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Acknowledgements

The 11th International Meeting of the Asian Pacific Society of Periodontology (APSP) was held in Bali, Indonesia, on 8 and 9 October 2015. More than 475 delegates from 20 countries (Japan, Korea, the Philippines, Thailand, China, Myanmar, Malaysia, Australia, India, Singapore, Brunei, Saudi Arabia, Nepal, New Zealand, Canada, Fiji, Mongolia, Vietnam, Bangladesh and Indonesia) attended this APSP meeting, which had as it's theme 'Current Issues on Periodontics'. The meeting was opened with an address by Dr Yulianti Kemal, Chairperson of the 11th International Meeting of the Asian Pacific Society of Periodontology Meeting. Additional greetings were given by Dr Yulianti Soeroso, Chairperson of the Indonesian Society of Periodontology; Professor Toshihiko Nagata, President of the Asian Pacific Society of Periodontology; Mr Yoshihiro Kaneda, Sunstar Group; and Mr Kenjiro Kobayashi, Lion Corporation.

The two day program was very full, with 23 presentations from speakers from 13 different countries. In addition, 110 posters were scheduled for presentation. Over the two days, special keynote speakers and representatives from many countries in the Asian Pacific region presented lectures on a wide range of topics which are recorded in this volume as a record of this meeting.

The poster sessions, sponsored by Sunstar, were very successful and in keeping with tradition from previous meetings, six prizes were awarded for the posters judged to be the best on the day. The abstracts for each of these posters are published herein.

This volume contains an impressive array of contributions from all around the Asian Pacific region and serves as a full record of all invited presentations. Each of the chapters covers a unique aspect of current issues in periodontology as we understood them in 2015. I am sure this will serve as a very important reference volume in the years to come.

The APSP wishes to acknowledge the Diamond sponsor Sunstar Inc. and Gold sponsor Lion Corporation. The exhibitors at the 11th APSP Meeting Dental Exhibition were: Batan, Dental Jaya, Dgdent-orthotechnology, FONDACO, GIgi Geligi, Kalbe, Lion Corporation, NIBEC Japan, SDS Sugiarto Dental Supply, Sunstar Inc and Thomasong.

I want to thank my co-editor Yulianti Kemal for her invaluable help in proofreading all of these manuscripts. Finally it is very important to acknowledge that this publication would not have been possible without the untiring efforts of our production editor, Ms Catherine Offler.

P. Mark Bartold
June 2016

Attendees at the 11th APSP Meeting

Lower (L to R): Shinya Murakami, Tin Tun Hla, Narongsak Laosrisin, Amarender Vadivelu, Toshihiko Nagata, Young Ku, Paul Lin, Stanley Lai

Upper (L to R): Isao Ishikawa, Li-Jian Jin, Yuichi Izumi, Nanette Vergel De Dios, Hisashi Watanabe, Yulianti Kemal, Mark Bartold, Yuniarti Soeroso, Warwick Duncan, AR Pradeep, Tara Taiyeb Ali, Errol Devamanoharan, Ki-Young Cho, Yun-Jeong Kim, Seong-Ho Choi, Benjamin Tan

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Chapter 1

Translational Research of Periodontal Regeneration Using Cell Sheet Engineering

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Introduction

The final goal of periodontal therapy is regeneration of the entire periodontal tissues including alveolar bone, periodontal ligament (PDL) and cementum. PDL cells are known as a source of regeneration of the attachment apparatus of the teeth. Based on this assumption, several procedures have been developed for regeneration, such as guided tissue regeneration (GTR) or use of enamel matrix derivatives. The former is aimed at making space for periodontal progenitor cells, whereas the latter is aimed at accelerating and differentiating periodontal progenitor cells from the periodontal ligament area. However complete regeneration of periodontal tissues is still difficult to achieve, especially in severe cases of periodontal defects. As a novel method of periodontal regeneration, transplantation of the necessary stem cells is proposed as a new paradigm (Bartold *et al* 2000).

PDL cells have been transplanted into periodontal defects as a stem cell replacement therapy (Dogan *et al* 2003, Nakahara *et al* 2004). These studies have shown that the transplantation of PDL cells is effective in regenerating periodontal tissues. Several trials have been performed using not only PDL cells, but also bone marrow-derived mesenchymal stroma cells (BMMSCs), adipose-derived stromal cells (ADSCs), gingival fibroblast cells, and alveolar periosteum-derived stromal cells (APSCs) (Kawaguchi *et al* 2004, Mizuno *et al* 2006, Mohammad *et al* 2007, Tobita *et al* 2008). Tsumanuma *et al* (2011) compared different tissue-derived stem cell sheets and reported on their efficacy in periodontal regeneration. Significantly higher periodontal regeneration occurred in the PDL cell group than in the bone marrow mesenchymal stroma cell and alveolar periosteal cell groups. PDL groups showed more newly formed cementum and well-oriented PDL fibers.

Cell sheet transplantation from temperature-responsive culture dishes

Okano *et al* (1993) developed an intelligent cell culture surface that responds to temperature changes in order to detach cells. A temperature-responsive polymer, Poly(N-isopropyl acrylamide) (PIPAAm) is grafted onto cell culture dish surfaces using electron beam irradiation. PIPAAm is a nanoscale material, and is not toxic to cells. This surface is hydrophobic under 37°C, but hydrophilic below 32°C. Thus, the attachment and detachment of cells on this culture surface can be controlled by simple temperature change. Cells can adhere and proliferate similarly to those on commercial tissue culture dish surfaces at 37°C, and cells

detach from the surface spontaneously by reducing temperature below 32°C enabling cell sheets to be obtained from the culture surfaces.

The application of this technology enables cells to be harvested as a sheet without any enzymes such as trypsin, thus cell-cell interactions, cell surface proteins and extracellular matrix proteins are preserved within the sheet. In addition, cell sheets contain intact cell adhesion molecules, and therefore can be transplanted without sutures to the necessary areas of the body.

Basic experiments of PDL cell sheet engineering

Initially, Hasegawa *et al* (2005) reported the characteristics of human periodontal ligament cell sheets transplanted into a dehiscence model in immunodeficient rats. The transplanted cell sheets were found to survive and proliferate in the defect. Newly formed fibers were obliquely anchored on the dentin surface. The results indicated that cell sheet engineering was applicable for periodontal regeneration. Following this, Akizuki *et al* (2005) also investigated periodontal regeneration following the application of PDL cell sheets in beagle dogs. Defects were surgically prepared on the buccal root surfaces of the first molars. The cell sheets were transplanted to the deficient defects. More than 60% of defects treated were regenerated with bone, cementum and PDL formation intact. Nagatomo *et al* (2006) reported on the stem cell properties of human periodontal ligament cells. About 30% of PDL cells underwent osteogenic differentiation when cultured in osteogenic differentiation medium. Flores *et al* (2008) transplanted PDL cell sheets onto the root surface in immunodeficient rats. A new layer of cementum and new attachment of PDL fibers was observed. Thus PDL cells cultured in osteogenic differentiation medium can regenerate both cementum and periodontal ligament. Iwata *et al* (2009) reported periodontal regeneration with the use of multilayered PDL cell sheets in dogs. The bone defects were filled with β-TCP. The experimental sites showed remarkable regeneration of periodontal tissues, confirming the efficacy of PDL cell sheets.

Preclinical study of PDL cell sheet transplantation

Prior to the clinical study of PDL cell sheet transplantation, a protocol for PDL cell sheet engineering was required to be prepared according to the guidelines of Japanese Ministry of Health, Labor and Welfare. Autologous cell transplantation and cell culture with autologous serum methods were selected to avoid potential infection from animal and other sources. Regarding the validation of the quality of the cell sheet, PDL cells were checked for the production of periostin and alkaline phosphatase (Iwata *et al* 2010).

PDL cell culture was performed in the Cell Processing Center (CPC) to avoid any contamination. Operators worked according to the standard operating procedure (SOP). Finally we had to show the safety of the cell sheet as free of bacterial contamination. Karyotype testing was performed in order to demonstrate no chromosomal abnormality related to tumorigenicity. Through the soft agar test, no evidence of tumorigenic potential of cell sheets were found. Regeneration properties of the cell sheet were shown according to the SOP. The cell sheets were demonstrated to maintain periostin and ALP activities (Washio *et al* 2010, Yoshida *et al* 2012). This clinical protocol was finally approved by the government committee in January 2011, but due to the catastrophic earthquake which occurred in Japan on 11

March 2011 our research project was delayed for a year because of interruptions to electrical power and continuation of the CPC.

Clinical study of PDL cell sheet engineering

After the approval of our protocol, a clinical trial was performed. The primary purpose of this clinical trial was to evaluate the safety and efficacy of autologous transplantation of periodontal ligament cell sheets in humans (Iwata *et al* 2015). The study was initiated to treat periodontal patients. The study type was interventional with single arms and not randomized. Blinding and control were not selected. Patients were aged more than 20 years old and a total of 10 patients participated in the study. All patients provided written informed consent according to the GCP. Criteria for patient selection were as follows:

1) Infrabony defects with a probing depth of more than 4 mm after the initial therapy.
2) Radiographic evidence of infrabony defects.
3) A redundant tooth (wisdom tooth) that contains healthy periodontal tissue as a cell source.

Exclusion criteria were as follows:

1) Relevant medical conditions contraindicating surgical interventions (e.g. diabetes mellitus, cardiovascular, kidney, liver, or lung diseases, or compromised immune system).
2) Pregnancy or lactation.
3) Tobacco smoking (more than 11 cigarettes a day).

The transplantation of PDL cell sheets was concluded in 2014, and there were no immediate or delayed transplant-related toxicity. The observation is still continuing, and to date clinical, laboratory, and radiographic evaluations of the patients have shown no serious transplant-related adverse events. Results showed an overall reduction of pocket probing depth and clinical attachment gains at three and six months after the procedure.

Discussion

This clinical study was successfully concluded, however a randomized controlled clinical trial will be required in order to obtain permission for clinical use. The final goal is still far away. A step by step confirmation procedure will produce the final outcome of optimal periodontal regeneration.

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Chapter 2

Emerging Regenerative Approaches for Periodontal Regeneration: From Cytokine Therapy to Stem-Cell Therapy

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Periodontal regeneration: Theory and current status

The basic principle of periodontal therapy is to mechanically remove the bacterial biofilms that cause periodontal disease, along with the necrotic cementum covering the root of the tooth. When performed correctly, this method, which targets the underlying cause, eliminates periodontal inflammation and arrests the periodontal necrosis process. However, lost periodontal tissue cannot be regenerated using this approach. Taking into account the worldwide high morbidity associated with periodontal disease, and that oral health plays a major role in the maintenance and promotion of quality of life in middle-aged and elderly individuals, there is an urgent need to establish a periodontal regeneration therapy with a high success rate.

Periodontal regeneration can be achieved with immature cells within the periodontal ligament, which is a 100 to 200 μm thick tissue located between the cementum and the alveolar bone. Interestingly, many cells in the periodontal ligament have been reported to constantly express high levels of various molecules such as RUNX-2 and alkaline phosphatase, which serve as indicators of osteoblast and cementoblast differentiation and play important roles in maintaining the function of generating alveolar bone and cementum. Furthermore, the periodontal ligament has been shown to contain not only osteoblasts and cementoblasts, but also undifferentiated mesenchymal stem cells (Seo *et al* 2004). Thus, by activating the stem cell function of cells present in the periodontal ligament of affected teeth, current treatment strategies attempt to regenerate damaged periodontal tissue.

A representative example of periodontal regeneration therapy currently in use is guided-tissue regeneration (GTR), developed in the 1980s (Nyman *et al* 1997). The affected periodontal area is covered by a GTR membrane that prevents entry of cells from the gingival epithelium and connective tissue, thus preventing the formation of a long epithelial attachment via downgrowth of the gingival epithelium. Subsequent delivery of periodontal ligament-derived cells (including periodontal stem cells) to the affected periodontal site allows induction of lost tissue regeneration. The enamel matrix derivative (EMD), used clinically since the 1990s, is secreted from Hertwig's epithelial sheath during tooth development and is said to possess the ability to promote cementum formation (Lindhe 1997). EMD, produced from the mandibular tooth germ of six month old pigs, is currently available commercially as Emdogain®.

Despite previous reports on the efficacy of

GTR and EMD as periodontal regeneration therapies, the limitations of operability and applicability still need to be addressed.

Induction of periodontal regeneration using cytokines

Cytokine therapy

Cytokine therapy has drawn attention as a next generation therapy that attempts to induce periodontal regeneration by local administration of cytokines. These cytokines would activate processes such as migration of periodontal ligament cells to the affected area, proliferation of cells at the site, as well as differentiation into osteoblasts and cementoblasts. Currently available commercial products in clinical use include GEM 21S®, a combination of platelet-derived growth factor and β-tricalcium phosphate (TCP; a biodegradable bone graft material) used to induce periodontal regeneration, and INFUSE®, a combination of bone morphogenetic protein(BMP)-2 and bovine type 1 collagen, used in sinus augmentation and ridge augmentation. Both of these have been approved by the United States Food and Drug Administration and are commercially available in the United States.

Induction of periodontal regeneration by basic fibroblast growth factor

Fibroblast growth factors (FGF), designated FGF-1 through -23, are a family of proteins found in the brain and pituitary tissue that promote the growth of fibroblasts. FGF-2 is known to induce the growth of not only fibroblasts, but also of several other types of cells such as vascular endothelial cells, neuroectodermal cells, osteoblasts, chondrocytes, vascular smooth muscle cells and epithelial cells. In particular, FGF-2 has drawn a great deal of attention in the field of regenerative medicine primary for two reasons: it's powerful pro-angiogenic effect, and it's capacity to induce the proliferation of undifferentiated mesenchymal cells, while leaving their pluripotency intact. One example of the clinical application of FGF-2 is Fiblast®, a therapeutic agent used in decubitus ulcers and other intractable skin ulcerations, approved for clinical use in Japan.

We previously examined whether FGF-2 induces periodontal regeneration using beagle dogs and non-human primates (Murakami *et al* 2003, Takayama *et al* 2001). We created experimental class II furcation defects in mandibular molars, filled the defects with a gelatinous carrier containing 0.1 to 0.4% FGF-2, and conducted tissue morphology assessments six and eight weeks after FGF-2 administration. We found that local administration of FGF-2 induced periodontal regeneration with statistically significant volumes of newly formed bone, trabecula and cementum. Sharpey's fibers were also reproduced at the defect site, thus confirming the reconstruction of fibrous attachments (Takayama *et al* 2001).

Furthermore, to examine the effects of FGF-2 *in vivo*, we developed a beagle dog three wall periodontal defect model and histologically measured the quantity of regenerating tissue, number of proliferating cells at 3, 7, 14 and 28 days, and angiogenesis at 7 days after administration of FGF-2. In addition, we used real-time polymerase chain reaction to analyze the expression of osteogenic genes in the regenerated tissue at 7 and 14 days (Nagayasu-Tanaka *et al* 2015).

We found that FGF-2 significantly diminished the clot, as well as formation of connective tissue, formation of new bone and angiogenesis at the root surface at 7 days. Further to this, we found that nuclear antigen-positive proliferating cells emerged from existing bone and periodontal ligament and spread to the entire defect site, thus

demonstrating that administration of FGF-2 led to the early emergence of, and an increase in, the total number of cells. Interestingly, we also confirmed that administration of FGF-2 resulted in early increased expression of BMP-2, osterix, alkaline phosphatase and osteocalcin compared to the control group. This finding indicated that the induction of bone formation by FGF-2 may in part be due to the enhanced expression of BMP-2.

Next, when we examined the effect of FGF-2 on *in vitro*-maintained periodontal ligament (PDL) cells, we found the following:

1) FGF-2 directly stimulated the proliferation of PDL cells.
2) FGF-2 induced the production of vascular endothelial growth factor (VEGF), and the cooperation of VEGF and FGF-2 induced the proliferation and migration of PDL cells in addition to promoting angiogenesis.
3) FGF-2 induced the synthesis of high-molecular mass hyaluronan and activated the migration of PDL cells.
4) FGF-2 induced the production of osteopontin and inhibited the apoptosis of PDL cells (Yanagita *et al* 2014, Shimabukuro *et al* 2005, Terashima *et al* 2008).

These results indicate that, in the initial stages of wound healing, FGF-2 induces the growth and migration of PDL and other periodontal stem cells while maintaining their undifferentiated state, increases the density of periodontal stem cells at the healing site and induces the formation of new tissue by filling the entire defect site with cells at an early stage. Furthermore, we believe that by inducing angiogenesis and the production of specific extracellular matrices to prepare a local environment suitable for periodontal regeneration, FGF-2 quantitatively and temporally promotes the regeneration of periodontal tissue such as alveolar bone and cementum (Murakami 2011).

Clinical trials with FGF-2

In 2001, a nationwide 13 center phase IIA clinical trial (double-blinded trial with concurrent control of dose responses, including a placebo) was initiated in Japan to examine the safety and efficacy of FGF-2 to induce periodontal regeneration, using a placebo and an investigational formulation containing 0.03%, 0.1% or 0.3% FGF-2. Local administration of 0.3% FGF-2 in double- or triple-walled alveolar bone defects induced statistically significant alveolar bone neogenesis, as shown by standardized radiographs (Kitamura *et al* 2008). Next, a nationwide, 24 center phase IIB clinical trial (a dose-response trial) was initiated in 2005 to examine the safety and efficacy of FGF-2 using a placebo and an investigational formulation containing 0.2%, 0.3% or 0.4% FGF-2. As with the phase IIA clinical trial, 0.3% FGF-2 was found to induce statistically significant alveolar bone neogenesis (Kitamura *et al* 2011, Murakami 2011) (Figure 1). No statistical significant difference in efficacy was observed between 0.3 and 0.4% FGF-2. In addition, no safety related issues were observed during either clinical trial period.

Induction of periodontal regeneration by stem cell transplantation

As previously stated, current periodontal regeneration therapies employ periodontal stem cells in the periodontal ligament of the affected tooth. However, the number of these stem cells decreases with age and their ability to form hard tissue declines (Zheng 2009). Therefore, in order to supplement this deficiency, mesenchymal stem cells obtained from another site could be transplanted to the periodontal defect site.

When using mandibular periosteum, a tissue fragment is harvested from the

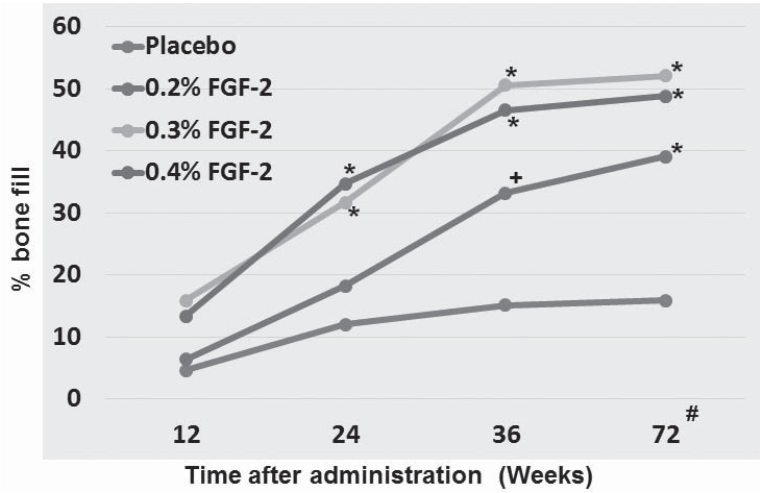

Figure 1. Percentages of new alveolar bone formation.
Data was obtained after breaking of the blind. * P<0.001: Compared to the placebo group of each time point. P=0.003: Compared to the placebo group of each time point.

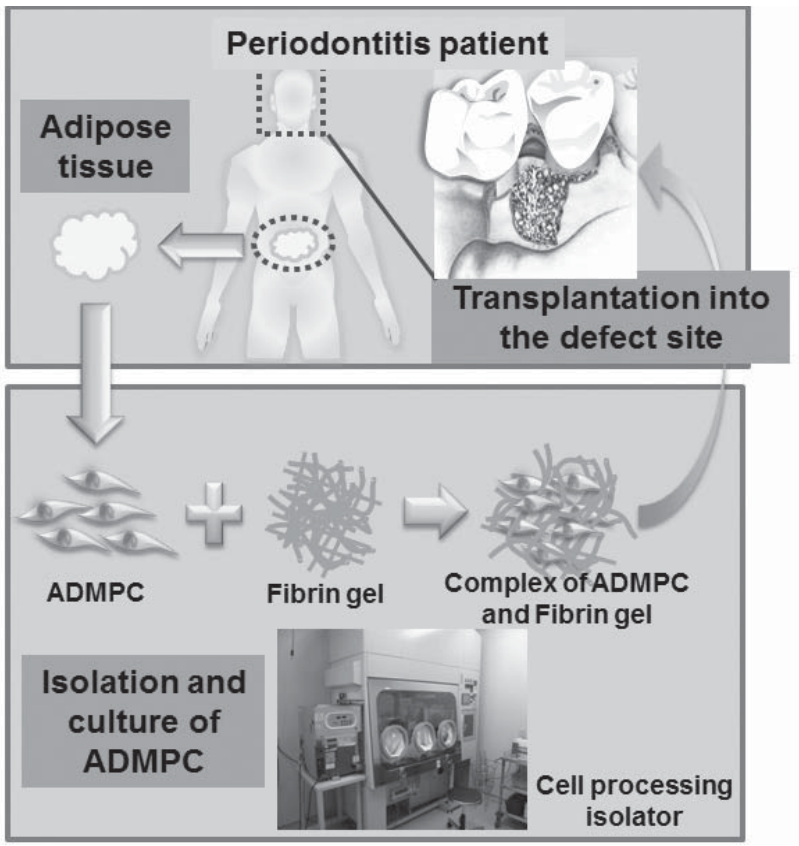

Figure 2. Periodontal regeneration by transplantation of adipose-tissue derived multi-lineage progenitor cells.

periosteum and cultured outside the body to form cells which grow in sheets (periosteum-derived cells, which are expected to possess high osteogenic potential). Transplantation of these cells along with hydroxyapatite to the periodontal tissue defect site is reported to induce periodontal regeneration (Okuda *et al* 2009). One study reported that periodontal ligament tissue was collected from the surfaces of wisdom teeth or other teeth and then cultured outside the body. The tissue was grown into sheets of cells, which were transplanted to the root surface of the exposed periodontal tissue defect, while β-TCP was transplanted in the adjacent defect site with the aim to regenerate periodontal tissue (Iwata *et al* 2009). This stem cell therapy is also reported to induce periodontal regeneration. In one example of the application of bone marrow cells for periodontal regeneration, a gel composed of bone marrow cells and platelet-rich plasma harvested from the iliac crest and other sources was transplanted to the periodontal defect site, thereby inducing periodontal regeneration (Yamada *et al* 2006). In another investigation, cells obtained from bone marrow were stimulated with basic fibroblast growth factor (FGF-2) outside the body to increase the number of cells and then mixed with a collagen gel scaffold and transplanted to the periodontal defect in order to induce periodontal tissue regeneration (Kawaguchi and Kurihara 2008).

In our department, we focused on adipose tissue, which is considered to be safer and less of a burden on the patient during harvesting. We are currently investigating the use of undifferentiated mesenchymal stem cells present in this tissue for periodontal regeneration (Figure 2). To date, we have found that adipose tissue-derived multi-lineage progenitor cells (ADMPC) possess the capacity to differentiate into osteoblasts, cardiomyocytes, hepatocytes, insulin-producing cells and periodontal ligament cells (Komoda *et al* 2010, Okura *et al* 2009, Okura *et al* 2010, Ozasa *et al* 2014). Additionally, ADMPC-conditioned medium contains molecules that induce periodontal ligament cell differentiation, one of which, insulin-like growth factor binding protein 6, has been shown to be involved in periodontal regeneration (Sawada 2015). Furthermore, transplantation of ADMPC along with fibrinogen gel in a defect site in a beagle dog model of periodontal disease (class II furcation defect) was found to induce significant periodontal regeneration at the transplant site (Ozasa *et al* 2014). A clinical study is currently in progress to assess the safety and efficacy of this therapy.

Cytokine therapy and cell transplantation therapy: Future prospects

In the present review, we introduced strategies for next-generation periodontal regeneration using FGF-2 therapy and ADMPC transplantation therapy. With regards to FGF-2 formulation, clinical trials are currently in progress to determine the safety and efficacy of FGF-2 alone. However, the next step would be the development of a novel FGF-2 carrier which possesses a "scaffolding" function with bone conductivity. There is also potential for application of FGF-2 therapy in bone augmentation and implant treatment. However, at present FGF-2 therapy remains a novel therapy. It is strongly hoped that close examination of its applicability, safety and efficacy will lead to further development.

In addition, while current clinical studies use fibrin gel as an ADMPC scaffold, there is greater anticipation for the development of optimal scaffolds, which possess space-making capacity and are customized for periodontal regeneration that support ADMPC growth and differentiation into osteoblasts and cementoblasts. There is also great

hope for the future establishment of a true customized periodontal regeneration therapy that combines cytokines, stem cells and scaffolding under optimal conditions.

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Chapter 3

The Use of Stem Cells for Periodontal Regeneration

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Introduction

Tissue engineering is a specialized field of science based on the principles of cell biology, developmental biology and biomaterial science, aimed at fabricating new tissue to replace lost or damaged tissues. Regenerative medicine is an emerging branch of medicine with the goal of restoring organ and/or tissue function using a biological approach. Stem cells in regenerative medicine can serve as gene delivery systems. Stem cells are unspecialized cells that can perform self-renewal as well as self-differentiation into functional phenotypes. A major value of stem cells in regenerative medicine is their potential to become different cell types. In other words, dental stem cells have a promising future for tissue regenerative medicine. Stem cells can be collected from the dental pulp of both deciduous and permanent teeth, periodontal ligament, apical papilla, dental follicle and gingiva.

Dental stem cells, moreover, can be differentiated into a range of tissues useful for regenerative medicine in dentistry, including dentin, pulp, bone, muscle, adipose tissue and neurons. Dental stem cells derived adult stem cells (DSCs) in the periodontal ligament (PDLSC), dental pulp (DPSC) and attached gingiva (GSC) can represent alternative sites for cell harvesting that are less invasive compared to bone marrow stem cells. Finally, DPSCs and PDLSCs also demonstrate their potential for regenerating human dental tissue *in vivo* and are capable of forming alveolar bone and periodontal ligament.

Background

Stem cells have recently been used in tissue engineering. The use of stem cells has become a current trend in the medical world. For instance, stem cells can be used to restore hard and soft periodontal tissues in the periodontal regeneration process as a treatment for periodontal defects. As a result the use of stem cells has been considered a promising treatment. Therefore, the use of stem cells in periodontal treatment has recently been developed to stimulate the regeneration of damaged periodontal tissues (Cavaleri *et al* 2009, Neel *et al* 2014, Taba *et al* 2005).

Furthermore, many studies with a biological approach have demonstrated that the use of stem cells has successfully stimulated the regenerative process in various tissues and organs. Thus, the use of stem cells has been considered as a prospective alternative to periodontal treatment. The technology of using stem cells is known as tissue engineering, which involves creation of new tissue with suitable cell and molecular biology for repairing and regenerating the

periodontium using a reconstruction form of isolated cells with biocompatible scaffolds and growth factors (Huang *et al* 2010, Lin *et al* 2008)

Stem cells in dentistry

The use of stem cells in dentistry has rapidly developed. Many studies on stem cells have been conducted to overcome various dental abnormalities in dentistry. Biological properties of stem cells are derived from embryonic stem cells, embryonic germ cells and adult stem cells/somatic stem cells. A stem cell has several abilities to renew itself and differentiate into multipotent cells as well as perform *ex vivo* and plasticity cultures. Based on their potential, stem cells can be differentiated into totipotent, pluripotent, multipotent and oligopotent/monopotent (Cavaleri *et al* 2009, Shruthi *et al* 2012).

The stem cell, moreover, can be considered as a totipotent cell since it is able to differentiate itself into all cell types of the main embryonic layers, namely ectoderm, endoderm and mesoderm. Consequently the totipotent cell can differentiate into a complete organism with a peripheral and central nervous system if implanted into a functional uterus. In mammals, a totipotent cell only can be found in zygotes and the early stages of division of the blastomere. In accordance with the development of differentiation, the totipotent stem cells in the zygote can divide themselves into the outer and inner layer cells. The cells in the inner layer can become all cell types in the body, but cannot exist without the outer layer cells which become the placenta (Huang *et al* 2012, Lin *et al* 2008).

In addition, stem cells located in the inner layer can be considered pluripotent cells. When pluripotent stem cells continue to divide these cells will begin to specialize further and then become specialized tissue progenitor. At this stage the cells are called multipotent cells. In other words, they can differentiate into multiple cell types within an organ. For example, blood stem cells or multipotent hematopoietic stem cells can develop into erythrocytes, leukocytes or thrombosis. Monopotent or oligopotent stem cells, on the other hand, can only develop into one or more specialized cell types. Mesenchymal cells can differentiate into bone, muscle, fat or certain other connective tissues (Huang *et al* 2010).

The current development of dental stem cells indicates that stem cells have shown great potential for periodontal tissue repair. Adult stem cells directly derived from dental tissues or dental stem cells have recently been developed. Dental stem cells have been divided into two groups. The first group consists of dental pulp stem cells (DPSCs), namely stem cells from human exfoliated deciduous teeth (SHED) and adult dental pulp stem cells (ADPSCs), while the second group consists of stem cells from the dental follicle (DFSCs) and periodontal ligament stem cells (PDLSCs) (Huang *et al* 2010, Nace *et al* 2014, Neel *et al* 2014).

Many studies have been conducted to produce mesenchymal stem cells that have osteogenic potential, but they are still not sufficiently developed to be used in clinical treatment. The use of scaffold, combined with growth factors and stem cells, needs to be further developed since stem cells have the properties needed for tissue engineering (Naito *et al* 2011).

A recent study using stem cells derived from human gingival tissue, called human gingival mesenchymal stem-derived cells (GMSC), showed some unique properties similar to mesenchymal stem cells (MSCs) derived from bone marrow and other postnatal tissues. Gingival MSCs are easy to isolate and proliferate more rapidly than bone marrow MSCs (Marynka-Kalmani *et al* 2010, Tomar *et al* 2010, Zang *et al* 2012). Research conducted by Zhang *et al* (2009) also showed

that a colony derived from GMSCs has the capacity for self-renewal. *In vivo* capacity differentiation can support stem cells as their properties further.

In addition, compared with MSCs derived from several adult dental tissues, such as DPSCs and PDLSCs, GMSCs reveal several profiles, such as similar cell surface molecules, high proliferation rate and increased population multiplication, thus *ex vivo* cells can easily be developed for several cell-based clinical applications. In other words, these findings show that human gingiva, which is easily accessible from the oral cavity or tissue samples discarded from some dental procedures, can serve as a unique source of postnatal stem cells with potential therapeutic functions in the regeneration and repair of tissues (Mitrano *et al* 2010, Samad 2011, Zhang *et al* 2009).

Research was conducted at Dentistry Faculty of Universitas Airlangga. This research was conducted with GMSCs using immunophenotyping technique on stem cells derived from gingiva with CD105, CD34 and CD90 markers. Next, measurement using flow-cytometry showed good results. Based on the results, CD105 and CD90 could decrease the passage to 14. Similarly, research conducted by Kamadjaya *et al* (2014) also showed the ability of human amniotic membrane mesenchymal stem cells (hAMSC) in maxillofacial reconstruction. Thus, these abundant mesenchymal stem cells can be considered multipotent cells useful for healing bone. Research conducted by Nike (2015) showed that human umbilical cord mesenchymal stem cells (hUCMSCs) can be considered potential materials for the treatment of abnormalities involving bone with certain markers, such as RUNX-2, osteoblasts and increased collagen type 1.

Conclusion

Stem cells are useful materials for various treatments in dentistry. Stem cells have recently been rapidly developed at the Dentistry Faculty of Universitas Airlangga. Thus, further investigation into the application of these useful stem cells is required.

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Chapter 4

Periodontal Regeneration: The Philippine Experience

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Introduction

Many studies have been performed to assess the efficacy of periodontal regenerative procedures. Systematic reviews have also been conducted and demonstrate that there is wide variability in the treatment outcomes using regeneration procedures. It is acknowledged that some benefits over that of conventional periodontal treatment procedures can be achieved, but many factors and complex biological processes seem to influence the treatment outcomes. Therefore, predictability of results is difficult to ascertain. In the same light, achieving complete regeneration as a treatment goal remains unrealistic (AAP Academy Report 2005).

Periodontal regeneration techniques were developed to restore periodontal structures lost through disease. These techniques are intended to regenerate the supporting apparatus of the tooth, namely the alveolar bone, periodontal ligament, and cementum on previously diseased root surfaces (American Academy of Periodontology 2001). At present, bone replacement grafts, guided tissue regeneration, root surface modification procedures and flap management techniques are the principal treatment modalities used for periodontal regeneration. Of these procedures, bone grafting and guided tissue regeneration were reported to have the most histologically documented evidence of periodontal regeneration according to the AAP Academy Report in 2005. Recently, a number of newly developed technologies were introduced to the profession as emerging approaches to periodontal regeneration. These included protein and peptide therapy, cell-based and stem cell therapy, genetic therapy, and the use of bone anabolics, scaffolds and laser (Cochran *et al* 2015). However, a consensus report from the American Academy of Periodontology Regeneration Workshops held in 2015 indicated that while these emerging technologies seem to be feasible approaches, the mechanisms of action are not yet well understood, and there is still presently insufficient evidence to warrant clinical recommendations (Cochran *et al* 2015).

