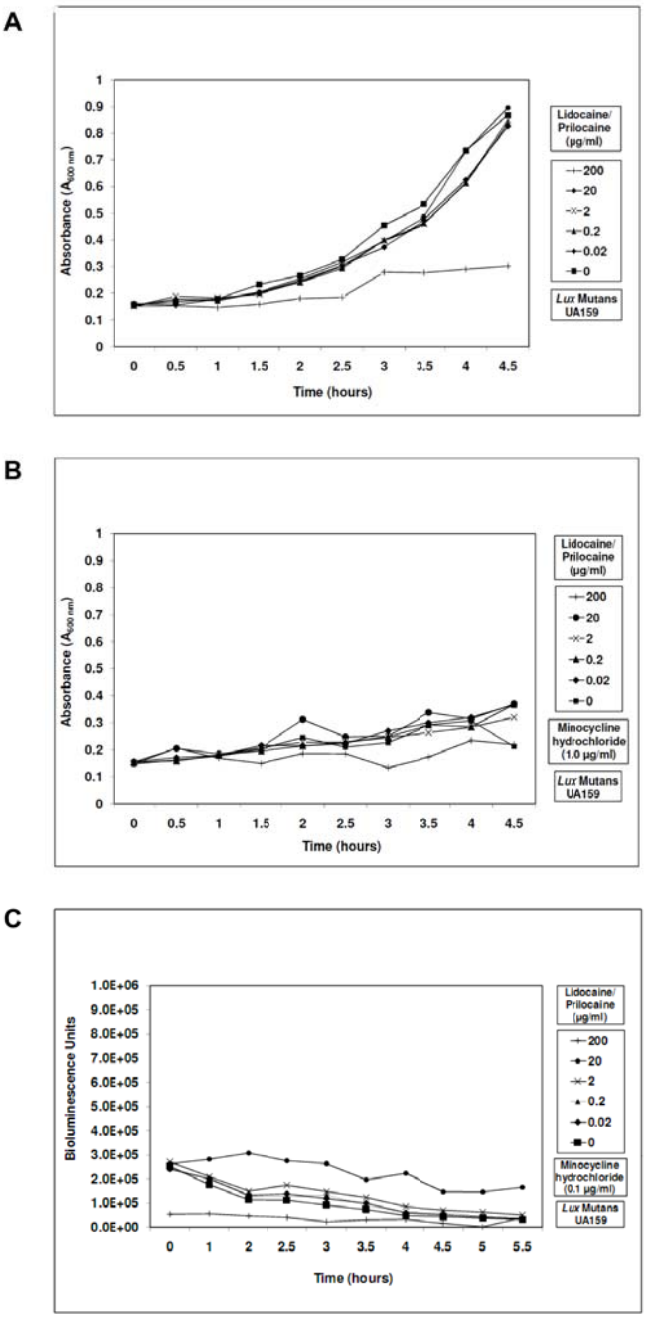

Figure 3. Panel A: Absorbance measurements of *lux* UA159 transformant treated with varying doses of lidocaine/prilocaine (0 – 200 µg/ml). Panels B and C: Matched experiment examining absorbance measurements (Panel B) and bioluminescence (Panel C) measurements of *lux* UA159 transformant treated with combination of Minocycline hydrochloride (1 µg/ml) and varying doses of lidocaine/prilocaine (0 – 200 µg/ml). Absorbances were measured at 600 nm. Replicate determinations (n = 4) were conducted for all experiments displayed in Panel C. Standard error bars have been placed over all data points, with some standard error bars indistinguishable from the data point itself.

Antimicrobial Activity of Lidocaine/Prilocaine is more Apparent when using Suboptimal Doses of Minocycline Hydrochloride

In order to more precisely test the sensitivity of the *lux* transformants to lidocaine/prilocaine, cultures of the ATCC 25175 transformant were treated with varied doses of lidocaine/prilocaine (0 – 100 μg/ml), combined with either near-optimal or suboptimal doses of minocycline hydrochloride (1 μg/ml and 0.1 μg/ml, respectively). In this experiment, we examined doses of lidocaine/prilocaine between 0-100 μg/ml, in the absence or presence of 1 μg/ml minocycline hydrochloride (Figure 4, Panels A and B, respectively). Consistent with the results of the UA159 transformant described above, we observe with the ATCC 25175 transformant, that lidocaine/prilocaine has weak antimicrobial activity when used alone at doses above 100 μg/ml (unpublished observations), and that no reductions in absorbance occurred using lidocaine/prilocaine at doses $\leq$ 100 μg/ml (Figure 4, Panel A). As described above in the case of the UA159 transformant, when lidocaine/prilocaine (25-100 μg/ml) is applied in conjunction with minocycline hydrochloride (1 μg/ml) in the ATCC 25175 transformant, we observe that lidocaine/prilocaine in this intermediate dose range does not have a contraindicative effect on the antimicrobial activity of minocycline hydrochloride, when minocycline hydrochloride is used at the near-optimal dose of 1 μg/ml (Figure 4, Panel B). Minocycline hydrochloride at that concentration will result in immediate and sustained reduction in absorbance. When minocycline hydrochloride is lowered to the suboptimal dose of 0.1 μg/ml, we observe that the combined effect of minocycline hydrochloride plus lidocaine/prilocaine at 100 μg/ml results in reductions in absorbance (Figure 4, Panel C). Other lower concentrations of lidocaine/prilocaine (< 100 μg/ml, when used with the suboptimal dose of minocycline hydrochloride (0.1 μg/ml) do not result in reductions in absorbance (Figure 4, Panel C). We conclude from these experiments that lidocaine/prilocaine at 100 μg/ml, when used in conjunction with suboptimal doses of minocycline hydrochloride, adds measureable bacteriostatic activity (Figure 4, Panel C).

Minocycline Hydrochloride and Antiseptic Chlorhexidine Display Additive Antimicrobial Activity in the UA159 Transformant

The UA159 transformant, when treated with chlorhexidine (0.01%) in conjunction with either high (1 μg/ml) or low (0.1 μg/ml) doses of minocycline hydrochloride, displayed reduced levels of cell mass accumulation, as measured by absorbance, that were additive when both antimicrobial agents were deployed (Figure 5, Panels A and B). When examining absorbance values at 5.5 hours, we find that the absorbance values of cultures treated with minocycline hydrochloride (1.0 μg/ml) plus chlorhexidine (0.01%) or minocycline hydrochloride (1.0 μg/ml) alone were statistically different from the absorbance values of untreated cultures ($p<10^{-6}$ in both cases with Bonferroni correction; Figure 5, Panel A). When comparing absorbance values at 5.5 hours for cultures treated with minocycline hydrochloride (1.0 μg/ml) with or without chlorhexidine (0.01%), we find that these values are nearly statistically distinct ($p=0.059$; Figure 5, Panel A). Similarly when examining data with minocycline hydrochloride at 0.1 μg/ml (Figure 5, Panel B), we observe statistical differences

in absorbances between cultures treated with minocycline hydrochloride (0.1 μg/ml) plus chlorhexidine (0.01%) versus untreated cultures (p=0.00017 including Bonferroni correction), and cultures treated with minocycline hydrochloride (0.1 μg/ml) with or without chlorhexidine (0.01%) (p=0.0018 including Bonferroni correction).

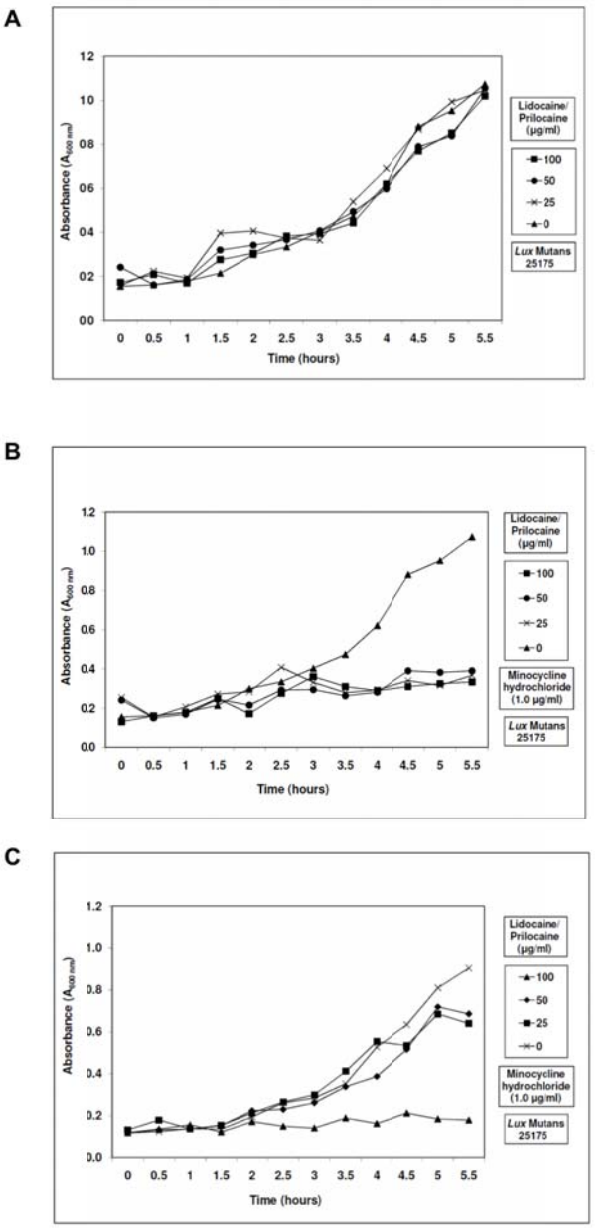

Figure 4. Panels A and B: Matched experiment examining the absorbance measurements of the *lux* ATCC 25175 transformant treated with varying doses of lidocaine/prilocaine (0 – 100 μg/ml), in the absence (Panel A) or presence (Panel B) of minocycline hydrochloride (1 μg/ml). Panel C: Absorbance measurements of the *lux* ATCC 25175 transformant treated with varying doses of lidocaine/prilocaine (0 – 100 μg/ml), in the presence of suboptimal doses of minocycline hydrochloride (0.1 μg/ml). Absorbances were measured at 600 nm.

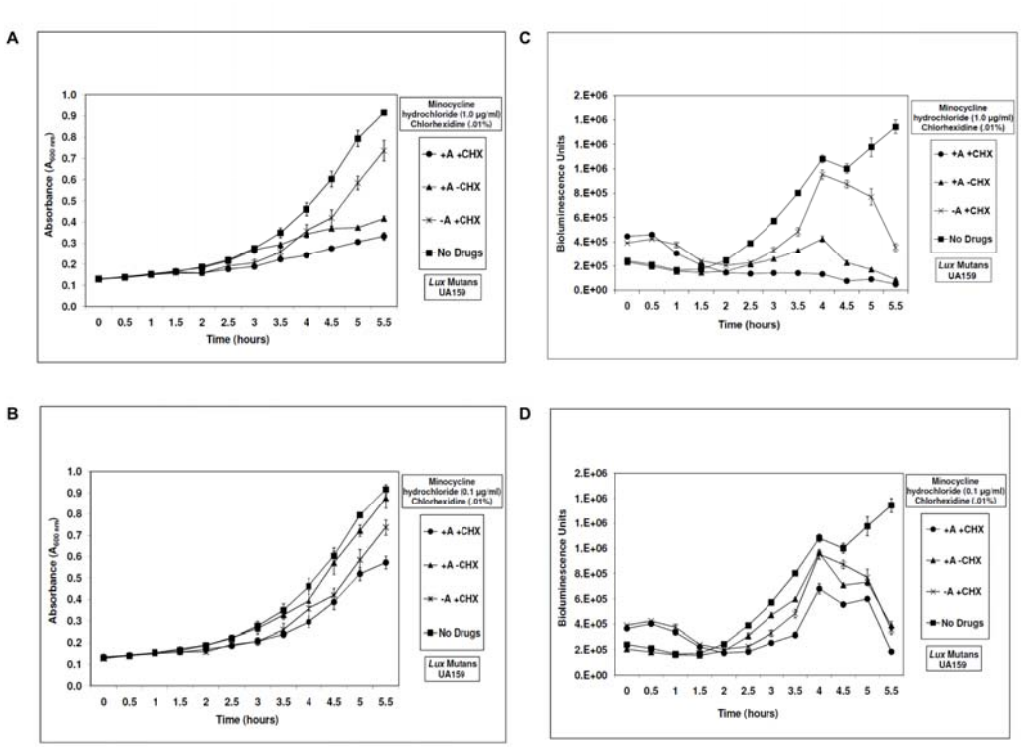

Figure 5. Panels A and C: Matched experiment examining absorbance (Panel A) and bioluminescence (Panel C) of the *lux* UA159 transformant treated with minocycline hydrochloride (1.0 µg/ml) or chlorhexidine alone or in combination. Panels B and D: Matched experiment examining absorbance (Panel A) and bioluminescence (Panel C) of the *lux* UA159 transformant treated with minocycline hydrochloride (0.1 µg/ml) or chlorhexidine alone or in combination. Absorbances were measured at 600 nm. Symbols A and CHX are reflective of Arestin and chlorhexidine.

Similar reductions in bioluminescence activity were also observed, with additive effects when both antimicrobial agents were deployed together (Figure 5, Panels C and D). When using bioluminescence values obtained at 4 hours for cultures treated with minocycline hydrochloride at 1.0 µg/ml (Figure 5, Panel C), and conducting multiple pair-wise comparisons, we find that all comparisons with the exception of the comparison between the untreated and chlorhexidine-treated cultures were statistically different with p values approaching zero ($p<10^{-10}$ including Boneferroni correction). Similar statistically-significant differences were found with bioluminescence values obtained at 4 hours using the lower dose of minocycline hydrochloride (0.1 µg/ml; Figure 5, Panel D). In these comparisons, statistically-significant differences were found between untreated cultures and cultures treated with minocycline hydrochloride alone (0.1 µg/ml) or minocycline hydrochloride plus chlorhexidine (0.01%) ($p=0.012$ and $p<10^{-8}$, respectively including Bonferroni correction), between cultures treated with minocycline hydrochloride (0.1 µg/ml) alone or with chlorhexidine (0.01%) ($p=10^{-6}$ including Bonferroni correction) or between cultures treated with chlorhexidine (0.01%) alone or with minocycline hydrochloride (0.1 µg/ml) ($p=0.00018$ including Bonferroni correction).

DISCUSSION

The treatment of periodontal disease commonly involves mechanical and surgical interventions, such as scaling and root planing (SRP). SRP is now often augmented with adjunct antimicrobial therapies to treat supragingival and subgingival plaque, including the use of chlorhexidine [18,21,22]. More targeted antimicrobial adjunct therapies have recently emerged as additional tools for the treatment of subgingival plaque and periodontitis. These include the slow-release minocycline hydrochloride, which has shown some success when used in conjunction with standard SRP procedures [18,22].

Adverse drug interactions are a growing concern for all aspects of patient care, including periodontology. Pharmacodynamic drug interactions, including competition by enzyme inhibition and substrate bioavailability, may potentially antagonize the effectiveness of specific drugs [64,65]. We have sought to identify potential antagonistic or complementary effects of two commonly used products, one the anesthetic lidocaine/prilocaine, the other the antimicrobial agent chlorhexidine, on the bacteriostatic activity of the antibiotic minocycline hydrochloride, used as an adjunct therapy after scaling and root planing. Even though the application of minocycline hydrochloride and lidocaine/prilocaine are temporally distinct, these two agents are applied subgingivally in the same periodontal sulcus within a short time of each other, and interaction is plausible. The two antimicrobial agents chlorhexidine and minocycline hydrochloride are designed for use during the extended period of time following SRP.

There are known antagonistic interactions between bactericidal and bacteriostatic compounds [64,65]. Bactericidal antibiotics are dependent on active cell replication, and bacteriostatic antibiotics, when used in conjunction with bactericidal agents, will inhibit replication and diminish the effectiveness of the bactericidal agent. For example, there are well-documented instances where simultaneous administration of penicillin with either tetracycline or erythromycin is less effective than using penicillin alone [64,65].

The potential interactions between minocycline hydrochloride and lidocaine/prilocaine, and between minocycline hydrochloride and chlorhexidine, were determined using the *lux* biosensor system, where *lux* gene recombinants from *Photorhabdus luminescens* were transformed into the oral bacteria *Streptococcus mutans*. This system can serve as sensitive real-time biosensors in the determination of antimicrobial activity and can rapidly monitor inhibition of bacterial metabolism. The application of the *lux* biosensor system in this study represents its first use in examining drug interactions in dentistry.

Streptococci were selected as host strains for transformation using *lux* gene recombinants, because they are among the most common colonizers in plaque biofilm, which would be reforming in the period following SRP when minocycline hydrochloride is designed to be effective, and in fact may be beneficial in shifting the reforming biofilm away from periodontal pathogens [56]. Some streptococci species are initiators of biofilms, and are required for the colonization and expansion of subsequent periodontal biofilm [66-69]. Mutans streptococci, including *S. mutans*, have also recently been identified in the subgingival plaque of patients with periodontitis [56]. Streptococci were also selected because of the availability of known transformation protocols for these strains and ease of growth in culture, and because minocycline hydrochloride is a broad-spectrum antibiotic that inhibits

common protein synthesis components found in both gram-positive streptococci and gram-negative periodontal bacteria [18-21].

Transformants that carry significant plasmid loads might be expected to have slowed growth kinetics because of the increased metabolic burden to replicate additional nucleic acids. Interestingly, this does not appear to be the case with the *lux* mutans transformants, where the saturating cell mass accumulation based on absorbance, and the duration of time necessary to obtain saturation, does not appear to be different from what occurs in nontransformed *S. mutans* (compare Figure 1, Panel C with Figure 2, Panel A).

The *lux A-E* operon reconstitutes an aldehyde-recycling pathway [60] and using bioluminescence can detect changes in metabolic activity before changes in cell mass occur. In this regard, we have determined that bioluminescence activity decreases as the culture enters stationary phase, reflective of the diminished metabolic activity of the culture at that stage, and occurs well in advance of the observed plateau in cell mass accumulation, which is an indirect measure of cell number (Figure 2, Panels C and D). Bioluminescence and the assessed metabolic activity of the culture approaches zero during prolonged periods of stationary phase (Figure 2, Panels C and D). Other similar systems using ATP-driven bioluminescence, also conducted in our laboratory [70], measures ATP content in bacterial cells using a luciferase-luciferin substrate, and like the *lux* biosensor system also demonstrates a dramatic decrease in metabolic activity during stationary phase of growth.

We have verified the two transformants used in this study as *S. mutans* derivatives, using the criteria of growth on MSA with bacitracin inhibitors, PCR using specific *S. mutans* primers and immunoreactivity using antibodies directed at unique *S. mutans* cell wall components. In spite of differences in utilization of metabolic substrates and acidification profiles (Table 1), both *S. mutans* transformants used in this study, *lux* UA159 and *lux* ATCC 25175, have near equivalent sensitivities to minocycline hydrochloride, where 1 μg/ml minocycline hydrochloride was noted to be the minimum effective dose for immediate and sustained reduction of accumulated cell mass as measured by absorbance (Figure 2). In addition, the influence of the *lux* recombinant plasmid in transformed cells on minocycline sensitivity is negligible, since both nontransformed and transformed mutans cells exhibit the same minimum effective dose of 1 μg/ml minocycline hydrochloride for sustained reduction of accumulated cell mass (compare Panel A versus Panel B of Figure 2). This reduction of accumulated cell mass is reproducible in an independently conducted experiment (Figure 2, Panel C) using an intermediate range of minocycline hydrochloride (0-10 μg/ml), where reduction in bioluminescence is also observed at the same dose of minocycline hydrochloride (Figure 2, Panel D; 1 μg/ml and 10 μg/ml minocycline hydrochloride curves are merged as overlapping line graphs).