Periodontal regeneration practices in the Philippines

The Philippines, despite its rising population of 103.5 million as of 26 August 2015, has only about 20 accredited practitioners of periodontics (Countrymeters 2015). As such, it is not surprising that periodontal regeneration procedures are also being performed by general dental practitioners and practitioners of other dental specialties. In previous surveys performed by the Philippine Society of Periodontology (PSP), only about 4 to 8% of dentists surveyed performed other periodontal procedures

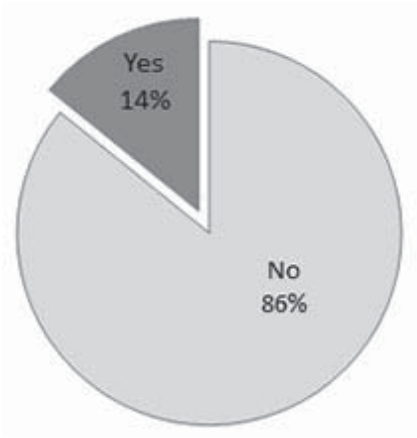

Figure 1. Percentage of respondents who perform periodontal regenerative procedures.

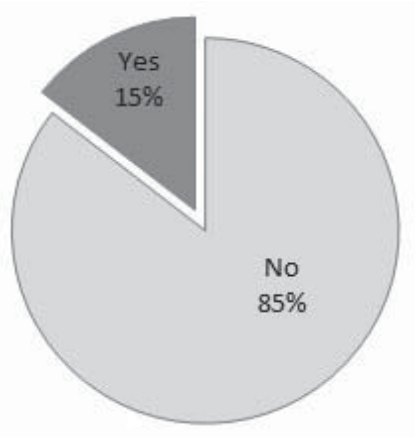

Figure 2. Percentage of respondents who perform bone augmentation procedures.

apart from scaling and prophylaxis in their clinical practices (Vergel de Dios *et al* 2000, Vergel de Dios *et al* 2010, Vergel de Dios *et al* 2014). With the introduction of dental implants as an alternative treatment modality in clinical practice to replace missing or lost teeth, we decided to once again conduct a survey to determine whether there has been an improvement in the utilization of periodontal regeneration by dental practitioners in the Philippines.

The results of the present survey showed that out of 303 respondents, 86% still do not perform periodontal regenerative procedures (Figure 1). Bone augmentation is performed by 15% of the respondents surveyed (Figure 2). The most commonly performed bone grafts are allografts (26%) while the use of other bone graft types (autografts, xenografts, and alloplasts) was about 20% for each (Figure 3). Bone grafts are either osteogenic, osteoconductive or osteoinductive and these have been used as fillers in intrabony defects (Bartold 2015, Darby 2011). The use of bone grafts in periodontal regeneration is based on the supposition that when placed on a treated root surface, the graft will stimulate the formation of a connective tissue attachment (Bartold 2015, Karring and Lindhe 2015). Earlier studies, both in humans and animals, have however shown that this concept is questionable. While placement of bone fillers may lead to bone deposition adjacent to pre-existing bone, results from studies have shown that healing occurs with a long junctional epithelium rather than a new connective tissue attachment (Caton and Zander 1976, Listgarten and Rosenberg 1979).

Systematic reviews have found that periodontal regeneration may be induced with the use of membranes, bone grafts with membranes and/or other regenerative materials (International Academy of Periodontology 2015). In order for regeneration to take place when performing guided tissue regeneration (GTR), the use of materials which would serve as a functional epithelial seal is required. The downgrowth of epithelial cells has to be prevented and sufficient time should be provided for periodontal cells to migrate to the healing site. When bone grafts are used in GTR, they serve a vital role by filling the defect and preventing the collapse of the membrane.

In our survey, 80% of those who perform bone augmentation procedures also use a periodontal membrane (Figure 4). Non-bioabsorbable membranes were

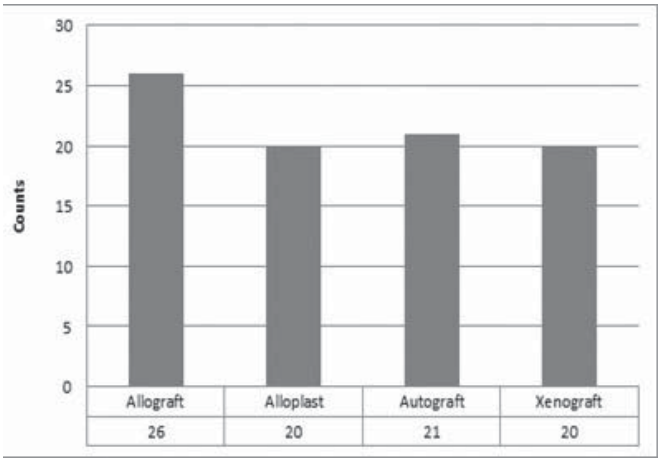

Figure 3. Type of grafts used by those who perform bone augmentation (N=44).

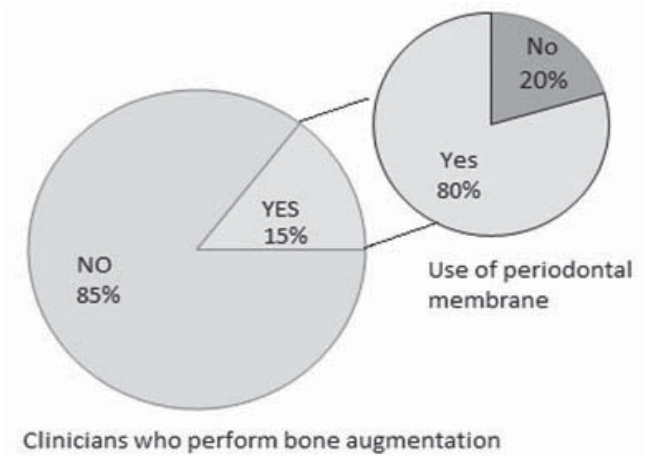

Figure 4. Proportion of clinicians who perform bone augmentation and use periodontal membrane.

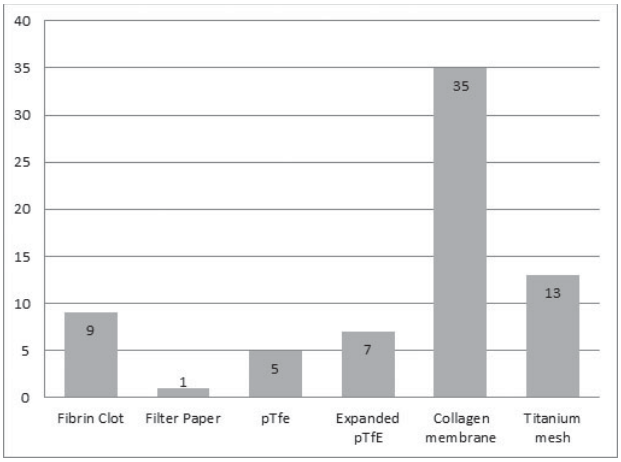

Figure 5. Periodontal membrane used by the clinicians who perform bone augmentation.

Reason	Counts
Ease of manipulation	24
Had most success using this type of membrane	20
No need for surgical re-entry	15
Cost of the membrane	11

Table 1. Reasons for preference of type of periodontal membrane.

the first to be developed and used for periodontal regeneration. Expanded polytetrafluoroethylene (pTFE), a commonly used non-bioresorbable membrane, has the advantage of having adequate stiffness to allow for proper adaptation to the bone defect and provide for space maintenance. However, the disadvantage in using this material is that a second surgical procedure has to be performed for its removal and it is more likely to be exposed in the oral environment.

Bioabsorbable materials were developed to address the disadvantages associated with non-bioabsorbable membranes. Examples of such materials are polyglycoside synthetic polymers, collagen and calcium sulphate. In the Philippines, collagen membranes were reported to be the most commonly used periodontal membrane (Figure 5). Collagen membranes were observed in both clinical trials and animal studies to be as effective as other types of membranes used in GTR procedures in terms of promoting new connective attachment and warding off the downgrowth of epithelium (AAP Academy Report 2005). As with other bioabsorbable membranes, collagen exhibits better tissue compatibility. It also has been found to have a haemostatic function by aggregating platelets, thus facilitating early clot formation and wound stabilization. Moreover, the membrane composition can be altered to regulate rate of resorption. Most importantly, placement of collagen membrane does not require surgical re-entry. The disadvantage, however, is the lack of rigidity (American Academy of Periodontology 2005, Darby 2011).

Reasons cited for membrane preference are ease of manipulation, successful treatment outcomes using the particular membrane, no need for surgical re-entry and the reasonable cost of the periodontal membrane material (Table 1).

In the last Asian Pacific Society of Periodontology meeting held in Japan in 2013, we reported that implant therapy has gained an acceptance among general dental practitioners (GDPs) in the Philippines (Vergel de Dios *et al* 2014). Among the present respondents, we observed that the most common indication for performing periodontal regeneration procedures was to augment deficient bone recipient sites prior to implant placement (Table 2). Implant training courses offered by distributors of dental implant systems continue to take place and are attended by GDPs. These training programs usually include exercises on bone augmentation procedures and may explain our observations.

It was also observed that regeneration procedures are performed to manage periodontal defects. It was determined that furcation involvement, particularly Class II defects can be managed predictably by periodontal regeneration (Reddy *et al* 2015). In a systematic review conducted by Needleman *et al* (2005), it was reported that GTR was superior to open flap debridement (OFD) in terms of clinical attachment gain. It was likewise reported that the combination of GTR and bone grafts resulted in greater probing depth reduction but results were

Criteria	Performs periodontal regeneration?		Total
	Yes (45)	No (258)	
Pre-implant placement	28	5	33
Pre-prosthetic treatment procedure	11	1	12
Pre-restorative treatment procedure	1	4	5
Remove tooth mobility in malpositioned teeth	1	1	2
Manage periodontal defects	16	1	17

Table 2. Most common indication for regeneration procedures.

Criteria	Total
Radiographic monitoring of bone fill	26
Gain of clinical attachment	20
Absence of clinical complications for 5 years	16
Absence of bleeding for 3 years	13
Absence of patient's symptoms for 1 year	12

Table 3. Criteria employed to measure/assess success of regeneration procedures.

similar to GTR alone for attachment gain.

The Consensus Report from the AAP Regeneration Workshop concluded that periodontal regeneration to manage intrabony defects on previously diseased roots is feasible (Reynolds *et al* 2015). The report further stated that improvement in clinical parameters, namely gains in attachment levels, reduction of pocket probing depths and increases in radiographic bone height, together with a general improvement of periodontal health, provide the evidence for success of periodontal regeneration procedures.

Practitioners of periodontal regeneration in the Philippines measure the success of the procedure by using clinical parameters as criteria. Most would use radiographic monitoring of bone fill and gains in clinical attachment as assessment tools to measure success. Others would include the absence of clinical complications and absence of bleeding for five and three years respectively as indicators for success (Table 3).

It is understood that periodontal regeneration is a biological process which is measured or defined histologically, but this manner of assessment cannot be applied in the clinical setting (International Academy of Periodontology 2015). Validation of the occurrence of regeneration cannot be made by improvements in clinical parameters. The working group thus stated that from this perspective, the outcome of periodontal regenerative procedures can best be considered as periodontal reconstruction rather than true periodontal regeneration. The working group likewise concluded that with periodontal regeneration, aside from improvements in clinical and radiographic parameters, the potential for prolonging the retention of a diseased tooth and improving its prognosis is greater.

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Chapter 5

Human Viruses as Risk Indicators for Periodontal Disease

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Background

Periodontitis is one of the most complex infectious diseases of the human body. Infectious agents must be able to colonize periodontal sites, overcome local host defences, proliferate in periodontal sites and participate in the breakdown of periodontal tissues (Slots and Genco 1984). Interplay between the periodontal infectious agent and the host immune responses reflects the dynamic which develops in a multistep process periodontitis. The oral cavity supports more than 700 bacterial species and the periodontal pocket area harbors more than 400 bacterial species (Parra and Slots 1996). Periodontopathic bacteria, such as *Porphyromonas gingivalis* and *Tannerella forsythia*, possess virulence factors involved in colonizing periodontal sites, neutralizing local host defences and destroying periodontal tissues, and individual periodontal lesions may harbor millions of genomic copies of herpesviruses as well as papillomaviruses, human immunodeficiency virus, human T-lymphotropic virus type 1, torquetenovirus, hepatitis B and C viruses, Epstein-Barr Virus and cytomegalovirus (Amaliya 2012, Holt and Ebersole 2005, Saygun *et al* 2008, Slots 2009, Slots and Genco 1984, Slots and Ting 1999). Herpesvirus-infected periodontal sites tend to exhibit more breakdown than herpesvirus-free sites, and herpesviral active infection is associated with an elevated risk of progressive periodontal disease (Slots *et al* 2005). Additionally, healthy periodontal sites of periodontitis patients may harbor more herpesviruses than healthy periodontal sites of individual with generally healthy periodontium (Dawson 2009). The host immune response attempts to control both pathogenic bacteria and viruses in periodontal sites. However, it is still unclear if various immune mediators, such as certain cytokines and chemokines exert a primarily protective or destructive role in periodontal disease.

Sufficient levels of proinflammatory substances protect against bacterial periodontopathogens, however excessive level will destruct periodontal tissue (Rateichak 2009). Successful immune control of a periodontal infection depends on a highly coordinated series of host defences (Cutler and Teng 2007). The host identifies pathogens as foreign bodies by recognizing pathogen-associated molecular patterns (Mahanonda and Pichyangkul 2007). Pathogen recognition receptors and signaling pathways subsequently activate cells of the immune system. Cytokines mediate the interaction and regulation of immune cells. Optimally, the host executes immune responses sufficient to control the pathogens, but also ensures suppression of excessive immune reactions in order to limit the pathological consequences of inflammation. If uncoordinated, the host

immune response by itself may cause pathosis.

Herpesviruses

The eight human members of the herpesvirus family infect parenchymal cells, connective tissue cells, epithelial cells, various hematopoietic cells and other cell types, and can cause a variety of illnesses by mechanisms that are direct, indirect or immunoregulatory (Pellett 2007, Slots 2005, Slots 2009). In neonates it causes gingivostomatitis or a subclinical infection and can then lie dormant in the trigeminal ganglion. In response to external stimuli such as stress, cold weather or other virus infection, the virus can be activated and cause herpes labialis (cold sores) (Wade 2009). Current evidence strongly implicates viruses, particularly cytomegalovirus and other herpesviruses, in the pathogenesis of periodontitis (Amaliya 2012, Slots 2007, Slots 2010). The majority of adults are carriers of Epstein-Barr virus and cytomegalovirus. Once infected, a person harbors the herpesvirus for life. Clinical and experimental evidence show that viruses can directly affect the homeostasis of microbial population as well as modulate the host response to bacterial biofilms. Active herpesvirus infection evokes strong innate and adaptive immune responses, which include both immune activation and immune suppression (Crough and Khanna 2009, Mocarski *et al* 2007, Rickinson and Kieff 2007). Key effector cells of the innate immune system are dendritic cells, monocyte/macrophage and natural killer cells, whose function is to limit the viral burden until cells of the adaptive immunity become available to suppress the infection. The effector cells recognize viral proteins via toll-like receptors, natural killer cell receptor or other pattern recognition receptors. Herpesvirus DNA reacts with toll-like receptor 9, which is significantly up-regulated in periodontitis lesions compared with gingivitis lesions (Kajita *et al* 2007a, Kajita *et al* 2007b). Cells of innate immunity employ cytokine secretion and cell-mediated cytotoxicity as the primary anti-herpesvirus effector mechanisms. Macrophages and polymorphonuclear leukocytes can destroy antibody-coated virions or virus-infected cells via reactive oxygen species, nitric oxide and activated caspases. Natural killer cells are an important source of interferon-gamma and are able to kill herpesvirus-infected cells via virus-specific antibody-dependent cell-mediated cytotoxicity or via antibody-independent mechanisms. In addition, natural killer cells share similarities with cytotoxic T cells and may play a role in adaptive immunity (Sun *et al* 2009).

Herpesvirus infections in immunocompetent individuals also induce antibody production against herpesvirus proteins and patients with periodontitis exhibit an elevated level of antibodies against herpesviruses, however it does not ensure a favorable clinical outcome (Hochman *et al* 1998, Kajita *et al* 2007a, Kajita *et al* 2007b, Svahn *et al* 2006).

Bacteria

Bacteria are the predominant microorganism in the human mouth. They are present in large numbers, of around 100 million per milliliter of saliva, and are the primary constituent of dental plaque, where they are around 1 billion per milligram. All types of bacteria are found; Gram-positive and Gram-negative, obligate aerobes, facultative anaerobes, obligate anaerobes, saccharolytic and proteolytic (Wade 2009). Bacterial infections evoke functionalities of both the innate immune system and the adaptive immune system. Bacterial species attach to specific toll-like receptors to establish some degree of specificity in the innate immune system and subsequently in the adaptive immune system (Hirschfield 2001, Kinane 2007). Bacterial pathogens detected by toll-like receptors

on the surface of macrophages activate NF-αB mediated transcription of cytokines and chemokines (Nagasawa 2007). Macrophage-released cytokines and chemokines recruit neutrophils in the innate immune system and serve as antigen-presenting cells for lymphocytes in the adaptive immune system. Neutrophils phagocytize and kill ingested bacteria by means of reactive oxygen species, anti-microbial proteins, degradative enzymes and other microbial pathways (Dennison 1997).

Extracellular pathogenic bacteria activate Th2 cells, which release anti-inflammatory cytokines and commit B cells to antibody production. *P. gingivalis* seems to primarily evoke a Th2-type response in periodontitis patients (Houri-Haddad *et al* 2007). Antibacterial antibodies of the IgG1 subclass play an important role in opsonization and complement activation (Dennison 1997). Lipopolysaccharide of *P. gingivalis* and of other periodontopathic bacteria can also activate complement via the alternative pathway and induce the release of pro-inflammatory cytokines (Dixon 2004). Complement assists antibodies by acting as an opsonin, by lysing bacterial cells and by attracting lymphocytes and neutrophils to the site of infection.

Intracellular bacteria trigger a Th1-mediated immune response, which includes the release of interferon-gamma, pro-inflammatory cytokines and the IgG2 antibody isotype (Elkins 2007, Titbal 2008). IgG2 serum antibody occurs at high levels in patients with localized aggressive periodontitis, and despite being a less efficient opsonin than IgG1 and IgG3, seems to protect against tissue destruction (Schenkein 2007). *P. gingivalis*, *A. actinomycetemcomitants* and other periodontal species have the ability to invade cells of the periodontium and thus may trigger a Th1, as well as a Th2, immune response (Christersson 2002, Colombo 2006, Lamont 2002, Li 2008, Rudney 2005, Tribble 2009, Vitkov 2005). *P. gingivalis*-specific T cells can produce both Th1 and Th2 cytokines irrespective of the type of antigen-presenting cells (Gemmel 2002).

Herpesviral-bacterial model of periodontitis

The finding of abundant herpesviruses in periodontitis lesions redefines the pathogenicity of the disease. The core of the development of periodontitis proceeds from bacteria to herpesvirus to bacteria (Slots 2000). Initially, bacteria in the dental biofilm induce gingivitis, which permits latent herpesviruses, embedded in the DNA of macrophages, T lymphocytes and B lymphocytes, to infiltrate the periodontium (Contreras 1999). Cytomegalovirus can replicate in gingival tissue, which may help to sustain the periodontal infection (Hai 2006). Re-activation of the latent herpesviruses may occur spontaneously or during periods of decreased host defence, as a result of drug-induced immunosuppression, concurrent infection, unusual and prolonged emotional stress, hormonal changes, physical trauma, etc. Not coincidentally, most herpesvirus-activating factors are also suspected risk factors/indicators for periodontitis (Reddy 2007). In response to the active herpesvirus infection, the host elicits a robust T-cell mediated immune response, comprised primarily of CD8+ T cells. To counteract the hostile host environment, herpesviruses in turn execute strategies to down-regulate antiviral host defences. Herpesviruses evade immune responses by disintegrating components of the MHC and interfering with antigen presentation, by silencing natural killer cells, by expressing a viral homolog of IL-10, by diverting potent cytokine responses and by inhibiting apoptosis (Slots 2005). The encounter between antiviral host defence and virally mediated anti-host

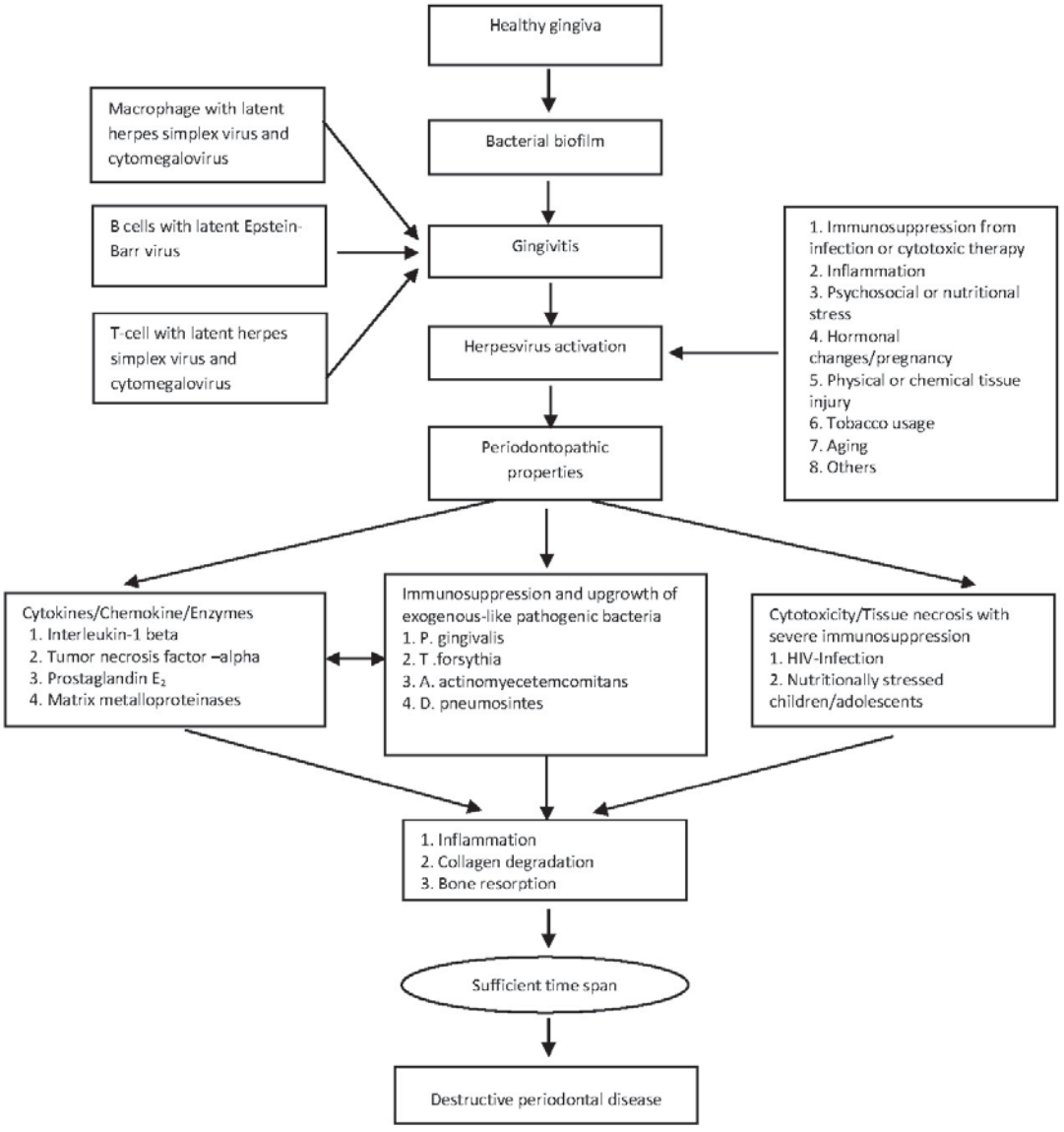

Figure 1. Herpesviral-bacterial model of periodontitis (Slots 2009).

responses results in a major release of pro-inflammatory cytokines that have the potential to activate osteoclasts and impair antibody-mediated host defences against exogenous-like bacterial species, such as *P. gingivalis* and *A. actinomycetemcomitans* (Botero *et al* 2008, Han 2007, Saygun *et al* 2008). The ensuing increase in pathogenic bacteria provides additional mechanisms of periodontal tissue destruction (Holt and Ebersole 2005). Both cytomegalovirus and other herpesviruses can exert an acute cytopathogenic effect on fibroblasts, epithelial cells, keratinocytes, endothelial cells, inflammatory cells and bone cells (Britt 1996). An active herpesviral infection in severely immunocompromised patients may directly destroy periodontal cells and tissue by cytotoxic mechanisms, as seen in patient with necrotizing ulcerative gingivitis and noma. Herpesvirus infections may participate in oral collagen degradation, as suggested by *in vivo* and *in vitro* studies, and potentially interfere with periodontal tissue turnover and healing (Botero *et al* 2008, Saboia-Dantas 2008).

In the herpesviral-bacterial model of periodontitis, herpesvirus-related cytopathogenic effects, immune evasion, immunopathogenicity, latency, re-activation from latency and tissue/site tropism comprise important aspects of periodontal pathosis. It is likely that the early stage of periodontitis in immunologically naive hosts involves an active herpesviral infection that primarily causes cytopathogenic effects, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. The proposed model may help to clarify at least some of the clinical features of periodontitis (Saygun 2004, Slots 2005). The propensity for site tropism of herpesviruses may explain why periodontal tissue destruction can differ markedly from tooth-to-tooth in the same patient or from surface-to-surface in individual teeth. A vigorous anti-herpesvirus host defence may ensure a prolonged period of periodontal stability, even in the presence of virulent bacteria. Herpesvirus re-activation from a latent state may trigger a burst of periodontal tissue damage and progressive disease. However, most immunocompetent individuals experience episodes of oral herpesvirus re-activation lasting only a few hours or a few days, the duration of which is too short to initiate or aggravate clinical periodontal disease (Mark 2008).

Conventional periodontal therapy can reduce the periodontal load of herpesviruses. Mechanical debridement has suppressed subgingival Epstein-Barr virus and cytomegalovirus to undetectable levels in patients (Saygun 2005, Wu 2006). After repeated debridement, many patients with periodontitis yielded no cytomegalovirus, but were found to have Epstein-Barr virus and herpesvirus-7, suggesting that cytomegalovirus is particularly susceptible to the effects of periodontal therapy (Rotola 2008). In patients with Papillon-Lefevre syndrome, mechanical debridement and systemic amoxicillin-metronidazole suppressed subgingival Epstein-Barr virus, cytomegalovirus and *A. actinomycetemcomitant* to undetectable levels and prevented further loss of periodontal attachment (Pacheco 2002). The decrease in post-treatment herpesvirus counts is probably caused by a reduction in gingivitis and thus in the number of virally infected inflammatory cells. Similarly, low herpesvirus counts in healthy periodontal sites are probably the result of a virtual absence of infected inflammatory cells. Cytomegalovirus was also not detected in healthy peri-implant sites (Nowzari 2008). The treatment data suggest that diseased periodontal sites are an important source of salivary herpesviruses. The potential of periodontal therapy to decrease herpesvirus levels in saliva may reduce the risk of herpesvirus transmission

and herpesvirus-related disease among close acquaintances.

The herpesviral-bacterial model of periodontitis provides a rationale for considering new approaches to disease prevention and treatment. A patient who exhibited refractory periodontitis and high Epstein-Barr virus subgingival copy count was treated with valacyclovir HCl at 500 mg twice a day for 10 days. The treatment suppressed subgingival Epstein-Barr virus to an undetectable level for at least one year and resulted in a dramatic clinical improvement. Vitamin C supplementation for 90 days may improve plasma vitamin C levels, reduce periodontopathic bacterial load and reduce viral load of Epstein-Barr virus but not cytomegalovirus (Amaliya 2012, Sunde 2008). Major advances in periodontal treatment to determine when antiviral intervention is appropriate are dependent upon screening of periodontal viruses using diagnostic DNA microarrays that are able to simultaneously detect herpes simplex virus and Epstein-Barr virus.

Future management of periodontal diseases may benefit from anti-viral immunotherapy; either prophylactic vaccines which harness the immune system of healthy subjects to prevent infection with decrease-causing viruses, or therapeutic vaccines, which stimulate the immune system into combating existing viruses and disease.

Conclusion

The etiopathogenesis of periodontitis includes virulence factors of herpesviruses and bacteria, host immune responses against viral and bacterial infections, and manipulation of host-cell processes by the infectious agents. Herpesviruses may induce periodontitis by activating specific tissue-destroying pathways of the immune system and by predisposing an individual to bacteria carriage or increased bacterial load. However, ongoing research is required to better understand the molecular contribution of herpesviruses versus bacteria to periodontal pathosis.

An active herpesvirus infections correlates with periodontitis disease activity and may be a major contributor to the periodontal immune response. Herpesviruses are potent inducers of proinflammatory cytokines that have the potential to activate osteoclasts and matrix metalloproteinases. An active herpesvirus infection can also impair antibacterial immune mechanisms and potentially cause an upgrowth of periodontopathic bacteria. Some periodontopathic bacteria may re-activate a latent herpesvirus infection. Synergism among herpesviruses and bacteria may play an important role in the onset and progression of periodontitis. The inflamed periodontium appears to be a major site for Epstein-Barr virus and cytomegalovirus accumulation and re-activation, especially in the progressive phase of periodontal disease. Immunosuppressive factors are potential triggers of herpesvirus re-activation and, perhaps for that reason, are also major risk factors for periodontitis.

Conventional periodontal therapy, systemic amoxicillin-metronidazole, anti-herpesvirus drugs or supplemental vitamin C can reduce the periodontal load of herpesviruses. Therapeutic treatment to control herpesviruses by vaccination will help develop fields for future investigation.

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Chapter 6

The Effect of Vitamin D and/or Dexamethasone on CPY19 Gene Expression in Osteoblast Cell Culture of Alveolar Bone from Aggressive Periodontitis Patients

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Introduction

The clinical signs of aggressive periodontitis (AgP) include rapid attachment and bone loss which can result in tooth loss. This condition is common in young adults and it has been suggested that it may be associated with changing esgtrogen levels. It has been established that the CPY19 gene is responsible for the conversion of androgen to estrogen. Investigators have demonstrated that CPY19 gene expression can be increased in osteoblasts by both vitamin D and dexamethasone.

The vitamin D receptor is present in more than 30 different tissues including the pancreas, myocardium and lymphocytes, and its widespread distribution signifies an important role for vitamin D in humans. While vitamin D is critical for calcium homeostasis, current studies also highlight the role of vitamin D deficiency in diseases other than metabolic bone disorders (Goswami *et al* 2008).

Dexamethasone, unlike indomethacin or ibuprofen, may diminish the pathological processes that likely contribute to inflammation and loss of vessel wall integrity, leading to hemorrhage. Interleukin-9 mRNA expression and protein secretion are very markedly inhibited by dexamethasone (Holzs *et al* 2005).

Osteoporosis, the most common metabolic bone disease in Western society, constitutes an imbalance of the bone remodeling process in favor of bone resorption, leading to decreased bone mineral density and bone quality, with an increased propensity to fracture (Enjuanes *et al* 2005).

Non-ovarian estrogen synthesis has proven relevant to bone metabolism in postmenopausal women, as well as in men. Aromatase, the cytochrome P450 enzyme involved in the conversion of androgenic steroids into estrogens, has been documented in bone, strongly suggesting that aromatase expression in this tissue plays an important role in both postmenopausal and male osteoporosis. Cytochrome P450 aromatase (hereafter called aromatase), together with NADPH–cytochrome P450 reductase, forms the so-called aromatization complex, found exclusively in estrogen-producing cells, and catalyzes the conversion of androgens (C19 steroids) into estrogens (C18 steroids).

Aromatase activity has been detected in several human adult and fetal tissues and pathologic samples. With regard to bone, aromatase activity and/or aromatase mRNA have been detected in tissue samples, in human cultured osteoblasts, in human osteosarcoma cell lines HOS, U2OS and MG63, in differentiating human osteoblastic cells such as SV-HFO, and in SV40-immortalized human osteoblasts. One study has shown that promoter usage and regulation of aromatase gene expression in osteoblast-like cells may be specific and different from those of other tissues such as placental, ovarian and adipose tissue (Enjuanes *et al* 2003).

In this context, evaluation of the contribution of local estrogen production in bone is of interest and to date no detailed studies of CYP19 expression in primary human osteoblasts have been carried out. Here we present a transcription study in primary normal human osteoblasts including assessment of alternative promoter usage. We evaluated the influence of calcitriol (1,25 dihydroxyvitamin D3; vitamin D), dexamethasone (DEX), estrogens (17b-estradiol; E2), testosterone, tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) on CYP19 gene expression in these cells. The results have shown, for the first time, that vitamin D stimulates aromatase transcription in human osteoblasts. Vitamin D and dexamethasone were potent stimulators of CYP19 transcription, while testosterone and 17β-estradiol stimulated moderately (Enjuanes *et al* 2003).

It is suggested that non-ovarian estrogen production is relevant in the maintenance of bone mineralization, pubertal growth spurts, epiphyseal fusion, skeletal maturation,and the prevention of osteoporosis, since males have exhibited failed epiphyseal closure, osteopenia and delayed bone age. Therefore, the understanding of the role estrogens play in both females and males has grown significantly and considerable emphasis has been placed on the regulation of extragonadal estrogen biosynthesis, including bone estrogen production (Enjuanes *et al* 2005).

Cytochrome P450 aromatase is the key enzyme for estrogen biosynthesis. Aromatase catalyzes the conversion of testosterone to estradiol, of androstenedione to estrone, and of 16-hydroxylated dehydroepiandrosterone to estriol. Aromatase is encoded by a single gene, CYP19, localized on 15q21.2. Aromatase activity and CYP19 gene expression have been detected in human cultured osteoblasts as well as in the human osteosarcoma cell lines HOS, U2OS and MG-63 (Enjuanes *et al* 2005).

The primary features of aggressive periodontitis include a history of rapid attachment and bone loss with familial aggregation. Secondary features include phagocyte abnormalities and a hyper responsive macrophage phenotype (Enjuanes *et al* 2003, Holzs *et al* 2005). Aggressive periodontitis can be localized or generalized. Localized aggressive periodontitis (LAgP) patients have interproximal attachment loss on at least two permanent first molars and incisors, with attachment loss on no more than two teeth other than first molars and incisors. Generalized aggressive periodontitis (GAgP) patients exhibit generalized interproximal attachment loss including at least three teeth that are not first molars and incisors. In young individuals, the onset of these diseases is often circumpubertal. Some investigators have found that the localized form appears to be self-limiting, while others suggest that it is not. Some patients initially diagnosed as having LAgP were found to have GAgP or to be periodontally healthy at a six year follow-up exam (American Academy of Periodontology 2003).

The goals of periodontal therapy are to alter or eliminate the microbial etiology and contributing risk factors for periodontitis,

thereby arresting the progression of disease and preserving the dentition in comfort, function, and appropriate esthetics and to prevent the recurrence of disease (American Academy of Periodontology 2003).

This study aimed to determine the effect of stimulation of vitamin D and dexamethasone combination against the number of osteoblasts expressed by CYP19 in alveolar bone of aggressive periodontitis patients.

Materials and methods

Human bone cells were obtained from specimens of alveolar bone from patients with AgP who had undergone surgery for bone grafting. Treatment of patients with AgP consisted of initial phase therapy in the form of scaling root planing in preparation for subsequent surgical procedures. Fragments of alveolar bone from AP patients were cultured (explant) in modified F-12 medium supplemented with FBS 20%, penicillin streptomycin 5%, and fungizone 2% (Freshney 1987). The osteoblast cells grown in culture were then divided into 4 groups: Group 1: non treated culture, Group 2: treated with vitamin D 10-6 mol/L3, Group 3: treated with dexamethasone 10-7 mol/L3, Group 4: treated with combination vitamin D and dexamethasone. Alveolar bone samples from non-periodontitis (NP) subjects were taken using rongour from the alveolar region of impacted third molar teeth (Figure 1). After 7 days, culture groups were observed by immunocytochemistry (Figure 2).

Results and discussion

CYP19 AgP expression was increased 1.75 times by vitamin D and 1.13 times by vitamin D+dexamethasone. Increased expression of CYP19 in aggressive periodontitis adds to existing estrogen from androgen sources, resulting in increased repair of alveolar bone damage (Table 1). Dexamethasone alone did not increase CYP19 AgP in this study.

Amongst the treatment groups, vitamin D (with or without dexamethasone), dexamethasone, testosterone and E2 all increased transcriptional expression levels of CYP19 in osteoblasts in serum-free conditions. The highest levels were obtained with vitamin D and dexamethasone. The effect of dexamethasone is in agreement with those obtained in previous studies, including the only one performed on primary human osteoblasts. However, activation by vitamin D and dexamethasone and inhibition by IL-1β and TNFα were consistently observed in samples from three other individuals (not shown). In our study we have shown, for the first time, that vitamin D alone induces a clear increase in CYP19 expression in osteoblasts. This increase is similar to, or even more relevant than, that obtained with dexamethasone alone. Vitamin D stimulation of CYP19 gene expression has been recently documented in ovarian cells. Here, we have shown that this effect of vitamin D also occurs in bone cells. In their study, Kinuta *et al* (2000) proposed that the action of vitamin D on estrogen biosynthesis may be partially due to its role in maintaining calcium homeostasis and partially to a direct regulation of the expression of the aromatase gene. As such, the stimulatory action of vitamin D on CYP19 gene expression in osteoblasts may constitute an additional relevant effect on bone turnover regulation, aside from its well-established stimulatory effect on genes such as osteocalcin and osteopontin (Enjuanes *et al* 2003).

There have been very few studies in cultured primary human osteoblasts from normal donors. These studies have found CYP19 expression only when cells are treated with dexamethasone, with or without other treatments. In contrast, we have observed CYP19 transcripts in serum-free cultures as well as under different treatments. The levels

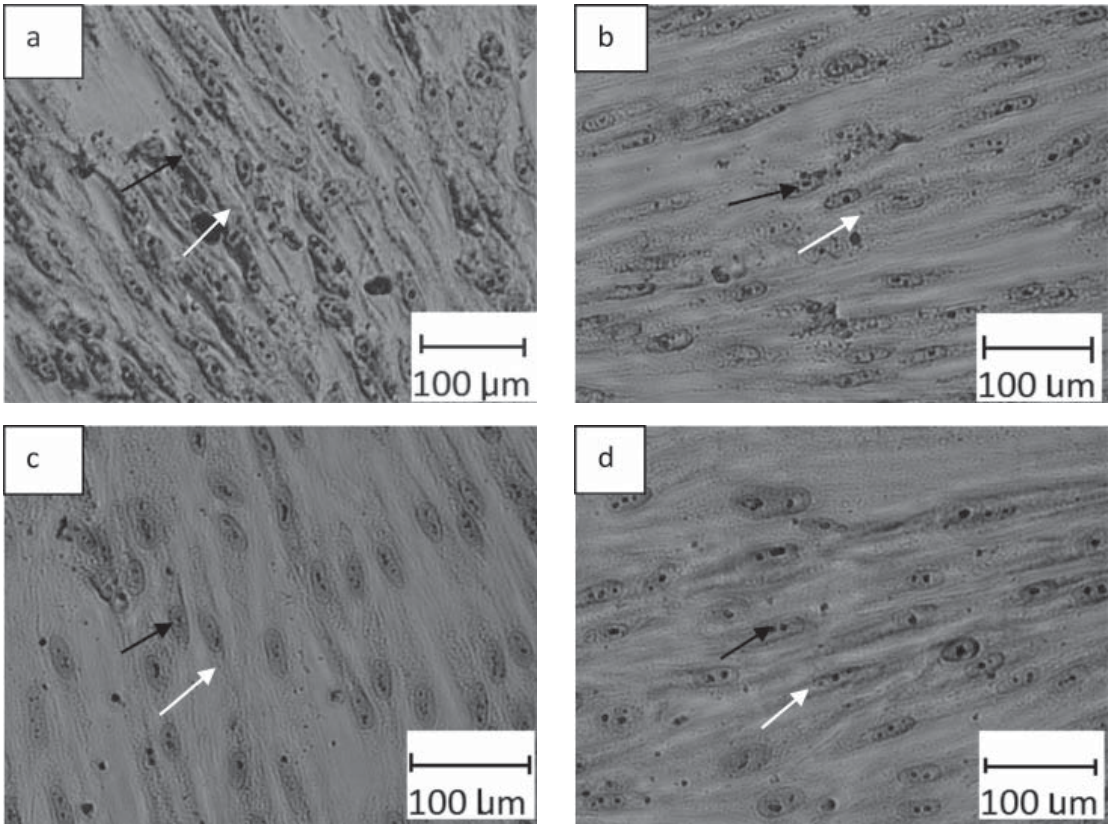

Figure 1. Expression of CYP19 in osteoblasts cultured from AP. (A) Without stimulation. (B) Stimulation by vitamin D. (C) Stimulation by dexamethasone. (D) Stimulation by vitamin D and dexamethasone. Black arrow indicates positive osteoblasts (expression CYP19), white arrow indicates negative osteoblasts (non-expression of CYP19).

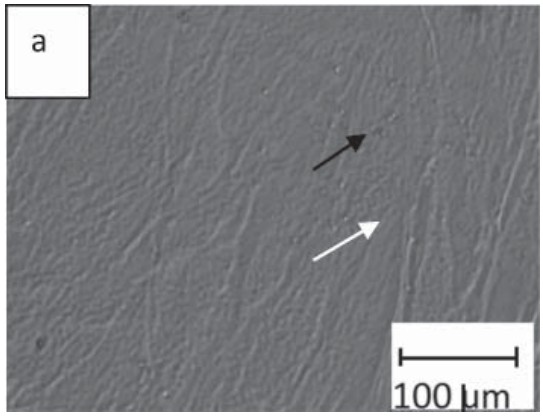

Figure 2. CYP19 expression in non-periodontitis sample as control.
Black arrow indicates positive osteoblasts (expression CYP19), white arrow indicates negative osteoblasts (non-expression of CYP19).

Osteoblast Culture	Without stimulation		Stimulated		
CYP19 AgP	Control NP	AgP	Vit D	Dex	Vit D + Dex
RR	1*	1.5	1.75	1	1.13
CC		0.79-2.86	0.95-3.22	0.47-2.14	0.56-2.32
P	0.05	0.22	0.07	1	0.75

Tabel 1. Risk ratio (RR) CYP19 gene, in osteoblast culture of AgP and NP samples stimulated by vitamin D, dexamethasone, combination of vitamin D and dexamethasone.
1* Constant from NP, RR>1, positive association between risk factor and treatment

of CYP19 gene expression were maintained up to the sixth passage, consistent with the observations by Shozu and Simpson (1998) on aromatase activity.

In aggressive periodontitis patients, scaling and root planning were carried out before extracting samples of alveolar bone for culture, therefore the health of alveolar bone was increased. From this research we noted that initial phase therapy such as scaling and root planing, as well as chemotherapy before treatment is very important (Seiler and Herold 2005). Systemic antibiotics reach the periodontal pathogens via serum at the base of deep pockets, in furcation areas, and within gingival epithelial and connective tissues. Diffusion of the antibiotic into the connective tissue and epithelium is important because *A. actinomycetemcomitans* invades these areas.

Conclusion

Vitamin D and vitamin D+dexamethasone increase the expression of CYP19 genes, in osteoblasts of cultured tissues from aggressive periodontitis patients. These findings indicate that vitamin D and vitamin D+dexamethasone regulate local estrogen synthesis in human osteoblasts. In conclusion, we observed that normal human osteoblasts in primary culture exhibit expression of the CYP19 gene in serum-free conditions, and that it is regulated by vitamin D and dexamethasone. Although many different treatments are able to stimulate aromatase expression, vitamin D and dexamethasone are the single molecules that exert the highest effect. The results of this study contribute to the knowledge and understanding of local estrogen synthesis by bone cells.

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Chapter 7

Etiology of Drug-Induced Gingival Overgrowth

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Introduction

Drug-induced gingival overgrowth (DIGO) is caused by three different types of drugs; phenytoin, an anticonvulsant drug for epilepsy patients; nifedipine, a calcium-channel blocker for patients with hypertension and cardiovascular diseases, and cyclosporine A (CsA), an immunosuppressive drug for organ transplant patients and patients with immune disorder such as rheumatoid arthritis and atopic dermatitis. DIGO is a major side effect of these drugs and its etiology has been discussed in several reviews (Ciancio 2005, Kataoka *et al* 2005, Mavrogiannis *et al* 2006, Seymour *et al* 2000, Trackman and Kantarci 2015). Our group has also investigated the etiology of DIGO (Asahara *et al* 2000, Ishida *et al* 1995, Kataoka *et al* 2000, Kataoka *et al* 2001, Morisaki *et al* 1993, Nishikawa *et al* 1991, Nishikawa *et al* 1996, Ogino *et al* 2005, Shimizu *et al* 2002). However, the exact mechanism of DIGO occurrence is not fully elucidated. In this chapter, we discuss the clinical observations of DIGO and then discuss etiological factors from the recent literature elucidating cellular and molecular mechanisms of DIGO occurrence.

Clinical characteristics of DIGO

DIGO usually appears at the interdental papilla and then progresses around the marginal gingiva. The gingival tissues are hard and fibrotic and gingival lobulations are frequently formed at the interdental papilla (Figure 1). Recent findings show that nifedipine- and especially phenytoin-induced lesions are highly fibrotic and that CsA-induced gingival overgrowth lesions principally exhibit the presence of inflammation and little fibrosis (Bullon *et al* 2007, Trackman and Kantarci 2015). Although such tendencies are observed in the case of patients presenting in Figure 1, it seems that not all the cases of DIGO show similar patterns. In fact, we have experienced several clinical cases of severe inflammation in nifedipine-DIGO in Japanese subjects. On the other hand, most cases of phenytoin-DIGO tend to exhibit highly fibrotic DIGO. Histological observation of gingival tissues in DIGO shows evidence of thick epithelial layers and increased collagen fibers (Figure 2).

Risk factors for DIGO

Seymour *et al* (2000) reported the risk factors associated with the appearance and severity of DIGO as follows:

1) Oral hygiene (plaque-induced inflammation).
2) Drug variables (dose, duration, serum concentration).
3) Age and gender.
4) Genetic factors.

Genetic factors can generally convey

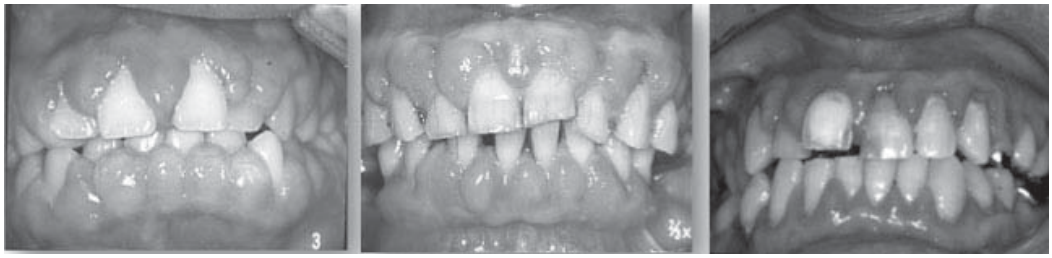

Figure 1. Clinical observations of DIGO in patients taking phenytoin, nifedipine and CsA.

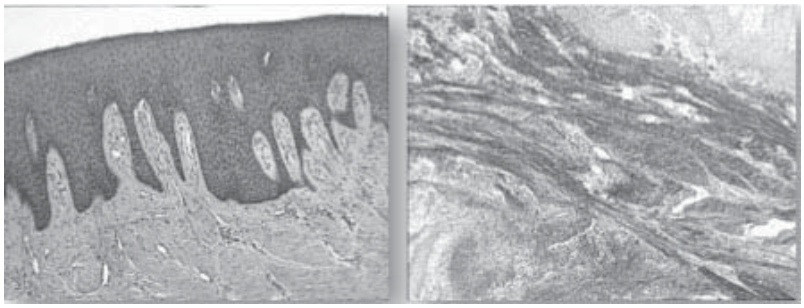

Figure 2. Histological observations of DIGO. Note the thickened epithelial layer (left panel, HE staining) and the increased and condensed collagen fibers (right panel, Mallory Azan staining). Sample was taken from gingival tissue in anterior teeth of 60 year old DIGO patients.

susceptibility to various diseases and recent reports have shown the relationship between single-nucleotide polymorphisms (SNPs) and DIGO. It has been reported that SNPs of CTLA-4, CYP2C, IL-10 and TGF-α may be associated with the appearance of DIGO (Kusztal *et al* 2007, Luo *et al* 2013, Radwan-Oczko *et al* 2008, Soga *et al* 2002). We also reported that α2-integrin +807 polymorphisms were related to DIGO (Ogino *et al* 2005). On the other hand, Nishimura *et al* (2002) found that impaired cathepsin-L activity may play a key role in the establishment of DIGO through studies using cathepsin-L deficient mice.

Although several studies demonstrate that age is a risk factor for DIGO, its evaluation is difficult because nifedipine is prescribed to middle aged or older patients, and phenytoin to relatively young patients. However, it is conceivable that children showed higher rates than adults in the case of Cs-A-DIGO (Seymour *et al* 2000). When gender was evaluated, males were shown to be three times more likely than females to develop clinically significant gingival changes in the case of patients taking Ca-channel blockers (Seymour *et al* 2000). We also reported that male rats are more prone to DIGO using an animal model (Ishida *et al* 1995). Association of drug variables such as dose, duration, serum and salivary concentration remain unclear. However, it is generally accepted that concomitant medication affects the initiation and progression of DIGO. The combination of nifedipine and CsA in organ transplant patients produces more DIGO than the single use of nifedipine or CsA (Wilson *et al* 1998, Wondimu *et al* 1996). As periodontal variables, plaque accumulation and gingival inflammation are major risk factors associated with DIGO appearance, it is controversial whether dental plaque is important for triggering or worsening of DIGO.

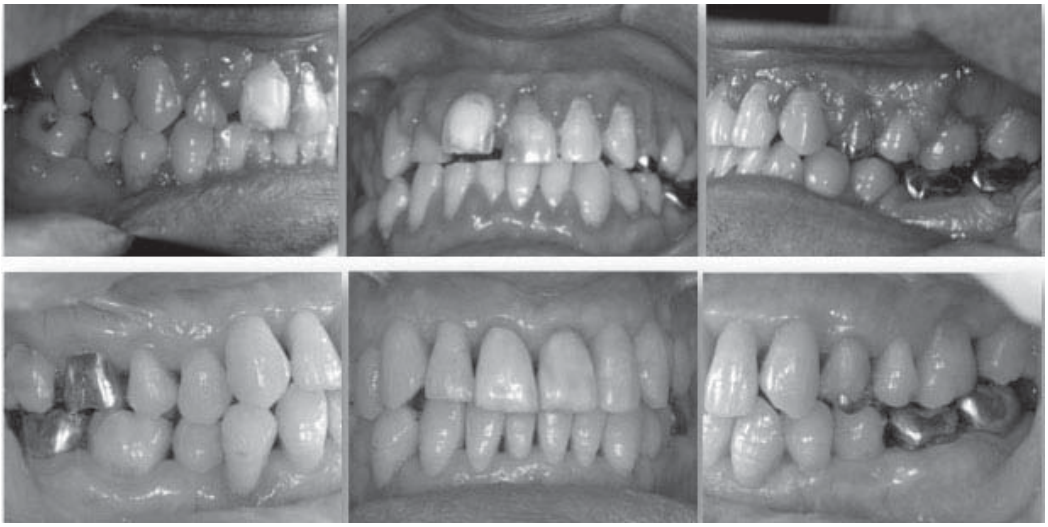

Figure 3. Treatment of DIGO in a kidney transplant patient who was 45 year old man taking CsA for 6 months. Although drug substitution was not carried out, initial therapy and gingivectomy using CO_2 laser completely removed his gingival problems. (A) Initial presentation. (B) 8 years following treatment.

Treatment of DIGO

In 2004, the Executive Committee of American Academy of Periodontology published the information paper "Drug-Associated Gingival Enlargement" in the *Journal of Periodontology*. In the paper, drug-substitution/withdrawal, non-surgical treatment, surgical periodontal treatment, and treatment outcomes and recurrence rate are discussed. It is then recommended that the treatment of DIGO is generally targeted at drug substitution and the effective control of local inflammatory factors such as plaque and calculus. Although surgical treatment is the most reliable option for DIGO, non-surgical treatment is also important as a first treatment option. Clinical studies show that proper oral hygiene may minimize the severity of DIGO and non-surgical treatment of DIGO based on strict plaque control could improve DIGO (Aimetti *et al* 2008, de Carvaloho Farias *et al* 2010, Seymour *et al* 2000, Thompson *et al* 2004). We have experienced a clinical case showing a marked improvement of CsA-DIGO in a kidney transplant patient (Figure 3). In this patient, strict plaque control procedures such as oral hygiene instruction, scaling and root planning was undertaken before and after surgical treatments (gingivectomy). These treatments could prevent not only gingival inflammation, but also DIGO recurrence. Recently, a number of reports recommend the use of laser surgery to remove excess gingival tissue (Mavrogiannis *et al* 2006). The laser gingivectomy is known to be useful in patients on anticoagulant therapy.

Etiology of DIGO based on cellular and molecular researches

As the etiology of DIGO seems to be different between cases with or without gingival inflammation, we propose two kinds of mechanisms concerning the occurrence of DIGO (Figure 4).

In cases without inflammation, the metabolism of collagen is precisely balanced by collagen synthesis and degradation to maintain normal tissue architecture. Collagen phagocytosis is thought to be an important pathway for the physiological degradation of

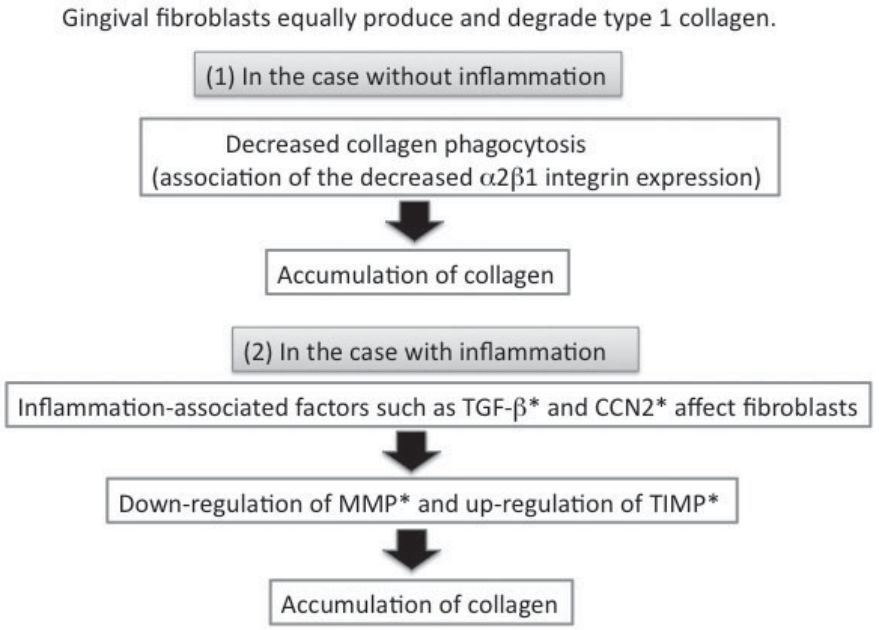

Figure 4. Etiology of DIGO based on collagen metabolism. Decreased collagen degradation may induce the imbalance of collagen metabolism. Two kinds of mechanism are speculated; with or without gingival inflammation. *TGF-α: transforming growth factor-α, *CCN2: connective tissue growth factor, *MMP: matrix metalloproteinase, *TIMP: tissue inhibitor of metalloproteinase.

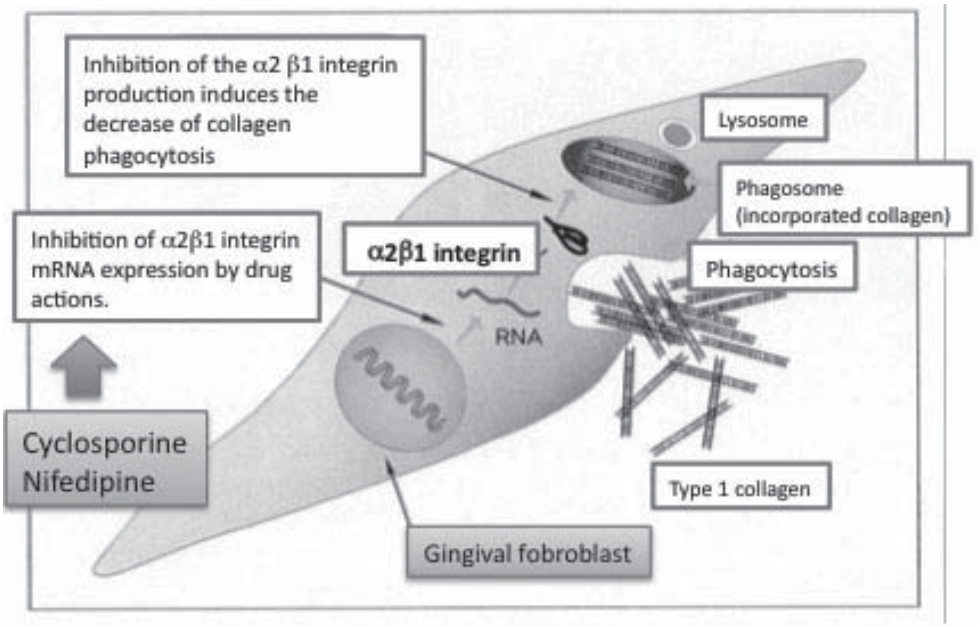

Figure 5. The role of α2β1 integrin in collagen phagocytosis in the case without gingival inflammation. DIGO may be caused by the inhibition of collagen phagocytosis derived from the drug-induced reduction of α2β1 integrin expression.

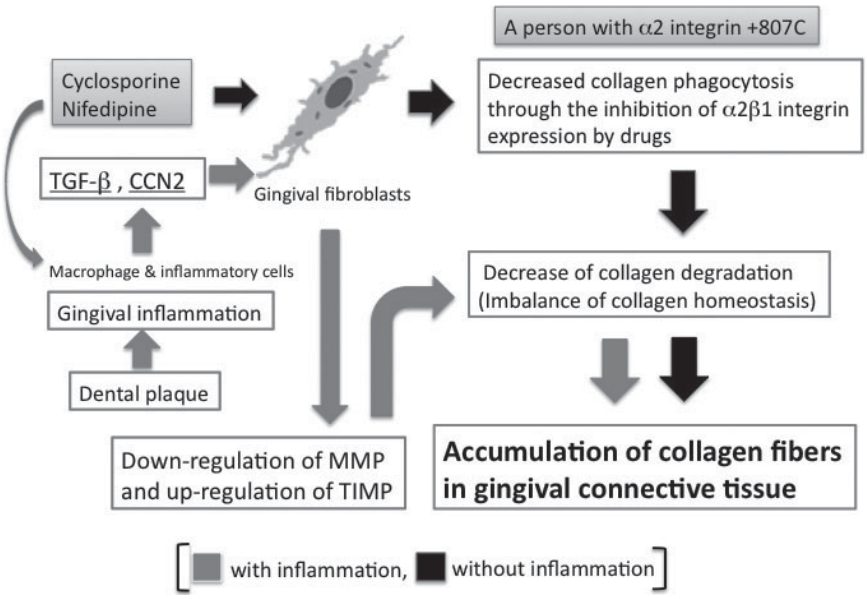

Figure 6. A possible mechanism of DIGO appearance with or without inflammation.

collagen in gingival connective tissue (Sodek and Overall 1988). Inhibition of collagen phagocytosis by fibroblasts is one of the mechanisms leading to DIGO (Kataoka *et al* 2000, McCulloch and Knowles 1993). α2β1 integrin serves as a specific receptor for type I collagen in fibroblasts, and the initial binding step of collagen phagocytosis relies on adhesive interaction between fibroblasts and collagen (Dickeson *et al* 1999, Kataoka *et al* 2003). α2β1 integrin plays a critical role in the phagocytic regulation of collagen internalization, and DIGO may be caused by a reduction of α2β1 integrin expression (Figure 5).

In cases with inflammation, there have been many reports demonstrating the association of inflammatory cytokines with DIGO. A synergistic enhancement of collagenous protein synthesis was found when gingival fibroblasts were exposed to nifedipine and interleukin-1β (IL-1β) (Johnson *et al* 2000). IL-6 has a role enhancing proliferation of fibroblasts and fibroblasts derived from CsA-influenced gingival tissues spontaneously secreting high level of IL-6 (Fries *et al* 1994, Myrillas *et al* 1999). In fact, the IL-6 family of cytokines increases in gingival crevicular fluid (GCF) of CsA-DIGO patients (Gürkan *et al* 2015). There have been many studies demonstrating the association of transforming growth factor-α (TGF-α) with gingival tissue proliferation. TGF-α levels in GCF and blood were found to be higher in DIGO patients (Buduneli *et al* 2001, Pakosz *et al* 2012). *In vitro* studies showed CsA-induced gingival fibroblast proliferation via induction of TGF-α and crosstalk between Shh and TGF-α signaling in CsA-enhanced cell proliferation (Chung and Fu 2013, Cotrium *et al* 2003).

Connective tissue growth factor (CTGF/CCN2) expression is positively related to the degree of fibrosis and is a reliable marker of fibrosis (Kantarci *et al* 2006, Uzel *et al* 2001). CTGF contributes to fibrosis development initiation by TGF-α and TGF-α-CTGF axis is activated in the Ca channel blocker-induced DIGO (Mori *et al* 1999, Pisoschi *et al* 2014, Trackman and Kantarci 2015). From histological measurements, most phenytoin-

induced DIGO is fibrotic. CsA-induced DIGO is highly inflamed and exhibits little fibrosis, while nifedipine DIGO is mixed (Trackman and Kantarci 2015, Uzel *et al* 2001). Interestingly, the expression of TGF-α and CTGF was elevated in phenytoin DIGO (Subramani *et al* 2013, Thompson *et al* 2010). TGF-α directly induces extracellular matrix (ECM) gene expression and promotes ECM production by simultaneously suppressing matrix metalloproteinase gene expression and inducing tissue inhibitors of matrix metalloproteinase gene expression (Eikelberg 2001, Subramani *et al* 2013). CsA is associated with fibrosis via the increase of TGF-α production in kidney and intestine (Shehata *et al* 1995, Vieira *et al* 1999). These findings indicate that CsA and TGF-α may be major regulators of gingival fibrosis, which is involved in DIGO pathogenesis.

Conclusion

Since prescription of these drugs will increase over time, the number of DIGO affected patients will also increase. As part of the present clinical approach, oral hygiene is very important for preventing DIGO and surgical procedures are important to remove marked DIGO. Etiological factors, divided into two categories of with or without gingival inflammation, are summarized in Figure 6. Novel information based on cellular and molecular approaches can provide the design for future DIGO therapies.

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Chapter 8

Overview of Risk of Maternal Periodontal Disease and Adverse Perinatal Outcomes: A Commentary

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Introduction

Adverse perinatal outcomes contribute to a huge disease burden in terms of growth and development of the child, inter-current respiratory diseases and delayed milestones. The term perinatal denotes the time interval between 20 weeks of gestation and the birth of the infant.

It is important to realize that there are numerous risk factors which can impact adverse outcomes. It is also challenging task to account for the incidence of perinatal outcomes which cannot be explained by currently known risk factors. In this context, numerous studies have been carried out during the last two decades to assess the risk of maternal periodontal disease to mediate adverse perinatal outcomes. Current statistical paradigms have limitations in their ability to draw conclusions due to variation in design of studies, coexisting confounders, and varying case definitions of periodontal disease. Ide and Papapanou (2013) concluded in a systematic review that there was a modest but statistically significant association between maternal periodontitis and the perinatal outcomes of low birth weight and preterm births, although the studies analyzed showed considerable heterogeneity in design and data recording.

The scope of this commentary will address the following:

1) Various risk factors for adverse pregnancy outcomes.
2) Review Bradford Hill's criteria for association/causation in the context of periodontal disease being a potential risk factor.
3) Review a selection of positive and negative studies with respect to periodontal disease being a risk factor
4) Comment on recent published literature emphasizing use of the periodontal inflamed surface area as variables in reference to pocket depth and clinical attachment level to quantify risk.
5) Review the biological mechanisms underpinning the association between periodontal disease pathology and its propensity to spill cytokines and inflammatory mediators into maternal circulation.
6) Outline the need for future research initiatives.

Risk factors mediating adverse birth outcomes

Perinatal outcomes can range from low birth weight, preterm or premature babies, still births and neonatal deaths, and small for gestational age. These adverse outcomes pose a huge challenge, not only to the neonatal services in both developed and developing

countries, but they also have huge financial and social implications. The March of Dimes report (2012) has indicated various comorbidities like blindness, cerebral palsy, hearing loss, neonatal jaundice and delayed brain development. Many prematurely birthed children have the risk of developing attention deficit hyperactivity disorder. 97 to 99% of the 3 to 4 million still births occur in low income and middle income countries. Lawn *et al* (2005) commented on the high preponderance of neonatal deaths in sub-Saharan Africa and south central Asian countries.

Periodontal disease is a dental biofilm-initiated chronic persistent infection which can spill over cytokines into the maternal blood stream. The various risk factors mediating adverse perinatal outcomes are genito-urinary infections, a short cervical length between 14 and 28 weeks, and positive fetal fibronectin between 22 and 34 weeks of gestation. In addition, gestational diabetes, genetics, smoking and alcohol consumption by the mother, pre-eclampsia, fetal growth restriction and congenital abnormalities can be potential risk factors. Maternal demographics can also be a risk, such as mothers aged less than 17 years or more than 35 years old; education less than year 12; indigent socioeconomic background; and short inter-pregnancy interval of less than 6 months. Nutritional status can also be a risk factor in women, such as pre-pregnancy weight of less than 50 kg or BMI less than 19, and those women working more than 80 hours a week. The incidence of preterm birth shows a tendency to occur more often in black women at 16 to 18%, whilst the incidence of PTB in white women is 5 to 9% (Goldenberg *et al* 2002). A history of spontaneous preterm delivery confers notable risk (Goldenberg *et al* 2000). Vaginal bleeding in the first or second trimester and maternal stress or depression can be risk factors for adverse perinatal outcomes. Periodontal disease is a chronic persistent infection initiated by the dental biofilm at the interface between the tooth and the gingival margin in the mouth, culminating in loss of the ligamentous attachment to the tooth and formation of potential spaces referred to as periodontal pockets.

The initiation of this bacterial insult results in chronic inflammation which may culminate into degeneration necrosis and fibrosis. The Limulus lysate assay detects endotoxin in the blood stream of patients with periodontal disease. Periodontal infection can trigger an acute phase response with an increase in C-reactive protein. Sanz and Kornman (2013) assert that although scaling and root surface debridement induce transient bacteremia, these procedures are reported to be safe during pregnancy as cited in the consensus report of the joint EFP/AAP Workshop on Periodontitis and Systemic Diseases.