In additional experiments, we demonstrate that lidocaine/prilocaine has weak antimicrobial activity at the higher doses examined (200 μg/ml) in mutans transformants (Figure 3, Panel A). Minocycline hydrochloride at the optimal concentration of 1.0 μg/ml added to all doses of lidocaine/prilocaine resulted in the sustained reduction of the mutans transformant both in cell mass accumulation and bioluminescence (Figure 3, Panels B and C, respectively). Thus, in these instances, the addition of lidocaine/prilocaine does not have a contraindicative effect on the antimicrobial activity of minocycline hydrochloride. In some experiments, we used reduced amounts of minocycline hydrochloride (0.1 μg/ml), where the bacteriostatic activity of minocycline hydrochloride was purposefully weak, in order to

confirm that the addition of lidocaine/prilocaine (at 200 μg/ml) had augmented antimicrobial activity. Interestingly, the dose of lidocaine/prilocaine used in the *lux* biosensor system (200 μg/ml) is much lower than the dose applied in the periodontal pocket (each cartridge contains 1.7 g of gel which contains 42.5 mg of lidocaine and 42.5 mg of prilocaine), where lidocaine/prilocaine is mixed with crevicular fluid and saliva, and one may presume that the effective dose of lidocaine/prilocaine in the periodontal pocket would be less than the applied dose.

The limited antimicrobial activity of lidocaine/prilocaine at the 100 μg/ml dose in the ATCC 25175 transformant (Figure 4, Panel C) has been found to be reproducible in additional experiments (unpublished observations). The ATCC 25175 transformant appears to retain similar sensitivity profiles for lidocaine/prilocaine when administered with the suboptimal dose of minocycline hydrochloride (0.1 μg/ml), but may be less sensitive than the UA159 transformant at the 100 μg/ml dose (unpublished observations). Thus again, lidocaine/prilocaine does not appear to be contraindicative to the bacteriostatic activity of minocycline hydrochloride, and may in fact augment the antimicrobial effect of suboptimal doses of minocycline hydrochloride in the *lux* biosensor system (Figure 4, Panel C).

The mechanism of the antimicrobial activity of lidocaine/prilocaine is unknown. The anesthetic composite of lidocaine and prilocaine has been found to retain antimicrobial properties in skin creams used to treat human skin flora [71,72]. Both lidocaine and prilocaine are sodium channel blockers [73], and voltage-gated sodium ion channels have been found in bacteria [74]. One potential mechanism of action may constitute blockade of ion flux and resultant dysregulation of osmolarity, ultimately resulting in slow disruption of the cell. Interestingly, chlorhexidine, another antimicrobial agent used in the treatment of supragingival and subgingival plaque, is a chemical antiseptic with both bactericidal and bacteriostatic properties that is believed to act through membrane disruption [75]. Like the combination of minocycline hydrochloride and lidocaine/prilocaine, the combination of the bacteriostatic antibiotic minocycline hydrochloride and the antiseptic chlorhexidine display additive antimicrobial effects in the *lux* biosensor system (Figure 5), and that the majority of pair-wise comparisons are statistically distinct. Statistically-significant differences were found 1) between untreated cultures and cultures treated with minocycline hydrochloride alone (0.1 μg/ml) or with minocycline hydrochloride plus chlorhexidine (0.01%) ($p=0.012$ and $p<10^{-8}$, respectively including Bonferroni correction), 2) between cultures treated with minocycline hydrochloride (0.1 μg/ml) alone or with chlorhexidine (0.01%) ($p=10^{-6}$ including Bonferroni correction) or 3) between cultures treated with chlorhexidine (0.01%) alone or with minocycline hydrochloride (0.1 μg/ml) ($p=0.00018$ including Bonferroni correction). Our results are consistent with other studies that indicate chlorhexidine may enhance the penetration and intracellular activity of antibiotics in bacterial cells [76,77]. This may have important implications for the periodontist when considering use of minocycline hydrochloride antibiotic as an adjunct therapy for SRP, followed by use of chlorhexidine for generalized control of supragingival plaque. Using the *lux* biosensor system, there appears to be no contraindicative effect when using minocycline hydrochloride and chlorhexidine in combination.

CONCLUSION

We have found that the *lux* biosensor system is an excellent monitor of the metabolic activity of the culture, and is especially useful when assessing single antimicrobial agents, or with combinations of multiple agents. We conclude that the anesthetic lidocaine/prilocaine, or the antiseptic chlorhexidine, does not interfere with the potent bacteriostatic activity of minocycline hydrochloride, and in fact has an additive antibacterial effect.

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Chapter 34

THE ROLE OF THE T_H17 PATHWAY IN THE PROGRESSION OF PERIODONTAL DISEASE

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CONCEPTS OF PERIODONTAL DISEASE PATHOGENESIS

Periodontitis is a chronic inflammatory disease which destroys the tooth-supporting tissues[1]. This disease is initiated by bacteria; in particular, faculative anaerobic Gram-negative microorganisms[2]. Several types of these pathogens initiate periodontal disease, and the host response determines the disease progression and ultimate tissue damage[3-4]. The early periodontal lesion (gingivitis) is characterized by the presence of large numbers of T cells and macrophages within the gingiva, while the presence of beta (B) and plasma cells characterize the advanced lesion[5]. These phenomena suggest that a shift in the type of host response occurs during the progression of periodontal disease[6]. However, there is little specific information available concerning the characteristics of this shift.

It is uncertain whether periodontal disease is a continuous process, or consists of episodes of exacerbation and remission[7-8]. It is generally accepted that periodontal health is a dynamic state where the activity of pro-inflammatory/antimicrobial cytokines and chemokines is balanced by anti-inflammatory cytokines and chemokines. When this balance is disrupted, severe inflammation and tissue destruction occur. Two models of the pathogenesis of periodontal disease have been proposed[9]. In the "linear model" of periodontal disease, specific bacteria initiate the disease. The inflammation is either resolved or progresses based on the host response to these bacteria[10]. The "circular model" contends that bacteria are required for both the initiation and progression of the disease. These bacteria constantly reshape the T-helper (T_H) cell response, which determines the ultimate fate of the infection. During the progression of the inflammation, alveolar bone resorption and soft tissue damage offer new niches for colonization of bacteria. These niches facilitate bacterial overgrowth, which reinforces this circular process and produces an ongoing vicious cycle.

In both models of pathogenesis of periodontal disease, innate and adaptive immune responses are featured. The initial response to bacterial infection is a local inflammatory reaction that activates the innate immune system[11-12]. In this response, neutrophils, macrophages and monocytes become activated by periodontal pathogens such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, and then produce a pattern of pro-inflammatory cytokines and chemokines[11-13]. Under normal circumstances, these innate immune cells are capable of preventing significant tissue invasion of the microbes. However, if these cells are unable to clear the infiltration of bacteria, the acquired immune responses become active. Cytokines produced by cells of the innate immune system are the first line of defense against pathogens. Their pattern recognition receptors, which are not specific for any particular epitope, allow them to respond to a wide variety of microbial invaders by producing cytokines that activate T and B cells of the adaptive immune system[14-16,17]. The failure to encapsulate this "inflammatory front" within gingival tissues results in the expansion of the inflammation adjacent to alveolar bone[12]. During the infectious process, inappropriate immune and inflammatory responses can reduce tissue damage or even undesired systemic reactions and outcomes[18-19].

PERIODONTOPATHOGENIC BACTERIA AND THE INNATE IMMUNE RESPONSE

A critical aspect of the host response is the detection of bacteria by Toll-like receptors (TLRs) on cells of the innate immune system. Activation of the innate immune response by the binding of various bacterial components to TLRs results in the production of cytokines and chemokines[20]. This process involves activation of the TLRs, which induces an intracellular signaling cascade that results in the activation of transcriptional factors and the production of various cytokines and chemokines[20].

The activation of TLR's depends on the type of microorganism and its specific component. For instance, *Porphyromonas gingivalis* fibriae promote monocyte or macrophage derived cytokine expression, such as IL-1-β, IL-8, or TNF-α[21 22-23], which are mediated through TLR2[24]. *P. gingivalis* type II fimA fimbriae are the predominant fimbrial phenotype associated with periodontal disease[25]. *P. gingivalis* lipopolysaccharide promotes the expression of a T_H2 pattern of proinflammatory cytokines and chemokines in monocytes/macrophages (which express IL-5, IL-10, and IL-13)[26,27] due to activation of a CD14/TLR-4 and/or a TRL-2 dependent pathway[28-30]. However, antigens to *P. gingivalis* produce IL-17[31].

THE T_H1/T_H2 PARADIGM

For almost two decades, the T_H1-T_H2 paradigm has prevailed in immunology[32]. Periodontopathogenic bacteria induce naïve CD4+ cells to differentiate into several lineages of T-helper (T_H) subsets with unique cytokine profiles and functions[5, 33]. When a naïve CD4+ T cell is exposed to an antigen in the presence of IL-12, it is driven to develop into a T_H1 phenotype[34]. The T_H1 cell uniquely expresses the IL-12 receptor subunit IL-12Rβ2, which

further commits the cell to proceed along this differentiation pathway. The process is dependent on the transcription factor STAT-4, which is activated by IL-12[35].

T_H1 cells activate macrophages, cytotoxic T cells and natural killer (NK) cells as well as driving anti-viral signals in target cells. T_H1 cells produce IFN-γ and lymphotoxin-α and are responsible for protection against intracellular pathogens such as viruses, mycobacteria and protozoa[36]. IFN-γ provides a positive feedback signal to reinforce T_H1 development by upregulating the IL-12 receptor. The key regulator of T_H1 lineage commitment is T-bet[37]. T-bet is upregulated in developing T_H1 cells[38], but not in T_H2 cells[39]. Thus, signals from T_H1 cytokines inhibit T_H2 and T_H17 differentiation.

Conversely, when a newly activated T_H cell is exposed to IL-4, differentiation to a T_H2 phenotype occurs[40]. This process is dependent on transcription factors STAT-6, GATA-3, and c-maf[41-43] . T_H2 cells produce IL-4, IL-5, IL-10, and IL-13[40]. IL-4 is the signature cytokine of the T_H2 population and promotes differentiation and expansion of this population. T_H2 cytokines are potent activators of B-cell IgE production, and eosinophil recruitment [40]. Thus, T_H2 cells mediate humoral responses and autoimmunity.

T cell subsets have distinct characteristics[44], which regulate their function in the immune response. Dysregulated T_H1 responses can promote tissue destruction and chronic inflammation, whereas dysregulated T_H2 responses can cause allergy and asthma [36, 45].

T-HELPER CELLS AND PERIODONTAL DISEASE

Lesions of advanced periodontitis are characterized by the presence of both T_H1 and T_H2 cytokines[46-48]. Several studies have reported that the expression of T_H1-type cytokines predominates over that of T_H2 cytokines within diseased periodontal tissue, indirectly suggesting T_H1 involvement in the inflammation[49-50]. There is likely an initial predominance of T_H1 cytokines followed by their decline and a rise in T_H2 cytokines at later stages of infection[11]. In this way, T_H2 cells have been associated with non-protective antibody responses and progressive periodontal lesions and T_H1 with stable lesions[51]. T_H1 cells are protective to the host through IL-12/IFN-γ-stimulated cell-mediated immunity[52-53] and by inhibition of osteoclastogenesis[54-55].

T cells play an important role in maintaining a balance between the host and the biofilm on the tooth and gingival surfaces[5, 56]. TLR4 agonists (*E.coli*) promote production of IL-12 and a T_H1 response in contrast to TLR2 agonists (bacterial lipopolysaccharides) which foster T_H2 responses[57].

Another method for control of T_H responses is by Treg cells, which are CD4+/CD25+ cells that antagonize T_H1 and T_H2 immune responses by secreting suppressive cytokines. Treg cells are formed in the presence of TGF-β and secrete IL-10 and additional TGF-β[58-59]. These cells are present in high amounts within tissues with periodontal disease[60-61].

T_H1 cells express the chemokines, CCR5 and CXCR3[62-66] , while CCR3 is expressed by T_H2 cells[65-68]. Several recent studies suggest that a T-helper cell response in addition to the T_H-1 or T_H-2 immune response contributes to the pathogenesis of periodontal disease.

THE T_H17 IMMUNOLOGICAL RESPONSE: A NEW PARADIGM FOR PERIODONTAL INFLAMMATION

There is evidence that the initiation and progression of periodontal disease cannot be explained, in its entirety, by the T_H1/T_H2 paradigm. Recent studies describe a new pathway, which links gingival inflammation and bone resorption[69-70]. This pathway is the T_H17 immune response[45, 71]. T_H17 cells are an important early response to catastrophic injuries that require immediate neutrophil recruitment[72]. IL-17 plays a crucial role in innate immunity because its secretion triggers production of numerous chemokines, resulting in neutrophil and macrophage recruitment[73-77] and subsequent pathogen clearance[78-79].

Proinflammatory cytokines IL-17 and IL-17F have been reported to be expressed by T_H17 cells[80-82]. IL-17A and IL-17F induce the production of various proinflammatory cytokines such as TNF-α, IL-1-β, and IL-6, and CXC chemokines[82-83]. T_H17 cells also produce IL-21, IL-22, and IL-26[84]. T_H17 cells rapidly initiate an inflammatory response dominated by neutrophils, and when unregulated, have been reported to maintain chronic inflammation. These responses result in recruitment, activation, and migration of neutrophils to the sites of inflammation and infection[82]. In addition to cytokines and chemokines, T_H17 cells also induce secretion of matrix metalloproteinases[73, 76, 85], which destroy extracellular matrix. In addition to the proinflammatory effects, T_H17 cells stimulate osteoblasts to express RANKL, activating osteoclasts, resulting in loss of bone. In this way, T_H17 cells link gingival inflammation to alveolar bone resorption.

It has been long recognized that there are discrepancies with the T_H1-T_H2 model of periodontal inflammation[86]. The recent discovery of a new "T_H17" subset has resolved many of these controversies, but has also raised many new questions and research directions[87-89]. The protective role for IL-17 is consistent with the protective role played by T_H17 cells in infectious inflammatory diseases, compared to events in sterile inflammatory situations where IL-17 is tissue destructive[90]. T_H17 cells protect against extracellular bacteria and fungi, which are not dealt with by T_H1 mediated immunity[82].

The development of T_H17 cells is different from that of T_H1 and T_H2 cells. IFN-γ, IL-12, and IL-4, which are important for T_H1 and T_H2 differentiation, have been shown to be dispensable for T_H17 cell differentiation *in vitro* and *in vivo,* providing the first clue that T_H17 cells are an independent lineage from T_H1 or T_H2 cells[45, 73]. IL-6 and TGF-β initiate T_H17 differentiation[39, 91-92] and TNF-α and IL-1-β amplify T_H17 cell differentiation. IL-21 is not only expressed by T_H17 cells, but also controls the generation of T_H17 cells[93-95]. In addition, IL-21 can substitute for IL-6 in generation of T_H17 cells[93]. IL-23 synergizes with IL-6 to also induce T_H17 differentiation[96]. While IL-23 is not required for initial differentiation of T_H17 cells, it may play a role in the survival and expansion of the T_H17 cell population has been reported to promote T_H17 development/expansion in the presence of IL-6 and TGF-β[39, 97], which may be especially relevant to periodontal disease as IL-1-β concentrations are elevated within gingiva at sites of chronic periodontitis and within bone at sites of chronic periapical lesions[49, 69, 98-100]. For example, one study reports that IL-17 was 6.2 fold higher within gingiva obtained from sites of periodontitis patients than from healthy sites[101].

In addition, IL-18 synergizes with IL-23 in the induction of IL-17-producing CD4+ T cells [37]. IL-6 and IL-21 signal through STAT-3[102-103], which is essential for T_H17

differentiation. IL-21 also acts as a feedback factor to further reinforce T_H17 development. IL-27 inhibits T_H17 development[104] and IL-2/STAT-5 also limit this process [53, 73, 102].

IL-27 inhibits IL-17 production, suggesting that IL-27 may have an important role in switching the early pro-inflammatory effects of IL-17 toward a T_H1 response[105]. Both T_H1 and T_H2 subsets negatively regulate T_H17 differentiation[45, 73].

Following the discovery of the T_H17 subset, the role of T_H1 destructive inflammation has been questioned [90, 106]. T_H17 cytokines have been found in diseased gingiva[49, 69-70, 98]. In addition, IL-17 and IL-23 concentrations have been reported to be higher within gingiva adjacent to sites of clinical attachment loss[100,69, 107]. Thus, the inability to sustain a T_H1 response may lead to disease progression. PGE_2 inhibits IL-12p35, but enhances IL-23 expression and may contribute to T_H17 development[108]. IL-17 regulates COX-2 and PGE_2 production[109-110]. PGE_2 is strongly associated with periodontal tissue destruction[111-112].

IL-23 mediates autoimmunity, and may be a link between periodontal inflammation and autoimmune diseases[90, 113-114]. IL-23 is not the direct initiator of T_H17 production, but is an important factor for expanding and maintaining T_H17 cells[115,116].

Since molecules involved in the induction of T_H17 cells have been identified, they could be targets for prevention, or treatment, of chronic periodontal inflammation.

THE T_H17 PATHWAY AND ALVEOLAR BONE RESORPTION COINCIDENT TO PERIODONTAL DISEASE

Although a functional immune system is vital to protect against infectious diseases, dysfunctional immune responses may have deleterious effects on the host, which, in periodontal disease, is manifested in alveolar bone destruction and numerous systemic effects. Several studies have suggested that alveolar bone resorption coincident to periodontal disease was caused by the immune response, rather than directly by the infectious organism[4, 117]. Whether alveolar bone resorption will occur in response to gingival inflammation depends on two critical factors[12]. First, the concentration of proinflammatory mediators within the gingival tissue must be sufficient to activate pathways leading to bone resorption. Second, the inflammatory mediators must penetrate the gingival tissue to reach a critical distance from alveolar bone. There is increasing evidence that T_H17-type cytokines participate in the pathogenesis of periodontal disease, but whether their role is host-protective or host-destructive is uncertain, but is likely both roles[118-120].

During the pathogenesis of periodontitis, IL-17 appears within gingival tissues during the early stages of the inflammatory process, and begins to disappear from the gingiva at later stages of the inflammation[69, 49, 69, 98]. As these data were obtained from gingiva in a cross-sectional study, it was not certain whether this “removal” of IL-17 was a sign of impending resolution of the inflammation. A recent study of gingiva obtained from sites of severe clinical attachment loss indicated higher concentrations of IL-17, IL-23, IL-1-β, IL-6 and TNF-α within those tissues[70]. Thus, it seems that IL-17 could potentially be a factor for progression of periodontal disease from gingivitis to periodontitis when other related cytokine concentrations are also elevated.

Most of our knowledge about the relationship between IL-17 and bone resorption comes from studies of rheumatoid arthritis, a disease very similar to periodontal disease. IL-17, in

conjunction with TNF-α, seems to be a primary factor in bone resorption at sites of inflammation at sites of rheumatoid arthritis[119-120]. IL-17 modulates the RANKL/OPG ratio: it increases RANKL (receptor activator of nuclear factor-kappa B ligand) expression and decreases osteoprotegerin (OPG) expression, resulting in enhanced formation of osteoclasts and bone resorption[121-122]. T_H17cells have been reported to also express higher levels of RANKL than T_H1 cells[106]. Similarly, IL-17 can induce osteoclast differentiation at other sites of inflammation, such as periodontal disease[12, 123].

In periodontal disease, there is an uncoupling of alveolar bone resorption from alveolar bone deposition, so that a net loss of alveolar bone occurs[124-125]. There is some evidence for IL-17 involvement in this destructive phase of periodontal disease[31,106].