Bradford Hill's criteria for association/ causation

Hill (2015), in a landmark paper, outlined the criteria to be incorporated into a study design to ascertain causation or association. Weed (2000) emphasized the causal criteria of strength of association, biologic plausibility, consistency and dose-response relationship and expressed that these criteria, although qualitative, have a relationship with meta-analysis. The current consensus is that epidemiological studies concur that there is a modest but statistically significant association between maternal periodontitis and low birth weight, pre-eclampsia and pre-term birth independent of other exposures. Different classifications used in researching periodontitis as a risk factor for preterm/low birthweight outcomes compound the realistic interpretation of research data. Standardization of case definitions of periodontitis will go a long way in validating study results. The paradox here is that although epidemiological

studies do indicate a positive association, intervention non-surgical therapy during the second trimester of pregnancy did not result in better pregnancy outcomes. It is important to note that these studies utilized conventional measures of periodontal inflammation such as pocket depth and attachment levels, although these studies were well powered. Another intervention study performed by Jeffcoat (2011) analyzed the success of periodontal treatment in terms of mitigation of inflammation and related it to incidence of full term birth. A logistic regression analysis showed a strong and significant relationship between successful periodontal treatment and full-term birth (adjusted odds ratio 6.02; 95% CI 2.57–14.03). Subjects refractory to periodontal treatment were significantly more likely to have preterm birth.

The above study emphasizes that follow up of periodontal treatment to ensure that signs of inflammation have subsided translates into better pregnancy outcomes. It has also been speculated that better design of clinical trials with regards to type, timing and frequency of intervention, apart from identifying subpopulations of susceptible pregnant women, would have far reaching tangible benefits to both mother and baby.

It has further been speculated that meta-analysis alone cannot capture causality as it is highly quantitative, although it can provide a reproducible weighted average of the estimate of effect. It would be prudent to consider the more qualitative inputs from the classic Hill's criteria such as strength of association, dose response relationship and biologic plausibility, along with meta-analysis in clinical decision making. Assessing of heterogeneity amongst studies in a meta-analysis has also been stated to be a cardinal consideration in the interpretation of study results.

Pregnancy loss or miscarriage has been defined as loss of the fetus occurring before 22 weeks of gestation, whereas still birth connotes fetal death at or after 22 weeks of gestation. Preterm premature rupture of membranes can occur anywhere between 23 and 36 weeks of gestation. In the absence of any gynecological contraindications, it might be more prudent to start nonsurgical periodontal therapy in week 14 of gestation rather than the present practice of elective scaling during week 20 of gestation. This would entail mechanically controlling the biofilm at a much earlier stage in pregnancy.

Novel paradigms to assess inflammatory burden

Parakkal (1979) summarized the characteristics to be measured for quantitative evaluation of periodontal disease and noted the following considerations to be of importance:

1) Loss of attachment measured from the cement enamel junction to the apical extent of the pocket epithelium.
2) Pocket depth measured from the gingival margin to the apical extent of the sulcus/pocket epithelium.
3) Potential pocket area measured from the gingival margin to the apical extent of the sulcus/pocket epithelium.
4) The area of attachment of the connective tissue to the root surface measured from the apical extent of the sulcus/pocket epithelium to the root apex.

Recent publications by Huda *et al* (2015) and Nesse *et al* (2008) have stressed that new paradigms to measure oral inflammation are needed, such as the periodontal inflamed surface area and oral inflammatory load assay. The authors feel that these indices may give more realistic values of the oral inflammatory burden with a greater propensity to correlate with systemic outcomes.

Periodontal inflamed surface area

Hujoel *et al* (2001) computed the dento-

gingival surface area. Nesse *et al* (2008) used a modified Hujoel's algorithm to deduce the periodontal inflamed surface area (PISA). Since bleeding on probing has shown dense infiltration of inflammatory cells, the PISA would seem a more logical instrument to use in future studies correlating periodontal disease and systemic outcomes. PISA is a composite index calculated using clinical attachment loss, gingival recession and bleeding on probing. It has also been argued that pocket depth and clinical attachment loss represent historical disease parameters and do not represent current disease activity.

Oral inflammatory load assay

Huda *et al* (2015) have advocated an assay using a 15 second saline rinse to calculate the neutrophil levels in the rinse sample. The authors published a study on pregnant females to calculate the oral inflammatory load. A statistical significance was noted when comparing healthy mouths with those afflicted with periodontitis. The authors recommend using the oral inflammatory load assay, along with a surrogate marker like C-reactive protein, in future studies linking maternal periodontitis and gingivitis with pregnancy outcomes. It is logical to comprehend that studying the oral neutrophil count is a novel way to elucidate the inflammatory burden, given that neutrophil mediated metalloproteinases can cause tissue destruction and also interact with CD 40 –CD 40 ligand system to perpetuate production of pro-inflammatory cytokines (Phipps 2000).

Biological rationale for inter-current periodontal disease in pregnancy

Li *et al* (2000) cited three mechanisms whereby periodontal disease can enhance the susceptibility of the host to systemic disease. They are shared risk factors, subgingival biofilm as a reservoir for gram negative bacteria and the inflamed periodontal tissues being a reservoir for inflammatory mediators.

Female sex hormones

Pregnancy does not initiate gingivitis per se, but can exacerbate pre-existing gingivitis. The hormones present during pregnancy can interact with receptors in the gingiva periodontal ligament and periosteum. Pregnancy, with its relatively long period of gestation, is a state of immunosuppression. Progesterone maintains the pregnancy but can act to perpetuate oral inflammation by causing stagnation in the microcirculation in the presence of the dental biofilm. The gingiva is the target organ for female sex steroids (Formicola *et al* 1970). Degranulation of mast cells can amplify inflammation in the presence of the dental biofilm with deleterious consequences.

Animal and human studies support the evidence that in untreated periodontal disease, periodontal microorganisms or their products spread through the blood stream to affect feto-placental unit. Physiological needs of fetal metabolism increases the utero-placental blood flow exponentially during the third trimester. Apart from nutrients, harmful molecules and micro vesicles from bacteria could cross the placental barrier and create an adverse local environment to threaten the full term completion of gestation.

Cytokinemia and acute phase proteins

The dental biofilm can initiate production of a cascade of pro-inflammatory cytokines like IL-1, IL-6, TNF-α and PGE_2 which can initiate maternal parturition. Another indirect mechanism to affect the feto-placental unit is the production of C-reactive protein by the liver (Pitiphat *et al* 2006). Horton *et al* (2008) showed that pregnant African-American women had a higher serum level of C-reactive

protein in the early period of gestation.

Various biomarker studies lend credence to an indirect biological mechanism reflecting the association of adverse perinatal outcomes with maternal periodontal disease such as sIcam1, matrix metalloproteinases and PGE_2. Oral bacterial antigens have the potential to upregulate inflammatory markers common to both periodontal disease and initiation of parturition. Tarannum *et al* (2011) demonstrated that gingival crevicular fluid PGE_2 levels are a predictor for preterm low birth weight.

Humoral antibodies against oral pathogens in cord blood

Madianos *et al* (2001) found that cord blood IgM levels of *Campylobacter rectus* were elevated in preterm infants. This study was pivotal in providing proof of principle involvement in implicating the role of oral organisms in providing an antigenic challenge to the fetus. This study was complimented by another landmark publication by Boggess *et al* (2005) who measured IgM and other inflammatory mediators in umbilical cord blood of 640 infants against oral organisms such as *Fusobacterium nucleatum*, *Peptostreptococcus micros* and *Campylobacter rectus* and concluded that that a higher IgM titre in cord blood against any of the aforementioned conferred a greater risk of prematurity.

Isolation of periodontal pathogens from amniotic fluid and placenta

Bearfield *et al* (2002) showed that periodontal bacteria can be isolated from the amniotic fluid in pregnant mothers. This clearly indicates the propensity of periodontal bacteria to translocate from the oral tissues and upset the homeostasis in the feto-placental unit.

Katz *et al* (2009) were able to localize *P. gingivalis* antigens using immunocytochemistry, as evinced by greater intensity of immunostaining in epithelial amniotic cells, decidual cells and chorionic trophoblasts in the placenta of preterm mothers as compared to placental tissues of mothers who delivered full term.

Stockham *et al* (2015), in their study on pregnant mice, found that inoculation of *Fusobacterium nucleatum* (Fn) subspecies nucleatum into the blood stream of mice elicited adverse pregnancy outcomes such as intrauterine growth restriction and fetal loss. It can be surmised that signatures of the Fn subspecies nucleatum, a bridging organism in biofilm evolution, identified in the placenta can spread from the blood stream to the feto-placental unit and mouth and induce adverse pregnancy outcomes along with increased alveolar bone loss.

Maternal D dimers and fibrin fragment E

There will be a disruption of the bi-laminar flow of blood in the capillaries during inflammation. Inflammation affects the microcirculation and causes vascular dilatation and increase capillary permeability. In *in vitro* studies in mice, Guo *et al* (2009) revealed that placental trophoblast cells can undergo apoptosis due to fibrin degradation products in the absence of thrombomodulin. It is conjectured that uncontrolled periodontal disease might confer risk to the mother due to increase in fibrin degradation products.

Olofsson *et al* (2003) stated that plasmin can activate proforms of metalloproteinases. The authors studied fibrinolytic activity of gingival crevicular fluid in 31 young individuals and found that all samples were positive as deduced by the fibrin gel lysis assay. The authors speculated that enhanced reactivity to dental plaque could predict future tissue destruction and spread of the

inflammatory process.

Periodontal disease risk: Literature reviews

A cross section of studies conducted with various adverse pregnancy outcomes in mothers with the risk of periodontitis are outlined below.

Preterm-birth and low birth weight and gestational periodontal disease

A systematic review of cross-sectional prospective cohort and case-control epidemiological studies led the researchers to conclude that maternal periodontal disease was modestly but independently associated with low birth weight less than 2500 grams and preterm birth of less than 37 weeks of gestation (Ide and Papapanou 2013). The authors found that heterogeneity in periodontal case definitions amongst studies impacts the results, noting that if periodontal disease is treated as a categorical variable significant associations are found, as opposed to diminished association when periodontal disease is defined as a continuous variable.

A systematic review with varied study designs comprising case-control, cross-sectional, cohort studies and three randomized controlled trials done in 12 countries between 1996 and 2006 was undertaken (Clothier *et al* 2007). The authors identified 24 studies exhibiting a positive association between maternal periodontitis and pre-term birth, low birth weight neonates or both. On the other hand, 14 studies did not indicate an association-effect relationship between periodontitis and pregnancy outcomes.

Matevosyan (2011), in a systematic review undertaken in Austria, concluded that periodontal disease is a risk factor for pre-eclampsia and pre-term birth. The studies reviewed included original research published between 1998 and 2010. The author computed an oral inflammation score from parametric and observational components of maternal periodontal disease. The author surmised that the oral inflammation score was significantly associated with pre-term birth after adding confounders to the statistical model (OR 2.3)

Heimonen *et al* (2009), in a cross-sectional study conducted in Finland, examined 328 Finnish women with singleton births. None of the periodontal parameters predicted preterm birth but the oral inflammatory burden index calculated from multiple oral infections experienced during pregnancy significantly correlated with preterm birth.

Kilpatrick *et al* (2008), in a systematic review tabled to the policy makers in the New Zealand Ministry of Health concluded that control of plaque for the treatment and prevention of periodontal disease has a potential benefit to both mother and child. The authors further stated that women from disadvantaged groups with periodontal disease had poorer birth outcomes. Significantly, the authors note that although current strength of evidence does not support non-surgical treatment in relation to better birth outcomes, intervention periodontal treatment is likely to be beneficial.

Xiong *et al* (2007), in a systematic review, identified 29 positive studies and 15 negative studies correlating periodontal disease parameters and pregnancy outcomes. The analysis included 5 clinical trials, 13 cohort studies and 26 case studies.

Khader and Ta'ani (2005), in a systematic review and meta-analysis of two case-control studies and three cohort studies of maternal periodontal disease, surmised that the adjusted odds ratio of a delivery of either preterm birth or low birth weight was 2.30 (95% CI, 1.21 to 4.38; $P < 0.005$).

Agueda *et al* (2008) conducted a cohort study in Spain which included caucasian, black and gypsy populations in a sample of

1296 mothers and found a modest association of pocket depth and clinical attachment level with preterm birth but did not discern any relationship with low birth weight.

Michalowicz *et al* (2006) in an intervention study did not find an association between pregnancy outcomes and periodontal disease. This was an intervention trial consisting of white, black and hispanic populations. The authors concluded that periodontal treatment during gestation did not significantly alter preterm birth low birth weight and fetal growth restriction.

Scannapieco *et al* (2003), in a systematic review without meta-analysis, examined six case-control, three interventional and three cross-sectional and longitudinal studies, and observed that few studies focussed on whether preventive and therapeutic periodontal procedures would make an impact on the outcome of adverse perinatal outcomes. The authors discerned that more studies are needed to define a causal role for periodontitis in mediating adverse birth outcomes.

Macones *et al* (2010) surmised that treatment of localized periodontal disease in pregnancy does not reduce the occurrence of spontaneous preterm delivery, as reflected in the "Periodontal Infections and Prematurity Study".

Kaur *et al* (2014) followed a cohort of 120 pregnant women with generalized gingivitis for which intensive oral hygiene instructions were given at baseline, 4 weeks and 8 weeks. Non-surgical scaling procedure was done at baseline. The authors found that all clinical variables pertaining to periodontal health had improved including reduction in cytokine levels. This study affirms the reduction of periodontal disease risk as a result of periodontal treatment during gestation.

An intervention study performed by Jeffcoat in 2011 analyzed the success of periodontal treatment in terms of mitigation of inflammation and related it to incidence of full term birth. A logistic regression analysis showed a strong and significant relationship between successful periodontal treatment and full-term birth (adjusted odds ratio 6.02; 95% CI 2.57–14.03). Subjects refractory to periodontal treatment were significantly more likely to have preterm birth. The above study emphasized that follow up of periodontal treatment to ensure that signs of inflammation have subsided translates into better pregnancy outcomes.

Pre-eclampsia and maternal periodontal disease

An association between periodontal organisms and pre-eclampsia has been reported. Pre-eclampsia denotes high blood pressure over 140/90 mm of Hg and proteinuria of more than 300 mg in 24 hours after the 20th week of gestation. A case-control study was conducted by Contreras *et al* (2006) in Colombia. An association between periodontal organisms and pre-eclampsia has been reported in mothers with inter-current periodontal disease. Ide and Papapanou (2013) conclude from the meta-analysis of observational epidemiological studies that there is a significant association between maternal periodontitis and pre-eclampsia. Shetty *et al* (2010) concluded that periodontitis during pregnancy confers a greater risk of pre-eclampsia.

Periodontal disease as a risk indicator for perinatal death

Shub *et al* (2009) found a high degree of association between maternal periodontal disease and perinatal death. Extreme prematurity showed a very strong association with pre-existing periodontal disease. Perinatal death was associated with periodontal disease (odds ratio (OR) 2.34, 95% confidence interval (CI) 1.05, 5.47). Periodontal disease

was more strongly associated with perinatal mortality due to extreme prematurity (OR 3.60, 95% CI 1.20, 12.04). Multivariate analysis showed this relationship to be consistent after inclusion of higher parity, country of birth, and advanced maternal age and maternal obesity in the model (OR 4.56, 95% CI 1.25, 21.27).

Need of the hour: Well designed studies

It is evident that numerous risk factors play a key role in mediating adverse perinatal outcomes. Although numerous studies have lent credence to periodontal disease being a risk factor some intervention studies in particular have been equivocal. Sanz and Kornman (2013), in a consensus report of the Joint EFP/AAP Workshop, advise a number of strategies for future research to delineate the role of periodontal disease risk. Amongst these, working group participants have emphasized the need to examine inflammatory components such as bleeding on probing as a parameter in future studies. In addition, at least two assessments are recommended during gestation. This is important as gram negative infection is present to a greater extent in the second trimester. Kornman and Loesche (1980) have shown that in the second trimester of pregnancy the ratio of anaerobic to aerobic organisms is of a high magnitude in the dental biofilm.

Analysis of surrogate biomarkers in cord blood, in addition to primary effect outcomes, may provide compelling data to delineate a direct causal role. It has also been suggested that the design of future clinical trials take into account the type and timing of intervention. In this context, monitoring of the periodontal healing response with respect to attenuation of inflammation will enhance a higher incidence of full term births (Jeffcoat *et al* 2011).

It is also possible that an interplay of risk factors amplifies the risk of triggering adverse events. In this context, heterogeneity is bound to exist in study cohorts with regard to social habits like smoking and alcohol consumption, genetic predisposition in certain races and pregnancy complications. Controlling of confounders, reducing systematic and random errors in research design and use of multiple bias modelling for analysis of observational data have been suggested.

The potential use of parameters reflecting real-time inflammation such as PISA and the oral inflammatory load assay need to be used in future studies correlating maternal periodontitis and adverse perinatal outcomes.

It might be more prudent to carry out nonsurgical intervention therapy at an earlier time point around the 14th week of pregnancy, in the absence of obstetric contraindications, to mitigate the oral inflammatory burden as a potential risk.

There is no doubt that health care professionals, including obstetricians, midwives, dentists, and pediatricians need to come together and explore ways and means to combat the scourge of adverse perinatal events through a focused antenatal monitoring program. The media also needs to play a role in spreading awareness regarding good oral hygiene during pregnancy and create materials pertaining to antenatal care.

Summary

This commentary has reviewed the risk factors for adverse perinatal outcomes, along with a selection of studies pertaining to maternal periodontitis and adverse perinatal outcomes. The need to mitigate these risk factors through periodic antenatal care is of paramount importance with the overarching goal to tilt the balance in favor of healthy birth outcomes. Research initiatives need to identify subgroups of population at risk who might benefit from making periodontal screening

a routine part of antenatal care. It has been emphasized that development of gingivitis and the sustained inflammatory response can drive the shift towards colonization by pathogenic microbes (Bartold and Van Dyke 2013). It is thus of paramount importance to reverse this oral inflammation during pregnancy. The need to use 'real time' inflammatory indices in maternal studies of periodontitis and gingivitis and relating them to pregnancy outcomes has been emphasized.

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Chapter 9

Periodontal Disease: A Local Immune Response

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Introduction

Periodontitis, one of the ancient diseases in humans, is characterized by loss of tooth attachment and bone loss. The most recent epidemiological data suggest that severe periodontitis affects 11% of the global population (approximately 800 million people) (Kassebaum *et al* 2014). Clinical and histological observations regarding the pathogenesis of human periodontitis, especially relating to the local periodontal immune response to plaque bacteria, have been described by many leaders in the field (Page *et al* 1997, Seymour 1991, Yamazaki *et al* 1995). The presence of dense infiltrated immune cells and inflammatory mediators in periodontitis lesions have been demonstrated. Gram-negative bacteria within the subgingival dental plaque biofilm, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Treponema denticola* have been implicated in the initiation and progression of the disease (Slots 1999, Socransky *et al* 1998). Despite this current knowledge, there are still many questions to be answered. This article focuses on current knowledge of the local adaptive immune response in periodontal disease.

A landmark study of human experimental gingivitis by Löe *et al* (1965) demonstrated the body's response to bacterial plaque biofilms resulted in gingival inflammation. Page and Schroeder (1976) outlined a sequence of histopathological stages that involve the transition from healthy gingiva to advanced periodontitis. These stages include initial, early, established and advanced lesions. As the plaque accumulated, the degree of gingival inflammation increased and this was characterized by the influx of polymorphonuclear neutrophils (PMN), destruction of connective tissue and increased numbers of lymphocytes. A notable observation at this time was the predominant presence of plasma cells in advanced periodontitis lesions. Seymour *et al* (1982) further demonstrated that most of the lymphocyte infiltrates in gingivitis were T cells. Since lymphocytes and plasma cells are key cells in the adaptive immune response and predominate in the periodontitis lesions, a central role for these cells in immunopathogenesis of periodontal diseases has been postulated (Berglundh *et al* 2007, Seymour *et al* 1993).

Local B cell response in periodontal disease

Many studies in the past have evaluated the immune response to periodontal bacteria using immune cells isolated from circulating blood, which could be misleading in the

understanding of what occurs in periodontitis where the immune response occurs in the local periodontal tissue not peripheral blood. To address this issue we measured and compared the frequency of B and T cells in peripheral blood versus periodontal tissues from individuals with different disease severities. Mean percentages of B cells and T cells in periodontitis tissue and clinically healthy gingiva are presented in Figure 1. In periodontitis tissue, the mean percentage of B cells (34.41 ± 4.38%) was significantly higher than T cells (24.98 ± 3.07%) ($p < 0.05$) with a B cell to T cell ratio of 1.5:1. In contrast, the mean percentage of B cells (6.12 ± 1.17%) in healthy gingiva was significantly lower than T cells (34.78 ± 2.57%) ($p < 0.05$) with a B cell to T cell ratio of 1:6. Our findings are in agreement with previously published results (Amunulla *et al* 2008, Lappin *et al* 1999). Comparison of infiltrated B cells and T cells between the two clinical groups reveals a significant higher mean percentage of infiltrated B cells and a significant lower mean percentage of infiltrated T cells in the severe periodontitis group ($p < 0.05$, Figure 1). These results confirm the early classic study by Seymour *et al* (1979) showing that the periodontitis lesion is dominated by B cells, whereas clinically healthy gingiva is dominated by T cells.

We also analyzed the lymphocyte frequency in peripheral blood and found no difference in mean percentages of circulating B cells (14.14 ± 1.12%) and T cells (50.90 ± 3.55%) in periodontitis patients as compared to those from healthy subjects (mean B cells 8.84 ± 1.31%; mean T cells 53.01 ± 4.48%). Similar profiles of circulating B cells and T cells were observed in periodontitis patients and healthy periodontal subjects with the B cell to T cell ratio of 1:5 (Figure 1). Hence, different profiles of infiltrated B cells and T cells present in periodontitis tissue reflect active local immune response.

B cells are recognized for their role in the humoral mediated immunity with the primary function of antibody production. Generally, B cells can be divided into three subsets, naïve B cells, memory B cells and antibody secreting cells (ASCs). Naïve B cells are cells that have not been activated by antigen. Upon

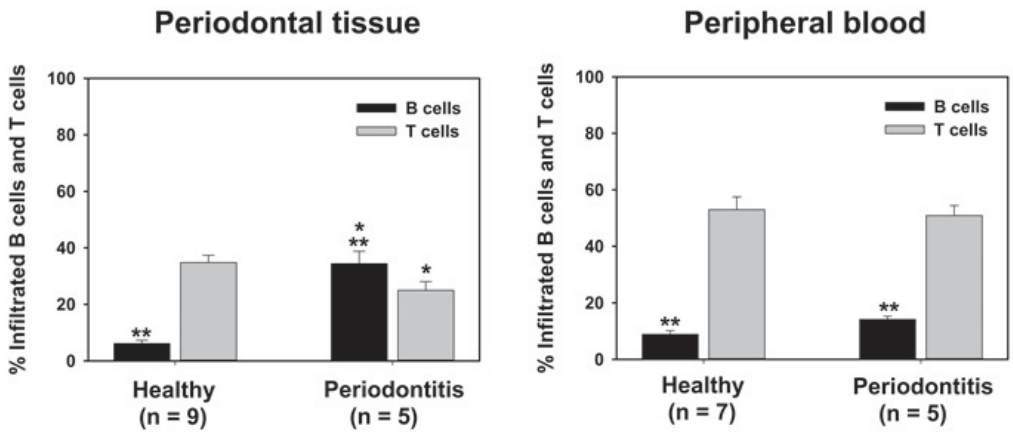

Figure 1. Mean percentages of infiltrated B cells and T cells in periodontal tissue and in peripheral blood. Cells extracted from periodontal tissue (left) and peripheral blood (right) in healthy and periodontitis were stained with monoclonal antibodies specific to B cells and T cells, and then analyzed by flow cytometry. Data were presented as mean ± S.E. *, $p < 0.05$, periodontitis group compared with healthy group, **, $p < 0.05$, B cells compared with T cells in each group.

Monoclonal antibodies	Populations
$CD19^+$	B cells
$CD19^+$ $CD27^-$ $CD38^-$	Naïve B cells
$CD19^+$ CD27+ $CD38^-$	Memory B cells
$CD19^+$ $CD27^+$ $CD38^+$	ASCs

Table 1. Monoclonal antibodies used for flow cytometric analysis (Fink 2012, Jacobi et al 2010, Odendahl et al 2005, Wrammert et al 2008).

antigen encounter, naïve B cells are activated and differentiated into effector B cells, either memory B cells or ASCs with IgG, IgA, IgD, IgE, or IgM (Delves *et al* 2011). Memory B cells, long-lived lymphocytes, are important in the secondary immune response. They provide a more rapid response to re-encountered antigens and a more efficient antibody production with high affinity Ig than the primary naïve B cell response (Delves *et al* 2011, Murphy *et al* 2008).

In the past, several investigations focused on activation stages of tissue B cells in periodontitis. The B cell activation markers studied included FMC7+, CD25+, and CD69+ (Champaiboon *et al* 2000, Gemmell and Seymour 1991, Seymour *et al* 1985). The frequency of activated B cells has been reported to be much higher in periodontitis than gingivitis (Yamazaki *et al* 1993). To date, there has been very little data published concerning infiltrated B cell profiles including naïve B cells, memory B cells, and ASCs, and their role in protection or pathogenesis of periodontal disease. This may be due to limited markers for the B cell phenotype. We have conducted a preliminary study of the local B cell response in periodontal disease using a combination of monoclonal antibodies and flow cytometry to identify different B cell subsets in severe periodontitis tissue compared with clinically healthy gingiva (Table 1).

Among these three populations, we found that ASCs (CD19+ CD27+ CD38+) were the major cell type in severe chronic periodontitis tissues (n =5, 53.76 ± 4.78) whereas memory B cells (CD19+ CD27+ CD38-) were the major cell type in healthy periodontal tissues (89.80 ± 2.87%) (Figure 2). Both clinical groups demonstrated low levels of infiltrating naïve B cells (CD19+ CD27- CD38-). At present, it is not clear if the observed ASCs in periodontal tissue are derived from germinal center activation in draining lymph nodes,

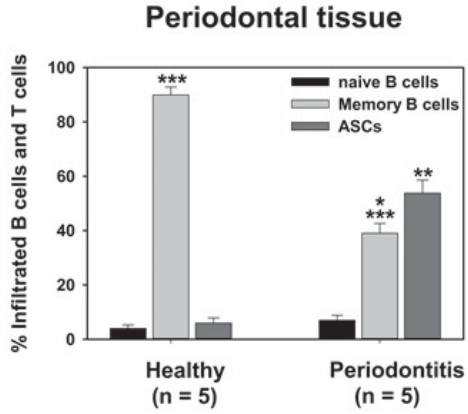

Figure 2. Mean percentage of B cell profiles in periodontal tissue. Cells extracted from periodontal tissues in clinically healthy gingiva and periodontitis were stained with monoclonal antibodies specific to naïve B cells, memory B cells, and ASCs, and then analyzed by flow cytometry. Data were presented using mean ± S.E. *, $p < 0.05$, memory B cells in periodontitis group compared with healthy group **, $p < 0.05$, ASCs in periodontitis group compared with healthy group, ***, $p < 0.05$, memory B cells compared with ASCs in each group.

or from polyclonal activation of pre-existing memory B cells resided in healthy periodontal tissues. It is also unclear what local factors promote a "survival niche" for ASCs and what the role of ASCs is in the pathological process. Further research on the generation of memory B cells and ASCs in periodontitis lesions and clinically healthy gingiva is needed.

A local T cell response in periodontal disease

Generally, T cell populations are divided into two main subsets, CD4+ and CD8+ T cells. CD4+ T cells or helper T (Th) cells have received much attention, not only regarding their role in effective immune defence, but also their participation in tissue inflammatory responses through the cytokines they produce. About three decades ago, Mosmann *et al* (1986) first described distinct functional subsets of Th cells in mice (Th1 and Th2) based on their cytokine profiles. Th1 cells secrete IFN-γ and IL-2 and mediate predominantly cell mediated immune responses. In contrast, Th2 cells mediate humoral immunity by secreting IL-4, IL-5 and IL-13. An elegant experiment conducted in a mouse model demonstrated that mice which were resistant to infection by the intracellular parasite *Leishmania major* developed a Th1 response whereas those susceptible to the infection develop a Th2 response (Mosmann and Coffman 1989). The simplicity of this Th1/Th2 paradigm of resistance/susceptibility in a mouse model subsequently encouraged further investigation into many human diseases including periodontitis. It was hypothesized by Seymour and co-workers that Th1 cells are associated with stable gingivitis lesions, while Th2 cells are associated with progressive periodontitis lesions (Gemmell *et al* 2002, Gemmell *et al* 2007). Some studies did not agree and showed mixed Th1 and Th2 responses in advanced periodontitis (Berglundh *et al* 2002, Fujihashi *et al* 1996, Prabhu *et al* 1996). Overall, the respective roles of Th1 and Th2 cells in human disease and periodontal disease remain inconclusive.

Other CD4+ T cell subsets, Th17 and Treg (regulatory T cells), have been described, both of which add complexity to the cytokine network. Th17 secrete a pro-inflammatory cytokine-IL-17 while Treg secrete IL-10 and TGF-β (Noack and Miossec 2014). Treg functions opposite to Th17 cells, which inhibit tissue inflammation and maintain self-tolerance (Awasthi and Kuchroo 2009). Both Th17 and Treg were identified in periodontitis. The role of Th17 cells has now been described in various models of inflammation mediated destruction and autoimmune disease (Tesmer *et al* 2008). For example, in rheumatoid arthritis, IL-17 mediates connective tissue destruction and bone resorption via induction of matrix metalloproteases and osteoclastic cytokine receptor activator of nuclear factor kappa-B ligand (RANKL) (Lubberts 2008). Most recent data suggest a pathway of IL-17 dependent inflammatory bone loss in periodontitis through suppression of molecule developmental endothelial locus 1 (Del-1), which expresses on endothelial cells. They reported that in human tissue biopsies, Del-1 mRNA expression dominated in healthy gingiva whereas IL-17 mRNA expression dominated in inflamed gingiva (Eskan *et al* 2012).

More recently, improved and more sensitive immunological methods have provided additional information about memory T cells. Different memory T cell subsets have been characterized based on the differential expression of surface markers (Table 2). The memory T cells found in human blood can be divided into central memory (TCM) T cells, effector memory (TEM) T cells, terminal effector memory T cells (TTE), and stem cell memory T (TSCM) cells which retain stem cell-like properties (Farber *et al*

	CD45RA	CCR7	CD28	CD95	CD69	CD103
TSCM	+	+	+	+	-	-
TCM	-	+	+	+	-	-
TEM	-	-	-	+	-	-
TTE	+	-	-	+	-	-
TRM	-	-	-	+	+	+

Table 2. Human memory T cell heterogeneity. Four circulating populations include stem cell memory T cells (TSCM), central memory T cells (TCM), effector memory T cells (TEM), and terminal effector memory T cells (TTE). Non circulating memory T cells permanently reside in tissue and express surface molecule involved in tissue retention (CD69) and tissue adhesion (CD103) termed tissue-resident memory T cells (TRM) (Farber et al 2014, Mahnke et al 2013).

2014). Another subset of memory T cells that permanently reside in non-lymphoid tissues has recently been identified; they are now widely referred to as tissue-resident memory T (TRM) cells (Ariotti *et al* 2012, Di Meglio *et al* 2011, Sathaliyawala *et al* 2013). The mechanism of TRM cell retention in tissues may involve CD103 and CD69. The αEβ7 integrin CD103 promotes cell adherence to E-cadherin expressed on tissue epithelial cells; whereas C-type lectin CD69 inhibits sphingosine-1-phosphate receptor 1 (S1P1), leading to tissue retention (Cepek *et al* 1994, Mackay *et al* 2015, Skon *et al* 2013). These TRM cells have been identified in skin, gut, brain, vagina, and lung. Accumulating data suggest that TRM cells are crucial as they provide a first line of defence against secondary infection by the same pathogen at local sites (Schenkel and Masopust 2014, Shin and Iwasaki 2013). For example, infection with influenza virus leads to the generation of both resident and transient circulating memory T cells in the lungs. However, lung TRM CD4 T cells and CD8 T cells show optimal protection against influenza challenge compared with circulating memory T cells (Teijaro *et al* 2011, Turner *et al* 2014, Wu *et al* 2014). In addition to protection, TRM cells could play a role in immunopathogenesis. Pathogenic autoreactive TRM cells induce fixed, recurrent skin lesions of psoriasis, and TRM cells specific to environmental allergens likely underlie the development and worsening of allergic asthma and contact dermatitis (Boyman *et al* 2007, Clark 2015).

Despite recent advances in understanding the role of TRM cells in mucosal tissues, there has been no study of periodontal tissue-specific memory T cell subsets. We have recently carried out a pilot study to investigate the presence of memory T cell subsets in two periodontal tissue specimens, one from clinically healthy periodontal subjects and the other from periodontitis patients. Flow cytometric analysis revealed that memory T cells expressed CD103, the phenotypic marker of TRM, and were found in both healthy and diseased tissues. However, increased percentages of CD103+CD4+ and CD103+CD8+ T cells were observed in periodontitis tissues (Figure 3). Therefore the presence of periodontal tissue-resident memory T cells in both health and disease requires further investigation for their role in protection or pathology.

Conclusion

Infiltrated adaptive immune cells have been well recognized for more than 60 years. These observations have lead to the conclusion that connective tissue destruction and bone loss in periodontitis results from

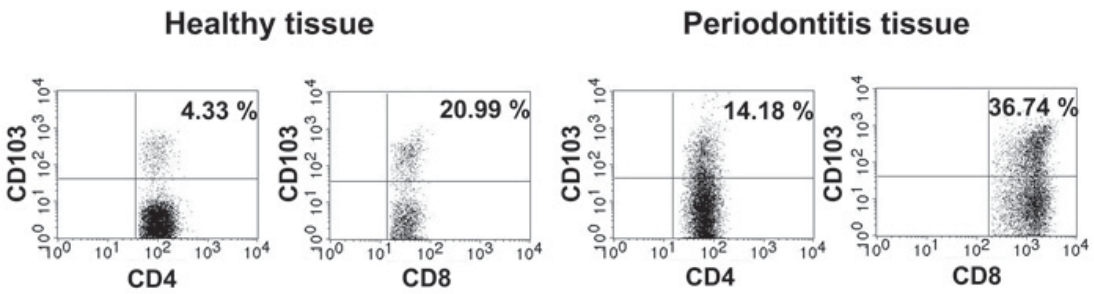

Figure 3. Flow cytometric analysis of CD103-expressing CD4+ and CD8+ T cells in periodontal tissues, healthy (left) and periodontitis (right). A representative result of gingival cells extracted from clinically healthy gingiva (n=3) and periodontitis tissues (n=3) were double stained with anti-human CD4 or anti-human CD8 and anti-human CD103 monoclonal antibodies. The gates were set with isotype control antibodies (data not shown). Percentage double-positive cells are in the upper right corner of each dot plot.

a chronic local immune response. Until now, the precise role of infiltrated adaptive immune cells in immunopathogenesis has been unclear. Human periodontal tissue specimens provide a unique opportunity to study the complex interplay between local host immune responses and plaque bacteria. Comprehensive analysis of infiltrated T, B and plasma cells and their functions will provide insight into how these cells are involved in tissue destruction and bone loss.

Acknowledgements

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Current Issues in Periodontics
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Chapter 10

Host Modulation for Managing Periodontitis

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Introduction

Periodontitis is a collection of diseases resulting from an interaction between the subgingival microbiota, the host immune and inflammatory systems, and modifying environmental factors. While the microbiota associated with periodontitis may be considered "pathogenic" in nature, it is an absolute requirement for the host to be susceptible to disease occurrence in order for periodontitis to develop clinically. Thus, patients who are susceptible through genetic or modifying factors react to the subgingival bacterial infection with resultant development of periodontal disease. In this context, periodontitis is considered to be of multifactorial origin in which bacteria are necessary but not sufficient for the disease to develop (Bartold and Van Dyke 2013).

A change in perspective for the pathogenesis of periodontitis

Early studies concerning the pathogenesis of periodontitis noted that the inflammatory reaction within the gingival tissues always occurred in the absence of any bacterial penetration of the tissues (Page and Schroeder 1976). Thus, it was assumed that soluble products from the subgingival bacteria initiated the inflammatory response that subsequently led to advancing tissue destruction (Page and Schroeder 1976). As a result of the initial inflammatory responses occurring in the gingival tissues, changes develop in the microenvironment of the subgingival biofilm that may then allow for the proliferation of specific microorganisms that are later identified as being associated with periodontitis as "periodontal pathogens". Therefore it has been proposed that periodontal "pathogens" may overgrow due to the inflammatory response, not specifically cause it (Marsh 1994). Interestingly these concepts are not new, with several authors noting the importance of the host response in driving the ultimate outcome of the host-parasite interactions noted in periodontitis (Clarke and Hirsch 1995, Mombelli *et al* 1991, Tanner *et al* 2007).

A new model for the pathogenesis of periodontitis

Based on the above concepts, a new model for the pathogenesis of periodontitis has been outlined (Bartold and Van Dyke 2013). This model, as have others, proposes that the presence of gingivitis is an essential initiating event for periodontitis to develop (Figure 1). Inflammation associated with gingivitis leads to an alteration of the subgingival microenvironment. This arises due to the

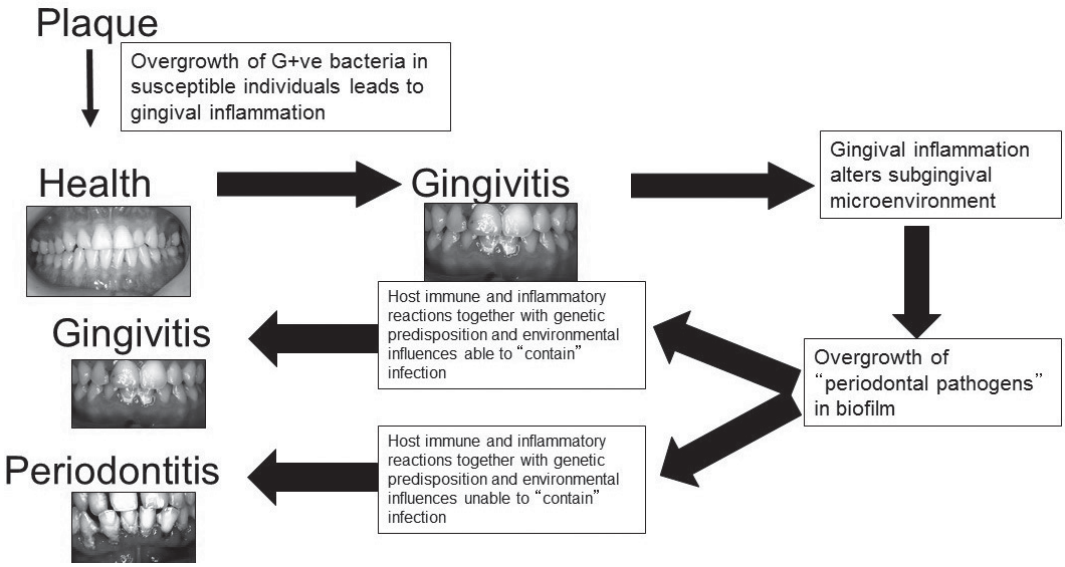

Figure 1. Revised proposal for pathogenesis of periodontitis circa 2013 (Bartold and Van Dyke 2013).

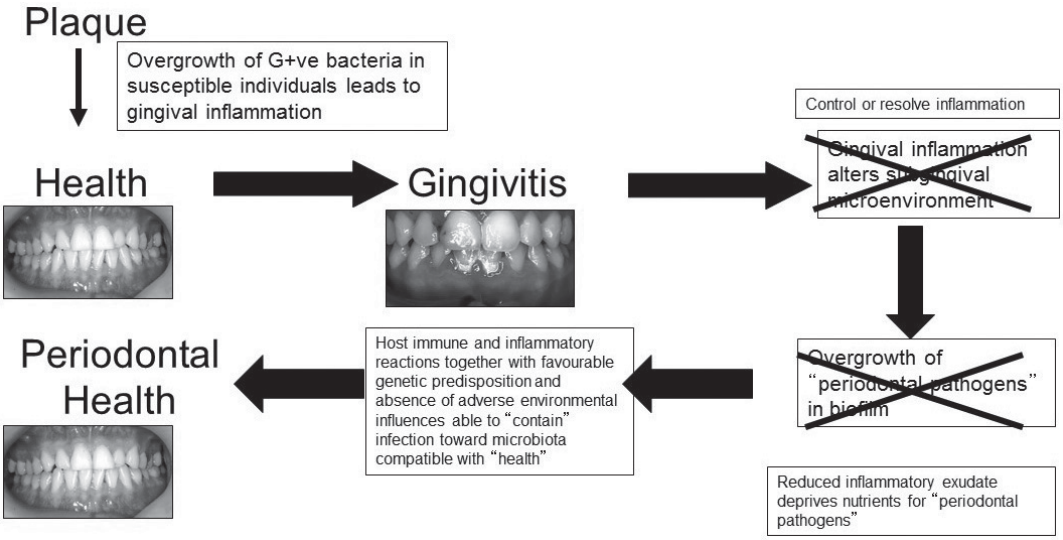

Figure 2. Proposal for controlling inflammation as a treatment approach for managing periodontitis (Bartold and Van Dyke 2013).

release of a plethora of cytokines, chemokines and breakdown products of connective tissue into the gingival sulcular fluid (Page and Kornman 1997). This response provides ideal conditions for "periodontal pathogens" to thrive and proliferate within the subgingival biofilm. If the host responses are robust, then the gingivitis lesion is contained and does not progress to periodontitis. On the other hand, if the host responses are poor as a result of genetically susceptibility, as well as the presence of adverse environmental factors (e.g. smoking), the condition can progress to overt periodontitis. With these concepts in mind it is possible to conceive how novel treatment strategies may be developed for the management of periodontitis based on host modulation (Figure 2). By controlling the inflammatory response associated with gingivitis the subgingival nutrient supply to the subgingival microbiota will not be conducive to periodontal pathogen overgrowth. Therefore, by reversing the inflammatory response, it may be possible to control the infection and return the subgingival microbiota to one predominated by commensal bacteria compatible with periodontal health.

Host modulation as an adjunct therapy for periodontitis

By understanding the impact of controlling periodontal inflammation, novel treatment strategies can arise based on modulating the host response in conjunction with conventional mechanical subgingival debridement. To date, a low dose tetracycline product marketed as Periostat® is the only product that has US FDA approval for such use. Periostat® exerts its adjunctive effect through a non-antimicrobial action in which the activities of several host derived matrix metalloproteinases responsible for tissue breakdown in periodontitis are inhibited. Clinical studies have produced variable results but in general it is accepted that this product demonstrates potential and it has been a significant step forward in the development of host modulation therapies in periodontics (Ryan and Golub 2000). Non-steroidal anti-inflammatory drugs (NSAIDS) that block prostaglandin E_2 production have also been studied as a host modulation adjunct to periodontal treatment (Salvi *et al* 1997). Equivocal results have been reported for the use of NSAIDS as an adjunct in the management of periodontitis and newer formulations have suffered from serious side effects leading to discontinuation of this line of investigation in periodontics (Bello and Holt 2014).

More recently anti-cytokine therapy such as drugs that target IL-1 and TNF have been studied. They have been shown to be able to reduce experimental periodontitis, through reduction of inflammation (Di Paola *et al* 2007). A recent systematic review concluded there is limited evidence for the use of anti-cytokine agents for the management of periodontitis (Han and Reynolds 2012).

A fundamental principal behind using host modulation therapy is to resolve the initial innate inflammatory response. A recent discovery is that natural resolution of inflammation is driven by mediators such as resolvins, lipoxoins and protectins. This has led to studies investigating how these pro-resolving mediators agents control inflammation and restore health. Resolvin E1 has been shown to be effective in resolving experimental periodontitis in animal models and can lead to regeneration of damaged bone (Hasturk *et al* 2007).

Another promising area of research in host modulation is the use of dietary supplements. In particular, the use of omega-3 polyunsaturated fatty acids are well recognized for their anti-inflammatory properties. There is emerging evidence that dietary supplementation with omega-3 polyunsaturated fatty acids in the

form of fish oil may be beneficial in the management of periodontitis (Chee *et al* 2015). Furthermore, it has been reported that this beneficial effect may be enhanced when omega-3 polyunsaturated fatty acid supplements are combined with aspirin (El-Sharkawy *et al* 2010, Elkhouli *et al* 2011, Naqvi *et al* 2014). Addition of aspirin to the omega-3 polyunsaturated fatty acid supplement treatment regime is understood to significantly increase the production of resolvins.

Combination anti-inflammatory and anti-bacterial therapies

Given the multi-factorial nature of periodontitis encompassing both bacterial infection and uncontrolled inflammation, the combined use of both anti-inflammatory and anti-bacterial agents would seem to be a logical approach in managing this condition. To date, only one study has addressed this issue and demonstrated that the combination of systemic antibiotic (tetracycline) and a non-steroidal anti-inflammatory drug (ibuprofen) with non-surgical root surface debridement resulted in a small but significant improvement compared to non-surgical debridement alone (Ng and Bissada 1998).

The macrolide antibiotic azithromycin has also been proposed to be of considerable use as an adjunctive aid in the management of periodontitis. Azithromycin possesses antibacterial and also exerts anti-inflammation properties thus allowing a combination of antibiotic and anti-inflammatory capabilities with one agent (Bartold *et al* 2013). A number of studies investigating the use of azithromycin as an adjunct to non-surgical root surface debridement have shown significant clinical improvement compared to non-surgical periodontal therapy alone (Zhang *et al* 2015). It must be noted that azithromycin should be used with caution in patients with risk of cardiovascular disease due to a small risk of increased cardiovascular death following the use of this antibiotic (Ray *et al* 2012).

Conclusions

It is generally accepted that preventive management approaches for the management of periodontal diseases are not always effective, particularly for the highest risk individuals. One reason for this may be the significant focus and importance placed upon plaque control and the role of specific "periodontal pathogens" and disregard for the importance of the host response, as well as genetic and environmental factors which can significantly modify the course and response of the periodontal diseases. Our current understanding of the etiology and pathobiology of the periodontal diseases highlights a need to investigate the use of host modulating agents for the management of periodontitis. Host modulation therapy is emerging as a significant treatment adjunct for periodontitis. Thus current evidence would suggest refocusing our attention from solely trying to control the infection to control the inflammation, to considering that controlling the inflammation may well control the infection.

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Chapter 11

Efficacy of Local Minocycline HCl 2% Gel as Adjuvant for Scaling and Root Planing in Chronic Periodontitis: A Prospective Randomized Open Blinded Endpoint Study

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Introduction

Chronic periodontitis is an inflammation of periodontal tissue caused by bacterial infection. The clinical symptoms are loss of attachment, bone destruction and tooth mobility. In Indonesia, a survey by the Indonesian Ministry of Health in 2010 showed that 157,485 people suffered from chronic periodontal disease (Kassebaum *et al* 2014). Since 1970, there has been an increasing awareness of bacterial involvement in the etiology of periodontal disease (Socransky 1970). Chronic periodontitis is caused by pathogens called the red complex, which includes *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*. These pathogens dominate the subgingival layers and are recognized as the most important pathogens in adult periodontitis (Slots *et al* 1979, Socransky *et al* 1998). The number of pathogens are usually higher in inflamed gingiva than in healthy (Socransky *et al* 1998). Mechanical debridement may not always be able to be carried out to an optimal level due to the variety and complexity of tooth anatomy, which may make it difficult to properly insert instruments into the periodontal pocket. Thus, a direct approach using antibacterial agents in systemic or topical administration has become an important part of periodontal disease management (Eickholz *et al* 2005, Petersilka *et al* 2002, Radvar 1996).

Routine use of systemic antibiotics is contraindicated due to the risk of antimicrobacterial resistance and possible systemic side-effects (Eickholz *et al* 2005). Therefore, local application of antibiotics directly at the subgingival area (into periodontal pockets) has become an alternative. Scaling and root planing, combined with local antibiotics, has been shown to have better results compared to scaling and root planing alone (Radvar *et al* 1996, van Steenberghe *et al* 1993, Timmerman *et al* 1996). A critical period of seven to ten days of routine antibiotic application is needed to provide a long term effect (Eickholz *et al* 2005).

One local antibacterial agent which is stable and has sustained action for at least seven days in periodontal pockets is the topical application of 2% minocycline (Radvar *et al* 1996, van Steenberghe *et al* 1993, Timmerman *et al* 1996). Minocycline is a member of the tetracycline class of antibiotics, and is effective in eradicating the

periodontal pathogens implicated in chronic periodontitis (Sunstar 2011). Subgingival application of minocycline can be used as an adjuvant therapy for chronic periodontitis after scaling and root planing (Ciancio *et al* 1980, Ciancio *et al* 1982, Nakagawa *et al* 1991, Perno *et al* 2001). Minocycline can reduce osteoid degradation by inhibiting osteoblast collagenase. Minocycline may also enhance bone formation by increasing the alkaline phosphatase and collagen synthesis produced by osteoblasts (Gomes *et al* 2007).

Unfortunately, minocycline gel is not yet available in many countries. In developing countries such as Indonesia, the scattered distribution of dental devices and experts requires development of practical, durable and effective treatment options for chronic periodontitis. Thus, in this study, we aimed to analyze the efficacy of minocycline HCl 2% gel administered subgingivally as an adjuvant treatment to scaling and root planing in a patient population.

Type of study

A prospective randomized open blinded endpoint study was conducted in a single center (Periodontic Clinic, Faculty of Dentistry, Universitas Indonesia) from November 2013 to November 2014. Ethics approval was given by the Research Ethics Committee of the Faculty of Dentistry, Universitas Indonesia. Patients were consecutively randomized into two parallel groups; minocycline group and control group.

Methods

Subjects

Patients were aged 30 to 55 years with localized chronic periodontitis, who had 4 to 6 mm proximal pocket depth (PD), clinical attachment loss (CAL) equal to or greater than 4 mm and gingival bleeding on probing. Inclusive criteria were patients that had not take any antibiotics in the last three months and had no periodontal treatment in the last six months. Patients were excluded if they were suffering from a systemic disease, were allergic to doxycyclin hyclate, had proximal tooth restorations, had proximal or cervical caries, pregnant or breastfeeding women, smokers, poor oral hygiene, malocclusion and patients on continuous medication.

Study drug

Minocycline HCl 2% gel (Periocline, Sunstar Japan) is a pale yellow coloured gel used for periodontal treatment. It contains 20 mg minocycline HCl (2% potent) per gram as microcapsules. It packed in a syringe (0.5 g) for every patient. The drugs were locally applied into the gingival pocket by inserting the gel at the periodontal pocket base, and then slowly pulling the ends of the syringe while continuing the injection.

Study procedures

At day zero of this study, patients who matched inclusion and exclusion criteria were examined for their oral hygiene. Subjects were examined for papilla bleeding index (PBI), PD and CAL. Subjects that fulfilled inclusion and exclusion criteria were consecutively randomized using a prepared randomization list. Patients with traumatic occlusion were given occlusal adjustments and evaluated for one week before receiving further treatment.

We performed supragingival scaling and root planing in all subjects at baseline (day 1). In the minocycline group, subgingival minocycline HCl gel 2% was applied. The same procedures were repeated at day 7 and day 14. Oral hygiene instruction was given to the patients after each procedure.

At day 14, the amount of plaque was scored

using Löe and Sillness Index (Löe 1967). Papilla bleeding index was scored using Muhleman Modification Index (Muhlemann and Son 1971). At day 21, plaque index, PBI, PD and CAL were scored. PD and CAL were examined using bite registration for probing. At month two, we measured the bleeding scores, PD and CAL in both groups.

The subjects were followed up for six months. At month 3 and month 6, subgingival plaque samples were taken for microbiologic test prior to clinical examinations. We scored the plaque index, PBI, PD and CAL during the follow up period. Radiographic images were taken to analyze the bone density and bone height using bite registration for radiographic film.

Outcome evaluation

In this study, we determined the efficacy of the applications of minocycline based on the rate of clinical and microbiological parameters. For clinical parameters, we measured PBI, PD and CAL at baseline, day 21, month 2, month 3 and month 6. In addition, we measured the increase in bone density at month 3 and 6 with radiographic imaging (Ellis *et al* 2002).

PBI were measured using a Hu-Freidy periodontal probe by carefully inserting the probe in the marginal gingival sulcus (Muhlemann and Son 1971). The mesial surface was measured from the labial/buccal site, while the bleeding on the distal surface was measured from the palatal/lingual site. The intensity of bleeding was measured after 20 to 30 seconds. The PBI was scored as follows: 0 for no bleeding; 1 for bleeding in form of point; 2 for bleeding in form of line; 3 for bleeding in form of triangle; and 4 for wide spread bleeding.

Periodontal pocket depth (PPD) was measured from the base of the gingival pocket with millimeter scale probe. Pocket depth was determined to the nearest millimeter. Pocket examination was measured from labial to buccal and from palatal to lingual (distal). Based on pocket depth, periodontitis can be described as: mild (pocket depths 1 to 3 mm), moderate (pocket depths 4 to 6 mm), and severe periodontitis (pocket depths more than 6 mm) (Cobb 1996, Novak 2006, Ranwey 1993).

CAL was measured from the cemento-enamel junction to pocket base. Measurement was done with periodontal probe in a millimeter scale. Increased or decreased attachment loss was defined as the difference in distance before and after treatment. We categorized the CAL as mild being 1 to 3 mm, as moderate when 4 to 6 mm and as severe being more than 6 mm attachment loss.

Plaque index was measured using an index of Loe and Silness (Löe 1967). We selected teeth 12, 16, 24, 32, 36 and 44. We examined the labial or buccal surface divided into facial, mesio facial, facial and disto facial, while the palatal or lingual were considered as single surfaces. The teeth were dried before plaque index scoring. Score 0 was noted if no plaque adheres to the tip of the explorer when a probe is passed along the cervical portion of the tooth surface. Score 1 was noted when a film of plaque adheres to the tip of explorer when a probe is passed along the cervical portion of the tooth surface. Score 2 was noted for a thin to moderate accumulation of plaque seen on the tooth surface at the cervical portion of the crown. Score 3 was noted when an abundance of plaque is seen on the tooth surface at the cervical portion of the crown. Individual plaque index was calculated as the average plaque score of mesiofacial, facial, distofacial, palatal surfaces. The individual scores of all teeth were then added and divided by the total number of teeth examined.

We performed radiographic evaluation from intraoral digital radiographs of alveolar bone loss in order to measure bone density

(International Association of Maxillofacial Radiology 1995, White *et al* 2004). Radiographic bone density were measured on teeth with 4 to 6 mm pocket depth using periapical projection on the mesial and distal. Radiographic bone density was measured with mean gray levels in an 8 bit computer ranging from 0 to 256 pixels (International Association of Maxillofacial Radiology 1995). We categorized the bone density with a cut-off point of pixel intensity value of 100, as low or high bone density (Hedstr *et al* 2010).

Statistical procedure

In this study, the sample size was calculated based on a power of 80% and alpha level of 5% using standard deviation of 0.8 mm and precision of 0,3736 (Piantadosi 2005). The minimal sample size was determined as 36 subjects for each group.

Statisticians were blinded on the treatment arms. Difference between means of both groups at each time point were tested using student t-test for numerical data (PD, CAL, bone density) and Wilcoxon rank sum test for non-parametric, categorical data (PBI, bleeding index, calculus index). Changes between different timeframes (baseline vs month 3 vs month 6) were tested using Wilcoxon sign tests. Differences between means of both groups were tested using ANOVA and Friedmann Two Way Anova. Data were analyzed using SPSS 20.

Results

84 patients in the study were randomized into 42 patients in each group. Two patients from the treatment group dropped out from the study before receiving any treatment. This resulted in 40 patients in the treatment group

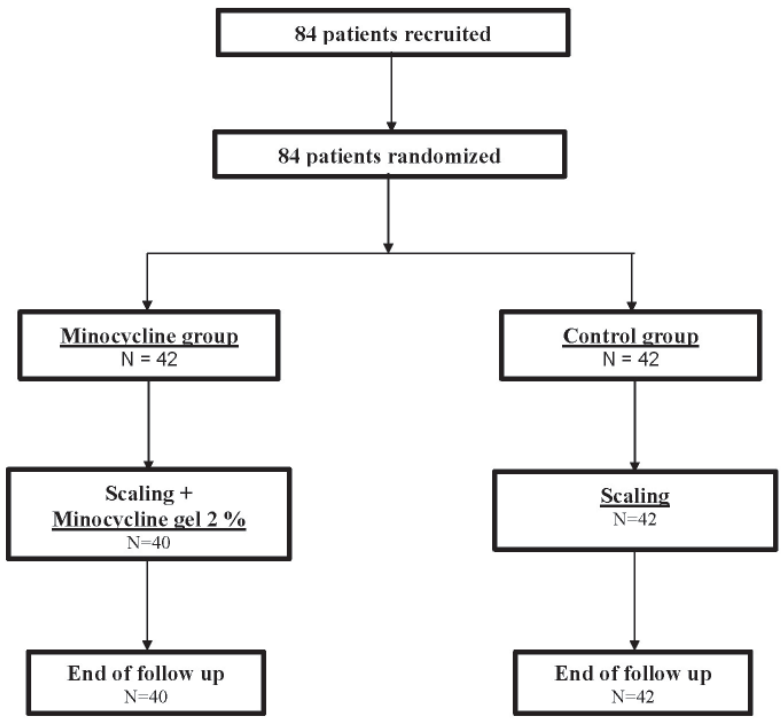

Figure 1. Study participant flow chart

Characteristics	Mean ± SD (unless stated otherwise)		P
	Minocycline group (N = 40)	Control group (N = 42)	
Female, n (%)	33 (78.6%)	31 (77.5%)	0.91
Age (years)	43.67 ± 6.88	44.24 ± 6.99	0.74
BMI (kg/m2)	25.83 ± 2.89	25.24 ± 3.89	0.44
Plaque index score	1.04 ± 0.54	1.08 ± 0.49	0.69
Calculus index score	1.26 ± 0.64	1.44 ± 0.84	0.42
Oral hygiene index score	2.31 ± 1.10	2.53 ± 1.13	0.35
Low bone density	78.67 ± 9.91	67.85 ± 13.09	0.00
High bone density	112.60 ± 9.86	105.01 ± 3.86	0.23

Table 1. Baseline characteristics of subjects.

(minocycline group) and 42 patients in the control group with mean age of 44 years that were followed for 6 months (Figure 1). From Table 1, we can see that the characteristics of test and control groups were similar except for patients with low bone density.

Clinical efficacy

Table 2 describes the CAL, pocket depth and papilla bleeding index based on baseline CAL. CAL, PD and PBI were observed in both groups for 6 months. CAL decreased rapidly in the minocycline group and was significantly different compared to the control group. PBI in the minocycline group were decreased and significantly different to control group with moderate and severe baseline CAL (>4 mm) at day 21 and month 2 follow-up ($p < 0.05$). This loss remained stable during the 6 months of follow-up. Pocket depths decreased in both groups and no significant difference ($p > 0.05$) was found.

In Table 3 we classified subjects based on their bone density and CAL at baseline. All patients with low bone density and moderate to severe CAL at baseline had increased bone density at month 3 and 6, although the increase was not significant. Similar findings were found in patients with low bone density and mild CAL (<4 mm) at baseline. The increase was significant for patients in the control group after month 3, and the increase was only significant for patients with minocycline at month 6.

Minocycline treated patients with high bone density and moderate to severe CAL at baseline had a significant reduction of bone density at month 3. A similar pattern was shown in minocycline patients with high bone density and mild CAL at baseline.

Discussion

In this study, we describe the efficacy of subgingival application of minocycline HCl 2% gel in an Indonesian population. Addition of minocycline HCl 2% to scaling and root planning in chronic periodontitis improved clinical outcomes in longer periods compared to scaling and root planning alone. The test drug also decreased the population of microbiological pathogens significantly.

Regeneration, repair, and a new attachment of periodontal tissues are the goals of treatment of periodontal disease. In our study, clinical attachment loss decreased rapidly in the minocycline group and was significantly

	B a s e - line	**P**	**21 days**	**P**	**2 mths**	**P**	**3 mths**	**P**	**6 mths**	**P**
Mild attachment loss <4.0 mm at baseline										
Clinical attachment loss										
Test	4.00 ± 0.00	1	2.67 ± 0.48	0.01*	2.58 ± 0.49	0.00*	2.72 ± 0.57	0.00*	2.72 ± 0.67	0.00*
Control	4.00 ± 0.00		2.87 ± 0.60		2.87 ± 0.63		3.25 ± 0.75		3.35 ± 0.78	
Pocket depth										
Test	4.00 ± 0.00	1.00	2.85 ± 0.47	0.34	2.73 ± 0.52	0.04*	2.72 ± 0.57	0.91	2.72 ± 0.67	0.76
Control	4.00 ± 0.00		2.73 ± 0.48		3.00 ± 0.47		2.75 ± 0.68		2.80 ± 0.63	
Papilla bleeding index										
Test	0.28 ± 0.13	0.24	0.07 ± 0.08	0.19	0.07 ± 0.11	0.49	0.05 ± 0.05	0.26	0.10 ± 0.10	0.88
Moderate to severe attachment loss >4.0 mm at baseline										
Clinical attachment loss										
Test	5.12 ± 0.34	0.97	3.50 ± 0.72	0.01*	3.41 ± 0.70	0.00*	3.29 ± 0.75	0.01*	3.33 ± 0.64	0.00*
Control	5.18 ± 0.52		4.50 + 1.71		4.65 + 1.66		4.31 + 1.63		4.45 ± 1.85	
Pocket depth										
Test	5.12 ± 0.34	0.68	3.50 ± 0.72	0.62	3.61 ± 0.94	0.64	3.29 ± 0.75	0.45	3.33 ± 0.64	0.49
Control	5.07 ± 0.61		3.41 ± 0.70		3.53 ± .03		3.13 ± 0.76		3.20 ± 0.75	
Papilla bleeding index										
Test	0.42 ± 0.42	0.10	0.03 ± 0.05	0.01*	0.07 ± 0.05	0.02*	0.06 ± 0.63	0.53	0.08 ± 0.05	0.10
Control	0.61 ± 0.42		0.10 ± 0.12		0.16 ± 0.17		0.07 ± 0.11		0.13 ± 0.14	

Table 2. Comparison of Mean ± SD of clinical data based mild attachment loss (<4 mm) and moderate to severe attachment loss (>4 mm) at baseline. Note: * difference between this time of follow up with the previous phase of follow up was significant with $p < 0.05$.

	Base-line	P	3 mths	P	6 mths	P
Low bone density and mild CAL <4.0 mm at baseline						
Test	78.78 ± 12.23	0.01	79.47 ± 13.89	0.07	81.21 ± 13.87$	0.04*
Control	64.41 ± 11.94		69.05 ± 11.77		68.65 ± 13.01#	
High bone density and mild CAL <4.0 mm at baseline						
Test	115.36 ± 12.47	NA	111.69 ± 5.00*	NA	117.71 ± 11.44^	NA
Low bone density and moderate to severe CAL >4.0 mm at baseline						
Test	78.60 ± 8.43	0.01	82.37 ± 10.47	0.01*	83.05 ± 11.07	0.55
Control	69.13 ± 13.48		71.48 ± 13.35		80.20 ± 18.79	
High bone density and moderate to severe CAL >4.0 mm at baseline						
Test	105.01 ± 3.86	0.28	100.87 ± 6.82	0.18	106.69 ± 4.08	0.49
Control	109.15 ± 4.84		109.35 ± 7.39		111.44 ± 10.26	

Table 3. Comparison of Mean ± SD of radiographic data based on bone density and CAL status at baseline. Note: There were no patients in the control group in category of High Bone Density (>100 PI) with baseline CAL <4.0 mm. * Differences between baseline and month 3 were significant ($p < 0.01$); Differences between month 3 and month 6 were significant ($p < 0.05$); ^ Differences between baseline and month 6 were significant ($p < 0.01$); # Differences between baseline and month 6 were significant ($p < 0.05$).

different compare to the control group. Papilla bleeding index in the minocycline group was decreased and significantly different in treatment group, with moderate to severe baseline CAL remaining stable for the 6 months of follow-up. These results are similar to two short-term, double-blind, parallel studies by Nakagawa *et al* (1991) and Van Steenberghe *et al* (1993) that evaluated the effect of subgingivally administered 2% minocycline in addition to mechanical debridement. Their studies showed that the treatment group had better response than patients in the placebo group. However, another study by Timmerman *et al* (1996) showed no statistically significant differences between test and control groups in probing depth and attachment level.

High quality radiographic imaging at the right time period is needed to evaluate the quality and quantity of alveolar bone after periodontal treatment (Van Steenberghe *et al* 1993). Among our patients who had bone density and low CAL ≤4 mm, minocycline significantly increased bone density after six months. Although not significantly different from the control group, the same pattern can be observed in patients who have low bone density with moderate to severe CAL ≥4 mm. Previous studies have stated that bone healing in chronic periodontitis patients took 4 to 6 months, while it might take 6 to 12 months to form mature bone (Ellis *et al* 2002, White *et al* 2005).

It should be acknowledge that our study had several weaknesses. Firstly, this study was

an open randomized trial without masking. This design was prone to observation bias that may have impacted the results. However, in this study we used hard clinical and radiological endpoints, thus the bias was reduced. Moreover, our effort to blind the statisticians who analyzed the results may help minimize the bias. Second, the study was conducted only in a single center located in a metropolitan city. Obviously, this impacted generalizability, as patient characteristics and severity of the disease in a metropolitan city may differ from other areas. However, patient characteristics in our study were similar with to the characteristics of chronic periodontitis populations in general.

Conclusion

Adjuvant minocycline HCl 2% gel to scaling and root planing has showed promising results. The use of minocycline HCl 2% might assist dentists in providing effective treatment options for their chronic periodontitis patients.

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Chapter 12

A Report of Three Periodontally Compromised Extranodal Non-Hodgkin's Lymphoma Cases

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Introduction

Lymphomas are a heterogeneous group of malignant neoplasms from lymphocytes, histocytes and their precursors, with a spectrum of behaviors. Lymphoma is only second to squamous cell carcinoma in frequency of occurrence of malignant lesions in the head and neck (DePena *et al* 1990). In China, the incidence of lymphoma is 1.39 per 100,000 for males and 0.84 per 100,000 for females (Ren and Lan 2003). It accounted for 2.08% of all cancer deaths and is the seventh most common cause of cancer-related death (Wang and Mi 2003, Zhang *et al* 2013). Lymphomas comprises of two main groups: Hodgkin's lymphoma, and non-Hodgkin's lymphoma (NHL). Hodgkin's lymphoma, often existing in lymph nodes, is comprized of typical Reed-Sternberg cells, while 40% of NHL may present outside the lymphoid system (Jordan and Speight 1996). NHL is subdivided into B-cell and NK/T-cell subtypes according to microscopic and immunohistochemical features, with the latter kind being far scarcer than the former. Studies showing NHL in periodontal tissue are limited. In the present chapter, three cases were first diagnosed as NK/T-cell NHL in periodontal clinic at a relatively early stage. The purpose of this chapter is to define the clinical features of NHL involved periodontal tissue, and to emphasize the vigilance of periodontal tissues presenting with inflammatory lesions.