During an inflammatory response involving bone, IL-17, IL-1-β, IL-6, and TNF-α, chemokines, and other mediators stimulate periosteal osteoblasts, enhancing their expression of RANKL on the osteoblast surface[122,125-127]. Bone resorption and deposition are regulated by the relative concentrations of RANKL, RANK (the RANKL receptor) and OPG on the surface of periosteal osteoblasts[126]. When RANKL expression is enhanced relative to OPG, RANKL is available to bind RANK on osteoclast precursors, enhancing the activation of osteoclast formation and bone resorption[126]. When OPG concentrations are high relative to RANKL expression, OPG binds RANKL, inhibiting it from binding to RANK[126]. Prevention of the binding of RANKL to RANK leads to reduced formation of osteoclasts and apoptosis of preexisting osteoclasts[126]. Relative decreases in OPG concentrations or increase in RANKL expression affect the RANKL/OPG ratio, which is indicative of the potential for bone resorption. The RANKL/OPG ratio is higher in individuals with periodontitis than in healthy persons[127-132]. An increased RANKL/OPG ratio may also be associated with the clinical severity of PD[131]. Thus, control of this ratio is likely to be crucial to control of alveolar bone loss coincident to periodontal disease and is likely regulated by the T_H17 immune pathway.

INDUCTION AND REGULATION OF THE T_H17 IMMUNE PATHWAY

A combination of TGF-β plus IL-6 has been reported to induce the differentiation of naïve T cells into T_H17 cells[39, 91-92]. T_H17 cells express a unique transcription factor, ROR-γt[133] which induces transcription of the IL-17 gene in naïve T-helper cells and is required for the development of IL-17 producing cells in the presence of IL-6 and TGF-β [134]. ROR-γt must act in cooperation with other transcription factors, including ROR-α, STAT3, IRF-4, and runt-related transcription factor (Runx1), for full commitment of precursors to the T_H17 lineage [96, 135-137]. Activation of ROR-γt also causes expression of the receptor for IL-23, indicating that IL-23 acts on T cells that are already committed to the T_H17 lineage. Exposure of developing T_H17 cells to IL-23 not only enhances their expression of IL-17 but also induces IL-22 and suppresses IL-10 and IFN-γ, which are not associated with the T_H17 phenotype[115]. Thus, IL-23 is essential for stabilizing the T_H17 phenotype.

IL-17 is neither a growth factor nor a differentiation factor for T_H17 cells and cannot amplify the T_H17 responses. However, IL-21, together with TGF-β, can amplify the T_H17 differentiation [93-95] and the expansion of the T_H17 cell population is defective in the absence of IL-21. TGF-β plus IL-21[138], TGF-β plus a combination of IL-6 and IL-23, or IL-6 plus IL-21[139] can induce the expression of ROR-c, the human counterpart of ROR-γt expressed in mice. Co-expression of the chemokine receptors CCR4 and CCR6[140] or expression of CCR2

in the absence of CCR5[141] also appears to define the T_H17 cells in humans. Inflamed tissues have large cells with a resemblance to plasma cells which produce IL17, suggesting that T_H17 cells acquire an activated phenotype at the tissue site[142].

The Treg cell population is driven to develop in opposition to T_H17, which is driven by TGF-β in the absence of STAT3. IL-2 is an important factor in expanding this lineage while simultaneously inhibiting T_H17 cell development[102]. There is a close relationship between Treg and T_H-17 cells, since TGF-β, which is required for the generation of Treg cells, is also necessary for the differentiation of T_H-17 cells[92] and the transcription factors required for the development of these two subsets might antagonize each other.

THE ROLE OF T_H17 CELLS IN OTHER DISEASES

Much of what we know about IL-17 in periodontal disease has been extrapolated from studies of other inflammatory diseases. Elevated IL-17 concentrations have been reported in individuals with a variety of autoimmune disease[143-144] and inflammatory bowel disease[145]. T_H17 cells can secrete IL-17 and IL-6 and TNF-α[146], and the presence of these cytokines has strong potential for initiating and intensifying systemic diseases. T_H17 cells have been shown to play important roles in many diseases ranging from inflammation and autoimmunity to infectious diseases and cancer[49, 98, 147-149]. IL-17 likely contributes to inflammatory pathologies such as atherosclerosis[150-151] and diabetes[152-154], which previously were attributed only to excessive levels of TNF-α and IL-6. For example, TNF-α and IL-6 are detectible in the sputum of COPD patients and are increased during exacerbations[155]. T_H17 cells attract numerous cells into inflamed tissues. These cells, acting in synergy with TNF-α and IL1-β, provides a potent inducer of CCL20, which is strongly chemotactic for lymphocytes. In this way, T_H17 cells are involved in psoriasis[156], multiple sclerosis[147], inflammatory bowel syndrome[145], and corticosteroid-resistant asthma[156-157].

Certain types of cancers use inflammatory mediators to induce angiogenesis and tissue remodeling[158]. Thus, IL-17 can promote tumor growth through the enhancement of angiogenesis-mediating factor production[159]. However, it has also been shown that IL-17 production can inhibit tumor cell growth due to the recruitment of CD8+ T lymphocytes with cytotoxic activity against the tumor[160].

Several studies have demonstrated a key role of IL-17 or IL-23 in the progression of arthritis[161-165]. Blockade of IL-17 after disease onset was able to prevent cartilage and bone destruction, leading to amelioration of the clinical symptoms of the disease [163]. Human IL-17 cells within the arthritic synovium expressed RANKL[106], which induces osteoclastogenesis[166]. Synovial fluids from human samples contained high levels of both CCL20 and IL-17, suggesting that both are required for disease progression[167-170]. IL-17 induced synovial fibroblasts to produce IL-6, IL-8, MMP-1, and MMP-3, contributing to the destruction of the joint[168]. In rheumatoid arthritis patients, the production of TNF, IL-1 and IL-17 by synovial cells is predictive of joint destruction[171].

Many reports have identified the presence of T_H-17 cytokines (IL-1-α, IL-1-β, IL-6, IL-17, IL-17F, and TNF-α) in psoriatic lesions[172-174]. A clinical trial using anti-IL-12/IL-23 p40 neutralizing antibodies has been conducted. Patients receiving the neutralizing antibodies had a significant improvement in psoriatic areas and disease index, demonstrating a crucial role for T_H-17 in the pathogenesis of that disease[175].

FUTURE QUESTIONS ABOUT THE T_H17 PATHWAY

Efforts are underway to test drugs that target the T_H17 pathway in humans[176-177]. Because periodontal disease cannot be characterized solely as a T_H1 or T_H2 response, discovery of the T_H17 cell subset may provide insight into the basis for this disease. Specific deletion of the T_H17 subset may be a more effective therapy than blocking IL-12, IL-17 or IL-23 alone. Specific deletion of the T_H17 cells would keep the IL-12/T_H1 immune pathway intact, which is an effective pathway for targeting many intracellular microbial infections and is important for the IFN-γ production that is required to resolve these infections.

Understanding the intricate interplay between cytokines and bone may allow for therapeutic intervention in diseases caused by an imbalance in bone remodeling. It seems that IL-17 blockade could be a treatment for periodontal disease; however, studies in rodents suggest that alveolar bone loss is exacerbated in animals infected with *P. gingivalis,* that were deficient in IL-17 receptors[178]. The amplification and propogation of the inflammatory response through gingival tissue is critical to the pathogenesis of periodontal disease. However, it is the spread of the response to the adjacent alveolar bone that drives the cellular machinery involved in bone loss. The RANKL-RANK-OPG axis is clearly involved in the regulation of bone metabolism in periodontitis and an increase in the relative expression of RANKL or a decrease in OPG can tip the balance in favor of alveolar bone loss. Interference with this axis may have a protective effect on alveolar bone loss. Thus, future therapeutic options are likely to have regulation of the RANK-RANKL-OPG axis as their goal.

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Chapter 35

NATURAL AVOCADO SUGARS INDUCE SECRETION OF ß-DEFENSIN-2 BY EPITHELIAL CELLS: EFFECTS ON *PORPHYROMONAS GINGIVALIS*

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ABSTRACT

Oral epithelia represent the first physical and chemical barrier against bacterial invasion and colonization of the underlying tissues. This protection results from the production of epithelial innate immune responses, including the secretion of cationic antimicrobial peptides with a large spectrum of activity against pathogenic microorganisms. Among these antimicrobial cationic peptides, ß-defensin 2 (hBD-2) is expressed in the gingival epithelia upon stimulation by microorganisms or inflammatory mediators such as interleukin-1β or tumour necrosis factor-α. The aim of the present study was to investigate the effect of AV119, a patented blend of two sugars from avocado, on the induction of hBD-2 in two epithelial cell lines and a primo-culture of gingival epithelial cells. Culture supernatant from epithelial cells treated with AV119 was also evaluated for its antimicrobial activity against the periodontopathogen

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Porphyromonas gingivalis. Cell ELISA assays revealed that AV119 induces the production of hBD-2 by all the epithelial cells tested. Minimal Inhibition Concentration assay also showed that the culture supernatant of epithelial cells treated with AV119 possesses antibacterial activity. In conclusion, our data revealed that AV119 component, through hBD2 induction and antibacterial activity, could be considered for potential use in the control of oral mucosal infections and reduction of microbial tissue invasion during periodontitis.

Keywords: ß-defensin, natural avocado sugars, epithelial cells, *Porphyromonas gingivalis*

INTRODUCTION

Periodontitis, the result of bacterial infection of the gingival sulcus, is the most common infectious disease in humans [1]. This infectious disorder is caused by a subset of periodontal Gram-negative anaerobic bacteria where the major pathogen is *Porphyromonas gingivalis* [2]. These microorganisms lead to the destruction of periodontal tissues, including both connective tissue and the alveolar bone surrounding the teeth. Oral epithelial cells, which are directly invaded by periodontal bacteria, act as mechanical and immunological barriers by producing cytokines and metalloproteinases that regulate the host defense against periodontal microorganisms. Antimicrobial proteins and peptides constitute a diverse class of host-defense molecules that act early in protection against invasion and infection by microorganisms [3]. Two main antimicrobial peptide families are defensins and cathelicidins [4]. Defensins are small cationic peptides with a vast spectrum of antimicrobial activity [1, 5-7]. Actually, there are 6 different α-defensins, including 4 peptides (HNP-1 to HNP-4) in neutrophils and 2 peptides (HD5 and HD6), and six β-defensins (hBDs) (hBD-1–6) expressed in numerous epithelial cell surfaces [8, 9]. Only the first three hBDs (hBD-1–3) have been characterized in some details [10]. hBD-1 is constitutively expressed by various tissues and may be modulated by inflammation [11]. hBD-2 and hBD-3 are expressed by the epithelial cells upon stimulation with proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and by microorganisms [6]. These peptides are regarded as primary effector molecules in defense against invading microorganisms [12]. Human hBD-1, hBD-2 and hBD-3 are expressed by epithelia of the gingival tissues [13] and hBDs may play a role in the control of the many commensal and putative periodontopathogenic bacteria.

hBD-2, a cysteine-rich, cationic, low-molecular-weight antimicrobial peptide, was first discovered in psoriatic lesions and was thought to be involved in cutaneous defense and inflammation. hBD-2 exhibits strong antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [14, 15]. Yin and Dale [16] have shown that both commensal bacteria and hBD2 activate protective responses of oral epithelial cells and play an important role in immune modulation in the oral cavity.Until now, bacteria did not show any resistance to antimicrobial peptides. Therefore, antimicrobial peptides become valuable antibiotic compounds and natural products that promote the increase of the secretion of antimicrobial peptides are of great interest.

The aim of the present study was to investigate the effect of natural sugars AV119, extracted from the avocado fruit (*Persea gratissima*), on the induction of production of hBD2

in and on antibacterial activity of their culture supernatant against the major periodontal pathogen *Porphyromonas gingivalis*.

MATERIAL AND METHODS

AV119 Extraction Process

AV119 is a patented extract from the avocado fruit (*Persea gratissima*) (Patent N° 0607651 FRANCE 08/31/2006 and EP2007/059136 08/31/2007) (Laboratoires EXPANSCIENCE). AV119 was prepared from sliced and dried avocado fruit. After elimination of fats, the dry matter was ground and extracted in a water-alcoholic solution. The insoluble material was eliminated by filtration and the final product was obtained by concentrating the solution to obtain 5% of active substance. The final solution was mainly composed of two rare sugars, D-mannoheptulose and perseitol, which together represent 80% of the dry matter.

Bacterial Strain and Growth Conditions

P. gingivalis ATCC 33277 was maintained on Colombia agar plates and grown in Brain Heart Infusion (BHI) broth (AES CHEMUNEX, France) supplemented with hemin (5 µg/ml) (Sigma, France), vitamin K1 (1 µg/ml) (Sigma, France) and yeast extract (0.5%) (AES CHEMUNEX, France). The bacterial cultures were incubated overnight at 37°C in an anaerobic chamber (80% N_2, 10% H_2, 10% CO_2).

Epithelial Cells

Two cell lines, KB cells (ATCC CCL-17), and Ca9-22 cells (TKG 0485, Japanese Cancer Research Resources Bank) and primary human gingival epithelial (HGE) cells were used in this study. The cell lines and HGE cells were grown in RPMI 1640 medium with Glutamax™ (Invitrogen, France), supplemented with 10% fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. Cells were maintained in a humidified incubator at 37°C under 5% CO_2 atmosphere.

MTT Assay for Cell Viability

Cell viability was estimated by the MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells [17]. Epithelial cells were seeded in a 96 well plate at 1×10^5 cells/ml. Twenty-four hours after plating, cells were treated with two concentrations of AV119 (0.01% and 0.03%) and incubated for additional 24 h at 37°C. Twenty microlitres of sterile filtered (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT, Sigma, France) stock solution in phosphate buffered saline (PBS) pH 7.4 (5 mg/ml) were added to each well reaching a final concentration of 0.5 mg MTT/ml. After 4 hs, culture supernatants were removed by aspiration, the insoluble formazan crystals were dissolved in 200 µl/well dimethylsulfoxide (Sigma, France) and the optical density (OD) measured spectrophotometrically using an ELISA microplate reader at a

wavelength of 570 nm. The OD_{570nm} of the formazan formed in the control cells was taken as 100% viability. Assays were performed in triplicate and repeated three times.

Cell ELISA Assay for hBD-2 Production

hBD2 production was measured following a modification of the protocol described by Arunachalam *et al.* [18]. Briefly, epithelial cells were seeded into 96-well culture plates at a density of 3×10^4 cells/well. After 24 h incubation, cell monolayers were treated with AV119 (0.01%, 0.03%) or 200 ng/ml TNFα (T0157 Sigma, France) and incubated for additional 48 h at 37°C under 5% CO_2 atmosphere. Untreated cells were used as control for the basal production of hBD2. The cell monolayers were then washed twice with PBS and fixed with 4% formaldehyde (Sigma, France) for 15 min. Primary Goat polyclonal antibody against hBD-2 (0.5 µg/ml) (Ab9871, Abcam, France) was applied for 1 h at room temperature. After three washes, the plates were treated with rabbit horseradish peroxidase-conjugated secondary antibody (Abcam, France, 1:1000 dilution) for 1 h at room temperature. After washing, the tetramethylbenzidine (TMB™)-substrate (Sigma, France) was added for 15 min and the OD was measured at 450 nm using an ELISA microplate reader. Results were expressed as OD_{450nm} percentage of the control cells. Assays were performed in triplicate and repeated five times.

Effect of AV119 on P. Gingivalis Viability and Growth

To determine the effect of AV119 on *P. gingivalis* viability and growth, serial dilutions of AV119 (ranging from 0.03 µg/ml to 20 µg/ml), hBD-2 peptide (ranging from 2.5 µg/ml to 25 µg/ml) and metronidazole (ranging from 0.03 µg/ml to 2 µg /ml) (Sigma, France) were prepared in BHI culture medium. *P. gingivalis* suspensions 10^5 to 10^6 CFU/ml were incubated with or without various concentrations of AV119, hBD2 or metronidazole for 5 days at 37°C under anaerobic conditions. Antibacterial activities were assessed by $OD_{660\ nm}$ measurement. Bacterial susceptibility to the various compounds was expressed as minimal inhibitory concentration (MIC) values. Each assay was performed three times in duplicate.

Antibacterial Activity of Epithelial Cell Culture Supernatants

The antibacterial activity of epithelial cell culture supernatants, harvested from cells treated with AV119, was determined against *P. gingivalis*. Briefly, epithelial cells were seeded into 96-well plates for 24 h at 37°C in a 5% CO_2 atmosphere to allow cells to adhere. The culture medium was replaced with antibiotic-free RPMI medium supplemented with 0.01% or 0.03 % of AV119 or TNFα (20 ng/ml). After 48 h, the cell supernatants of two wells (400 µl) were harvested and tested for antimicrobial activity against equal volume of *P. gingivalis* suspension (~ 5 x 10^3 CFU/ml). After 5 h of incubation in an anaerobic atmosphere, bacterial growth was determined by plate counting method. A negative control consisted of bacteria treated with culture supernatant from untreated epithelial cells (RPMI 1640 medium without AV119).

Statistical Analysis

All the experiments were performed at least three times for each assay conducted in duplicate or triplicate. Results are expressed as means ± SDs. Statistical analyses of all data between the treated and untreated cells were performed by the Student t test. A p value <0.05 was considered statistically significant.

RESULTS

Effect of AV119 on Cell Viability

To assess the effect of AV119 on epithelial cell viability, dose-response curves were generated using data from the MTT assay. Treatment of cells with AV119 for 24 h did not affect the cell viability of HGE cells (Fig. 1). However, treatment of KB and Ca9-22 cells with 0.03% of AV119 resulted in slight but negligible decrease (7%, p <0.001) of cell viability compared to untreated cells (Fig. 1). For the following experiments, both 0,01 and 0,03 % of AV119 were tested.

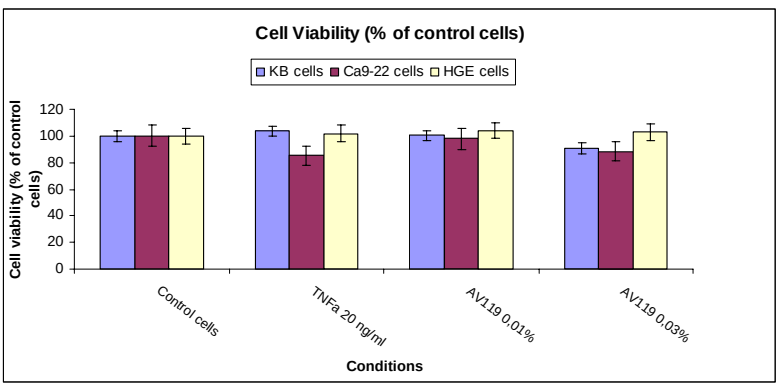
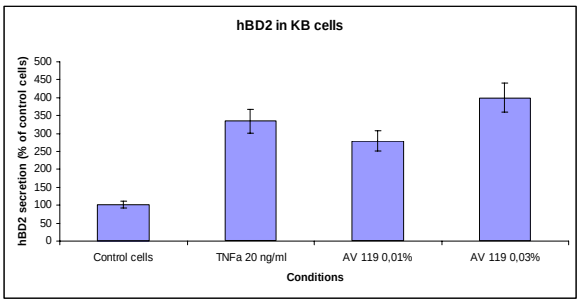


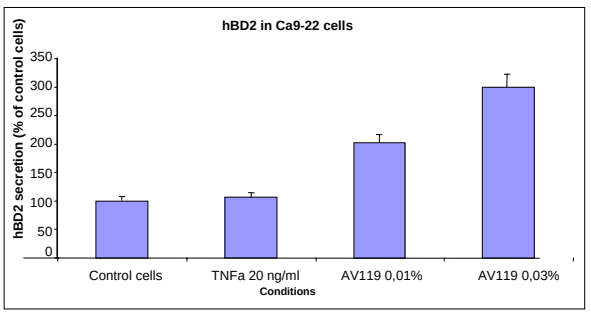
Figure 1. Cell viability of KB, Ca9-22 and human gingival epithelial cells (HGE) treated for 24 hours with 2 concentrations of AV119. The cell viability was determined by an MTT assay as described in Materials and Methods. The absorbance was measured with an enzyme-linked immunosorbent assay reader at a wavelength of 570 nm. Each assay was performed in triplicate, and the data are presented as percent cell viability in terms of control (untreated cells). Data shown are the mean ± SD of three independent experiments, and the values marked with asterisks (*) are significantly different from the control (p <0.05).