Case reports

Case 1

A 34 year old male was referred to the Periodontal Department of Peking University School of Stomatology in December 2012 with the complaint of ulceration on the lower anterior gingiva for the past 20 days. In November 2011, he presented at a local hospital because of right nasal obstruction and facial swelling, accompanied by night sweats and fever of 38°C. The local doctor considered it an infection and prescribed a course of antibiotics. Although the facial swelling vanished after taking medication, the patient still felt nasal discomfort. 20 days prior he noticed gingival ulceration around lower anterior teeth and had persistent fever of 37°C. The ulcer was biopsied and diagnosed as inflammatory hyperplasia by a local hospital.

The patient presented to our clinic with poor oral hygiene (Figure 1A). The plaque index was 1 to 2, and dental calculus was 2+. Ulceration and necrosis were present around the lower central incisors and right lower lateral incisor. The coarse fringe ulcer was 7 x 3 mm on the labial side and 7 x 3 mm on the lingual side. These three teeth had class II/III

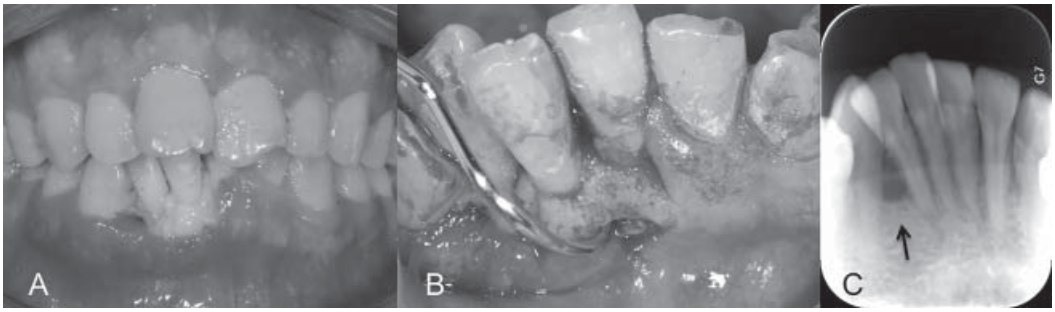

Figure 1. Intraoral view at first visit. (A) General oral health was poor with necrosis in lower anterior area. (B) Gingival fistula present through labial and lingual side in the bone resorption area. (C) Curving bone loss present on radiograph.

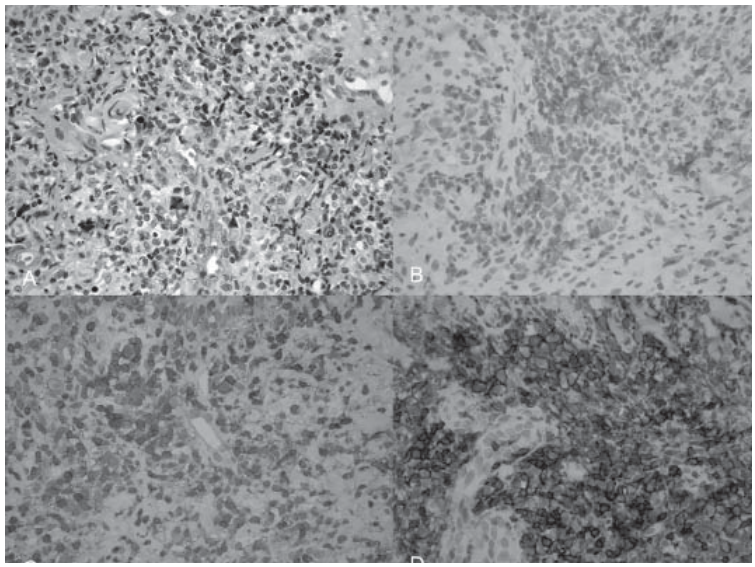

Figure 2. Pathological sections. (A) Haematoxylin-eosin staining demonstrated hyperplasia and lymphocytic infiltration combined with scattered large atypical cells. (B) CD56 immunohistochemical staining showed few large cells. (C) CD45RO immunohistochemical staining is obvious. (D) Diffused CD3 staining could be found.

mobility and 2 to 4 mm gingival recession.

The localized radiograph showed curving bone resorption on the right alveolar crest of tooth 42 and horizontal bone loss of other lower anterior teeth all nearly to the apical one third (Figure 1C). Lymphoma was highly suspected and the ulcer was then re-biopsied on both sides by an experienced periodontist (Figure 1B).

The biopsy showed mucous inflammation with local lymphocytic hyperplasia, which was partly aggregating near a blood vessel. On these pathological tissues, there was covering squamous epithelia with ulceration and severe necrotic exudation. Lymphocytic infiltration was combined with scattered large atypical cells, whose nucleus was big and pleomorphic in lightly stained cytoplasm. Some lymphocytes invaded into thickening blood vessel (Figure 2A). Immunohistochemical staining manifested LCA (plenty+), CD3 (diffused+), CD20

(focal+), CD45RO (plenty+), CD56 (only few large cells+), CD68 (histocyte +) (Figure 2B-D).

Special examination showed Granzyme B (+) and EBV-EBER (+) expressing cytotoxicity. CD8 and TiA1 were (a few+). Ki67 was (large cells+). Other auxiliary examinations such as hemogram had no significant abnormality.

Nasopharyngeal CT showed symmetrical recessus pharyngeus, nasal patency and slightly right-curving deflection of nasal septum as well as mucosal thickening of right maxillary sinus. Basis cranii, parotid gland, salivary gland, and thyroid gland were normal while lymphadenovarix was absent. Chest CT scan demonstrated normal lung sac without obvious pulmonary tumor or tubercule. There were no tumescent lymph nodes in mediastinum or underarm nor hydropericardium or pleural effusion present.

The patient was finally diagnosed as non-Hodgkin's lymphoma involving right nasal cavity and right cervical lymph node. The lesion was an extranodal malignant NK/T cell lymphoma, nasal type based on WHO pathological classification of IIB stage with I IPI score.

A few sequestra could be seen on the first follow-up. The patient later returned to the local hospital for treatment with GEMOX (gemcitabine plus oxaliplatin) combined with pegaspargase for six courses at intervals of one month. At the second follow-up after the chemotherapy in April 2013, the local lesion had not spread but had somewhat diminished and CBCT showed no further loss of hard apparatus.

Case 2

A 24 year old female presented at the clinic in March 2001 complaining of lower anterior gingival ulceration over the preceeding week. Approximately 12 days prior she had noticed a rapidly expanding red lesion in a lower anterior site. The lesion was misdiagnosed as necrotizing gingivitis by another hospital, and prescribed a mouthwash after local irrigation but the symptoms did not improve.

Intraoral exam showed a 13 x 12 mm ulcer on the lower anterior labial gingiva extending to vestibule covered with a white pseudomembrane which did not wipe off (Figure 3A). The base of the ulcer was uneven and surrounded by a red, hard border. The labial gingiva was necrotizing, revealing white alveolar bone proper. The two lower central incisors were distinctively loose, and displayed proximal radiolucency on radiograph (Figure 3B).

Bacterial smear and Gongo red staining showed no spirochete or fusiform bacteria. Laboratory tests showed the patient was HIV negative without abnormality in hematological analysis. Bacterial culture showed *Enterobacter cloacae*, sensitive to ciprofloxacin and ceftazidime pentahydrate. The immunohistochemical outcome of biopsy was LCA (+), CD45RO(+), L26 (-), supporting the ultimate diagnosis of extranodal T cell type non-Hodgkin's Lymphoma.

The patient revisited our clinic one, two, three and five years after radiotherapy respectively (Figures 3C and D). The macroscopic impairment started to reduce in the first year and had almost disappeared two years later. The patient now is free of disease.

Case 3

The third case was a young male aged 20 presenting in September 2001. His complaint was gingival pain over the previous three months. About three months ago, the gingiva surrounding his lower posterior teeth, left maxillary posterior teeth and lower anterior teeth became sore and ulcerative necrosis was present. Metronidazole was self-prescribed but had no effect. He had a fever of 38°C for

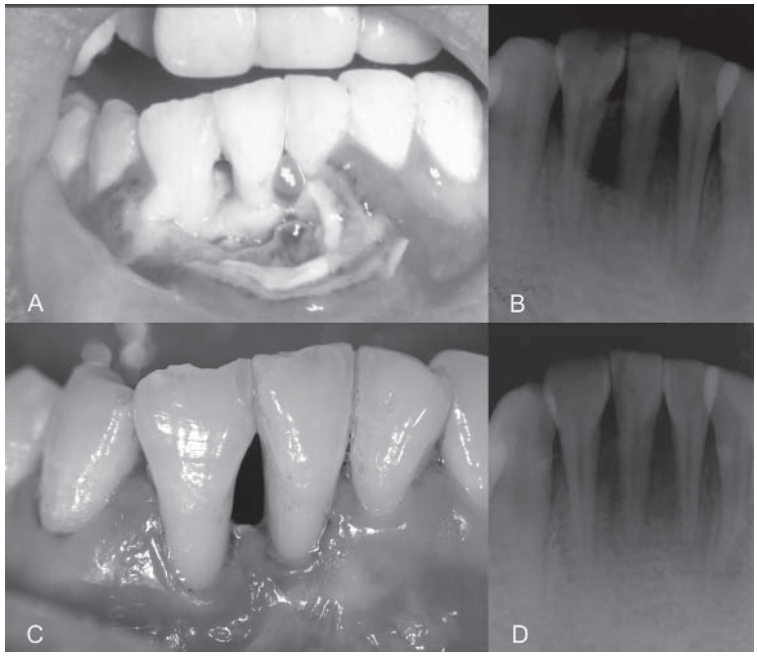

Figure 3. (A) Massive ulceration and pseudomembrane were seen on the labial gingival of lower anterior teeth. (B) Proximal radiolucency demonstrated in the local radiography. (C) Soft tissue lesion greatly alleviated and the situation stable with only mild inflammation after five years. (D) The partial recovery of alveolar crest in compromized area could be observed in follow-up radiography.

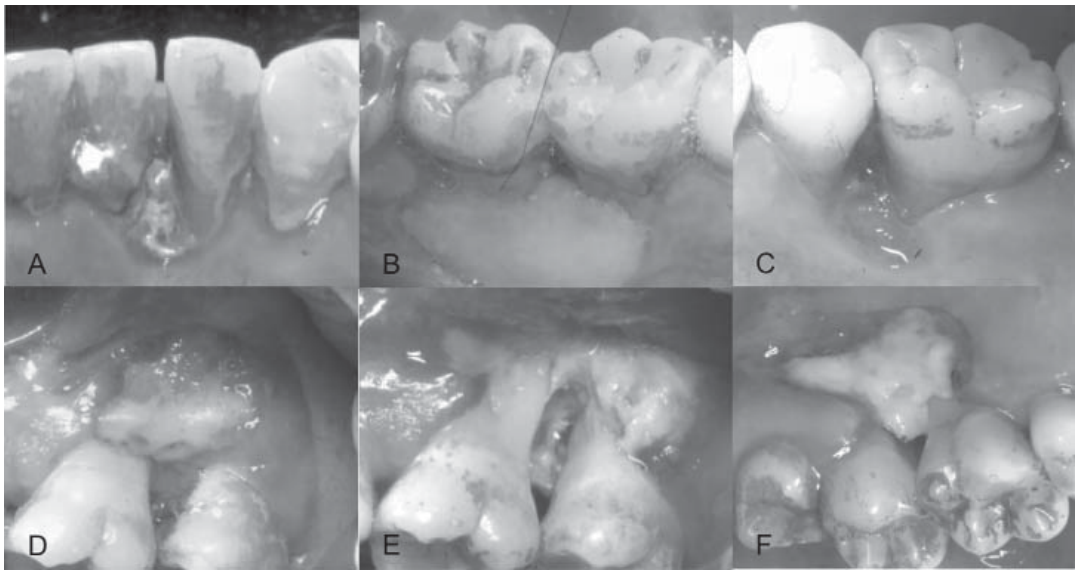

Figure 4. (A) Ulceration on the lingual gingiva of lower anterior teeth. (B) Widespread ulcer on the lingual gingiva of left lower molar teeth. (C) Ulcer on the lingual papilla of right lower molar teeth. (D) The ulcer lesion on the buccal side of left upper molar teeth on first visit. (E) The extent of left upper molar buccal lesion significantly increased after one month. (F) A new ulcer lesion formed on the lingual gingiva of left upper molar teeth and connected with the buccal lesion one month following the first visit.

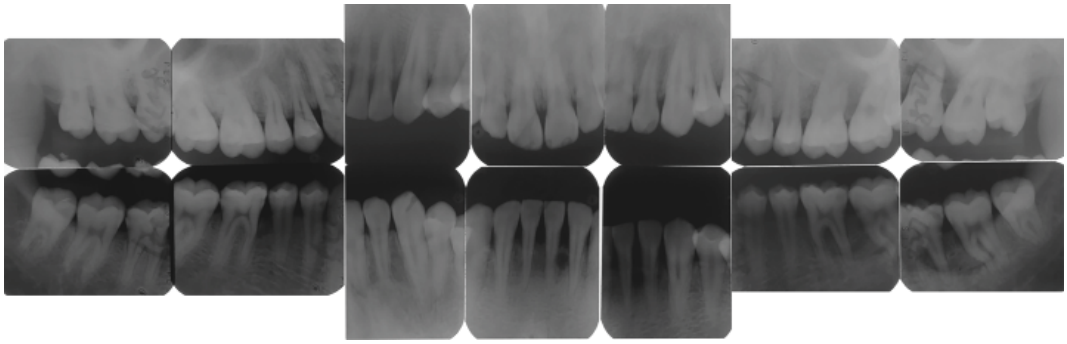

Figure 5. Full-mouth apical radiography. Bone loss and radiolucency distinctively present in lesion compromized bilateral molar area.

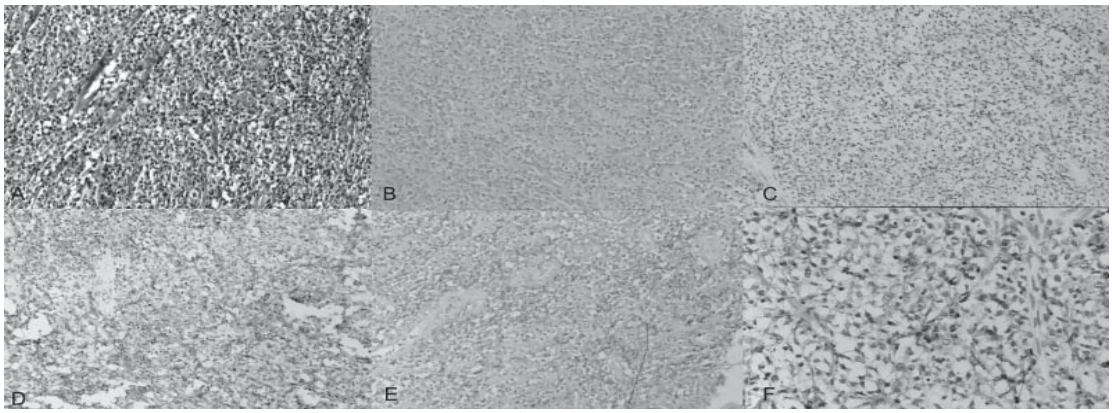

Figure 6. (A) H-E staining. (B) CD43- under 20 times magnification. (C) CD20- under 20 times. (D) LCA20+ under 20 times. (E) S100+ under 20 times magnification. (F) CD45 + under 20 times magnification.

no apparent reason. The local hospital found no cause, so prescribed anti-inflammatory medicine. He attended our hospital for continuous fever.

His oral hygiene was poor, with large amounts of dental calculus. The buccal gingiva at 26 and 27 showed a 7 x 6 mm firm ulcer without tenderness. A lingual gingival ulcer around 36 and 37 was 13 x 6 mm. On the lingual side of teeth 31, 32, 45, and 46, two ulcerative sites were apparent (Figure 4). Necrosis was not only universal on these ulcer sites, of which the margin was punched out, but also alveolar bone was exposed. Full-mouth radiography illustrated heavy bone resorption of 36 and 37 almost exceeding the root whole length and 26 to apical first third (Figure 5). Other teeth had bone loss to differing extents. Three weeks later, the buccal lesion had increased and a new ulcer emerged from the palatal aspect of teeth 26. Blood smear displayed regular leukocytic morpha without korocyte proliferation. Multidisciplinary consultation was carried out with the endodontic, maxillofacial surgery and pathologic departments. Biopsy after inflammatory control by supra-gingival scaling and irrigation was carried out. The immunohistochemical consequence was LCA (+), CD45 (+), S100(+), CD20(-), which gave rise to the diagnosis of T-cell non-Hodgkin's lymphoma (Figure 6). The patient received radiographic therapy immediately after diagnosis, however unfortunately passed away

the following year.

Discussion

Only 3.5% of all oral malignancies are NHL (Chan and Chan 2005). It was reported that 10 to 20% cases of NHL are primary extranodal NHL. 2 to 3% of these extranodal cases may arise primarily in the oral cavity and jaws (Freeman *et al* 1972). Waldeyer's ring is commonly the most prevalent area for extranodal non-Hodgkin's lymphomas of the head and neck region, followed by hard palate, gingiva and tongue. Other sites include posterior hard palate, gingiva and buccal vestibule. Oral non-Hodgkin's lymphoma is more prone to be diffused rather than an insular primary focus. The oral cavity, including the palate, gingiva, tongue, buccal mucosa, floor of the mouth and lips, are the primary sites of approximately 2% of all extranodal lymphomas (Ferry and Harris 2005). The most common clinical appearance of NHL in the mouth is a non-healing, painless ulceration (Richards *et al* 2000). NHL can affect both bony and soft oral tissue, with the most frequent localization being the palate and the mandible. However, it is rare to find extranodal NHLs in the gingiva (Castellano *et al* 2002).

The most common subtype of NHL is disseminated large B cell lymphomas, while NK/T cell type occupied close to 6.5%. Mature T cells and NK cell neoplasms make up only about 12% of NHL cases worldwide (Non-Hodgkin's Lymphoma Classification Project 1997). The incidence of NK/T-cell lymphoma is 2 to 10% of the cases of primary non-Hodgkin's disease, and its occurrence is more frequent in Asian populations, especially southern China and South East Asia (Hahn *et al* 2002, Li *et al* 1998, Van Prooyen Keyzer *et al* 2000, Wan *et al* 2004).

The probable symptoms of oral NHL are persistence of pain with mucous ulceration, neurological disorder, tumor mass on gums and unexplained tooth mobility, which should alert the dentist to the need to perform a biopsy. In the head and neck region, extranodal NHLs predominantly affect the midface, causing extensive destruction of the cartilages and surrounding soft tissues as a result of both inflammatory processes and its angiocentric, angioinvasive and angiodestructive behavior (Chan *et al* 1997, Chan and Chan 2005, Van Prooyen Keyzer *et al* 2000). This causes invasion and destruction of blood vessel walls leading to widespread necrosis (Chan *et al* 1997, Freeman *et al* 1972, Van Prooyen Keyzer *et al* 2000).

Michaud *et al* (2008) examined hematologic malignancy for an association with history of periodontal disease with verified radiographic bone loss, and reported an increase with non-Hodgkin lymphoma (NHL), leukemia, and myelomas but only NHL yielded a significant relationship. However, Hiraki *et al* (2008) could not demonstrate an association between lymphomas in general and tooth loss. In the present report, all three cases expressed necrotizing gingiva ulcers combined with bone loss to dissimilar degrees. They all had previous medical attendance history for biopsy but were not correctly treated because their clinical signs could easily be misdiagnosed as necrotizing gingivitis, pyogenic granuloma or pericoronitis. Two of the three patients were referred for a second biopsy for microscopic and immunohistochemical staining due to the persistence of a periodontal expert with abundant pathological experience in order to be properly diagnosed. It was reported that multiple biopsies are sometimes necessary for NK/T cell lymphomas. 56.5% of patients underwent a single biopsy procedure to make a correct diagnosis. Two biopsies were required in 20.9% of patients, while 22.6% patients required three or more for diagnosis (Wu *et al* 2008). The accuracy of differential diagnosis is based on sufficient knowledge of

the presentation of oral neoplasms.

Patients with ENKTCL have poor survival outcomes, with the cumulative probability of survival at five years ranging from 37.9 to 45.3% (Cheung *et al* 1998, Cheung *et al* 2002, Chim *et al* 2004, Lee *et al* 2005).The prognosis was strongly influenced by the stage at which NHL was diagnosed and remedied, therefore early detection for NHL is extremely crucial. Although the long-range survival rate of NK/T cell lymphoma is low, the patient described in the first case received chemotherapy at an early stage and the tissue destruction is now stable. 35% of cases metastasized to oral soft tissues. The gingiva and tongue are the most common sites in the oral cavity for metastasis, with adjacent apparatus the third common. 35% of cases have oral lesions appeared as the first sign of malignant disease, pre-dating diagnosis of the primary focus (Arendt 1985).

The principal modes of therapy are chemotherapy and/or radiation treatment, depending on the morphological type, grade of lymphoma, and clinical stage. Although an optimal therapy has still not been found, local low-grade lesions can be left untreated unless associated with clinical expression (Al-Hakeem *et al* 2007). Patients with intermediate to high-grade NHL lesions are recommended to undergo radiotherapy if localized, or a combination of radiotherapy and chemotherapy if diffused. Others have reported the importance of complete excision of localized lesions during biopsy and periodontal plastic surgery of tissue defects to manage esthetic concerns. However recurrence is common, even several years after therapy and long term follow-up is indispensable. Cautious risk factor revaluation is essential to rule out relapse, along with managing potential complications.

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Chapter 13

Adipokines in Gingival Crevicular Fluid Correlating With Tear Fluid in Periodontitis, Obesity and Diabetes Mellitus

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Introduction

Chronic periodontitis (CP) is a complex and multifactorial disease that results from the interaction of the host-defense mechanisms with plaque microorganisms. This interaction leads to the primary clinical features of periodontitis, which are gingival inflammation, attachment loss, periodontal pockets and alveolar bone loss (Marsh 1994, Socransky and Haffajee 1999). Obesity is characterized by the abnormal or excessive deposition of fat in the adipose tissue. It is a risk factor for several chronic diseases, most notably hypertension, type 2 diabetes, dyslipidemia and coronary heart disease (Must *et al* 1999). It is associated with oral diseases, particularly chronic periodontitis and has been recognized as second only to smoking as the strongest risk factor for inflammatory periodontal tissue destruction (Nishida *et al* 2005, Saito *et al* 2005). Diabetes mellitus (DM) comprises a group of metabolic disorders manifested by abnormally high levels of glucose in the blood (Mealey and Ocampo 2007). Diabetes is now viewed as an inflammatory condition and its development is preceded by a low grade systemic inflammation, with elevated plasma concentrations of pro-inflammatory mediators (Freeman *et al* 2002). Periodontitis is described as the sixth complication of diabetes (Loe 1993). Adipokines are inflammatory mediators associated with an inflammatory cytokine profile leading to destruction of periodontium along with monocytes/macrophages, lymphocytes, chemokines and other cell types involved in inflammatory processes (Pradeep *et al* 2012). Similar pathways are involved in the pathophysiology of CP, obesity, DM and related inflammatory diseases. These include vaspin, chemerin and lipocalin 2 (LCN2). Vaspin is a visceral adipose tissue-derived serine protease inhibitor. It is a 395 amino acid, 45.2KDa adipokine showing approximately 40% homology with alpha1-antitrypsin. It is expressed throughout the body, particularly in white adipose tissue/ fat cells, having an effect on liver and skeletal muscle (Hida *et al* 2005). It has been identified as a new biomarker for obesity and type 2 DM (suggesting impaired insulin sensitivity and human metabolic syndrome), with serum levels correlating with body mass index (BMI) (Youn *et al* 2008). Chemerin is a 137-amino acid protein secreted in liver and adipocytes. Active chemerin binds to the G protein coupled receptor ChemR23, which is expressed on macrophages and periodontal dendritic cells, and induces cell migration (Wittamer 2005). It is associated with adiposity, insulin resistance and metabolic

syndrome risk factors. It is associated with several inflammatory markers in obesity and type 2 diabetes (Ress *et al* 2010, Weigert *et al* 2010). Lipocalin 2 [LCN2] [24p3] [neutophil gelatinase associated lipocalin (NGAL)] is a 25-kDa secretory glycoprotein, originally identified in mouse kidney cells and human neutrophil granules (Wang *et al* 2007). It is also found in liver, lung, kidney, adipocytes and macrophages. It is implicated in diversified functions like apoptosis and innate immunity (Wang *et al* 2007). It is involved in cell survival, inflammation and matrix degradation (Gupta *et al* 2007). A strong positive correlation was observed between serum LCN2 concentrations and BMI (Wang *et al* 2007).

To date, no study has reported on human vaspin, chemerin and lipocalin 2 (LCN2) levels in GCF and tear fluid in obese or diabetic subjects with CP. In this context, this first of its kind clinico-biochemical study was designed to estimate and correlate the levels of vaspin, chemerin and LCN2 in GCF and tear fluid in healthy and CP subjects, with and without obesity/type 2 DM.

Materials and methods

This study was conducted from September to December 2013. The study was performed in full accordance with the ethical principles. The study protocol was approved by the Institutional Ethical Committee and Review Board of the Government Dental College and Research Institute, Bangalore, India. After ethical clearance was granted, 40 subjects each (20 males and 20 females) for the obesity and DM studies were selected from the outpatient section of the Department of Periodontology, Government Dental College and Research Institute, Bangalore, India. Written informed consent was obtained from those who agreed to voluntarily participate in the study.

Inclusion criteria consisted of subjects in the 25 to 40 years age group, with at least 20 natural teeth, diagnosed with chronic periodontitis who had not received periodontal therapy within the preceding six months.

Exclusion criteria consisted of subjects suffering from aggressive periodontitis, hypertension, eye infection, gross oral pathology, rheumatoid arthritis, heart disease, tumors, or any other systemic disease that can alter the course of periodontal disease. Pregnant or lactating females, or those subjects who had taken any medication affecting periodontal status such as cyclosporins, phenytoin or calcium channel blockers or antibiotics, anti-inflammatory drugs (NSAIDS) or had received periodontal therapy in the preceding six months were excluded from the study. Patients with a history of smoking and those wearing contact lenses were excluded. For diabetes studies (chemerin), obese individuals were not included. For obesity studies (vaspin/LCN2), diabetics were excluded.

A full mouth periodontal probing and charting was done for each subject and intraoral periapical radiographs were taken for each subject using the long cone technique. BMI charting was done according to the WHO Guidelines (World Health Organization 2000) and diabetic status evaluated based on glycated hemoglobin levels (HbA1c) and post-prandial plasma glucose (PPPG) criteria of the American Diabetes Association (American Diabetes Association 2012).

Subject grouping

The subjects were classified into different groups based on probing pocket depth (PPD), gingival index (GI), bleeding on probing (BOP), clinical attachment loss (CAL), radiographic evidence of bone loss (BL), BMI, HbA1c and PPPG.

For obesity studies (vaspin/LCN2): Group 1 (healthy, non obese) consisted of 10 subjects

with GI = 0, PPD ≤3 mm, CAL = 0, no radiographic crestal bone loss and BMI >18.5 kg/m^2 and <22.9 kg/m^2. Group 2 (healthy, obese) consisted of 10 subjects with GI = 0, PPD ≤3 mm, CAL = 0, no radiographic crestal bone loss and BMI >25 kg/m^2. Group 3 (non obese, CP) consisted of 10 subjects with GI ≥1, PPD ≥5 mm, CAL ≥3 mm, radiographic evidence of bone loss and BMI >18.5 kg/m^2 and <22.9 kg/m^2. Group 4 (obese, CP) consisted of 10 subjects with GI ≥1, PPD ≥5 mm, CAL ≥3 mm, radiographic evidence of bone loss and BMI >25 kg/m^2.

For type 2 DM studies (chemerin): Group 1 (healthy) consisted of 10 subjects with GI = 0, PPD ≤3 mm, CAL = 0, no radiographic crestal bone loss, HbA1c <6.5% and 1 to 2 hour PPPG <140 mg/dl. Group 2 (CP) consisted of 15 subjects with GI ≥1, PPD ≥5 mm, CAL ≥3 mm, radiographic evidence of bone loss, HbA1c <6.5% and 1 to 2 hour PPPG <140 mg/dl. Group 3 (CP with well-controlled type 2 DM) consisted of 15 subjects with GI ≥1, PPD ≥5 mm, CAL ≥3 mm, radiographic evidence of bone loss, HbA1c <7% and 1 to 2 hour PPPG <180 mg/dl.

Site selection

One examiner carried out all the clinical examinations, intraoral radiographic examinations, group allotment and sampling site selection. Another examiner, who was blinded to the groups allotted, collected the samples on the following day. This was done to monitor the contamination of GCF with blood at the time of probing. In order to obtain intra-examiner reproducibility, all the clinical assessments were performed with a University of North Carolina (UNC)-15 periodontal probe (Hu-Friedy, Chicago, IL, USA). Sampling in CP subjects was done from sites with the greatest CAL, signs of inflammation and showing radiographic evidence of bone loss. In healthy subjects, samples were pooled from 2 sites.

GCF collection

Initially, the subjects were made to sit comfortably on the dental chair in an upright position, after which air drying of the selected test site was performed. This was followed by isolation using cotton rolls. Supragingival plaque was then removed gently using a Universal Gracey curette #4R/4L to avoid contamination of the paper strips (Periopaper, Ora Flow Inc., Amityville, NY, USA) and GCF was collected using the intracrevicular 'superficial' method developed by Loe and Holm-Pederson (1965). The absorbed GCF volume of each strip was determined by electronic impedance using Periotron 8000 (ProFlow Inc., Amityville, NY, USA). The periopaper strips were placed in 400 µl of phosphate buffer saline kept in a sterile vial. These were then stored at -70^{o}C until the assay procedure. Periopaper strips spoilt with blood or saliva were rejected. After GCF collection, periodontal treatment (scaling and root planing) was carried out for periodontitis subjects at the same appointment.

Tear fluid collection

0.5 µl of minimally stimulated tear fluid was collected from the inferior tear meniscus of each eye using a glass capillary micropipette (Sigma-Aldrich Company, Bangalore, India). A total of 1 µl of tear fluid (0.5 µl from each eye) was eluted into one tube holding 9 µl of assay buffer to give it a 1:10 final dilution. This was followed by a two minute centrifugation and transportation in an insulated cooler to a -70° C freezer where they were stored until the time of assay.

Adipokine analysis

The samples were assayed for human

vaspin, LCN2 and chemerin using their respective enzyme linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (ABO Swiss Co. Ltd, China). The GCF sample tubes were centrifuged after homogenization for five minutes at 1500 g for 30 seconds to elute the solution. The elute was then used as sample for ELISA estimation from GCF samples. A 96 well microplate pre-coated with polyclonal antibody specific for each adipokine was used. Standards and samples were pipetted into the wells and any vaspin/LCN2/chemerin present was bound by immobilized antibody and captured by biotinylated antihuman vaspin/LCN2/chemerin polyclonal antibody (as per the kit). Addition of HRP conjugated streptavidin was done. After washing, a substrate solution was added. The colors developed in proportion to the bound adipokine quantity and were monitored using a microplate reader until an optimum optical density was reached. A stop solution was then added and the optical density was read at 450 nm. Reference calibrated standard curves, made using the optical density values of the standards (provided with the kits) was used to estimate the concentrations of each adipokine (vaspin/LCN2/chemerin).

Statistical analysis

The data were analyzed using a statistical software program (SPSS Inc. version 10.5, Chicago, IL, USA). For comparison of adipokine levels in GCF and tear fluid between the groups, Analysis of Variance (ANOVA) and Scheff's test were performed. The power of the study was calculated before the study was initiated. Based on the power of the study and the confidence interval of 95% ($p < 0.05$), sample size was determined. Pearson's correlation coefficient was used to find out whether the correlation between GCF and tear fluid concentrations for each molecule and other clinical parameters was statistically significant or not. p value <0.05 was taken as statistically significant. The mean intra-examiner standard deviation of differences in repeated PPD measurements and CAL measurements were obtained using single passes of measurements with a UNC-15 probe (Hu-Friedy, Chicago, IL, USA) (correlation coefficients between duplicate measurements; $r = 0.95$).

Study Group	**Group I (n=10)**	**Group II (n=10)**	**Group III (n= 10)**	**Group IV (n=10)**
Age (in years)	34.4 ± 4.06	35.6 ± 3.74	35.1 ± 3.98	33.8 ± 3.96
GI	-	1.89 ± 0.46	2.41 ± 0.21	2.05 ± 0.48
PPD	2.2 ± 0.75	2.1 ± 0.73	8 ± 1.24	7.3 ± 0.94
PAL	-	5.67 ± 1.04	6.2 ± 0.91	5.6 ± 0.84
GCF conc (ng/ml)	0.66 ± 0.018	0.95 ± 0.22	1.34 ± 0.42	1.83 ± 0.06
Tear fluid conc (ng/ml)	0.75 ± 0.02	1.27 ± 0.51	1.50 ± 0.06	1.98 ± 0.08
BMI (kg/m^2)	20.84 ± 1.17	28.16 ± 1.45	18.88 ±1.42	31.95 ± 4.6

Table 1A. Descriptive statistics of study population (mean ± SD) for vaspin.