Natural Avocado Sugars Induce hBD-2 Production in Epithelial Cells

The production of the antimicrobial peptide hBD-2 by KB, Ca9-22 and HGE cells was determined by a cellular ELISA assay after treatment of cells with AV119 (0.01%, 0.03%) or TNF-α (20 ng/ml). ELISA results revealed that AV119 and TNF-α (positive control for hBD2 induction), dose dependently increased the production of hBD-2 in the three epithelial cell types. In particular, 0.03% AV119 increased significantly hBD-2 production in KB cells

(+4 X, $p < 0.05$) as well as in Ca9-22 and HGE cells (+3 X, $p < 0.05$) compared to untreated cells (Fig. 2A, B and C).





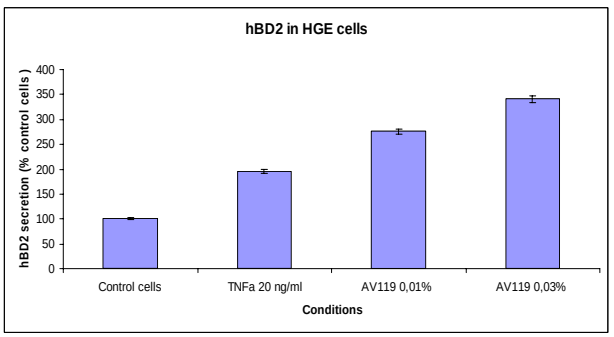


Figure 2. hBD-2 secretion by epithelial cells. The production of hBD-2 was assessed by cell ELISA assay. KB cells (A), Ca9-22 (B) and HGE (C) were treated with TNF-α 920 ng/mL) or AV119 (0,01% or 0,03%). Induced hBD-2 production was compared to hBD-2 in untreated (control) cells. The data are presented OD_{450nm} percent in terms of control untreated cells. The percentages indicate hBD-2 production as measured in three independent experiments. Data shown are the mean ± SD of three independent experiments. The significant differences in hBD-2 production were determined by the Student's t-test. (*$p < 0.05$).

Effect of Natural Avocado Sugars on P. Gingivalis Growth

The antibacterial activities of various concentrations of AV119, hBD-2 and metronidazole against *P. gingivalis* were examined by MIC determination. AV119 showed no significant effects on *P. gingivalis* viability or growth at the concentrations tested (0.01% and

0.03%), indicating that AV119 has no inherent antibacterial activity. In contrast, hBD-2 and metronidazole MIC values for *P. gingivalis* were respectively ≤ 2.5 µg/ml and 0.122 µg/ml.

Antibacterial Activity of Epithelial Cell Culture Supernatants

The antimicrobial effect of the supernatants of AV119-treated KB and Ca9-22 cells was measured by plate counting. Plate counts from the supernatants of KB cells treated with AV119 and TNFα revealed higher antimicrobial effect on *P. gingivalis* ($2 \times 10^2 \pm 0.02$ and $1.9 \times 10^2 \pm 0.10$ respectively) compared to untreated KB control cells. A lower antimicrobial activity was observed with supernatants of Ca9-22 cells treated with AV119 and TNFα reduction ($3.00 \times 10^2 \pm 0.20$ and $4.10 \times 10^2 \pm 0.1$ respectively) (Table 1). The positive control with the antibiotic metronidazole showed the most efficient antimicrobial activity.

Table 1. Antimicrobial activity against P. gingivalis in the supernatant of KB cells treated with AV119

Cellular supernatant	Number of culturable bacteria (UFC ml^{-1})
Untreated KB cells	$5.8 \times 10^2 \pm 0.05$
KB cells treated with TNF (20 ng/ml)	$1.9 \times 10^2 \pm 0.10$
KB cells treated with AV119 0.03%	$2.00 \times 10^2 \pm 0.02$
Untreated Ca-22 cells	$4.47 \times 10^2 \pm 0.37$
Ca9-22 cells treated with TNF (20 ng/ml)	$4.10 \times 10^2 \pm 0.1$
Ca9-22 cells treated with AV119 0.03%	$3.00 \times 10^2 \pm 0.20$
Media	Number of culturable bacteria (UFC ml^{-1})
BHI + Metronidazole (0.12 µg/ml)	3 x 10

DISCUSSION

Detection and elimination of periodontal pathogens by gingival epithelial cells is a crucial step in the maintenance of the homeostasis of oral health. Constitutive or induced expression of antimicrobial peptides provides a first line of defense against colonization by pathogens [19]. These cationic peptides are important in protection against invading microorganisms by modulating the innate immune response [10]. Among the antimicrobial peptides, numerous studies have focused on the expression of ß-defensins in gingival epithelial cells [20, 21] suggesting a role of these peptides in the oral mucosal immunity. hBD-2 has been shown to be expressed in all tissue samples and to play a role in the initial mucosal defense system. In

most epithelia, it has been reported that hBD2 is present only in inflamed tissue [22]. In the oral cavity, clinically healthy gingival tissue is permanently stressed by microorganisms. In order to maintain a balanced equilibrium between epithelial tissues and the bacterial invasion, cells increase their basal production of antimicrobial peptides including hBD2. Hence, hBD2 is potent therapeutic agent in oral diseases. Its antimicrobial activity could be useful in the prevention and treatment of periodontal diseases.

Identification of natural products, endowed with active properties that can increase the secretion of hBD2 is of high importance in oral health care. In the present study, we investigated the effect of two concentrations of natural sugars derived from avocado fruit (AV119) on the secretion of the antimicrobial peptide hBD-2 by 2 epithelial cell lines and by primary culture of HGE cells. We showed that AV119 increases the secretion of hBD2 by the all tested epithelial cells. The effect of AV119 on the signaling pathways involved in hBD2 secretion is still uncharacterized. However, the signaling pathways involved in hBD-2 induction in response to commensal and pathogenic bacteria [23] have been partially characterized. It has been stated that JNK and p38 pathways were involved in the induction of hBD2 and that hBD-2 induction, in both oral and skin keratinocytes, was blocked by inhibitors of NF-kappaB [23]. In addition, Krisanaprakornkit *et al.* [24] have demonstrated that phorbol ester induces hBD-2 via the p44/42 extracellular signal-regulated kinase pathway. The upregulation of hBD-2 secretion in cells treated with AV119 could be related to a potential effect of avocado sugars on these pathways. A recent study, using skin keratinocytes examined the intracellular signaling pathways and the nuclear responses that contribute to HBD-2 gene expression upon treatment with AV119 [25]. The reported data suggest that the activation of protein tyrosine kinases and protein kinase C could be involved and could lead to hBD-2 gene activation. These signaling pathways were obtained in a skin keratinocyte model and should be investigated in a gingival model.

The antibacterial activity of culture supernatants obtained from AV119 treated cells was also investigated against the major etiologic agent of periodontitis, *P. gingivalis*. Results of the MIC assays did not reveal any antibacterial effect of AV119 on *P. gingivalis*. Similar results have been obtained when AV119 was tested against *Staphylococcys aureus*, *Pseudomonas aeruginosa* or *Streptococcus pyogenes* [26]. These results exclude the direct antimicrobial effect of AV119 but not that of the culture supernatant of AV119 treated epithelial cells. Indeed, an antimicrobial effect on *P. gingivalis* was observed when KB and Ca9-22 cells were treated with 0.03 % AV119. These results are in agreement with those of Joly *et al.* [27], who demonstrated that hBD-2 possesses antimicrobial activity against Gram-negative bacteria. Nevertheless, these series of experiments do not exclude the fact that other antimicrobial peptides could be secreted by epithelial cells following AV119 treatment. The antibacterial effect of hBD1, hBD3, cathelicidin LL37 and other cytokines against *P. gingivalis* was not observed in similar conditions [28].

In conclusion, these results show that it is possible to stimulate antimicrobial peptides secretion with natural sugars AV119, and this could help in the maintenance of a healthy mucosal surface by preparing epithelial cells to subsequent exposure to oral pathogens. Avocado natural sugars are potential agents for use in managing gingival and periodontal diseases.

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Chapter 36

PERIODONTITIS AS A TRIGGER FOR CUTANEOUS DISORDER, CHEILITIS GRANULOMATOSA; REVIEW OF THE JAPANESE LITERATURE

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COMMENTARY

It is evident that periodontitis is the cause at least in part of the cases of cheilitis granulomatosa. Considering that periodontitis is extremely common, it is paradoxical that cheilitis granulomatosa is relatively rare, although mild cases might well be overlooked. Since most of the bacterial species found in periodontitis are not virulent by themselves, the notion of 'endogenous infection' might be reconsidered for the pathogenesis of cheilitis granulomatosa. Since only a small fraction of bacteria (~1%) can be cultured by conventional culture system, it should be necessary to employ PCR-based molecular approaches for identifying bacteria in diseases of unknown etiology. In the future, development of DNA-array system for identifying bacteria (or organisms) might be a promising approach for identifying the bacteria.

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ABSTRACT

Cheilitis granulomatosa is characterized by the non-inflammatory swelling of the lips, and is considered as the incomplete expression of the Melkersson-Rosenthal syndrome, which consists of the triad of recurrent orofacial swelling, relapsing facial paralysis, and fissuring of the tongue. Rapid improvement after the treatment of periodontitis was first reported in 1961 by Kawamura et al in Japan, and 46 such cases have been reported since then in the Japanese literature. We experienced a typical case of cheilitis granulomatosa. The swollen lip showed marked improvement following the treatment of apical periodontitis. A 57-year-old woman presented with a swelling of the lower lip for the period of two months. Skin biopsy of the lip disclosed non-caseous giant cell granuloma. Neither facial nerve palsy nor fissuring of the tongue was present, excluding the diagnosis of Melkersson-Rosenthal syndrome. Patch testing for metal allergy was negative for all dental metallic ions, except for only mild irritation reaction for Zinc ion. The patient was first treated with topical corticosteroid ointment and oral tranilast, which inhibits the release of chemical mediators from leukocytes, for 4 months. Although the treatment was ineffective, rapid and remarkable improvement of the swelling was noted soon after the treatment of apical periodontitis. Thus, it is highly likely that the periodontitis was the cause of cheilitis granulomatosa in this case. In this article, we review such 46 cases in the Japanese literature.

INTRODUCTION

The Melkersson-Rosenthal syndrome is characterized by the triad of recurrent orofacial swelling, relapsing facial paralysis, and fissuring of the tongue. The complete form with simultaneous occurrence of the above three symptoms is rare, and the most observed feature is the swelling of the orofacial region [1]. The incomplete expression of Melkerson-Rosenthal syndrome was first reported by Miescher in 1945 as cheilitis granulomatosa, for the localized, episodic non-inflammatory swelling of the lips [2].

The etiology and pathogenesis of cheilitis granulomatosa is still unknown. Genetic factors, infectious agents, allergies, and vasomotor disturbances have been postulated, but there have been no clear evidence for any of these [3-6].

The importance of dental infection as the cause for Melkersson-Rosenthal syndrome/cheilitis granulomatosa was first described by Rintala *et al* in 1973 in the English literature [7]. Worsaae *et al* then reported in 1982 that elimination of such foci is associated with regression or disappearance of orofacial swelling in 11 out of 16 patients [3].

REVIEW OF THE JAPANESE LITERATURE

In the Japanese literature, the association of cheilitis granulomatosa and the periodontitis was described much earlier. In 1961, Kawamura et al [8] described a 39-year-old male patient with cheilitis granulomatosa successfully treated along with the management of the chronic apical periodontitis, and concluded that the association of the periodontitis and cheilitis granulomatosa. At first, the patient was treated by the ‘old-fashioned’ irradiation therapy with Cobalt 60 (200 Roentogen), but the swelling of the lower lip was unchanged. Then the patient was administered by vitamin D and INAH. During the drug therapy, the patient complained

of the teeth pain when biting. The removal of the teeth with apical lesions resulted in marked reduction of the swelling of the lip within a few days. The second case in Japan was reported by Baba et al in 1964 [9]. A 39-year-old man presented with swollen lower lip. Four days after the teeth with apical lesions were removed, the swelling of the lip markedly improved. In 1968, Dr. Katsusuke Yokoyama at the University of Tokyo reported an extensive study regarding cheilitis granulomatosa [10]. He experienced 17 cases and was able to evaluate 16 cases. One was lost to follow-up just after the initial examination. All of the 16 cases had periodontitis and the treatment of the dental lesions resulted in the reduction of the swelling of the lips (Table 1). Since then, the association of the periodontitis and cheilitis granulomatosa is well-recognized in Japan, and 46 such cases were reported. As noted in the Table 1, treatments which were not effective for the management of cheilitis granulomatosa include anti-histamines, tranilast (mast-cell membrane stabilizer inhibiting the release of histamine), and oral antibiotics. These treatments were also effective when administered along with the treatment of periodontitis. Therefore, these treatments are not effective alone and the management of the periodontitis is essential for controlling cheilitis granulomatosa. Administration of corticosteroid is very effective even without the treatment of periodontitis. Corticosteroid may be carefully considered against the possible systemic side-effect. Minor plastic surgery has been performed in some recalcitrant cases, particularly for long standing lesions. If the lesion is old and the granulomas might be hard and solid, plastic surgery might be an option of the treatments. Some of the Japanese dermatologists stress the importance of metal allergy as the cause for the development of cheilitis granulomatosa. Positive patch testing for dental metal ions and the removal of those metals resulted in improvement of cheilitis granulomatosa. Some of these anectodal case reports must be carefully reevaluated, since it is also possible that the removal of the metals might have resulted in the improvement of associated periodontitis itself.

TYPICAL CASE REPORT OF THE ASSOCIATION

Here is a summary of a typical case of cheilitis granulomatosa with marked improvement after the treatment of apical periodontitis, which had been already published by Kawakami et al [28].

A 57-year-old woman presented with asymptomatic swelling of the lower lip for the period of two months. The treatment with topical corticosteroid ointment and oral tranilast showed no improvement during the preceding one-month period. On examination, diffuse swelling and erythema of the lower lip was noted. Facial nerve palsy or fissuring of the tongue was not observed, excluding the diagnosis of Melkersson-Rosenthal syndrome. The results of laboratory tests, including hematological and biochemical investigations, liver and adrenal function tests, immunoglobulin levels, and serum angiotensin converting enzyme measurement, were within normal range. Cutaneous patch testing with a metal allergy series revealed only mild irritation to Zinc ion. Strong positive patch testing suggests the presence of allergic reaction toward metal ion(s). Some of the previous studies had reported the presence of the positive patch test and the removal of the metal crowns resulted in the improvement of the swelling of the lips of cheilitis granulomatosa. In this case, however, the test was negative. Histological examination of biopsy specimens from the lower lip revealed noncaseating epithelioid cell granulomas with inflammatory cell infiltrates, which is in

agreement with the histology of the typical cheilitis granulomatosa. These clinical and histopathological findings led to the diagnosis of cheilitis granulomatosa. We asked her if she had seen a dentist. Although she had been seeing a dentist for ten years, obviously no treatment had been administered for possible periodontitis. We advised her to see another dentist, who found infections in the roots of the right upper 5^{th} and 7^{th}, right lower 6^{th}, and left lower 7^{th} molar teeth. In spite that the treatment of the swollen lip with topical corticosteroid and oral tranilast had been ineffective, one month after treatment of two lesions of apical periodontitis (right upper 5^{th} and right upper 7^{th} lesions), remarkable improvement of the swelling was noted, despite lack of treatment of the two lower lesions. Four months after the start of dental treatment, when treatment of the upper lesions had been completed, the swelling of the lower lip disappeared, although treatment of the lower two lesions was still ongoing. This patient had four foci of periodontitis, and treatment of the upper two lesions resulted in rapid improvement of cheilitis granulomatosa. The presence of apical periodontitis was clearly revealed on X-ray films. It is likely that the swelling of the lip in the present case was due to the apical periodontitis in either or both of the upper teeth.

BACTERIAL SPECIES OF PERIODONTITIS

A variety of bacterial species have been isolated from the periodontitis lesions: *Peptostreptococcus spp.*, *Eubacterium spp.*, and *Streptococcus spp.*. Less commonly, *Enterococcus spp.*, *Propionibacterium spp.*, and *Prevotella spp.* are observed in foci of periodontitis [31]. Recently, *Propionibacterium acnes* has been a focus of attention as a cause for sarcoidosis [32]. By quantitative PCR analysis, significantly higher copy numbers of *P. acnes* are reported to be found in the lymph nodes of patients with sarcoidosis [33]. *P. acnes* can produce components with strong chemoattractivity. In addition, *P. acnes* can activate the complement system by both the classical and alternative pathways. It is also evident that *P. acnes* induces pro-inflammatory cytokines, such as IL-1β, IL-1α, IL-8, and TNF-α [34]. Therefore, *P. acnes* might be a strong candidate as a causative agent for cheilitis granulomatosa.

In addition, the periodontopathic bacteria can give rise to virulence factors such as leukotoxins, cytolethal distending toxin, lipopolysaccharide, and proteases [35]. Cardiovascular diseases (atherosclerosis, heart attack, and stroke), complications of pregnancy (spontaneous preterm birth), and diabetes mellitus have been suggested to be associated with periodontitis [36]. Some of the above virulence factors and as yet unknown virulence factors may contribute to the production of cytokines and/or inflammatory mediators for these systemic disorders as well as cheilitis granulomatosa/Melkersson-Rosenthal syndrome.

Although periodontitis is caused by multiple bacteria, it is most likely that the interaction between the bacteria and host contributes the inflammation of periodontitis.

Table 1. The list of cheilitis granulomatosa patients improved after the treatment of periodontitis. In the column of the treatment, NA denotes 'not available'

patient No.	Investigator	age	sex	duration	other treatment(s) along with the treatment of periodontitis	non-effective treatment	dental lesion(s)	reference
1	Kawamura	39	M	2M	isoniazide	radiation with cobalt60,	chronic apical 'lesion'	8
2	Baba	39	M	1Y	oral corticosteroid (3mg of metazolone)	NA	apical 'lesion'	9
3	Yokoyama	62	M	6M	NA	NA	chronic apical periodontitis	10
4	Yokoyama	50	M	6M	NA	NA	chronic apical abscess	10
5	Yokoyama	33	F	1M	NA	NA	chronic apical periodontitis, chronic apical abscess	10
6	Yokoyama	57	M	3Y	oral antibiotics	NA	chronic apical periodontitis	10
7	Yokoyama	48	F	1Y	oral antibiotics, and corticosteroid	NA	chronic apical periodontitis, chronic apical abscess	10
8	Yokoyama	36	M	3Y	NA	NA	chronic apical periodontitis	10
9	Yokoyama	51	M	15Y	oral antibiotics	NA	chronic apical periodontitis, chronic marginal periodontitis	10
10	Yokoyama	56	F	2Y	oral antibiotics	NA	chronic marginal periodontitis	10
11	Yokoyama	58	F	3Y	NA	NA	chronic marginal periodontitis	10

Table 1 (Continued)

12	Yokoyama	20	M	8M	antibiotics	NA	chronic marginal periodontitis	10
13	Yokoyama	29	F	5Y	NA	NA	chronic marginal periodontitis	10
14	Yokoyama	37	F	2Y	NA	NA	chronic apical periodontitis	10
15	Yokoyama	34	F	3M	NA	NA	chronic apical periodontitis	10
16	Yokoyama	47	M	2Y	NA	NA	chronic apical abscess	10
17	Yokoyama	23	M	3M	oral antibiotics	NA	chronic apical granuloma	10
18	Yokoyama	40	F	2Y	NA	NA	chronic apical periodontitis	10
19	Oogase	29	M	6M	oral antibiotics	ABPC	chronic periodontitis	11
20	Yurashige	53	F	5Y	NA	NA	suprodontis	12
21	Inamura	50	F	5M	NA	NA	chronic apical and marginal periodontitis	13
22	Inamura	60	M	2M	NA	NA	periodontitis	13
23	Inamura	54	F	8M	oral antibiotics	NA	periodontitis	13
24	Yano	51	F	1Y	NA	oral antibiotics	chronic periodontitis	14
25	Kounoe	38	M	2Y	oral corticosteroid	NA	periodontitis	15
26	Kondo	53	M	1Y	repeated injection of 4mg of triamcinolone acetonide		chronic marginal periodontitis	16
27	Ezura	61	M	1Y	NA	NA	chronic marginal periodontitis	17
28	Takeshita	39	M	4M	oral antibiotics	NA	periodontitis	18
29	Hattori	31	M	2Y	NA	NA	chronic apical periodontitis	19

Table 1 (Continued)

30	Yokoya	25	M	1Y	oral anti-histamine	NA	apical 'lesion'	20
31	Yokoya	47	M	5Y	oral corticosteroid	NA	chronic periodontitis	20
32	Yokoya	30	M	7M	oral anti-histamine	NA	chronic apical periodontitis	20
33	Yokoya	30	M	5M	oral corticosteroid	NA	chronic marginal periodontitis	20
34	Yokoya	19	F	1W	NA	NA	chronic apical periodontitis	20
35	Yoshida	53	M	1Y	NA	200mg of minocycline hydrochloride, and 300mg of tranilast	chronic apical periodontitis	21
36	Hasegawa	50	M	9M	tranilast	NA	chronic apical periodontitis	22
37	Hasegawa	45	F	1M	NA	tranilast	removal of the dental metal that contains Zn++ which showed positive patch testing	22
38	Tatsumi	45	F	8M	NA	NA	chronic periodontitis	23
39	Tsukamoto	34	M	5M	NA	tranilast	apical cyst	24
40	Tsukamoto	47	M	6M	NA	NA	apical granuloma	24
41	Tadokoro	24	F	6M	NA	NA	suprodontis	25
42	Sato	50	F	1M	NA	NA	apical 'lesion'	26

Table 1 (Continued)

43	Hattori	51	F	6M	NA	10mg of oral predonizolone, 120mg of fexofenadine hydrochloride, and other anti-histamines	chronic apical periodontitis	27
44	Kawakami	57	F	2M	NA	oral tranilast and topical corticosteroid ointment	chronic apical periodontitis	28
45	Kawa	6	M	4M	injection of corticosteroid, tranilast	NA	chronic apical periodontisis	29
46	Izumiyama	47	F	10M	NA	NA	apical periodontitis	30

THE NOTION OF ENDOGENOUS INFECTION

Koch's postulate for exogenous infection has long been the 'Gold-standard' for identifying the cause of a particular infectious disease. However, it has become increasingly clear that many disease-causing bacteria are difficult to culture and do not meet Koch's postulate, for examples, *Helicobacter pylori* in gastritis, *Campylobacter jejuni* in Guillain-Barre syndrome, *Chlamydia pneumoniae* in arteriosclerosis, *Propionibacterium acnes* in sarcoidosis, and *Bartonella henselae* in bacillary angiomatosis. Since 99% of bacteria cannot be cultured by conventional culture system [37], it should be necessary to employ PCR-based molecular approaches for identifying bacteria in diseases of unknown etiology. In the future, development of DNA-array system for identifying bacteria (or organisms) might be a promising approach for identifying the causes.

Escherlish, who discovered *E. coli*, might have been aware of or predicted this situation. He introduced the concept of 'endogenous infection' to describe and focus upon the pathologies due to normal flora. Endogenous infection is distinct from opportunistic infection in that the host is not immunologically compromised. Endogenous infection has been defined by Nakatani as follows: 1) the disease is caused by one of the normal host flora; 2) certain species of bacteria proliferate abnormally within the flora or expand in ectopic locations; 3) the disease is caused by non-virulent bacteria; 4) no latent period exists; 5) the disease is not contagious; 6) obtaining immunity to block recurrence is difficult; and 7) preceding precipitating factors exist along with the bacteria themselves [38].

Since most of the bacterial species found in periodontitis are not virulent by themselves, the notion of 'endogenous infection' might be reconsidered for the pathogenesis of cheilitis granulomatosa.

SARCOIDOSIS VS CHEILITIS GRANULOMATOSA

Generally, the formation of granuloma is the pathological process against bacterial agents. Macrophages are trying to eat bacteria but the process is not sufficient to kill those agents. Since *Propionibacterium acnes* has become a promising causative agent for developing sarcoidosis, the bacteria might also be a candidate pathogen for cheilitis granulomatosa. Sarcoidosis is a systemic disorder affecting lung, heart, skin, lymph nodes, and so on. On the other hand, cheilitis granulomatosa is limited to the orofacial swelling, adjacent to the dental lesion(s). In many cases, the treatment of the dental lesion(s) rapidly results in the improvement of the swelling in cheilitis granulomatosa, whereas in sarcoidosis no such foci have been reported.

CONCLUSION

Thus, it is evident that periodontitis is the cause at least in part of the cases of cheilitis granulomatosa. Considering that periodontitis is extremely common, it is paradoxical that cheilitis granulomatosa is relatively rare, although mild cases might well be overlooked. From 46 Japanese reported cases, no particular associated diseases, such as diabetes mellitus, allergic disorders, liver diseases or others, were noted. What is the missing piece linking between the two conditions, periodontitis and cheilitis granulomatosa? Are there any specific genetic background? Specific bacterial species? Special cytokines or virulence factors? Allergic host reaction? Molecular approaches should be tried to analyze the enigmatic disorder.

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Chapter 37

GENETIC POLYMORPHISMS AND PERIODONTITIS

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ABSTRACT

Periodontitis is a complex disease which is associated with multiple factors, including host immune responses, and genetic, behavioral and environmental factors. It is generally accepted that genetic polymorphisms can modulate host immune responses to bacterial challenge, hence influencing subjects' susceptibility to periodontitis. Genetic association with periodontal disease experience has been a subject of interest for more than a decade. With the completion of Human Genome Project, genetic association studies emerged in many fields of research including research into periodontitis, one of the most common human diseases. This chapter summarizes past and current research approaches with respect to periodontal disease experience and genetic polymorphisms, and suggests anticipated directions of future studies.

HISTORY OF RESEARCH ON GENETIC POLYMORPHISMS AND PERIODONTITIS

Early Days

Back in the 1970s, researchers had already realized that inheritance could possibly be involved in pathogenesis of what was then termed early onset or juvenile periodontitis. Evidence came from studies on families, from studying syndrome-associated juvenile periodontitis and also from research involving animal models. Some researchers considered juvenile periodontitis as possibly an X-linked disease with a decreased penetration dominant

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trait but a relatively consistent gene expressivity (Melnick et al., 1976; Marazita et al., 1994), whereas others suggested it was an autosomal recessive disease (Saxen, 1980; Saxen and Nevanlinna, 1984; Long et al., 1987). Although the inheritance pattern was unclear at that stage, the concept of heredity as one of the etiologic factors in this form of periodontitis was recognized. A common consensus was reached that genetic factors play a role in determining early onset periodontal disease susceptibility by modulating host immune responses rather than by causing the disease directly. Along with juvenile periodontitis, studies on twins also provided solid evidence of genetic predisposition on pathogenesis of adult chronic periodontitis. Michalowicz and colleagues reported clinical, radiographic, and bacteriologic findings in reared-together and reared-apart adult twins, including 21 pairs of reared-apart monozygous twins, 17 pairs of reared-apart dizygous twins, 83 pairs of reared-together monozygous twins and 43 pairs of reared-together dizygous twins. They concluded that about 50% of population variance in both severity and distribution of chronic periodontitis was influenced by genetic factors (Michalowicz et al., 1991). Another large study of twins confirmed these earlier findings and extended these by reporting that the concordance rates for chronic periodontitis were higher for monozygous twins (0.38) than dizygous twins (0.16), noting in addition, that the mean difference in age at diagnosis for concordant pairs was less for monozygous twins (0.94 years) than for dizygous twins (5.38 years) (Corey et al., 1993). Thus genetic predisposition and risk for periodontitis was confirmed, however any particular gene or genotype association with periodontitis remained unclear due to limited genotyping technology available at that time.

Association Study Era

In 1997, Kornman and co-workers reported a possible genetic basis for different levels of host immune responses in adult periodontal disease, which was considered as a major advance in genetic studies in Periodontology (Kornman et al., 1997). They found that a specific genotype of polymorphic interleukin-1 (IL-1) cluster to be associated with severity of periodontitis. Their study showed a strong and clear association between IL-1 cluster genotype and periodontitis. IL-1 and other pro-inflammatory cytokines and inflammatory mediators, such as tumor necrosis factor and prostaglandin E2, have been found that can be stably induced to be secreted under the influence of lipopolysaccharide (LPS) a product of the plaque biofilm. The 1997 study of Kornman and co-workers provided genotype evidence of these phenotypic traits. A simple test performed on a drop of peripheral blood was developed to screen IL-1 cluster genotype for determining periodontitis susceptibility in patients (Kornman et al., 1997). It was hoped that such a test could become a routine in screening periodontitis patients for high susceptibility to the disease, hence allowing recognition of those with high susceptibility to severe periodontal destruction so that special active and supportive periodontal care could be delivered for those at greater susceptibility. However, after diverse data from different populations emerging from differently designed studies were published, hope in the promise of periodontitis susceptibility testing was dampened, and it came to be realized that genetic study in Periodontology was still in its infancy. After 1997, genetic association studies became one of the ‘hot spots’ in periodontal research in the following years. The number of such studies on different candidate genes, most of which related to cytokines and inflammatory mediators, in different populations investigating

associations with different types of periodontitis, was large. The results were diverse, even opposite to each other in some studies. The main problems of genetic studies in this association studies era will be discussed in following sections. Restriction fragment length polymorphisms polymerase chain reaction (RFLP-PCR) was the main method for genotyping in that era. Along with the burst of genetic association studies, some attention was devoted to the incorporation of genetic variance as a risk factor in periodontitis risk assessment. Page and Kornman put genetic risk factors into their working hypothesis of periodontitis pathogenesis, in which genetic variance can modulate host immune responses to bacterial challenge hence influencing the inflammatory and tissue events in response to the bacterial challenge resulting in the clinical manifestations of disease (Page and Kornman, 1997).

Post Human Genome Project Era

In 2003, a milestone in genetic research history, even human history, was established with the completion of the human genome project (HGP). Without question the HGP has provided detailed knowledge of the human genome, and such knowledge it is expected will revolutionize the traditional ways to diagnose, treat and prevent many diseases. Some genetic tests based on the results of the HGP have been starting to benefit clinical practice, for example screening subjects with high risk for breast cancer. However, completion of the HGP does not mean that associated variance with most diseases will be established soon. One reason is that many diseases, such as diabetes and periodontitis, are complex diseases, in other words, these are multi-factorial diseases which are likely associated with the effects of multiple, not single, genes in combination with lifestyle and environmental factors. Therefore, new strategies other than direct association studies and new technologies other than or in addition to direct PCR need to be developed. Recent emergence of genotyping and sequencing technologies such as SEQUNOM MassARRAY system, Illumina GoldenGate system and gene chips have made large-scale population and genome-wide investigation possible. Research on diabetes recently commenced employing different combined strategies other than direct association studies (Sladek et al., 2007; Nejentsev et al., 2009). It is coming to be realized that not only DNA sequence variance can affect gene expression and phenotype, and are heritable at the same time, other changes such as DNA methylation and chromatin remodeling can affect gene expression and also last for generations, which is called epigenetics. The association between epigenetic change and pathogenesis of disease is drawing more and more attention in recent years. Although there is the potential for dramatic progress in genetic studies using approaches mentioned above, most genetic studies in Periodontology seem to have remained as traditional association studies. There are only limited number of studies that have utilized advanced genotyping or statistical methods in searching disease associated single nucleotide polymorphisms (SNPs) (Chai et al., 2010a & 2010b). Periodontal researchers are encouraged to incorporate the use of recently developed technologies and concepts in searching for genetic variance in periodontal disease susceptibility, so that they can provide more reliable data from different populations and help in fostering greater understanding of genetic roles in the pathogenesis of periodontitis.

STRATEGIES FOR GENETIC ASSOCIATION STUDIES

Before the completion of the Human Genome Project and the emergence of dense genetic maps, investigators used linkage studies and positional cloning to identify DNA mutations that caused rare disorders, such as cystic fibrosis (Riordan et al. 1989; Rommens et al. 1989) and Huntington's disease (Gusella et al., 1983; THsDCR Group, 1993). However such approaches were unsuccessful when attempting to identify loci that contribute to complex diseases. In 1996, Risch and Merikangas suggested that for complex human diseases association study could be more powerful than linkage study in identifying the elusive susceptibility loci that geneticists seek (Risch and Merikangas, 1996). Furthermore, the common variant/common disease (CV/CD) hypothesis suggested that common DNA variation, as opposed to rare mutations, could be responsible for a proportion of common diseases (Lander, 1996; Collins et al., 1997; Chakravarti, 1999). Though that hypothesis remains controversial, resources for association studies, such as dense genetic maps of SNPs across the human genome, were channeled to enable investigators to identify disease-causing loci that could potentially have a major impact on public health (Collins et al., 2003). Nowadays association studies have become the focus of most study designs for identifying loci involved in complex diseases, such as cardiovascular diseases, diabetes, cancer and also periodontal diseases.

Direct Association Study

There are two approaches for studying candidate genes: direct and indirect. Direct association study means the putative causative SNP is genotyped directly. Although the direct approach using non-synonymous SNPs has proven successful (Cohen et al., 2004), it is facing some challenges. The major challenge is envisaging or determining *a priori* which SNPs are likely to be causative or to predict the phenotype of interest. However, our current knowledge about the pathogenesis of most complex diseases and related SNP functions is so small that the selection of the candidate SNPs has turned out to be tough in most situations.

Indirect Association Study

The indirect approach, on the other hand, is much more like a linkage study in that the study design assays many, presumably, neutral markers and makes no assumption on the location of the causative gene or locus (Crawford et al., 2005). The indirect approach is most often a case-control study drawn from the general population, using a measure of allelic association or site correlation, known as linkage disequilibrium (LD), to detect historical recombination. However, this strategy also has some problems. Sample selection looses statistical power, particularly for rare alleles, haplotypes at multiple loci cannot be resolved, precluding some powerful mapping strategies, and finally clinical samples are less readily stratified by phenotypic differences and environmental factors, yet such analyses may be critical to understanding disease susceptibility (Kruglyak and Nickerson, 2001).

Genome-Wide Association Studies and Other Combined Strategies

Genome-wide association study (GWAS) is one strategy based on the CD/CV hypothesis. This approach utilizes current high-throughput, array-based technologies to investigate DNA throughout the genome (Khor and Goh, 2010). Most GWAS arrays are for investigation of SNPs. Current arrays with 500,000 to 1 million SNPs have provided reasonable representation (>80% in Caucasians) of the common variants across the genome. GWAS is an unbiased approach since it makes no prior assumptions about the biological process or the mode of inheritance of the trait. The whole genome is scanned for association, which enables the discovery of genetic variants in genomic regions that would not have been suspected based on current knowledge. A good GWAS requires accurate phenotyping to reduce trait heterogeneity, a substantial sample size, a reliable genotyping platform, and the proper statistical strategies for handling large amounts of data and performing complex data analysis on large volumes of data, as well.

Another current strategy is the resequencing of extremes. This strategy is based on an opposite hypothesis, that is a common disease/rare variant (CD/RV) hypothesis in which common disease is considered to be due to the aggregate contribution of multiple different rare variants rather than being due to common variants (Sandhu et al., 2008). In this strategy, individuals at both extremes of the phenotypes are selected for further sequencing, which entails detailed examination of their DNA. It was not until recently, following the development of high throughput sequencing platforms, that resequencing could be carried out in large populations. Unlike GWAS, resequencing requires a selection of candidate genes or candidate regions for sequencing, and this selection is usually based on previous knowledge of the disease pathogenesis. Therefore this approach is unlikely to find out novel causal gene(s) or novel pathways for any disease under investigation. However, it can detect some rare variants other than common SNPs which cannot be detected through GWAS due to their low frequencies.

With respect to periodontitis, limited resources and heterogeneity of the disease manifestations usually prevent large-scale GWAS being conducted. On the other hand, our current limited understanding of the molecular pathogenesis of periodontitis also makes it tricky for periodontal researchers to select proper candidate genes or regions for resequencing. Nejentsev and colleagues targeted resequenced regions selected from results of GWAS and found out that a new variant may be protective in diabetes (Nejentsev et al., 2009). Such a combined strategy of resequencing on the basis of GWAS results may enlighten the pathogenesis of or susceptibility for, periodontitis and the outcomes to its treatment.

CURRENT UNDERSTANDING OF GENETIC POLYMORPHISMS AND PERIODONTITIS

Summary of Meta-Analyses and Systematic Reviews

In the past decade, much research into genetic polymorphism in periodontitis has been performed. The focus mostly has been directed to genetic association with infection processes and aspects of immunoregulation, such as cytokines, cell-surface receptors, chemokines,

enzymes and other biologics that are related to antigen recognition. The results have been diverse, even studies on the same locus or same gene having shown opposite results sometimes, therefore it is difficult to draw a general conclusion without systemic review or meta-analysis of available appropriately designed and well conducted studies. Several systemic reviews or meta-analyses have been performed and published in recent years and an overview of these should hopefully consolidate some general ideas of the current status of the study of genetic polymorphism in Periodontology.

Cytokine gene polymorphisms have been the most extensively investigated genetic polymorphisms in Periodontology, due to the importance of cytokines in the pathogenesis of periodontitis. The number of such studies makes it possible to use meta-analysis or systemic review to establish a gross view of current understanding derived from genetic studies on cytokine polymorphisms in relation to clinical expressions and behaviour of periodontitis. In 2007, a systemic review on the association between composite IL-1 genotype (IL-1A-889 and IL-1B+3954) and periodontitis progression and treatment outcomes was published (Huynh-Ba et al., 2007). The systematic review screened 122 possible studies and finally included 11 longitudinal studies that reached the requirements for study design and conduct. Possibly due to the small number of studies included, no association between IL-1 composite genotype and periodontitis progression or treatment outcomes was confirmed in this systemic review. The authors also suggested a more cautious interpretation of the results obtained by using commercially available test kits. A meta-analysis which retrieved 53 studies, covering 4178 cases and 4590 controls (Nikolopoulos et al., 2008), analyzed the most investigated cytokine polymorphisms in periodontitis including IL-1A-889, IL-1A+4845, IL-1B-511, IL-1B+3954, IL-6-174 and additionally TNFA-308. A significant association of IL-1A-889 and IL-1B+3954 with chronic periodontitis was reported, with a weak association between IL-1B-511 and chronic periodontitis being noted. However there was great heterogeneity between different study populations, therefore the conclusions of this meta-analysis need cautious interpretation in certain populations. For example, IL-1 composite genotype has very low frequency in Han Chinese (Armitage et al., 2000), which makes the significant association of little importance when explaining pathogenesis of periodontitis in 19% of the world's 2009 population.

Another meta-analysis performed was on human leukocyte antigen polymorphism in both chronic and aggressive periodontitis among Caucasians (Stein et al., 2008). The authors selected 12 suitable studies from 174 publications and found HLA-A9 and HLA-B15 to be significantly associated with aggressive periodontitis, while HLA-A2 and HLA-B5 were suggested to be potential protective factors against aggressive periodontitis among Caucasians. There are currently no other systematic reviews or meta-analyses on other genetic polymorphisms and periodontitis, probably because of the limited number of suitable studies for inclusion.

PROBLEMS OF CURRENT GENETIC STUDIES AND PROSPECT

From the overview of the systematic reviews and meta-analyses, it is not difficult to realize that although the number of genetic studies in Periodontology is large, only a small proportion have been of a high standard which can provide complete data necessary for meta-analysis. The main problems are to do with ethnic heterogeneity, clinical classification and

diagnosis of periodontal conditions, choice of controls, size of study groups, and data presentation and handling.