Study Group	Group I (n=10)	Group II (n=10)	Group III (n= 10)	Group IV (n=10)
Age (in years)	31.90 ± 5.685	31.80 ± 4.04	31.80 ± 4.96	31.90 ± 5.42
GI	-	-	1.823 ± 0.71	2.04 ± 0.76
PPD	1.9 ± 0.87	1.8 ± 0.78	6.80 ± 1.54	6.8 ± 1.54
PAL	-	-	5.8 ± 1.54	5.6 ± 3.11
GCF conc (ng/ml)	57.9 ± 6.41	99.4 ± 8.05	103.4 ± 6.44	129.2 ± 7.25
Tear fluid conc (ng/ml)	44.84 ± 6.6	72.6 ± 9.30	77.52 ± 8.73	102.16 ± 9.08
BMI (kg/m^2)	19.9 ± 1.27	27.5 ± 1.9	20.19 ± 1.4	27.46 ± 1.90

Table 1B. Descriptive statistics of study population (mean ± SD) for LCN2.

Study Group	Group I (n=10)	Group II (n=15)	Group III (n= 15)	Group IV (n=10)
Age (in years)	34.7 ± 6.76	37.7 ± 4.23	40.93 ± 2.37	33.8 ± 3.96
GI	-	2.04 ± 0.49	2.17 ± 0.32	2.05 ± 0.48
PPD	2.1 ± 0.73	6.26 ± 1.16	6.93 ± 1.22	7.3 ± 0.94
PAL	-	5.13 ± 1.88	5.86 ± 0.83	5.6 ± 0.84
GCF conc (ng/ml)	95.99 ± 5.40	158.2 ± 9.75	243.5 ± 30.73	1.83 ± 0.06
Tear fluid conc (ng/ml)	76.83 ± 4.83	122.70 ± 5.75	168.05 ± 9.46	1.98 ± 0.08

Table 1C. Descriptive statistics of study population (mean ± SD) for chemerin.

	Vaspin		LCN2		Chemerin	
	GCF	**Tear fluid**	**GCF**	**Tear fluid**	**GCF**	**Tear fluid**
Groups 1-4	F-value =2753.213 p-value <0.001*	F-value =681.792 p-value <0.001*	F-value =72.844 p-value <0.001*	F-value =70.649 p-value <0.001*	F-value =285.86 p-value <0.001*	F-value =490.60 p-value <0.001*

Table 2. Results of ANOVA comparing the mean GCF and tear fluid vaspin concentrations between four groups. *Significant at p value <0.001.

	Correlation co-efficient	**p-Value**
Group 1	0.971	0.001*
Group 2	0.969	0.001*
Group 3	0.980	0.001*
Group 4	0.917	0.001*

Table 3A. Pearson correlation co-efficient between GCF and tear fluid concentrations of vaspin. *Significant at p value <0.01.

	Correlation co-efficient	**p-Value**
Group 1	0.998	0.001*
Group 2	0.997	0.001*
Group 3	0.995	0.001*
Group 4	0.997	0.001*

Table 3B. Pearson correlation co-efficient between GCF and tear fluid concentrations of LCN2. *Significant at p value <0.01.

	Correlation co-efficient	**p-Value**
Group 1	0.261	0.001*
Group 2	0.505	0.001*
Group 3	0.835	0.001*
Group 4	0.917	0.001*

Table 3C. Pearson correlation co-efficient between GCF and tear fluid concentrations of chemerin. *Significant at p value <0.01.

Results

The descriptive statistics along with the mean GCF and tear fluid concentrations for all groups are tabulated in Tables 1A-C for vaspin, LCN2 and chemerin respectively. The mean vaspin and LCN2 concentrations in both GCF and tear fluid were highest for group 4, followed by group 3, group 2 and least in group 1. The mean chemerin concentrations both in tear fluid and GCF were highest for Group 3 followed by Group 2 and least in Group 1.

ANOVA test was carried out to find out the equality of means between the groups for each molecule. A significant difference in the GCF and tear fluid levels of vaspin/LCN2/chemerin was found between the groups (Table 2).

The correlation of GCF and tear fluid levels of vaspin/LCN2 to BMI was statistically significant ($p < 0.05$) in all the four groups. The tear fluid and GCF levels of chemerin were found to be significantly ($p < 0.05$) positively correlated with all the clinical parameters. The Pearson's correlation coefficient test found significant correlation between GCF and tear fluid levels for all the molecules (Tables 3A-C).

Discussion

This is the first study conducted to date which has assessed the roles of vaspin, LCN2 and chemerin as inflammatory markers in CP and obesity/type 2 DM. The variability of their concentrations within the different groups can be attributed to the difference in inflammatory burden present in the different stages of disease process current at the time of collection of GCF and tear fluid samples.

Mean GCF and tear fluid concentrations of all the three adipokines were found to be highest in groups with both CP and obesity/DM. This was followed by CP groups. The values for obese healthy groups fell between those for healthy and CP. The concentrations were lowest in healthy subjects with no obesity/DM. This suggests that periodontal disease can act as a source of systemic inflammatory burden and these adipokines can serve as markers of periodontal inflammation.

While vaspin and lipocalin-2 can be considered as biomarkers of inflammation in CP and obesity, chemerin can be considered as the inflammatory link between CP and type 2 DM. These adipokines may also serve as markers of inflammatory conditions of the eyes like conjunctivitis, dacrocystitis.

Chairside kits can be developed for easier and faster diagnosis using these adipokines as biomarkers. Multicentre interventional studies should be carried out to find out the role of these adipokines in periodontitis and obesity/diabetes in various ethnic group of population. Therapies targeting these adipokines can be developed. Further research has to be carried out to explore the clinical implications of lowering the levels of theses adipokines on improvement in diseases complications.

Conclusion

Within the limitations of the present study, it can be postulated that increased concentrations of vaspin, lipocalin-2 and chemerin can be detected in GCF and tear fluid in chronic periodontitis with or without obesity or type 2 DM. These adipocytokines can thus be regarded as 'inflammatory biomarkers' of chronic periodontitis, obesity and diabetes mellitus. The role of these adipokines in ocular inflammatory conditions like conjunctivitis and dacrocystitis may also be explored. However further longitudinal, multicentric, interventional studies are needed to validate them as an inflammatory markers in these inflammatory diseases.

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Chapter 14

Proactive Periodontal Care in General Dental Practice: An Update and Perspective

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Introduction

Oral health and functions such as speech, eating, drinking and facial expressions are deeply integrated into one's general wellbeing, quality of life, happiness and daily social interactions (Beaglehole *et al* 2009, FDI 2015). Periodontal disease, as one of the most common diseases in humans, significantly affects people's oral health and normal oral functions and has close links to major non-communicable diseases (NCDs) such as diabetes and cardiovascular disease (Jin *et al* 2011, Pihlstrom *et al* 2005, Tonetti and Kornmen 2013). The High-level Meeting of the UN General Assembly on the Prevention and Control of NCDs (2011) recognized that oral disease remains one of the major global health burdens, and is particularly high in disadvantaged and poor populations in both developing and developed countries, with significant socio-economic impacts (Jin 2015b, Petersen *et al* 2005, United Nations General Assembly 2011). The recently launched FDI Data Hub provides the currently available dataset of oral diseases for easy reference (FDI 2014). According to the recent Global Burden of Disease Study (2010), severe periodontitis is the sixth most common disease in humans, with an overall prevalence of 11.2%, affecting over 743 million patients worldwide (Kassebaum *et al* 2014, Marcenes *et al* 2013). Indeed, severe periodontitis predominantly causes multiple tooth loss and edentulism in the adult population worldwide, and markedly accounts for a significant socio-economic burden and high healthcare costs (Chapple 2014, Pihlstrom *et al* 2005). Unfortunately, public awareness of periodontal health remains low, which in some extent is due to the 'silent' nature of this complex disease and the neglect of oral health in healthcare policy and agenda (Horton 2009, Jin 2015a, Jin 2015b). Therefore the key for tackling this global problem lies in strong promotion of disease prevention, enhancement of public awareness of oral/periodontal health and proactive care strategy for optimal oral and general health. This paper addresses the current understanding of periodontal etiopathogenesis and its key care strategy, and highlights some fundamental issues for the promotion of periodontal health and proactive care of periodontal patients in general dental practice, through a scientific approach and multidisciplinary teamwork.

Current understanding of periodontal pathogenesis and care strategy

The past two decades have seen exciting advances in periodontal science, care strategies and innovative techniques. It has been progressed from the distinct evolution of periodontal etiopathogenesis and renewed paradigm (Kornmen 2008, Meyle and Chapple

2015, Page *et al* 1997). Currently, the biological basis of periodontal health and healthcare recognizes the emerging notion of host-microbe symbiosis. The molecular profiles and underlying mechanisms of periodontal health, gingivitis and periodontitis, have recently been further defined and elaborated (Meyle and Chapple 2015). It is now understood that the human gingiva is constantly 'armed' and protected by an innate defense system, and beneficial oral species contribute to the protective host response and tissue homeostasis, while certain so-called 'keystone' bacteria like *P. gingivalis* in pathogenic biofilms are claimed to account for the shift of microbial biofilms and disturbance of host defense mechanisms subsequently contributing to periodontal pathogenesis (Berezow *et al* 2008, Curtis *et al* 2011, Darveau 2010, Hajishengallis *et al* 2011, Hajishengallis *et al* 2012, Jin 2011, Roberts and Darveau 2002, Zenobia *et al* 2013). Notably, host macro- and micro-environments are critically associated with the components of oral microbiota and constant microbe-host crosstalk (Marsh and Devine 2011). As such, the transformation from beneficial species to pathogenic through host-driven changes such as increased inflammation may significantly account for disease initiation and development, due to genetic and epigenetic factors, immuno-inflammatory responses, as well as various host and environmental factors (e.g. smoking, diet, socio-economic determinants, general health status like uncontrolled diabetes, and stress) (Bartold and van Dyke 2013, Marsh and Devine 2011). Therefore, in addition to effective plaque control, it is crucial to control and resolve periodontal inflammation especially in susceptible individuals (Bartold and van Dyke 2013). The emerging host modulatory therapy is promising to provide better care to periodontitis-susceptible patients (American Academy of Periodontology 2002, Kinane and Bartold 2007, Preshaw 2008, Sanz *et al* 2011).

Proactive periodontal care in daily practice

Lower awareness of periodontal health and 'symptom-driven dental visits' are regarded to be the great challenges in management of periodontal patients in general dental practice, although there are various underlying personal and socio-economic factors (Jin 2015a). From a clinical point of view, a number of patients often seek care for the consequences or complications from severe periodontitis (e.g. orthodontic therapy for aesthetic problems due to pathological tooth migration, and prosthodontic or implant treatment for multiple tooth loss or even complete edentulism in patients with uncontrolled advanced periodontitis), rather than early detection of their periodontal disease and timely control of the 'root' of their problem. Increased legal cases may arise from litigation relating to periodontal neglect (Jin 2015a, Zinman 2001). This issue needs to be addressed critically in public health campaigns, dental education and professional dental care for promotion of optimal oral/periodontal and general health. In daily practice, dental professionals should not only be concerned about and address their new patients' chief complaint(s), but also critically and comprehensively assess their medical status, health-related common risk factors such as tobacco use and diabetes, and overall oral and periodontal conditions.

Dentists usually offer the treatment their patients ask for, but more importantly detect and provide professional care for underlying problems (i.e. the causes of their problems) that patients often ignore and/or do not necessarily request treatment for (e.g. gingivitis, periodontitis, peri-implant mucositis and peri-implantitis). Salvador Dali, the famous Spanish artist, once said, 'I do not understand why, when I ask for a grilled

lobster, I am never served a cooked telephone.' As patients often ask for 'a grilled lobster' such as a denture, dentists should seriously consider whether 'a cooked telephone' (e.g. effective control of untreated moderate to severe periodontitis via intensive periodontal therapy) should be served, prior to offering 'a grilled lobster'! These on demand dental services need to be questioned and challenged, and proactive periodontal care implemented for effective management of periodontal patients. Moreover, it is generally accepted that periodontal care should be considered as the foundation of general dental practice, and this issue has been critically addressed in the recent 1st Workshop of the International Academy of Periodontology (Jin 2015a, Zinman 2001). For delivery of proactive periodontal care, the following four steps are essential:

1) Risk assessment, appropriate diagnosis and prognosis.
2) Formulation of individualized treatment plans and elaboration of treatment options as appropriate.
3) Delivery of well-sequenced treatment, re-evaluation of treatment responses with a multidisciplinary care approach when needed.
4) Long-term supportive periodontal care including care of dental implants in periodontal patients.

Risk assessment, diagnosis and prognosis

Over the years, accumulated scientific evidence has shown that host susceptibility to periodontal disease varies greatly in disease severity during an individual's lifetime, rate of disease progression during its natural history, and in the extent of treatment response to preventive and therapeutic measures (Hirschfeld and Wasserman 1978, Löe *et al* 1986). This notion is in line with the emerging concepts of personalized medicine and dentistry (Garcia *et al* 2013). In this regard, individualized risk assessment, appropriate diagnosis and prognosis are crucial for managing periodontal patients in daily practice. A number of systemic and local risk factors and indicators have been documented, and several risk assessment systems have been proposed (Jin *et al* 2011, Lang and Tonetti 2003, Nunn 2003, Page *et al* 2002). The Periodontal Risk Assessment (PRA) model has been increasingly used in dental practice and has been validated in a longitudinal study (Matuliene *et al* 2010). It shows that a high-risk patient is more susceptible to recurrence of periodontitis and periodontally-related tooth loss after active periodontal treatment than those with a moderate or low risk, and interestingly, the risk for disease recurrence among high-risk patients may be partially reduced through strictly following a tailor-made supportive care protocol (Matuliene *et al* 2010).

Currently, risk assessment and control have become more important than ever before, following the promotion and implementation of a well-defined common risk factor approach (FDI 2013a, Sheiham and Watt 2000). Major NCDs such as cardiovascular disease, diabetes, cancer and respiratory disease account for over 60% of human mortality worldwide, and these NCDs share common risk factors with common oral diseases such as periodontal disease (Ezzati and Riboli 2012, Sheiham and Watt 2000, UN General Assembly 2011). Therefore, incorporating oral health into general health agendas and policies is fundamentally important for health promotion and disease prevention (Petersen and Ogawa 2012). Advocacy for optimal oral and general health through a strong global healthcare approach is highly required (Jin 2013). The recent FDI Istanbul Declaration in 2013 makes an official call upon all national, regional and global health authorities for the

need 'to recognize oral health as an essential component of global health, and promote a reinforced inter-professional collaborative approach in the development of global and national policies' (FDI 2013b). Obviously, this crucial notion should be further incorporated into dental education, continuing professional development programs and daily dental practice.

Formulation of individualized treatment plans

Considering the current concept of periodontal etiopathogenesis and the emerging personalized medicine/dentistry concepts, formulation of treatment plans for periodontal patients should be individualized through a holistic approach, with the aim of recovery and maintenance of healthy and comfort oral functions, yet fulfilling aesthetic requirements and contributing to general wellbeing (Garcia *et al* 2013). The exact determination and undertaking of treatments would be based upon the patients' medical status, risk profile, clinical conditions, multidisciplinary care and good teamwork, as well as financial concerns.

Treatment in appropriate sequence and with a multidisciplinary approach

On the basis of formulating an individualized treatment plan, it is emphasized that the treatment should be undertaken with appropriate phase sequences, including systemic concerns, cause-related therapy, regenerative and reconstructive treatments and long-term supportive care (Salvi *et al* 2008). Herein, the 'Periodontal Clearance' concept should be appreciated and executed, namely that periodontal disease and other active infection/inflammation such as caries, endodontic and periapical infections should be well controlled prior to proceeding with regenerative, corrective, reconstructive and prosthodontic treatment (Jin 2010). This basic rule is one of the crucial elements for achieving desired treatment outcomes.

At the beginning of treatment, proactive periodontal care requires adequate communication with patients. For instance, to deliver effective oral health education and oral hygiene instruction (OHI), it is very crucial to convince patients that without proper health education and OHI, it is common to have deficiencies in being able to adequately manage their condition through effective plaque control at home and active control of risk factors, and to motivate them to fully understand the importance of such actions, thereby take the necessary actions for oral and general health. Other than effective plaque control, proactive promotion of healthy lifestyle should be incorporated into oral health education and OHI, such as smoking cessation, control of sugar intake and eating healthy foods. It has recently been suggested that certain foods could be beneficial in reducing inflammation, such as fish, vegetables, olive oil, nuts, fruits and tea (Arthritis Foundation National Office USA 2015).

Periodontology is a unique specialty in dentistry in that it has close links to nearly all other dental disciplines and medicine. Multidisciplinary care and teamwork through interactive collegiality and appropriate referral are therefore essential for successful patient management, especially for severe periodontitis patients and those with medically compromised conditions (Dowell and Chapple 2002, Glicksman 2001, Goldberg *et al* 2001, Jin 2015a).

Long-term regular supportive care

It is evident that long-term regular supportive care is of great importance for effective management of periodontal patients. Generally, six monthly recall for periodontal

supportive care remains the norm in clinical practice. The emerging personalized dentistry concept may be incorporated into proactive periodontal supportive care, through assessing individualized risk profiles. The PRA model could be the starting point in implementing a strategy for the delivery of more cost-effective periodontal care (Matuliene *et al* 2010). A tailor-made supportive periodontal therapy protocol could then be developed and undertaken. Herein, patient compliance is highly important, particularly for susceptible patients.

Summary

The following notes are crucial for proactive periodontal care in general dental practice with the aim of achieving oral and general health:

1) Periodontal disease remains a major global oral health burden with huge socio-economic impacts, and it is crucial to enhance the awareness of this complex and challenging problem worldwide.
2) Beneficial species contribute to protective host response, and host-microbe symbiosis/interactions armed by innate defense system are therefore crucial to periodontal health.
3) The 'keystone' bacteria like *P. gingivalis* in pathogenic plaque biofilms could disturb host defense mechanisms and contribute to periodontal pathogenesis, while a microbial community could be significantly modulated by host and environmental factors, and inflammatory components may therefore critically contribute to the shift of microbial biofilms and initiating disease occurrence.
4) Dysregulated host immuno-inflammatory response critically accounts for the severity of periodontal destruction, and the emerging host modulatory therapy is potentially promising for the effective management of susceptible periodontitis patients.
5) In addition to controlling plaque biofilms, resolution of periodontal/peri-implant inflammation is crucial for high-risk individuals susceptible to advanced periodontitis.
6) The emerging personalized medicine/dentistry concepts should be incorporated in proactive periodontal care. The key issues to be addressed include risk assessment and controlling risk factors through good patient communication and the common risk factor approach, formulation of individualized treatment plan, delivery of treatment in an appropriate sequence through evidence-based multidisciplinary approaches, and undertaking long-term regular supportive care for optimal oral and general health.

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Chapter 15

Current Updates on the Orthodontic-Periodontic Interrelationship

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Introduction

The main objective of periodontal therapy is to achieve complete periodontal health and maintain the integrity of the attachment apparatus of teeth. Similarly, the objective of orthodontic treatment is to gain facial and dental esthetics with the restoration of masticatory function. Orthodontics and periodontics have a very complex relationship within the mouth. Dental plaque is a highly bacterial complex organized within a biofilm form and is considered the main causative factor in dental caries and periodontal disease. Orthodontic treatment with fixed appliances is considered one of the risk factors for plaque accumulation. Tooth movement in fixed orthodontic therapy is made possible due to the remodeling characteristics of the periodontium. Every orthodontic intervention has an important periodontal dimension and thus orthodontic biomechanics and treatment planning are fundamentally determined by periodontal factors, including the length and shape of the root, remaining alveolar bone support and the structure and anatomy of the gingiva (Diedrich *et al* 2004).

Clinically, in periodontally susceptible or compromised patients who present with a malocclusion, orthodontic intervention can be only initiated once the periodontal inflammatory/infectious process is controlled and a stable periodontal condition is achieved. Periodontal microbiota control is achieved through elimination or significant reduction of periodontal pockets. After periodontal treatment has been performed, orthodontic treatment provides clear benefits in order to achieve and maintain periodontal homeostasis. Similarly, awareness of the pathologic or other undesirable changes which can occur in the periodontium as a result of orthodontic procedures would assist in better treatment and management of patients.

Altered tooth position may affect periodontal health. Placement of orthodontic appliances causes microbiological changes, as well as clinical changes in the periodontium. Using fixed multi-banded appliances, orthodontic treatment in adults has become a very popular treatment option. It is in connection with adult orthodontics that periodontal factors are becoming more important to the orthodontist. Orthodontic treatment in adult patients, especially those with compromised periodontal status, requires essential periodontal consideration and treatment to maintain the periodontium in a healthy state during and after the orthodontic treatment. Minor periodontal surgery such as pericisions may be required to prevent relapse after orthodontic treatment. In addition, since periodontal diseases can secondarily cause malocclusion, orthodontic treatment can be

an essential adjunct for successful periodontal therapy.

Orthodontic treatment in adults is best undertaken by a team of both periodontists and orthodontists. Interdisciplinary co-operation with clinical excellence in both disciplines may transform patients with unattractive dentitions, who show spaced, extruded, or otherwise migrated teeth in the inflamed and compromised periodontium, into people with attractive, esthetic dentitions and good smiles.

Effects of orthodontics on the periodontium

Orthodontic therapy involves the movement of teeth using controlled forces with the aid of either removable or fixed appliances. Properly controlled forces are critical for tooth movement. When the orthodontic force is applied on the tooth, local changes in the blood flow occur, leading to bone resorption on the pressure side and bone deposition on the tension side (Gottleib and Orban 1931, Oppenheim 1911). Optimal orthodontic force causes the compression of the blood vessels in the periodontal ligament, leading to tissue hypoxia which causes the release of cellular mediators such as prostaglandins and cytokines. These mediators play an essential role in the activation of both osteoblasts and osteoclasts. However, heavy orthodontic force, if applied on the tooth, occludes the blood vessels of the periodontal ligament, leading to ischemia and tissue necrosis, which ultimately results in the undermining resorption of alveolar bone and slower tooth movement (Reitan 1960).

Bacterial plaque is the main etiological factor for the initiation and progression of periodontal diseases (Cochran 2008). Orthodontic therapy is sometimes considered as a predisposing factor for periodontal disease, as orthodontic appliances may compromise oral hygiene maintenance, resulting in increased bacterial accumulation adjacent to the gingivae (van Gastel *et al* 2007). However, there are conflicting reports in the literature regarding the role of orthodontic therapy in the initiation and progression of periodontal disease. Some investigators state that the problem is not only due to bacterial accumulation resulting from orthodontic appliances, but also due to the transition of subgingival plaque to more periopathogenic flora, leading to the conversion of gingivitis to periodontitis (Sallum *et al* 2004, Turkkahraman *et al* 2005, van Gastel *et al* 2008). If a thorough oral hygiene maintenance protocol is followed prior to and during active orthodontic therapy, minimal or no change in gingival bleeding index or plaque quantity will be evident (Ari-Demirkaya and Ilhan 2008, Erkan *et al* 2007). Some studies have even shown that if there is adequate plaque control, patients with a reduced periodontium can undergo orthodontic treatment without aggravating their periodontal condition (Re *et al* 2000, Speer *et al* 2004). If inflammation is not fully controlled before initiating orthodontic treatment, orthodontic therapy may exacerbate the inflammatory processes and accelerate periodontal destruction, even in patients with sound oral hygiene practices during orthodontic therapy (Wennstrom *et al* 1993).

Bands, brackets, and orthodontic wires in orthodontic appliances act as plaque retentive areas and may be limiting factors in effective plaque control. Additional accessories for orthodontic appliances such as springs, coils and ligature wires may further facilitate plaque accumulation. As plaque accumulation persists, a shift from aerobic to anaerobic bacteria will occur. This bacterial shift is similar to that which occurs during the transition from periodontal health to disease. Within a short period of time following commencement of orthodontic therapy there will be increase in the number of periopathogenic bacteria

including spirochetes, fusiform bacteria, facultative anaerobes, lactobacilli and *Prevotella intermedia* (Pan *et al* 2007). This change in bacterial composition occurs within 12 days of commencement of orthodontic therapy, where the number of cocci and motile rods increase (Davis *et al* 2014). By 6 weeks, the amount of cocci decreases, and there is a subsequent increase in spirochetes and motile rods (Huser *et al* 1990). By 3 months, bacteria associated with the red and orange complex are established (Davis *et al* 2014). All of these microbial shifts may be purely due to plaque accumulation, without the direct effects of brackets, bands or any other functional components of orthodontic appliances.

Following placement of fixed orthodontic appliances, increased plaque accumulation in orthodontic patients is associated with an increase in bleeding on probing and gingival index (Karkhanechi *et al* 2013, Ristic *et al* 2007). In the majority of the patients, a small amount of gingival inflammation is visible following placement of a fixed appliance, which could be transient in nature and does not lead to attachment loss (Alfurji *et al* 2014). Some reports indicate that fixed orthodontic treatment may result in localized gingivitis, which rarely progresses to periodontitis (van Gastel *et al* 2007). Gingival inflammation around orthodontic bands leads to pseudo-pockets, which usually disappear with removal of the brackets. A number of studies have shown a reduced risk of gingivitis in the absence of plaque, orthodontic forces, and tooth movements (Dannan 2010, Naini and Gill 2008). If orthodontic forces are kept within adequate limits in healthy but reduced periodontal tissue supported regions, the chances of gingival inflammation will be minimal.

With respect to probing depths, there are conflicting reports in the literature, with some studies reporting no effect of orthodontic treatment on probing depth, while others have reported increased probing depths as a consequence of orthodontic therapy (Karkhanechi *et al* 2013, Liu *et al* 2011, Naranjo *et al* 2006, Ristic *et al* 2007, van Gastel *et al* 2011). In general it is accepted that deeper probing depths observed in orthodontic patients are most likely attributed to pseudo-pockets or deeper probe penetration into weakened connective tissues, as opposed to attachment loss of the supporting periodontium (van Gastel *et al* 2011). Deep pseudo-pockets will form in children due to moderate hyperplastic gingivitis within one to two months of appliance placement, occurring most commonly at interproximal and posterior sites (van Gastel *et al* 2011). This response is attributed to increased plaque retention secondary to inadequate plaque control practices. This inflammatory enlargement is noted even in patients with good oral hygiene, and is resolved as early as 48 hours after band removal, suggesting that the appliances themselves have an influence on periodontal health unrelated to plaque-induced periodontal disease (Zachrisson 1972).

Orthodontic patients have been shown to experience greater attachment loss and bone loss as compared to control groups who do not undergo orthodontic therapy, although this difference may not be clinically significant. However, one study has shown that orthodontic patients experienced 0.23 mm more bone loss when measured radiographically. It has been found that closed extraction spaces, retracted canines and serial extraction cases were at higher risk for bone loss (Zachrisson and Alnes 1974). Orthodontic therapy may cause or exacerbate gingival recession through the movement of teeth, as well as the effects of orthodontic appliances on the gingiva. A recent systematic review suggested that tooth movement out of the alveolar housing may be associated with a higher tendency for the development of gingival recession, although definitive evidence is lacking and

clinical significance of the recession may be minimal (Joss-Vassalli *et al* 2010). In addition, certain situations appear to be high risk for gingival recession, which includes facial tooth movement and thin gingival biotype with inadequate width of keratinized gingiva.

Root resorption is a common consequence associated with orthodontic treatment. Severe root resorption after orthodontic treatment compromises the outcome of successful orthodontic treatment. Orthodontic root resorption is unique as compared to other types of root resorption. Brezniak and Wasserstein (2002) suggested a new and more descriptive term for orthodontic root resorption based on the actual process, termed orthodontically induced inflammatory root resorption (OIIRR). OIIRR is a sterile inflammatory process that is extremely complex and composed of various disparate components including forces, tooth roots, bone, cells, surrounding matrix, and certain known biological messengers (Brezniak and Wasserstein 2002).

Response of the periodontium to orthodontic force

Tooth movement by orthodontic force application is characterized by remodeling changes in the periodontal ligament (PDL), alveolar bone, and gingiva. These tissues, when exposed to varying degrees of magnitude, frequency and duration of mechanical loading, express extensive macroscopic and microscopic changes. Orthodontic tooth movement differs markedly from physiological dental drift or tooth eruption. Orthodontic tooth movement is mediated by coupling bone resorption and deposition in compressed and stretched sides of the PDL, respectively. Orthodontic forces, by virtue of altering the blood flow and localized electrochemical environment, disturb the homeostatic environment of the PDL space. This abrupt alteration initiates biochemical and cellular events that reshape the bony contour of the alveolus (Toms *et al* 2002). The duration and character of force have great influence in orthodontic mechanotherapy, alterations in which can produce varied tissue reactions.

Most contemporary fixed orthodontic appliances use light continuous forces as part of the mechanotherapy to effect tooth movement. However, a continuous force can subside rapidly and thus be interrupted after a limited period of time, such as during torqueing movements by an edgewise archwire or labial movement of blocked-out maxillary lateral incisor with the help of ligation. It is not always possible to distinguish between continuous and interrupted movements, but the latter act for only comparatively short durations (Gusmao *et al* 2011). Nevertheless, it appears that this kind of a force, that starts in a continuous mode and then becomes interrupted, is biologically favorable, particularly when the initial magnitude is low. In such a case, hyalinized zones might develop in sites of compressed PDL, but as soon as this necrotic tissue is eliminated and the tooth moves, the force decreases quickly. Intermittent force results in small compression zones in the PDL, short hyalinization periods, and lengthy rest periods when the appliance is removed intermittently. During this time, the tooth moves back to the tension side and remains in normal function. This mode of treatment can improve the periodontal circulation and promote an increase in the number of PDL cells, because its fibers usually retain a functional arrangement. Reitan (1960) defined this condition as “semi-hyalinization”, meaning that in the compressed PDL not all fibers become compressed, and only some cells undergo necrosis. Consequently, osteoclasts might be formed directly along the bone surface subjacent to hyalinized tissue, and bone resorption is less disturbed

by hyalinization. This situation might affect smooth and uniform movement of teeth (Thilander *et al* 2000).

Periodontal problems in orthodontic practice

Orthodontic practitioners may encounter several periodontal problems before starting orthodontic therapy, during the treatment phase and even after the completion of orthodontic treatment. There is conflicting evidence in the literature regarding malocclusion as a risk factor for periodontal disease, and whether correction of it has any benefit to periodontal health. Some investigators have found a positive correlation between malocclusion and periodontitis and some have found little or no correlation between the two (Alfurji *et al* 2014, Bollen 2008, Gusmao *et al* 2011). Therefore, correct diagnosis of the conditions requiring management and formulation of an ideal treatment plan is an essential aspect of the long term success of any treatment. Any periodontal disease or condition should be treated first before initiation of the orthodontic therapy. Orthodontic treatment superimposed on poor periodontal health may further aggravate the periodontal breakdown and may further complicate the treatment outcome. If any advanced periodontal surgery has been performed on the patient, at least six months should be allowed for the healing and resolution of the inflammation before commencing orthodontic tooth movement (Geisinger *et al* 2014). If a patient is diagnosed with periodontal disease, scaling, curettage and soft tissue grafts can be performed prior to orthodontic tooth movement. However, pocket elimination therapy, and resective or regenerative osseous surgery can be done after the completion of orthodontic therapy (Gkantidis *et al* 2010). Open flap debridement prior to orthodontic therapy will eliminate inflammation and enhance the attachment at more coronal level. Sometimes, guided tissue regeneration (GTR) will even correct flaring of central incisors without orthodontic intervention. This suggests GTR should be performed prior to orthodontic treatment and the need for orthodontics should be reassessed after the completion of healing period (Gkantidis *et al* 2010). Nevertheless, other reports suggest that GTR can also be performed after orthodontic treatment when it is assumed that the repositioned teeth will create a better environment for the performance and effectiveness of the technique (Passaneizi *et al* 2007, Rabie *et al* 2001).

Gingival hyperplasia is a common complication associated with orthodontic treatment, usually appearing one to two months following orthodontic appliance placement. Persistent hyperplasia or inflammation of the gingiva can interfere with orthodontic treatment and increases the risk of relapse. These lesions can also lead to attachment loss. Therefore, periodontal maintenance by professional scaling during active orthodontic treatment is essential, particularly in the case where orthodontic intrusion is attempted, as this process may convert supragingival plaque to subgingival and create angular defects (Dannan 2010). To prevent such complications during orthodontic treatment, the frequency of periodontal maintenance by scaling and root planning should be twice that of patients not undergoing orthodontic treatment (Dannan 2010). For patients with pre-existing periodontal disease and reduced periodontal support, provided careful pre-orthodontic hygiene treatment of existing advanced periodontal disease is undertaken and the forces are kept within physiological limits, no increased progression of marginal periodontitis should occur due to orthodontic tooth movement (Geisinger *et al* 2014).

Increased tooth mobility and relapse following debanding are common problems

associated with orthodontic treatment, occurring more frequently among adult patients than adolescents. Adult patients require longer retention periods as they have less ossification in the remodeling period and sometimes may require permanent retention, particularly in those cases with advanced alveolar bone loss prior to orthodontic therapy. Relapse is due to the tendency of transseptal fibers to pull the tooth into its original position. This can be prevented by circumcrestal fiberotomy (CSF) which is particularly indicated in a rotated tooth, crowded mandibular anterior teeth, midline diastemas and palatally blocked lateral incisors (Dannan 2010). It should be performed a few weeks before debanding. If CSF is planned during debanding, a retainer should be given immediately and if it is planned prior to debanding, a minimum 3 weeks should be allowed for healing before debanding (Block and Hoffman 1995).