Ethnic Heterogeneity

There is a clear understanding that in the presence of large biological and environmental variability, genetic effects can differ across different populations, or even among generations within the same population (Ioannidis et al., 1998). Frequencies of the genetic marker of interest may also show large heterogeneity between races (Thomas and Witte, 2002). Variations in genotype frequencies across diverse populations may affect the number of individuals at increased risk for a disease, and population substructure imbalances may create spurious differences in genotype frequencies of the compared groups in gene-disease association studies (Kornman et al., 1997; Meisel et al., 2003; Scapoli et al., 2005). The current research-based understandings are derived from different populations and ethnic backgrounds, therefore it is not surprising, from the different results presented, that the current knowledge is not robust. This should warn periodontal researchers to try to ensure reasonable homogeneity of any selected study population, and also to be cautious when trying to replicate the same study of a certain gene marker in a different population. One example of this limited approach are studies conducted on Caucasian populations which suggested the IL-1 cluster composite genotype may associate with the severity of periodontitis (Kornman et al., 1997), however, the prevalence of such composite genotype is very low in those of Chinese heritage (Armitage et al., 2000), suggesting it plays a limited role in the pathogenesis of the periodontitis in Chinese.

Clinical Classification

Classifying periodontal diseases has been a longstanding dilemma largely influenced by paradigms that reflect the understanding of the nature of periodontal diseases during a given historical period (Armitage, 2002). Microbial plaque deposition, smoking, systemic diseases and other environmental factors influence the clinical expression of the periodontal disease condition, and its response to treatment, dramatically. In addition, it is probable that aggressive and chronic periodontitis share a common pathogenic pathway, therefore the periodontist is challenged regarding into which classification a patient would properly fall. Different classification criteria chosen by different studies can also result in different and sometimes conflicting findings.

Choice of Controls

Though control selection is well known to be very important in any genetic association study, it appears there is no clear definition of appropriate controls for a case-control study in periodontitis. Many of the reported association studies showed diverse criteria for control selection. Hence it is not necessarily easy to compare results from different research reports on a certain gene polymorphism given a wide diversity in the controls against which the genetic features of the diseased subjects were compared.

Size of Study Groups

One major concern related to genotype studies is the sample size of the groups of study subjects. Size of the study group clearly contributes to differences in statistical power of the study in its ability to interpret the results, especially in a complex disease like periodontitis. The results of small studies might differ significantly from the results of larger studies, but large studies with thousands of participants might not be easily performed (Ioannidis et al., 1998 & 2001). Experience from other clinical domains suggests that small studies may mistakenly yield more favorable outcomes than larger studies. The sample sizes in genetic association studies in relation to periodontitis have varied from around 50 (Caffesse et al., 2002) to more than 1000 (Meisel et al., 2003), thus making it difficult to combine relevant study results to draw a general view as to the interpretation of the results.

Data Presentation

Expressing differences in results in terms of p-values only is extremely popular in genetic association studies on periodontitis. However, the overuse and misuse of the venerable p-value has been criticized (Visintainer and Tejani, 1998; Sterne and Smith, 2001). It has been suggested that the data should be presented and evaluated not only by p-value but by confidence interval (CI), relative risk (RR) or odds ratio (OR) because these can provide more useful information than those which just use statistics applicable to hypothesis testing. Moreover, most of the current available periodontal data from existing studies do not have quality control measures, e.g. Hardy-Weinberg Equilibrium (HWE) testing, and genotyping success rates have been rarely presented calling for great caution in the interpretation of results.

PROSPECTS

Proper experimental design, including sensible control selection protocols, appropriate sample size calculations, quality control measures, and careful data analysis approaches are necessary before commencing periodontal genetic polymorphism studies, and failure to pay attention to these considerations would limit advances in our current understanding. Periodontal researchers are also encouraged to use combined strategies in genetic investigations of periodontal disease and to utilize newly developed technologies for investigating possible associated SNPs. Periodontitis is a multi-factorial, multi-gene involved disease, thus it is also important that suitable statistical strategies are employed allowing environmental factors which are relevant to genetic predisposition and gene-gene interaction to be taken into account. It is very likely that there is still a journey of discovery to be taken in relation to the understanding of genetic polymorphisms and periodontitis.

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Chapter 38

IMMUNE RESPONSES TO HEAT SHOCK PROTEINS IN CHRONIC PERIODONTITIS AND CORONARY HEART DISEASE

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ABSTRACT

Coronary heart disease (CHD) shares a number of features with chronic periodontitis (CP) including risk factors such as smoking and diabetes; an aetiopathogenesis implicating a number of microbial species, as well as chronic inflammation. However, the link between these two conditions remains unclear. The prevalence of CHD increases with age and is higher in males than females. CP is a chronic inflammatory condition which destroys the supporting tissues of teeth and also increases in prevalence with age. Immune responses against heat shock proteins (HSP) can be cross-reactive among bacterial and human antigens. There is evidence that microbial HSP65 and human HSP60 is involved in periodontal disease and CHD and may therefore provide a mechanistic link between CP and CHD. The aim of this study is to investigate immune responses to the human HSP60 and microbial HSP65 in patients with CP and CHD and relate these to the level of inflammation. We collected serum samples from 100 male subjects divided into 4 groups, each matched for age: (a) Healthy control group with minimal gingivitis, (b) CP, (c) CHD with gingivitis (d) CHD with CP. ELISA was used to determine the levels of serum anti-HSP and C-reactive protein (CRP) in the 4 groups. Peripheral blood mononuclear cells were also isolated from these 4 groups and stimulated with HSPs. Significant lymphoproliferation was seen in CHD with or without CP when stimulated with human HSP60. CRP and serum anti-human HSP60 IgG were elevated in the patients groups compared to the healthy control group, but not serum anti-microbial HSP 65 IgG,. In view of the potential confounding effects of smoking in CP and CHD, a group of current smokers (n=24) were also recruited to investigate whether smoking affects HSP immune responses.There was no significant difference in HSP-induced lymphoproliferation between smokers and non-smokers in any of the four groups. There

was a significant correlation between CRP and lymphoproliferative responses to Human HSP60.

This study shows that serum anti-human HSP60 IgG and serum CRP are raised in CHD with or without CP. In CHD with or without CP, serum CRP levels correlated significantly with human HSP60-induced lymphoproliferation, but not with anti-HSP antibody levels.

INTRODUCTION

Cardiovascular disease arising as a result of atherosclerosis is a major cause of death in western societies and has been associated with periodontal disease (Beck et al 1996, DeStefano et al 1993). Both atherosclerosis and chronic periodontitis share risk factors, most relevantly smoking, and this has led some groups to conclude that shared risk factors are acting as confounders and that there is no link between the two conditions (Hujoel et al 2001); however further evidence for an association continue to appear in the literature (Desvarieux et al 2005).

The periodontium in the diseased state is a complex polymicrobial niche (Lang et al 1998). Over 300 species of bacteria have been isolated and characterised from plaque deposits (Moore & Moore 1994). In 1mg of dental plaque, more than 10^8 bacteria are present (Lang et al 1998). In a neglected dentition, common oral hygiene procedures may result in bacteraemias with consequent systemic inflammation (Carroll & Sebor 1980, Silver et al. 1977).

Chronic periodontitis (CP) is an inflammatory disease characterised by connective tissue destruction and bone resorption. The aetiology of CP is associated with a number of bacteria, autoimmunity or microbial cross-reactivity (Listgarten et al 2003). There is evidence from cross-sectional studies implicating *Porphyromonas gingivalis, Tannerella forsythia* as well as other microbial species including Gram-negative and Gram-positive bacteria, some of which are unculturable (Kumar et al 2003). These findings support the hypothesis that the aetiology of CP is polymicrobial. Serum concentration of CRP, a marker of systemic inflammation, is raised in patients with chronic periodontitis (Amar et al 2003, Buhlin et al 2003}.

HSPs are the most highly conserved group of proteins known in phylogeny with respect to biochemical function, mode of regulation and structure (Ellis 1995, Jindal et al 1989, Thole et al 1988). They are expressed in all eukaryotic and prokaryotic cells including Gram-positive and Gram-negative bacteria (Ellis 1995). The high degree of homology between microbial and human HSPs has led to the hypothesis that tissue damage can occur as a result of cross-reactivity between bacterial and human HSPs. Humoral and T cell response to HSPs have been demonstrated in CP (Hasan et al 2005, Buhlin et al 2003, Tabeta et al 2000, Lopatin et al 1999, Schett et al 1997a, Ando et al 1995).

Chronic infection may also play a role in coronary heart disease (CHD) (Danesh et al 1997, Zhu et al 2000, Saikku et al 1988). As well as DNA from *Chlamydia pneumoniae,* studies have also demonstrated the presence of DNA from periodontotopathic organisms such as *Porphyromonas gingivalis, Tannerella forsythia,* and *Actinobacillus actinomycetemcomitans* in atheromatous plaques (Ford et al 2005, Haraszthy et al 2000). Furthermore, antibodies to periodontal pathogens are associated with CHD (Pussinen et al 2003).

Animal models provide evidence for shared pathogenic mechanisms between atherosclerosis and periodontitis. When apolipoprotein-E-deficient (-/-) mice are immunized with a major periodontopathogen, *Porphyromonas gingivalis,* thereby increasing the burden of pathogen, atherogenesis was enhanced (Ford et al 2007). Host HSP could be detected in atherosclerotic lesions and lesion progression correlated with anti-GroEL antibody levels suggesting that molecular mimicry between GroEL and host HSP60 is involved (Ford et al 2007).

Many studies have focused on identifying a single pathogen, however it has been postulated that the risk factor is the cumulative pathogen burden rather than mono-infections (Epstein 2002). Given the wide range of microbial species implicated in both conditions, a role for HSPs seems probable and in CHD has been suggested by a number of findings: autoantibodies to microbial HSP65 have been shown to mediate macrophage lysis (Schett et al 1997b) and endothelial cytotoxicity (Schett et al 1995) and mucosal administration of microbial HSP65 reduced the presence of atherosclerosis and inflammation in the aortic arch of LDL receptor deficient mice (Maron et al 2002).

The objectives of this investigation are to determine whether serum anti HSP60/65 IgG and IgA are associated with chronic periodontitis and/or CHD in male Caucasian patients and to determine if inflammation, as reflected by CRP levels, correlates with these immune responses.

MATERIAL AND METHODS

Subjects

Patients with CP and CHD were recruited from the department of Periodontology at Guy's, King's and St Thomas's Institute and the Coronary Care Unit St Thomas's Hospital (Table 1). All subjects were male caucasians, aged between 35 and 69 years of age and had at least 15 standing teeth. CHD patients with angiographic evidence of atherosclerosis but no history of myocardial infarction or previous heart bypass operation were recruited. A small number of smokers were also recruited and matched in age and condition to the 4 groups (Table 2). Subjects were excluded from the study if they had systemic disease including diabetes mellitus, recurrent aphthous stomatitis and autoimmune disease, a history of malignancies, previous treatment for periodontitis, or a history of antibiotic therapy within the past six months prior to recruitment .

Subjects were allocated into:

1) Healthy control with minimal gingivitis group
2) Chronic periodontitis only group (CP)
3) CHD group with minimal gingivitis(CHD-G)
4) CHD group with Chronic Periodontitis (CHD-CP).

In each subject, probing depths and recession of all teeth (rounded down to the nearest millimetre) were determined using a William's probe. Recession was measured as the distance from the cemento-enamel junction to the gingival margin. Measurements were rounded down to the nearest mm from 6 sites: mesio-buccal, mid-buccal, disto-buccal, mesio-

lingual, mid-lingual and disto-lingual, probing attachment loss was then calculated as the sum of probing pocket depth and recession.

Table 1. Clinical data and optical densities at 1:100 for serum IgA antibodies to human HSP60 and microbial HSP65 of patients showing median (interquartile range)

Group & Number of patients	Healthy controls n=25	CP n=25	CHD-G n=25	CHD-CP n=25
Age (years) ANOVA p=0.239	48.0 (44-58)	49.0 (43-53)	52.0 (48-56)	54.0 (50-55)
Probing attachment level (mm)	0.9 (0.8-1.0)	3.2 (2.8-4.2)	1.0 (0.8-1.2)	3.2 (2.4-4.8)
Anti-microbial HSP65 IgA ANOVA p=0.362	0.38 (0.28-0.54)	0.33 (0.24-0.66)	0.30 (0.24-0.41)	0.47 (0.27-0.75)
Anti-human HSP60 IgA ANOVA p=0.559	0.62 (0.41-1.07)	0.58 (0.43-0.84)	0.50 (0.36-0.76)	0.53 (0.37-0.94)

Table 2. Clinical data from smokers in each of the 4 groups. showing median (interquartile range)

Present Smokers	Healthy controls n=6	CP n=6	CHD-G n=6	CHD-CP n=6
Age (yrs) ANOVA P=0.331	47 (43-57)	48 (44-51)	50 (47-52)	53 (47-51)
Probing attachment level (mm) p=0.3	0.8 (0.7-1.0)	3.0 (2.7-4.1)	0.9 (0.7-1.1)	3.0 (2.2-4.3)

Periodontitis was defined as probing attachment loss ≥4mm in at least 4 teeth and healthy control group as probing attachment loss < 2mm in all teeth. Probing attachment loss was calculated as the sum of the probing depth and recession. Ethical committee approval was obtained (code no 98/12/04) and subject consent obtained. 50ml of venous blood were withdrawn from each subject before periodontal probing or angiography.

HSP

Recombinant HSP65 derived from *Mycobacterium bovis* was prepared at the National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands and used at a predetermined optimal concentration of 10μg/ml. Human HSP60 was purchased from Stressgen (Victoria, Canada). The two HSPs were detoxified using Detoxi-gel columns (Pierce, Oxford, UK) and the endotoxin level was determined by Limulus Amoebocyte Lysate assay (Sigma-Aldrich, Poole, Dorset, UK). The concentration of endotoxin was <0·007 U/μg or 7 pg endotoxin/μg for both HSPs.

Serum ELISA Measurements

Antibodies to the HSP (human HSP60 and microbial HSP65) were detected by enzyme-linked immunosorbent assay. 96-well flat-bottomed polystyrene microtitre plates were coated (Immulon 4 HBX USA) with HSP60 (Stressgen, Victoria, Canada) or microbial HSP65 diluted (1μg/ml) with phosphate buffered saline (PBS, pH 7.4) and left overnight at room temperature. HSPs were detoxified using Detoxigel columns (Pierce, Oxford, UK) and the endotoxin level was determined by Limulus Amoebocyte Lysate assay Sigma-Aldrich, Poole, Dorset, UK). Uncoated sites were then blocked with 0.5% wt/vol BSA (Sigma-Aldrich Irvine U.K.) in PBS (200μl/well) for 60 minutes at room temperature. In addition, human sero-negative and sero-positive samples were identified by running the whole series of patient serum samples and obtaining the most positive and negative samples as positive and negative controls respectively. Serum from rabbits previously immunised with microbial HSP65 and human HSP60 (kindly provided by Dr L Bergmeier and Dr A Hasan, KCL Dental Institute, London) was also used as positive control. Serum samples obtained from patients including positive and negative controls were then diluted as follows in PBS containing 0.5% BSA and 0.05% tween 20; 2 fold dilution of serum at 1:100 for anti-human HSP60 IgG, anti-microbial HSP 65 IgG and anti-HSP60 IgA, 2 fold dilution of serum at 1:50 for anti-microbial HSP65 IgA. 100μl of each diluted sample or positive/negative control were then added in duplicates to each well and serially diluted to 1:6400, except for anti-microbial HSP65 IgA which had a final dilution of 1:3200.

Plates were then incubated at room temperature for 2 hours and washed 4 times with PBS containing 0.05% Tween 20. Secondary antibody goat anti-human IgG, or IgA Fc specific, alkaline phosphatase conjugates (Sigma-aldrich, U.K.) were diluted in diluent buffer to an optimal concentration. 100μl were then added to each well and incubated at room temperature for 2 hours. Anti-human IgG alkaline phosphatase conjugate was used at 1:2000. Anti-human IgA alkaline phosphatase conjugate was used at 1:1000 for detection of serum anti-human HSP60 IgA and at 1:500 for the detection of serum anti- microbial HSP65 IgA. Plates were then washed 4 times with wash buffer and were developed with para-nitrophenylphosphate in diethanolamine buffer (pH 9.8) at room temperature. The absorbance was determined using a microplate reader (Anthos 2001, Anthos labtec instruments U.K.) for IgG, and (Opsys MR DYNEX technologies) for IgA at 405nm with wavelength correction at 620nm. Inter-assay variation was monitored using a standard positive serum in each assay (Direskeneli et al 1996). The variation of the titre of this positive serum was within one dilution step.

Separation of Cells

Peripheral blood mononuclear cells (PBMC) were separated from blood by density gradient centrifugation and cultured as described previously (Pervin et al 1993). Briefly, 10^5 cells were cultured in RPMI with or without antigens, including ovalbumin (Sigma, Poole, UK) as an unrelated protein control, in quadruplicate in 96-well round-bottomed plates for 6 days. Cells were cultured in RPMI-1640 and 10% autologous serum for 1 h at 37 °C in 5% CO^2. In the final 6 h of culture the cells were pulsed with [3H]-thymidine (0·5 μCi or 18·5 mBq per well; Amersham International, Amersham, UK). The results were assessed by calculating the stimulation index (SI), which is the ratio of antigen-stimulated to antigen-unstimulated cultures (Pervin et al 1993).

Detection of C-reactive Protein by ELISA

50μL of pre-diluted standard and blank were added to a 96 well plate precoated with anti-serum CRP IgG (Kalon Biological Ltd). Serum samples were diluted 1:1000 with assay diluent (Kalon Biological Ltd) and dispensed in duplicates to designated wells in CRP precoated plates. The Plate was then incubated at room temperature for 60 minutes. Plates were washed 4 times with wash buffer (Kalon Biological Ltd). 100μL of CRP tracer (affinity purified sheep anti-CRP labelled with alkaline phosphatase, Kalon Biological Ltd U.K) were then dispensed to each well and incubated uncovered for 30 minutes at room temperature. Plates were washed again 4 times with washing buffer (Kalon Biological Ltd, U.K).

100μl of substrate solution (4-nitrophenylphosphate in substrate buffer Kalon Biological Ltd) were then dispensed to each well and incubated at room temperature for 30 minutes. The reaction was stopped with 100μl of (120g/L) sodium hydroxide. Optical densities were read at 405nm with micoplate reader (Anthos 2001, Anthos labtec instruments U.K.). A standard curve was constructed with standard points and curve fitted with four parameter logistic curve fitting software. Test serum values were then read off the standard curve.

Statistical Method

Kruskal-Wallis ANOVA test was used to assess differences between the groups. ELISA for serum anti-HSP IgG or IgA was analysed by using data consisting of optical density readings for the performed test. The mean optical density was then analysed at 1:100. The significance level was set at $p<0.05$. Post-ANOVA pair wise comparisons between the healthy with CP group; healthy with CHD-G group CP, and with CHD-CP group was carried out using the Mann-Whitney U test, using the Bonferroni correction test to set the significance level at $p<0.02$. Inter-relationships between serum anti-HSP60 IgG and serum CRP levels were tested using Spearman ranked correlation coefficients (r_s). Non-parametric data is displayed in graphs as median (1st and 3rd quartile, minimum, maximum).

RESULTS

Clinical Data

We recruited 120 patients (non-smokers) divided into 4 groups, The median (interquartile range) age of the subjects in the control group was 48 (44-58) years, in the CP group 49 (43-53) years, in the CHD-G group 52 (48-56) and in the CHD-CP group 54 (50-55) and there was no statistically significant difference between the groups, (ANOVA p=0.239, Table 1). In order to investigate the effect of smoking on lymphoproliferative responses, we recruited a small number of current smokers (Table 2). The current smokers were matched for age, gender, and disease status with non-smokers from the 4 groups. There were no significant differences in the age and periodontal status of non-smokers in each of the 4 groups when compared with smokers in the 4 groups (p>0.05).

For the non-smokers, the median (interquartile range) probing attachment level in the control group was 0.9 (0.8-1.0) mm, in the CP group 3.2 (2.8-4.2) mm, in the CHD-G group 1.0 (0.8-1.2) mm and in the CHD-CP group 3.2 (2.4-4.8) mm (Table 1). In view of the confounding effect of smoking in studies associating periodontitis to cardiovascular disease, the data from smokers and non-smokers have been kept separate in all figures and tables.

IgA Antibodies to Microbial HSP65 and Human HSP60

The OD at 1:100 dilution of serum for anti-microbial HSP65 IgA was 0.38 (0.28-0.54) in the control group, 0.33 (0.24-0.66) in the CP group, 0.30 (0.24-0.41) in the CHD-G group and 0.47 (0.27-0.75) in the CHD-CP group, and was not significant ANOVA, p=0.362 (Table 1). Similarly, there was no difference in serum anti-human HSP60 IgA in the 4 groups (Table 1). The median (interquartile range) OD at 1:100 dilution of serum was 0.62 (0.41-1.07) in the control group, 0.58 (0.43-0.84) in the CP group, 0.50 (0.36-0.76) in the CHD-G group, and 0.53 (0.37-0.94), or in the CHD-CP group ANOVA (p=0.559) (Table 1).

IgG Antibodies to Microbial HSP65 and Human HSP60

The median (interquartile range) optical density at 1:100 dilution of serum for anti-microbial HSP65 IgG was 0.34 (0.17-0.63) in the control group, 0.46(0.25-0.85) in the CP group, 0.35 (0.23-0.71) in the CHD-G group and 0.59 (0.2-0.94) in the CHD-CP group (Figure 1). There was no significant difference amongst the 4 groups in serum anti-HSP65 IgG titres by ANOVA (p=0.661).

Serum levels of anti-microbial HSP65 IgG were significantly associated with serum levels of anti-human HSP60 IgG in only 2 groups: the CP group (r_s= 0.538, p= 0.005) and the CHD-G group (r_s =0.524, p=0.007) (Table 3). No statistically significant relationship was revealed between serum anti-microbial HSP 65 IgG and serum anti-human HSP60 IgG in the control group (r_s=0.005, p=0.983) and the CHD-CP group (r_s=0.255, p=0.219) (Table 3).

Lymphoproliferative Responses to Human HSP60 in Smokers and Non-smokers

The results of the lymphoproliferative assays are expressed as stimulation indices (the ratio of antigen-stimulated to unstimulated cultures). An analysis of smokers and non-smokers in each group yielded no significant differences in lymphoproliferative responses between any of the four groups (figure 3). All patient groups responded significantly to human HSP60, in contrast to the healthy controls which yielded a median (interquartile range) stimulation index of 2.0 (0.45) ($p<0.001$). Smoking does not appear to influence HSP60-induced proliferation in CP or CHD. In all groups of non-smokers except the controls, HSP proliferative responses correlated with CRP levels ($p<0.02$).

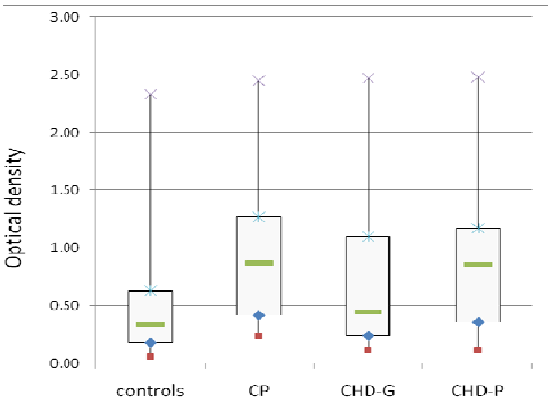

Figure 1. Boxplots showing median, interquartile range, minimum and maximum OD for serum anti-microbial HSP65 IgG in 4 groups of patients The median (interquartile range) OD of 1:100 dilution of serum for anti-human HSP60 IgG was 0.26 (0.17-0.34) in the control group, 0.87 (0.42-1.27) in the CP group, 0.33 (0.24-1.1) in the CHD-G group and 0.86 (0.36-1.17) in the CHD-CP group and this was statistically significant by ANOVA ($p<0.001$) (figure 2). Post-ANOVA analysis revealed a significant difference between the controls and the CP group ($p<0.001$). There was also a significant difference between the control and the CHD-G group ($p=0.019$), but no significant difference between the chronic periodontitis and the CHD-CP group ($p=0.907$).

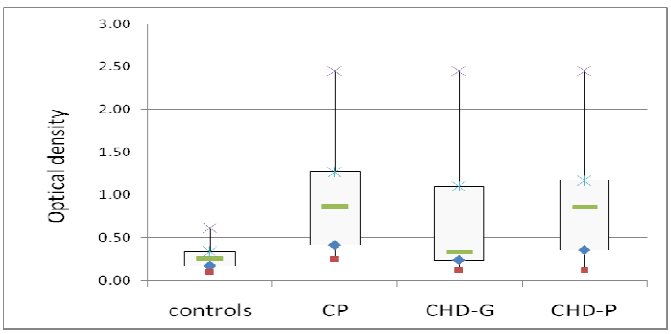

Figure 2. Boxplots showing median, interquartile range, minimum and maximum OD for serum anti-human HSP60 IgG in 4 groups of patients

Table 3. Correlation between serum anti-human HSP60 IgG and serum CRP levels to serum anti-microbial HSP 65 IgG

		anti-microbial HSP65 IgG	CRP
Control n=25	r_s	0.005	0.115
	p	0.983	0.583
CP n=25	r_s	#0.538	-0.194
	p	*0.005	0.352
CHD-G n=25	r_s	#0.524	-0.099
	p	*0.007	0.637
CHD-CP n=25	r_s	0.255	#0.417
	p	0.219	*0.038

significant correlation coefficient (r_s) * Significant p value

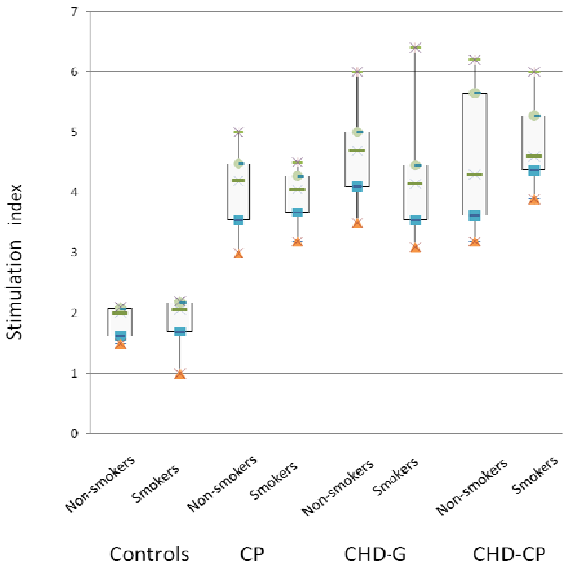

Figure 3. Boxplots showing median, interquartile range, minimum and maximum stimulation index of Human HSP60-induced lymphoproliferative responses in non-smokers and smokers (n=6 for each group). CP and CHD with or without CP responded significantly to HSP60 (ANOVA p<0.001)

CRP

The median (interquartile range) of serum CRP levels in the 4 groups was 0.64 (0.18-1.86) mg/L in the control group, 1.27 (1.01-2.83) mg/L in the CP group, 2.15 (1.03-2.94) in the CHD-G group and 2.0 (1.31-3.78) in the CHD-CP group (figure 4). There was a statistically significant difference amongst the groups by ANOVA (p=0.002). Post-ANOVA analysis revealed significant differences between the control group compared to the CP group (p=0.007) and the control group compared with the CHD-G group (p=0.001). However there was no significant difference between the CP compared with the CHD-CP group (p=0.383). It is tempting to interpret this to mean that CP influences CRP levels, however we do not have

data on whether these responses may fluctuate with time, and what other innate or adaptive mechanisms determine the outcome; nor do we know how this impacts on CHD.

Serum CRP levels are not significantly associated with serum anti-human HSP60 IgG levels in the control group (r_s =0.115, p=0.583), in the CP group (r_s =-0.194, p=0.352) and in the CHD-G group (r_s =-0.099, p=0.637) (Table 3). However in the CHD-CP group there was an association between serum CRP levels and serum anti-human HSP60 IgG levels (r_s =0.417, p= 0.038) (Table 3).

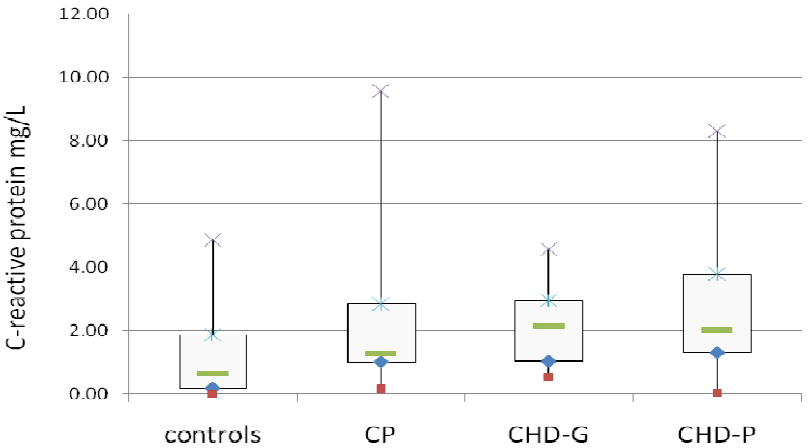

Figure 4. Boxplots showing median, interquartile range, minimum and maximum level of serum C-reactive protein amongst the 4 groups of patients

DISCUSSION

The results show that serum anti-human HSP60 IgG but not serum anti-microbial HSP65 IgG was elevated in patients with chronic periodontitis compared to the control group. No significant differences were noted in the level of serum anti-human HSP60 IgA and serum anti-microbial HSP65 IgA between the control group and patients with chronic periodontitis. These findings contrast with a study showing an elevation of serum anti-HSP65 IgG (Schett et al 1997a) and another study showing depression of serum anti-HSP65 IgA with no difference in serum anti-human HSP60 IgG and serum anti-microbial HSP 65 IgG in CP patients compared to the control group (Buhlin et al 2003). However these studies are difficult to compare directly as there are significant differences in demographical and clinical parameters. In one of these studies the patient group had a mean age of 37.5 ± 12.6 years range 26-65 years (Schett et al 1997); with this age range it is likely that more than one form of periodontitis was included. Our study included only patients with moderate-advanced chronic periodontitis or gingival health/mild gingivitis. The median age (interquartile range) of our patients with chronic periodontitis is 49 (43-53). Younger aged patients with advanced periodontitis may represent a different diagnostic category or a different spectrum of response from older patients suffering from chronic periodontitis (Ranney 2000), and may mount different immune responses (Gunsolley et al 1990).

The recruitment of patients who are undergoing treatment for chronic periodontitis (Buhlin et al 2003) is problematic and may yield conflicting results as treatment of CP has a modulating effect on humoral immune responses to HSP (Yamazaki et al 2004) and instrumentation may immunise patients with plaque antigens. To minimise the confounding effect of treatment in our results we recruited untreated cases.

Our findings are in agreement with studies where the mean ages of untreated chronic periodontitis were similar (Tabeta et al 2000, Lopatin et al 1999). These studies demonstrated a significant elevation in serum anti-human HSP 60 IgG compared to a control group (Tabeta et al 2000). Serum anti-microbial HSP65 IgG levels were either similar (Lopatin et al 1999) or elevated (Tabeta et al 2000) in chronic periodontitis patients compared to control patients.

It was important to match our patients for age, as clearly both CP and CHD occur with increasing frequency in older populations. In addition, the diagnosis of periodontal diseases is unfortunately age-dependent, so the distinction between early onset forms of periodontitis and chronic adult periodontitis becomes harder to establish when patients present at the age of 30-35. Until the behaviour of the disease can be determined it is difficult to be certain the correct diagnosis has been made without resorting to special tests and culture studies. We were therefore careful to match the age of patients to those presenting at the Coronary care unit, rather than those attending a Periodontal clinic where the patients tend to be three decades younger.

In this study whilst we demonstrated a statistically significant elevation in serum anti-human HSP60 IgG in the CHD-G group, there was no statistical difference in serum anti-microbial HSP65 IgG. A number of studies have demonstrated elevation in serum anti-microbial HSP65 IgG in patients with CHD (Ciervo et al 2002, Mahdi et al 2002, Prohászka et al 2001, Birnie et al 1998, Hoppichler et al 1996, Xu et al 1993) which was sustained over a number of years (Xu et al 1999) and predicted cardiovascular events (Veres et al 2002, Xu et al 1999). Most of these studies analysed subjects aged 60 years or over (Veres et al 2002, Ciervo et al 2002, Mahdi et al 2002, Xu et al 1999, Hoppichler et al 1996, Xu et al 1993) or did not include a healthy control group (Birnie et al 1998). Furthermore, the periodontal status of these patients was not determined. When a younger age group (less than 60 yrs old) was analysed for anti-HSP65 IgG, no significant difference was found compared with the control group (Xu et al 1993). In studies where the age group was comparable to our study, serum anti-HSP60 IgG was demonstrated to be elevated in the patient group compared to control (Bason et al 2003, Burian et al 2003, Prohászka et al 2001, Zhu et al 2001). Additionally, serum antibodies from these patients recognised human HSP60 peptide fragments (Bason et al 2003, Wysocki et al 2002). Older subjects have been shown to be more prone to chronic infections resulting in an increase in markers of inflammation including anti-microbial HSP65 (Kiechl et al 2001). The presence of anti-microbial HSP65 antibodies may reflect the cumulative effect of infections which increases with age. However this is still compatible with the hypothesis that cross-reactivity between microbial HSP65 and human HSP60 may lead to tissue damage in CP and CHD (Mayr et al 1999).

Serum anti-human HSP60 IgA when present with *Chlamydia pneumoniae* has been identified as a risk factor for future coronary events in patients who were initially healthy (Huittinen et al 2003, Huittinen et al 2002). Whilst we found there was no statistically significant difference in serum anti-human HSP60 IgA or serum anti-microbial HSP65 IgA between the control and the CHD-G group, it remains to be established whether antibody levels remain elevated or fluctuate in relation to intercurrent infections. It is clearly important

to determine how innate, humoral and cellular immune responses change with time, infection, or coronary events in CP or CHD.

Cross reactivity between anti-microbial HSP65 antibodies and human HSP60 antibodies has been suggested to mediate macrophage lysis (Schett et al 1997b) and endothelial damage (Schett et al 1995). Serum anti-microbial HSP65 have been shown to recognise specific epitopes on human HSP60 in subjects with atherosclerosis (Perschinka et al 2003). In our study there was a moderate correlation between serum anti-human HSP60 IgG and serum anti-microbial HSP65 IgG in patients with CP and CHD-G, and therefore the possibility of cross-reactivity still exists. Shared epitopes within human HSP60 may help account for the association between CP and CHD and reveal a mechanistic link for tissue destruction. Once the epitopes recognised in CP and CHD have been mapped, the role of T and B cell epitopes in relation to inflammatory and anti-inflammatory responses can be elucidated.

Smoking is a risk factor for chronic periodontitis and CHD (Genco et al 2002). In addition smoking has been suggested to be a confounding variable in the association of CP with CHD (Hujoel et al 2000). Smoking is known to modulate immune response and in chronic periodontitis there is a direct correlation between serum cotinine levels and serum levels of intracellular adhesion molecule- a risk factor for CHD (Palmer et al 1999). Exposure of human monocytes and endothelial cells to freshly prepared filtrates of tobacco smoke induces expression of the inducible HSP70 (Vayssier-Taussat et al 2001). The role of smoking on the cellular and humoral immune responses to HSP in patients with chronic periodontitis and CHD has not been fully elucidated although, exposure to smoking reduces the release of TNF-α in human alveolar macrophages (Yamaguchi et al 1993) and PBMC (Ryder et al 2002). We found no difference between smokers and non-smokers in any of the subject groups suggesting that at least HSP-induced proliferation is not affected by smoking. It however remains to be determined how smoking affects HSP-induced T cell responses and inflammatory cytokine production in CHD and CP.

Serum CRP levels in patients with CP and CHD when compared to a healthy control group were significantly elevated. Previous studies have shown that serum CRP levels are elevated in CP (D'Aiuto et al 2004, Buhlin et al 2003, Ide et al 2003). Cross-sectional studies have shown that CRP levels are also raised in patients with angiographically recorded coronary heart disease (Garcia-Moll et al 2000, Rifai et al 1999) and in patients with symptoms of angina but normal coronary angiogram (Cosin-Sales et al 2003). In a prospective multiple risk factor intervention trial CRP was found to be an independent risk factor for coronary heart disease mortality in healthy but high risk individuals (Kuller et al 1996). Furthermore, CRP could be used to predict the risk of acute myocardial infarction and mortality of patients with angiographically recorded coronary heart disease (Bickel et al 2002, Zebrack et al 2002).

No significant correlation was shown between CRP and serum anti-HSP60 IgG levels in subjects, except in the CHD-CP group. This is in agreement with previous reports in the literature which do not demonstrate significant relationship between CRP and serum anti-human HSP60 levels (Veres et al 2002, Zhu et al 2001). These conflicting findings may simply reflect the complex nature of innate immunity and its diverse relationships to both inflammatory and anti-inflammatory mechanisms.

CONCLUSION

This study demonstrated that serum anti-human HSP 60 IgG but not serum anti-microbial HSP65 IgG is elevated in patients with CP with or without CHD indicating there are autoantibodies to human HSP60 in CP and CHD and together with our previous findings of autoimmune T cell response to human HSP provides further support for cross-reactivity in the pathogenesis of CP and CHD. Serum CRP levels were also significantly raised in the patient groups but there was no significant relationship between serum CRP levels with serum anti-human HSP60 IgG levels. If we are to determine successfuly whether the association between CP and CHD is real, HSP-induced inflammatory and anti-inflammatory mechanisms need to be further elucidated in relation to innate immunity.

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Chapter 39

CHRONIC PERIODONTITIS AND THE RISK OF ORAL CANCER

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ABSTRACT

Morbidity and mortality from oral cancer are high and this has not improved in decades in spite of extensive research. A significant portion of research is concentrated on chemoprevention. However, advances in this field have not translated into a visible change in mortality and morbidity. In addition, existing chemoprevention strategies have two important obstacles: toxicity and reversal of the effects after cessation of treatment. Chronic infection and inflammation have been linked to carcinogenesis in a few organs. For oral cancer, substantial evidence has accumulated for the role of *human papillomavirus* (HPV). However, the development of an effective preventive vaccine strategy for oral cancer is still years away and the target population is largely unexplored. Therefore, safe and practical additional approaches are necessary to change the status quo of oral cancer. Periodontitis is a chronic oral infection caused by inflammatory reactions in response to gram negative anaerobic bacteria in the endogenous dental plaque. It leads to irreversible destruction of tissues around teeth clinically detectable as periodontal pockets and alveolar bone loss. Periodontal pockets have been suggested as reservoirs of HPV. Chronic proliferation and ulceration of the pocket epithelium may help HPV's initial infection and persistence. Our preliminary results from existing data at Roswell Park Cancer Institute suggest a robust independent association between the history of periodontitis and incident oral cancer. Our next step is to test the synergy between periodontitis and HPV for the risk of oral cancer. If this is true, it will translate to practical and safe prevention and treatment strategies. This chapter will review the evidence supporting the association between chronic periodontitis and oral cancer as well as HPV-periodontitis synergy.

INTRODUCTION

Oral cancer is a significant cause of morbidity and mortality and this has not changed for decades. It is estimated that 34,360 new cases of oral cancer and 7,550 deaths will occur in 2007 [1]. The incidence is open to debate because of the well-known field cancerization phenomenon in the head and neck region. Those with primary cancer of the oral cavity and pharynx are also at high risk for developing cancer of the esophagus, larynx, lung, and stomach. Oral cancer is notorious for its high rates of second primary cancers, recurrence, and distant metastases [2-4]. It often leaves the patients with disfiguration and loss of vital functions such as swallowing and breathing. Consequent psychological and social impairments are also very debilitating. The treatment is aggressive and often increases the morbidity without improving the survival significantly [5].

Even though oral cancer is largely preventable, the majority of funded research has been on treatment and diagnosis. However, advances in these fields have not translated into a visible change in grim oral cancer statistics. Primary prevention strategies against risk factors, mostly tobacco, alcohol and diet, are limited and have not been very effective. Most of the existing prevention strategies are secondary and tertiary. Preventive surgical resection, radiotherapy, photodynamic therapy and topical cytotoxic therapies had limited success [6, 7]. Chemoprevention, including retinoids, selenium, vitamin E, interferon- α (IFN-α), cyclo-oxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, have two important obstacles: toxicity and reversal of the effects after cessation of treatment [8-10]. Chronic infections with viruses, especially certain human papillomavirus strains (HPV 16 and 18) are another target for prevention. The vaccine targeting HPV types 6, 11, 16 and 18 became recently available for cervical cancer. However, the vaccine is prophylactic and is not effective for those who were previously exposed to the virus. It is expensive and requires 3 injections over a 6 month-period. Since the longest trial was 5 years, we don't know the length of immunity from the vaccine nor its long-term safety. Even though the vaccine is effective against infection, its effectiveness to prevent invasive cancer is not known [11-12]. Information concerning the role of viruses in oral cancer is fragmented and the development of an effective vaccine strategy may still be years away. The target population who would derive the most benefit from the vaccine is largely unexplored. Therefore, safe and practical additional approaches are necessary to change the status quo of oral cancer.

Although most studies assessing the infectious etiology of cancer are focused on viruses, the evidence on bacterial infections is also convincing [13-14]. Associations with *Helicobacter pylori* infection with gastric cancer and primary B-cell gastric lymphoma [15], *Chlamydia pneumonia* infection with lung cancer [16], *Salmonella typhi* infection with gallbladder cancer [17] and *Streptococcus bovis* infection with colon cancer [18] are a few examples. Recently, there are also a growing number of basic science studies identifying key molecular mechanisms [19-24].

Opposing effects of acute vs. chronic infection on cancer have been described. While chronic infection usually promotes cancer development, acute infection was shown to counteract cancer development. For example, a few studies showed that subjects who contracted *Salmonella typhi* but did not become carriers were not at higher risk for hepatobiliary carcinoma but only those who became chronic carriers were at high risk [25-

27]. The process of acute infection is self-limiting. Pro-inflammatory cytokines give way to anti-inflammatory cytokines as healing progresses. In chronic infection, on the other hand, active tissue destruction and repair proceed simultaneously. Angiogenesis and fibrosis are the chief components of this process and chronic inflammatory cells and mediators persist in the environment [28, 29]. Therefore, chronicity of the infection/inflammation appears to be the key factor for carcinogenesis.

CHRONIC PERIODONTITIS

Periodontitis is a chronic oral infection caused by inflammatory reactions in response to microorganisms in the endogenous dental plaque (Figure 1). The average prevalence of periodontitis in the general population is 30%; about 12% is a severe form [30-33].

The dental plaque is a biofilm. It is critical to understand the characteristics of the biofilm to effectively control periodontitis as well as its systemic sequela. Biofilms are sessile community of various types of bacteria embedded in a polymeric matrix irreversibly attached to a surface. The majority of bacteria in the body exist in biofilms. A main characteristic of biofilms is quorum sensing which is communication and group behavior of bacteria residing in the biofilm. The bacteria in the biofilm differ profoundly from their free floating counterparts and are resistant to antimicrobials as well to host defense [34]. Mechanical removal of the biofilm, by brushing and flossing, is required. In the presence of periodontitis, however, biofilm in the periodontal pockets is inaccessible to personal oral hygiene practices and professional treatment is needed. It is important to note that biofilm properties of the dental plaque contribute to the persistence of periodontitis in the absence of treatment. Treatment of chronic periodontitis is safe and is based on mechanical removal of the biofilm. Chemotherapy alone is not effective [30].

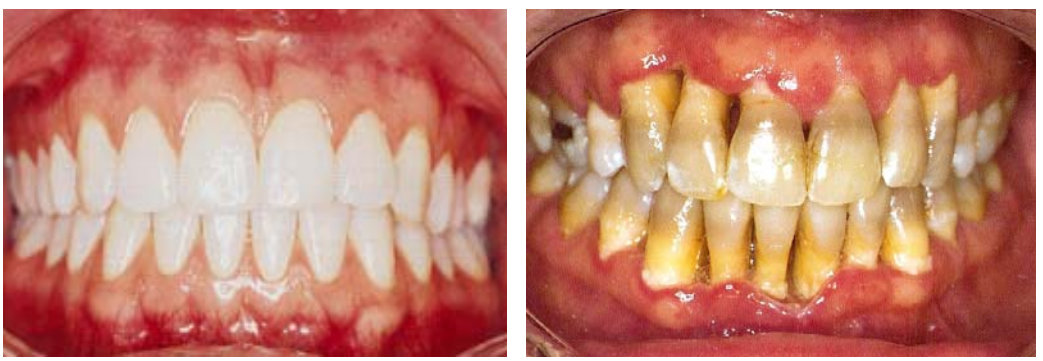

Figure 1. Comparison of healthy periodontium (A) with periodontitis (B).

Periodontitis results in a chronic release of inflammatory chemokines, cytokines, prostaglandins, growth factors and enzymes in saliva and to a lower degree in blood, all of which are also associated with carcinogenesis. The extent and severity of periodontitis are associated with the level of these inflammatory markers. The ensuing chronic inflammation is what leads to local pathologic anatomic changes, namely, periodontal pocket formation and alveolar bone loss [30]. As the disease progresses, epithelial attachment at the bottom of the

periodontal pocket migrates apically along the root surface and the pocket depth increases. The pocket epithelium is characterized by continuous proliferation, formation of rete-ridges and ulcerations. In the connective tissue, there is increased angiogenesis, chronic inflammatory infiltrate, fibrosis and tissue loss (figure 2). Furthermore, periodontal pathogens and inflammatory mediators travel with saliva and blood from the affected tissues to distant sites and adversely affect systemic health. Multiple studies have shown that oral pathogens and cytokines are aspirated to lungs by saliva and are transported to arterial plaques by blood [31-38]. Most importantly, treatment of periodontal infections significantly prevents systemic adverse events [39-43]. We anticipate that the same mechanisms may play a part in the field cancerization of oral cancer.

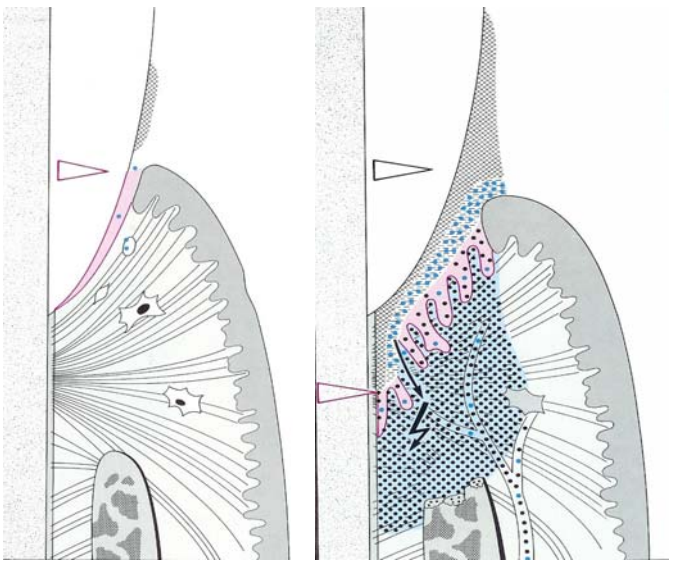

Figure 2. Tissue changes from periodontal health (A) to chronic periodontitis (B).

Poor oral hygiene (dental biofilm) is the cause of periodontitis but only small percentage of subjects with poor oral hygiene develops periodontitis [30]. A link between poor oral hygiene and the risk of oral cancer has also been suggested for a long time [44-54]. However, a definite association has not been established due to limited methodology of existing studies. We anticipate that among subjects with poor oral hygiene, those who develop periodontitis are at higher risk for oral cancer.

We are currently conducting an NIH funded study to test the hypothesis that chronic periodontitis is associated with increased risk of head and neck cancers using existing data at Roswell Park Cancer Institute (RPCI), Buffalo, NY. We have analyzed a subset of this population with a case-control study design. These preliminary analyses were restricted to subjects newly diagnosed with primary squamous cell carcinoma of tongue between June 15, 1999 and November 17, 2005. The control group consisted of subjects admitted during the same time period but not diagnosed with cancer. Children (age <21), edentulous, and those with prior history of cancer, cancer therapy and oral dysplasia were excluded. A total of 94 cases and 153 controls met the inclusion criteria. Histological diagnoses of cancer cases were available electronically from RPCI Tumor Registry. Cumulative history of periodontitis was measured by alveolar bone loss (ABL) from panoramic radiographs using an operator-

interactive program on digitized radiographic images [55-58]. Accuracy and reliability of this technique have been established [59, 60]. ABL was measured in millimeter (mm) on mesial and distal sites of all teeth. One trained and calibrated examiner, blind to cancer status, performed all ABL measurements. Number of missing teeth, fillings, cavities and endodontic (root canal) treatments were also diagnosed from the radiographs. The independent effect of ABL on oral cancer was estimated from multiple logistic regression analysis by odds ratio (OR) and 95% confidence interval (CI). Each millimeter of alveolar bone loss was associated with 4.47 fold increase in the risk of tongue cancer (OR=4.47, 95% CI=2.80-7.15) after adjusting the effects of age at diagnosis, gender, race/ethnicity, smoking status, alcohol use and number of teeth. The remaining oral variables cavities, fillings, crowns and endodontic treatments were not significantly associated with the risk of tongue cancer (Table 1).

Table 1. Adjusted Odds Ratios for the Risk of Tongue Cancer (N=247)

	OR*	95% CI#	p
Mean ABL (per mm)	4.47	2.80 - 7.15	<0.001
At least 1 Site with ABL ≥4 mm (per site)	9.67	4.01 - 22.82	<0.001
Missing Teeth (per tooth)	1.02	0.98 - 1.05	0.357
Decayed Teeth (per tooth)	0.95	0.83 - 1.09	0.493
Filled Teeth (per tooth)	1.04	0.97 - 1.11	0.253
Crowns (per tooth)	0.91	0.83 - 1.01	0.080
Endodontic Treatments (per tooth)	0.89	0.74 - 1.06	0.197

*Odds ratios adjusted for age at diagnosis, gender, race, alcohol use, smoking status and number of teeth; # 95% Confidence intervals.

In summary, this study, analyzing existing patient records at a local comprehensive cancer center, confirmed our suspicions that the history of periodontitis may in fact be associated with oral cancer independent of smoking. However, the study had the classical shortcomings of secondary data analyses: Data on confounding variables was limited. In addition, the diagnosis of periodontitis was based on radiographs. Radiographic assessment of periodontal disease is not sensitive, does not reflect present disease status nor differentiate gingivitis from periodontitis. The encouraging results of this preliminary study provide the basis for further studies with quantitative clinical measures and comprehensive assessment of confounding.

BIOLOGICAL MECHANISM

The question of how infections can influence cancer has interested scientists for over one and a half centuries, but only now are the general principles and the real complexity of this subject emerging. Chronic infections, such as periodontitis, can play a direct or indirect role in carcinogenesis:

1) Direct Toxic Effect of Microorganisms

Microorganisms and their products such as endotoxins (LPS), enzymes (proteases, collagenases, fibrinolysin and phospholipase A) and metabolic by-products (H_2S, NH_3 and

fatty acids) are toxic to surrounding cells and may directly induce mutations in tumor suppressor genes and protooncogenes or alter signaling pathways that affect cell proliferation and/or survival of epithelial cells [27]. *Porphyromonas gingivalis* is a major periodontal pathogen that is capable of invading epithelial cells. In a recent study, *P.gingivalis* induced a transient increase in keratynocyte DNA fragmentation; however, after prolonged incubation, *P. gingivalis* blocked apoptosis in keratynocytes [61]. Another example of direct bacterial stimuli is the association between *Helicobacter pylori* and primary B-cell gastric lymphoma, where chronic infection causes persistent B-cell activation culminating in chromosomal rearrangements that ultimately cause cancer [15]. Numerous other studies support the evidence that commensal bacteria can directly affect various stages of cell cycle in carcinogenesis [62-64].

2) Indirect Effect through Inflammation

Chronic infection may stimulate carcinogenesis through an indirect mechanism involving activation of surrounding host cells (neutrophils, macrophages, monocytes, lymphocytes, fibroblasts and epithelial cells) to generate:

- Reactive oxygen species (hydrogen peroxide and oxy radicals), reactive nitrogen species (nitric oxides), reactive lipids and metabolites (malondialdehyhe, 4-hydroxy-2-nonenal) and matrix metalloproteases (MMPs) which can act as endogenous mutagens and can induce DNA damage in epithelial cells [28].
- Cytokines, chemokines, growth factors and other signals which provide an environment for cell survival, proliferation, migration, angiogenesis and inhibition of apoptosis [65]. This environment:
    - Help epithelial cells to accumulate mutations.
    - Drive these mutant epithelial cells to proliferate, migrate and give them a growth advantage

Recently, a key molecular mechanism connecting inflammation and cancer was identified. It was shown that a pro-inflammatory gene is involved in cancer development and that inactivation of this gene could dramatically (80%) reduce tumor development [22]. A subsequent study showed that cancer metastasis was halted with inhibition of a pro-inflammatory protein, nuclear factor-kappa B (NF-B) or an inflammatory mediator, tumor necrosis factor alpha (TNF-) [23]. Another study showed how prostaglandin E_2 (PGE_2) promotes colorectal adenoma growth [24]. Numerous other studies have confirmed the associations of various players in inflammation to carcinogenesis in several organs [66-68] including the oral cavity [69-72].

3) Biological Interactions of Periodontitis with Other Carcinogens

In addition to a plausible biological mechanism for an independent association, periodontitis may also biologically interact with other carcinogens such as tobacco, alcohol and certain viruses to increase the risk of oral cancer. For example, breaks in the mucosal barrier in the presence of periodontitis can lead to enhanced penetration of carcinogens [73].

An increased production of acetaldehyde (a known carcinogen) from ethanol was also shown in subjects with poor oral health [74, 75]. In addition, periodontal pockets may act as reservoirs for *human papillomavirus* (HPV), a suspected causal factor of oral cancer [76-78].

PERIODONTITIS-HPV SYNERGY

HPV is a DNA virus with a specific tropism for squamous epithelia. More than 120 different HPV types have been isolated to date. Low-risk HPVs, such as HPV-6 and HPV-11, induce benign hyperproliferations of the epithelium such as papillomas or warts which rarely progress to cancer. High-risk oncogenic types such as HPV-16 and HPV-18 are associated with squamous cell carcinoma (SCC). HPV-16 and HPV-18 are capable of transforming epithelial cells. This transforming potential is largely a result of the function of two viral oncoproteins, E6 and E7, which functionally inactivate two human tumor suppressor proteins, p53 and pRb, respectively. Expression of high-risk HPV E6 and E7 results in cellular proliferation, loss of cell cycle regulation, impaired cellular differentiation, increased frequency of mutations, and chromosomal instability [79]. HPV-16 and HPV-18 are a central cause of cervical cancer and are also being investigated for head and neck cancers [80-85].

The average prevalence of HPV DNA in normal mucosa is 10% [80]. In a review of 60 studies, the prevalence of HPV DNA in head and neck SCCs was 25.9%. This was 23.5% in oral, 35.6% in oropharyngeal, and 24.0% in laryngeal SCCs. HPV-16 accounted for a larger majority of HPV+ oropharyngeal SCCs (86.7%) compared with HPV+ oral (68.2%) and HPV+ laryngeal (69.2%) SCCs. Conversely, HPV-18 was rare in HPV+ oropharyngeal SCCs (2.8%) compared with HPV+ oral (34.1%) and HPV+ laryngeal (17.0%) SCCs. Aside from HPV-16 and HPV-18, other oncogenic HPV DNAs were rarely detected in HNCs [82]. Probability of HPV DNA being detected in the oral mucosa increases with increasing degree of dysplasia. Overall, HPV DNA is 2 to 3 times more likely to be detected in precancerous lesions and 4.7 times more likely to be detected in HNCs than in normal mucosa [80].

HPV infection is a necessary but not sufficient cause of cervical cancer. Although majority of the population is infected with HPV at least once in their lives, most HPV infections are cleared rapidly by the immune system and do not result in pathology [86, 87]. Persistence of HPV infection is the strongest risk factor in the development of cancer. Thus, the identification of factors that influence the persistence of HPV infection is critical to facilitate efforts to prevent carcinogenesis. Certain bacterial co-infections in the cervix were shown to act synergistically with HPV infection. For example, several studies have shown that concurrent infection with *Chlamydia trachomatis* is associated with HPV persistence and increased the risk of cervical cancer [88, 89]. Studies suggest that viruses and bacteria, mostly studied in isolation, may in fact cooperate synergistically and should probably be considered as a pathogenic consortium in future investigations [90-96]. In addition, elevated levels of inflammatory cytokines IL-6, IL-1, and TNF-α were shown to modulate HPV E6/E7 gene expression and proliferation in human epithelial cells [97-102].

The strictly epitheliotropic nature of HPV, with a specific anatomic site preference, limits its range of infectivity. HPV infects exclusively the basal cells of the epithelium. Naturally occurring HPV infection begins when the virus gains access to permissive basal cells through wounds or abrasions [79]. In the presence of periodontitis, the periodontal pocket epithelium is usually ulcerated facilitating the access of the virus. In addition, replication of virus is

associated with proliferation of the basal and parabasal cells. The junctional epithelium, located at the bottom of the periodontal pocket, has a very high mitotic rate and consists of basal and parabasal cell layers. It shares properties similar to the squamous-columnar junction of the cervix uteri, where the majority of HPV-associated cancers arise [103]. Integrin $\alpha_6\beta_4$ and syndecan-1, candidate receptors for HPV, are expressed during wound-healing and are found in the pocket and junctional epithelium in the presence of periodontitis [79, 91].

It is not clear where latent HPV resides in the head and neck region. This is an important factor in the effectiveness of therapeutic methods. Latent state would require the presence of infected cells which fail to differentiate [79]. Theoretically, the junctional epithelium fully fits these criteria. It has only basal and suprabasal cell layers and does not differentiate. HPV types 6, 11 and 16 DNA is detected in gingival tissue, gingival cervicular fluid and subgingival plaque from periodontitis sites [104-107]. These observations suggest that periodontal pockets may serve as a reservoir of HPV in the oral mucosa. This could explain the development of gingival papillomas and condylomas frequently found in patients with AIDS and in patients with organ transplants receiving cyclosporin treatment [107]. In these patients, local reactivation of latent HPV infection could occur in gingival tissues.

In summary, chronic periodontitis may facilitate HPVs initial infection and persistence by: 1- providing anatomical predisposition, 2- stimulation of chronic inflammation, 3- causing epithelial damage, and 4- being a source of bacterial co-infections. Evidence of periodontitis-HPV synergy is important to understand the natural history of HPV infection and oral cancer. It has practical implications for intervention since there is no treatment for HPV infection but there is a safe treatment for periodontitis. If this synergy is real, it will provide us a clinical high-risk profile for HPV status as well as for oral cancer.

CONCLUSION

In spite of long years of research, the research of specific microbial etiology has not translated into effective intervention and prevention strategies for oral cancer. In most cases, the infectious agents are ubiquitous, but only a small fraction of infected individuals develop cancer. Our approach is to clinically identify those who are at high-risk of developing oral cancer among infected individuals**.** We hypothesize that chronic periodontitis increases the risk of oral cancer independently as well as by helping HPVs initial infection and persistence. If this hypothesis is true, it will shed light to the etiology of oral cancer and the results will translate into immediate and safe prevention and treatment strategies. Subjects with periodontitis may be targeted as a "high-risk" population, and survival from oral cancer may be improved by early diagnosis and treatment. Preventing or treating periodontitis may result in a decreased incidence, morbidity and mortality of oral cancer.

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