Oral hygiene maintenance in orthodontic patients

Maintaining good oral hygiene is a challenging task among patients undergoing fixed appliance orthodontic treatment. Brackets and other components present a favorable situation for rapid plaque accumulation and increased acid production, leading to demineralization and incipient carious lesions as well as increased risk of gingivitis and periodontitis. Similarly, it is established that poor patient oral hygiene affects orthodontic treatment outcomes, impacts quality of orthodontic treatment and even prolongs treatment time (Beckwith *et al* 1999). It has been reported that patients with poor oral hygiene at the commencement of orthodontic therapy will have a 0.67 times increase in treatment time than patients with good oral hygiene at commencement (Beckwith *et al* 1999). Destructive processes in the periodontium are also observed in patients with poor oral hygiene during orthodontic treatment (Morrow *et al* 1992). The accumulation of supra- and sub-gingival plaque and the establishment of a proinflammatory state can lead to destructive processes, as well as increasing the potential for developing other periodontal diseases. Placement of orthodontic bands may alter the physical and chemical environment to favor the growth of these periodontal pathogens (Pan *et al* 2007). It has been reported that fixed orthodontics do not cause periodontal damage if basic principles are followed in compliant patients with good oral hygiene (Ari-Demirkaya and Ilhan 2008, Erkan *et al* 2007).

In general, a significant percentage of orthodontic patients experience hygiene challenges and many demonstrate adverse effects from poor hygiene control during treatment (Boyd 2000). Frequent prophylactic programs and good oral home care for patients who are undergoing orthodontic treatment is of paramount importance to prevent the possible adverse effects of plaque accumulation around orthodontic appliances. As the orthodontist will follow up patients regularly for the activation of the appliance they will be the best person to judge the need for professional care at every visit. Early diagnosis and prompt treatment to prevent further periodontal breakdown is very important at this stage. The Cochrane Group recently reported that power toothbrushes with an oscillation rotation action remove more plaque and reduce gingivitis better than manual toothbrushes in the short term, as well as reduce gingivitis scores in studies over three months long (Robinson *et al* 2005). Thus, for orthodontic patient electric toothbrushes are preferred over manual brushes.

Enamel demineralization, which is characterized by typical white spot lesions on the enamel surface, is one of the complications associated with orthodontic treatment when

oral hygiene is poor. The development of white spot lesions is attributed to prolonged plaque accumulation around the brackets. To inhibit white spot lesions, twice daily use of over the counter (0.05%) neutral sodium fluoride rinse or twice daily 0.4% stannous fluoride gels is recommended. Another effective home-care tool is the use of an oral irrigator to remove loosely adherent plaque. When the above home care regimen, alongside flossing, regular use of interproximal brushes and brushing with a fluoridated toothpaste twice daily, is insufficient to maintain adequate periodontal health for orthodontic movement, routine use of 0.12% chlorhexidine mouth rinse could be implemented as a last resort.

Conclusion

The success of orthodontic treatment in periodontally compromised cases requires the periodontist to ensure the periodontal tissues are healthy before the orthodontist commences their treatment. Cooperation between the periodontist and orthodontist is important for the treatment of periodontic-orthodontic combined problems and can help to prevent the failure of the treatment. Integration between the two specialties may be required before, during, and after the orthodontic therapy. Active participation of the periodontist is essential, either in the management of periodontic-orthodontic problems or in specific interventions aiming to prevent orthodontic treatment relapse. The recognition and identification of patients with periodontal disease in the orthodontic office remains key for the success and long term maintenance of teeth.

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Current Issues in Periodontics
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Chapter 16

Orthodontic Treatment of Patients with Generalized Aggressive Periodontitis Using a Plasma/Serum IgG Test to Screen for Periodontitis

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Introduction

Aggressive periodontitis (AgP), an uncommon and often severe destructive periodontal disease, is mostly characterized by rapid attachment loss and bone destruction. Such advanced periodontal disease can result in pathological extrusion, labial inclination of the incisors, and/or posterior bite collapse, producing esthetic and functional problems for the patient, which often require orthodontic correction. Orthodontic treatment for patients with AgP demands special consideration due to the advanced progression of their periodontal disease. Close cooperation between the orthodontist and the periodontist is therefore considered to be important for determining the optimal timing of the treatment to ameliorate both periodontal and orthodontic problems. At Okayama University, a blood IgG antibody titer test and a microbiological examination for periodontal pathogens were used to diagnose the type of periodontal disease and to determine the timing for the initiation of orthodontic treatment. Comprehensive orthodontic treatment for a patient suffering from generalized AgP, with a longitudinal quantitative evaluation of the patient's periodontal condition and pathogens will be described. In addition, the importance of an efficient screening test for the successful orthodontic treatment of patients with advanced periodontal disease will also be discussed (Ishihara *et al* 2015).

Malocclusion and periodontal disease

Advanced periodontal disease could result in tooth migration and subsequent malalignment of the teeth (Towfighi *et al* 1997). Such types of pathologic malocclusion are associated with destruction of tooth-supporting alveolar bone and will worsen unless the inflammatory conditions and occlusal interference are eliminated. A combined periodontic-orthodontic treatment is one of the approaches to solving both functional and aesthetic problems associated with severe periodontal disease (Brunsvold 2005).

The application of orthodontic treatment in patients with advanced periodontal disease demands special consideration because of the potential for disease progression (Mathews and Kokich 1997). Advanced periodontal disease may result in pathologic extrusion, labial inclination of the incisors, or posterior bite collapse. These problems have the potential to cause further aesthetic and functional issues for the patient.

The orthodontic correction of such occlusal problems could eliminate risk factors

that might further aggravate periodontal breakdown (Cirelli *et al* 2006, Eliasson *et al* 1982). Although alveolar bone loss does not preclude orthodontic treatment, orthodontic treatment becomes much more complex in patients who have reduced alveolar bone support and often requires compromises with regards to the final treatment results (Re *et al* 2000). In addition, lighter orthodontic force has to be applied to teeth because greater pressures could negatively affect the periodontal tissues around teeth with compromised bone support.

However, orthodontic treatment in the presence of periodontal disease can aggravate and exacerbate periodontal problems (Kokich 2011). Orthodontic tooth movement is based upon the tissue reaction of the periodontal ligament to orthodontic mechanical loading in order to cause a remodelling process within the alveolar bone. In response to the orthodontically-applied force, both bone formation and resorption is enhanced and bone remodelling is activated. However, in the presence of inflammation and/or traumatic occlusion, bone resorption activity is specifically activated, which results in the further loss of the dental alveolar bone during orthodontic treatment. In other words, patients with periodontal disease are at risk of developing further periodontal tissue damage during orthodontic treatment. It is therefore essential that any existing periodontal problems be brought under control before the initiation of the orthodontic treatment (Kokich 2011). The orthodontist should then maintain a satisfactory standard of oral hygiene during the orthodontic treatment.

Monitoring of periodontal conditions using a blood IgG titer test and microbiological counts

It is important orthodontists identify periodontal problems before the initiation of orthodontic treatment and determine an appropriate treatment plan and the proper timing to ameliorate these problems. Furthermore, they should apply the orthodontic, periodontal and prosthetic treatments sequentially (Kokich 2011). Periodontal conditions are commonly assessed by several clinical parameters, including intraoral radiographic findings, periodontal probing depth, clinical attachment level and bleeding of the periodontal pockets. Intraoral radiographs are the gold standard for analyzing alveolar bone height and/or condition of the alveolar bone. However, a two-dimensional analysis using a conventional intraoral radiograph does not reflect the real bone situation. CBCT provides a precise, three-dimensional image of the bone defect. Periodontal charting of the periodontal pocket depth, mobility and bleeding on probing is also important for evaluating the periodontal condition.

In addition, microbiological examinations for periodontitis, including a blood IgG antibody titer test to determine periodontal pathogens and microbiological counts, have become available (Kudo *et al* 2012). The amount of serum IgG against each pathogenic bacteria is measured by ELISA as described previously (Takahashi *et al* 2001). As serum IgG antibody levels correspond to the amount of periodontal bacteria, the elimination of periodontal inflammation can be evaluated by the decrease of the serum IgG titers to the respective pathogens. Therefore, the serum IgG antibody titer test is useful for determining the optimal timing that subsequent orthodontic treatment can be initiated. This method of analysis is also useful for the monitoring of periodontal conditions during and after orthodontic treatment.

Clinical application of the blood IgG titer analysis and microbiological count

The patient was a 21 year old female whose chief complaint was impaired mobility. She had no systemic problems. She had experienced ongoing gingival inflammation, which began seven years previously, and had recognized the impaired mobility of her upper incisors four years previously. Significant recession of the attached gingiva was evident in the lower anterior segments and the lower left first molars with a deep probing pocket depth. The initial periodontal examination showed a deep probing pocket depth in almost the entire arch with sulcus bleeding. Despite the increased pocket depth, a corresponding amount of bacterial plaque was not evident throughout the affected area. Full-mouth radiographs demonstrated severe vertical bone loss. In particular, a remarkable infrabony defect was observed in her upper and lower molars. Advanced bone loss was also evident in the upper and lower incisors.

Quantitative evaluation of the microbiota of the patient was performed using a real-time PCR. The total number of bacteria was 106, with 104 *Aa* bacteria. A blood IgG antibody titer assay against *Aggregatibacter actinomycetemcomitans* Y4 was 4.85 times higher than that of healthy controls. Furthermore, the titers against *Aa* ATCC29523, *Pg* FDC381, *Td* ATCC35405 were less than one. Based on the above findings, the patient was diagnosed with generalized AgP, possibly

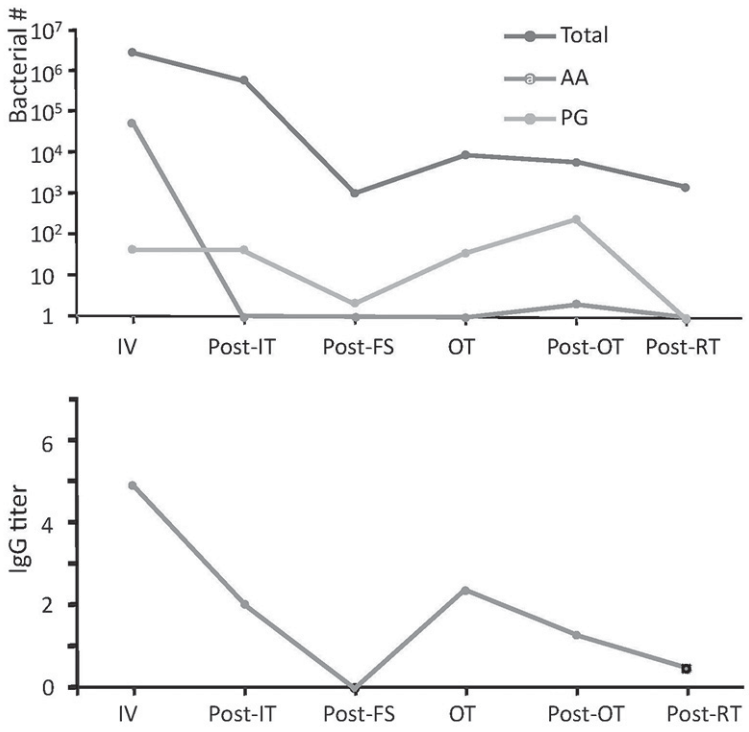

Figure 1. A periodontal examination during orthodontic treatment. (A) Quantitative evaluation of the microbiota of the patient was performed using a real-time PCR. The vertical axis indicates the numbers of bacteria in the subgingival plaque samples. (B) Blood IgG antibody titers against *Aa*.
AA = Aggregatibacter actinomycetemcomitans; PG = Porphyromonas gingivalis.

caused by *A. actinomycetemcomitans* Y4. In the transition phase of each active treatment step, we assessed the periodontal pocket depth, bleeding on probing, blood IgG antibody titer, and performed microbiological examinations to evaluate the periodontal condition (Figure 1).

Aggressive periodontitis is an uncommon and often severe destructive periodontal disease that is primarily characterized by the rapid loss of attachment and bone destruction in specific areas of the dental arch during the early stages. The patient was given oral hygiene instructions and careful local debridement was performed to assess her periodontal tissue response. The initial periodontal therapy included supragingival scaling and root planing, local antibiotic therapy and flap surgery at the sites with deep pocket depths.

During periodontal therapy, the percentage of sites with a pocket depth of $\geq$6 mm decreased from 13 to 3% (in comparison to the data from the initial examination). The bleeding ratio on probing also decreased from 50 to 30%. The microbiological count and the blood IgG titer analysis revealed that both the number of *Aa* and the IgG titer against *Aa* were undetectable in the pre-orthodontic treatment period. Based on the above improvements after periodontal therapy, we determined that her periodontal condition was well controlled and subsequently initiated orthodontic treatment.

The patient's chief orthodontic complaint was crooked teeth. Severe crowding of the upper and lower incisors and an anterior crossbite of both lateral incisors were observed. The molar relationship was angle class I on both sides. The patient was diagnosed with a class I malocclusion with lip protrusion, severe incisor crowding, and advanced bone loss. We planned to extract the patient's maxillary right lateral incisor, mandibular left first premolar, and the distal root of the mandibular right first molar because of the extensive periodontal damage. The planned orthodontic treatment aimed to achieve upper and lower alignment to establish better occlusal contact, anterior guidance, and aesthetics through the leveling and retracting of the incisors.

During the orthodontic treatment, periodontal management was performed, including professional mechanical tooth cleaning every month. We also evaluated the blood IgG titer against *Aa* and performed microbiological examinations to confirm that the periodontal conditions were well maintained.

The active orthodontic treatment period was 28 months. Acceptable occlusion was obtained with normal overjet and overbite. A post-treatment cephalometric evaluation demonstrated that the upper and lower incisors were significantly lingually inclined. Intraoral xrays showed no progression of alveolar bone resorption. Acceptable occlusion and periodontal disease screening values were maintained over the 36 month follow-up period. Prosthetic treatment was subsequently applied to the maxillary anterior segment to stabilize the mobile teeth. Periodontal regeneration therapy and plastic surgery were also performed. Follow-up examinations are currently performed every two to three months because the patient's periodontal condition remains stable. Blood IgG titer tests and microbiological examinations are performed at the follow-up examinations.

Conclusion

Blood IgG antibody titer tests and microbiological examinations could be used as supportive periodontal analyses to allow careful monitoring of periodontal conditions before, during, and after orthodontic treatment.

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Chapter 17

Prosthodontic Therapy in Periodontally Compromised Patients

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Introduction

The use of dental prostheses in periodontally compromised patients should provide a degree of rigidity, result in a more favorable distribution of the masticatory load along the entire arch rather than on individual teeth, and prevent overloading of abutment teeth which may have reduced periodontal support. There are three kind of dental prostheses in periodontally compromised patients; implant supported (fixed) dental prosthesis, tooth supported (fixed) dental prosthesis, or removable dental prosthesis. Periodontally compromised patients usually suffer tooth loss and the remaining teeth often have limited periodontal support. Therefore, which one of these prosthesis types is the best for periodontally compromised patients?

Scientific evidence has found that implants will serve as a long-term predictable prosthesis, and patients are highly satisfied with this form of treatment. Modern comprehensive dental care for a periodontally compromised patient could include the use of dental implants, unless the patient declines this form of treatment. It seems to be the opinion of the dental profession that dental implants are the first choice for replacing missing teeth. Factors such as bone quality, surgical trauma or bacterial contamination during implant surgery have been associated with early failures (Esposito *et al* 1998). Overload after prosthetic restoration is another possible cause of implant failure. Factors associated with late failures are less well understood and seem to be related to both the peri-implant environment and host parameters (Berglundh *et al* 2002).

Are periodontally compromised patients indicated for dental implant treatment?

Several studies have compared dental implant survival rate in periodontally compromised patients to non-periodontally compromised patients. Eight systematic reviews have shown a history of treated periodontitis as a risk indicator for implant outcomes (Al-Zahrani 2008, Karoussis *et al* 2007, Klokkevold 2007). Three studies reported a statistically significantly greater risk of peri-implantitis in patients with a history of treated periodontitis compared to those without a history of periodontitis with odds ratios ranging from 3.1 to 4.7 (Ferreira *et al* 2006, Karoussis *et al* 2003, Roos *et al* 2006). Karoussis and colleagues (2007) showed that 10 year survival rate of implants was lower (90.5 versus 96.5%) and the rate of peri-implantitis was five times higher (28.6 versus 5.6%). In a ten year study, Leonhardt *et al* (2002) found that bone loss around implants

was 1.7 mm, and the percentage of sites with bleeding on probing was 61%. In a literature review, Kanno *et al* (2008) concluded that periodontally compromised patients could be treated successfully with implants. However, implant therapy should be reconsidered if the oral infection cannot be satisfactorily controlled. Implant treatment in patients with generalized aggressive periodontitis is not contraindicated, provided that adequate infection control and an individualized maintenance program are undertaken (Jen 2007, Kim 2012).

Evidence has showed that severe periodontitis can be successfully treated and further bone destruction can be prevented. The long-term prognosis of peri-implantitis treatment is not well documented. If further periodontal destruction can be arrested by successful periodontal treatment, there is no obvious reason to replace a tooth (with no or little caries experience) with an implant even in cases with significant bone loss (Lundgren *et al* 2008).

There are differences between a natural tooth and an implant with markedly reduced bone support. If un-splinted, teeth may become mobile, but if well distributed they will successfully function as support for extensive bridges, with as little as 25 to 30% remaining periodontal support seeming to be sufficient, even in the long term (Laurell *et al* 1991, Nyman 1982). The prognosis of implants with markedly reduced but healthy bone support in carrying an extensive bridge is unknown. Therefore, natural teeth should be taken into account when treating periodontally compromised patients and should be considered as a functioning unit, including individual teeth as possible abutments for fixed partial dentures or as a part of removable partial denture, before implants (Lundgren *et al* 2008).

History of periodontitis as a risk indicator for peri-implantitis

In recent years, studies have shown that bacterial colonization occurred immediately after dental implant placement (30 minutes) and was stable for 2 weeks (Fürst *et al* 2007, Quirynen *et al* 2005, Quirynen *et al* 2006). The composition of the microorganisms after three months is predicted to be the same as after one year (Salvi *et al* 2008). The composition of the biofilm on implant surfaces closely follows those from teeth surrounded by healthy tissues. It can be predicted that the microorganisms in the oral cavity may impact biofilm formation on newly placed implants. Heitz-Mayfield and Lang (2010) showed that the microorganisms in patients with clinical signs of peri-implantitis are the same as those of periodontitis. They are the gram-negative anaerobic bacteria; *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium sp, Prevotella intermedia, Aggregatibacter actinomyecetemcomitans, Staphylococcus aureus,* enteric rods and *Candida albicans* (Botero *et al* 2005, Heitz-Mayfield and Lang 2010, Hultin 2002b, van Winkelhoff 2000).

S. aureus can be associated with some cases of refractory periodontitis. Increasing evidence showed the important of these bacteria in the initiation of some peri-implantitis cases (Dahlen and Wilkstrom 1995, Fine 1994). While the initial host response to the bacteria in peri-implant mucositis closely follows that in gingivitis, persistent biofilm accumulation may cause a more pronounced inflammatory response in peri-implant tissue than in the dento-gingival unit. This is possibly due to structural differences, such as vascularity and fibroblast-to-collagen ratios (Heitz Mayfield and Lang 2010). The periodontitis lesion is walled off by a connective tissue fiber compartment, preventing the inflammatory cell infiltrates

penetrating into the alveolar bone marrow. This is contrary to that of a peri-implantitis lesion in which the infection progresses into the bone marrow rapidly. This condition was demonstrated in dog and monkey studies (Lindhe *et al* 1992, Schou *et al* 1993). This means that in most cases, once established, peri-implant infections will probably continue to progress. This progression is possibly influenced by the roughness and topography of implant surfaces that favor the colonization of specific microorganisms.

Tooth supported fixed partial dentures in periodontally compromised patients

Periodontally compromised patients indicated for tooth-supported fixed prosthodontic treatments are as follows: patients with mobility of their remaining teeth; jaw relationship permitting the establishment of anterior occlusal contacts; favorable distribution of abutment teeth; existing restorations in need of replacement; contraindicated for implant treatment (e.g. medical reasons); lack of appropriate bone dimensions and intruding anatomical structures (inferior alveolar nerve, maxillary sinus); financial considerations; or patient preference to maintain their own teeth (Kourkouta *et al* 2007).

Periodontally compromised patients who are not indicated for tooth-supported fixed prosthodontic treatments are as follows: patients with lack of oral hygiene motivation; unrealistic aesthetic demands; a jaw relationship that does not permit establishment of anterior occlusal contacts; unfavorable abutment distribution; inadequate dental laboratory support; or financial considerations (Kourkouta *et al* 2007).

What is the capacity of reduced periodontal tissues to support fixed bridgework?

In a study by Nyman (1982) of 60 fixed bridges inserted in patients treated for advanced periodontal disease, 57% of bridges had an area of periodontal ligament around abutments less than 50% of the normal ligament area of the pontics, but all bridges had functioned properly for 8 to 11 years and the periodontal tissues around the abutment teeth had not suffered further loss of attachment.

Long term follow-up studies of tooth supported fixed partial dentures in periodontally compromised patients showed that once splinted, mobile teeth can survive and function. Even though the periodontal support is minimal (as little as 20 to 30%), they can be used as abutments if periodontal disease has been treated successfully and an effective recall program is in place (Kourkouta 2007).

Pjetursson *et al* (2007), in their meta-analysis studies, showed an estimated five year survival of conventional tooth-supported FDPs, cantilever FDPs, solely implant-supported FDP, combined tooth-implant-supported FDPs, and implant-supported single crowns (SC) of around 91 to 95%. After 10 years of function the estimated survival rate decreased. It was shown that 38.7% patients with implant-supported FDPs had complications after the five year evaluation, compared to 15.7% for conventional FDPs and 20.6% for cantilever FDPs. For conventional tooth-supported FDPs, the most frequent complications were biological complications like caries and loss of pulp vitality. Compared with tooth-supported FDPs, the technical complications were higher in implant-supported reconstructions. It was concluded that planning of dental prosthetic treatment should preferentially include

conventional end abutment tooth-supported FDPs, solely implant-supported FDPs or implant-supported SCs. Only for reasons of anatomical conditions, patient preference and as a second option should cantilever tooth-supported FDPs or FDPs supported by combination of implants and teeth be chosen.

Removable partial denture in periodontally compromised patients

Medical, surgical and financial considerations and patient attitude can lead to the use of a removable partial denture (RPD) as the chosen prosthetic restoration, even in the "dental implant era", especially in developing countries.

If the prognosis for the supporting teeth is poor, a tissue-tooth supported RPD should be considered, as any embracing part of the clasp assembly and a correct denture base can contribute to the stability of metal contact between teeth and the metal framework. Stress relieving clasp-assemblies such as the RPI/RPY/double RPY should be considered. Increased periodontal support can be achieved with a high number of abutment teeth which reduces injurious lateral and torsional stresses on abutment teeth. When the terminal tooth is periodontally weak, more than one adjacent tooth should be used for added support (Phoenix 2003). Clinical experience and many reports in the literature have shown that removable partial dentures are suitable for use in periodontally compromised patients. Appropriate design and adequate oral hygiene may decrease the occurrence of periodontal disease.

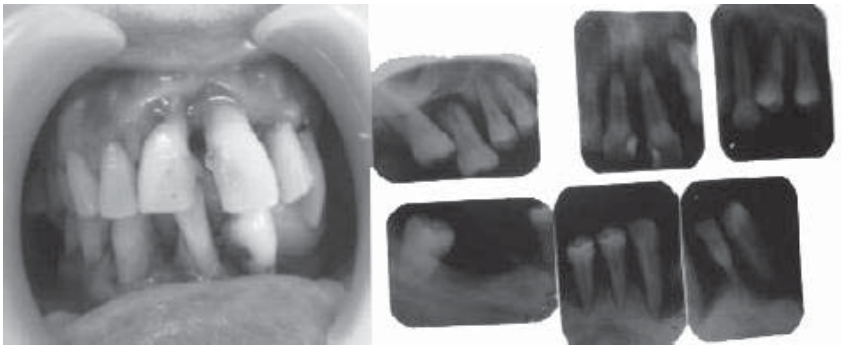

Figure 1. Type 1 periodontally compromised patient (Generalized Aggressive Periodontitis) in 30 year old female (Courtesy of Benso Sulijaya).

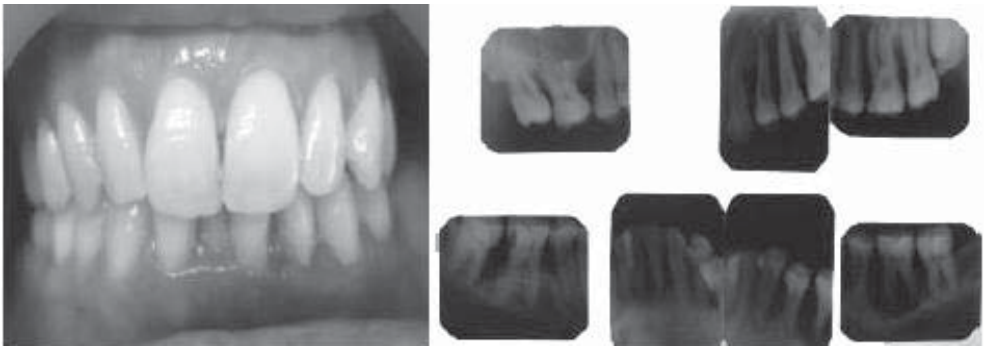

Figure 2. Type 1 periodontally compromised patient (Generalized Aggressive Periodontitis) in 30 year old female (Courtesy of Benso Sulijaya).

Periodontally compromised patients

The authors' clinical experience has shown there are 2 types of cases usually diagnosed as periodontally compromised patients.

Type 1. Generalized Aggressive Periodontitis

Patients are aged less than 35 years old. Clinical appearance shows loss of some teeth, mobility of almost all teeth from 3 to 4 degrees, tooth migration in almost all the teeth. Radiographic appearance shows generalized severe bone loss, as little as 10 to 20% (Figures 1 and 2).

Type 2. Severe Localized/Generalized Periodontitis

Patients usually aged more than 35 years old. Clinical appearance shows loss of none or few teeth, few mobile teeth or some of only 2 to 3 degrees, no or few migration of teeth. Radiographic appearance shows severe bone loss (as little as 10 to 30%), only at the region with occlusal trauma. Teeth are mobile only at the region with trauma from occlusion. This type of periodontitis could be localized or generalized, as a result of localized or generalized trauma from occlusion caused by either disproportionate crown to root ratio, occlusal interference during occlusion/articulation, or "cusp to cusp" occlusal inter-relationship (Figures 3 and 4).

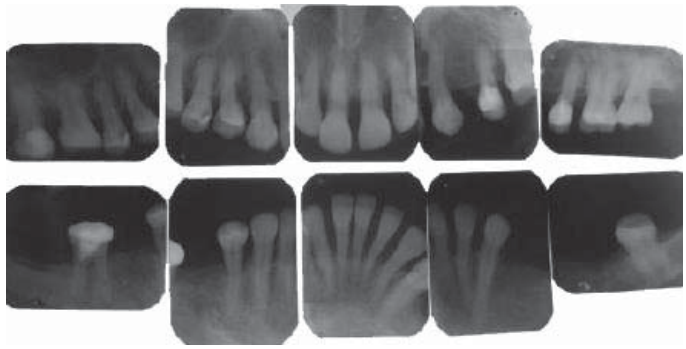

Figure 3. Type 2 periodontally compromised patient (Severe Generalized Periodontitis) in 56 year old female. Radiographs show severe bone loss as a result of occlusal trauma caused by disproportionate crown to root ratio.

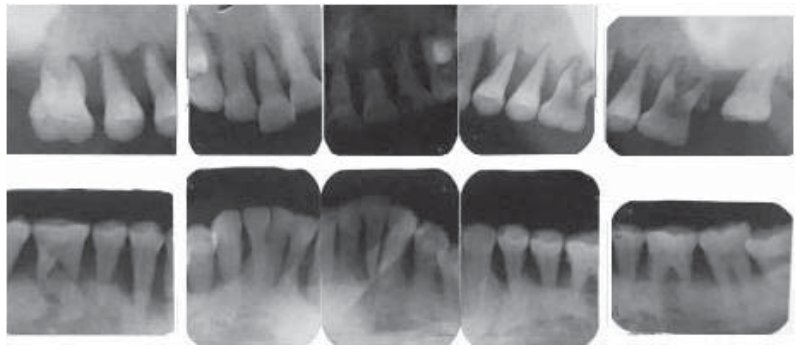

Figure 4. Type 2 periodontally compromised patient (Severe Generalized Periodontitis). Radiographs show generalized severe bone loss as a result of occlusal trauma caused by disproportionate crown to root ratio.

What kinds of dental prosthesis are chosen for periodontal compromised patients?

Scientific evidence has found that implants will serve as long-term predictable prostheses with high patient satisfaction. Modern comprehensive dental care for periodontally compromised patients could include consideration of dental implants, unless the patient declines this treatment option. It seems to be the opinion of the dental profession that dental implants are the first choice for replacing missing teeth.

Medical history, periodontal disease susceptibility, smoking, bruxism, oral hygiene, bone deficiency, gingival biotype, width of edentulous space, and soft tissue anatomy are important factors when performing risk assessment of an implant candidate. Weber *et al* (2008) recommended testing for the IL-1 gene polymorphism in implant candidates who are smokers, since in heavy smokers the presence of a functionally significant IL-1 gene complex polymorphism is associated with an increased risk for peri-implant bone loss.

In Type 1 periodontally compromised patients, few patients fulfil the requirements for implant candidates. The periodontal disease susceptibility, lack of appropriate bone dimensions, and lack of keratinized gingiva should be considered when making an implant supported dental prosthesis in this type of periodontitis. Minor movement of orthodontic treatment in combination with a removable partial denture or tooth supported fixed partial denture are safer than an implant supported partial denture.

In Type 2 periodontally compromised patients, the bone deficiency only happens at areas suffering from trauma from occlusion, but can be localized or generalized. The severity of bone loss is so advanced that the patients are usually diagnosed as Localized or Generalized Aggressive Periodontitis. Although the periodontitis is generalized, as a result of generalized trauma from occlusion, the quality of the bone is not as poor as that in Type 1. Therefore, in Type 2 patients, tooth migration is less than in Type 1. Some Type 2 periodontally compromised patients have intraoral conditions that fulfil the requirements for implant candidates and an implant supported fixed dental prosthesis could be carried out. In cases where bone loss is severe and the intraoral conditions do not meet the requirements for implant candidates, a removable partial denture is the chosen treatment. Clinical experience has shown that removable partial dentures are acceptable for use in periodontally compromised patients. If appropriate design and oral hygiene are accomplished, further periodontal destruction can be arrested and the teeth will successfully function. Even though the remaining periodontal support is as little as 25 to 30%, it seems to be sufficient, even in the long term.

Conclusion

Modern comprehensive dental care for periodontally compromised patients should include consideration of dental implants, unless the patient refuses or is contraindicated for dental implants.

The classification of the patient and the correct diagnosis for each individual tooth based on a meticulous examination should form the basis for pre-therapeutic prognosis and treatment planning of prosthodontic therapy in periodontally compromised patients. Medical history, periodontal disease susceptibility, smoking, the presence of IL-1 gene phenotype, bruxism, oral hygiene, bone deficiency, gingival biotype, width of edentulous space, and soft tissue anatomy are important factors for risk assessments in implant candidates. High risk patients with

implant supported dental prosthesis can use removable or tooth supported fixed dental prosthesis with a special design that performs as a dental splint of the mobile teeth. Proper infection control is a critical factor for the long term success of prosthodontic therapy in periodontally compromised patients, whichever prosthesis is used.

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Chapter 18

Immobilization of Periodontally Affected Teeth

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Introduction

The goal of dentistry is to obtain a healthy, functional and stable dentition in accordance with the patients' desires, capabilities and health status. In order to achieve this goal, the therapy requires a well-coordinated combination of various levels of periodontal and restorative treatment. With modern techniques it is now very much possible to maintain periodontally compromised dentitions on a very long term basis, i.e. 10 years upwards.

There are varying definitions of periodontal splints, some of which are:

- A "splint" can be defined as any apparatus, appliance, or device employed to prevent motion or displacement of fractured or movable parts.
- A "periodontal splint" is a rigid or flexible device that maintains in position a displaced or movable part, also used to keep in place and protect an injured part.
- A rigid or flexible material used to protect, immobilize or restrict motion in a part.
- The joining of two or more teeth into a rigid unit by means of fixed or removable restorations or devices.

It is evident from the above definitions that splinting essentially refers to the tying together of teeth, either unilaterally or bilaterally, to convey increased stability to the entire dentition. The relatively recent advent of bonding dentistry has led to a complete change in dentistry's approach to splinting, with successful results in clinical practices the world over.

There are three reasons which make splinting an acceptable treatment modality within the range of periodontal therapies. The first of these reasons is the author's firm belief that splinting is an invaluable tool in the management of a compromised periodontal status and this belief is substantiated by his personal experience, and reports and long standing works of dentists like Dr Ralph Pollack (1999). The second reason is the overwhelming logical basis of the laws of pure physics working when splinting is applied to the compromised dentition. The third reason is the axiom of "clinicians find out what works and the academicians find out why it works".

Smulker and Lemmer (1980) advocate splinting when mobility is sufficient to hinder function or cause discomfort. In an excellent paper, Lynde and Nyman (1984) demonstrated long term stability and maintenance of splinted dentitions that had more than 50% attachment loss on abutment teeth, and even though Antes law was not satisfied, in the absence of inflammation severely periodontally compromised dentitions could be maintained successfully for extended periods of time up to 20 years.

Indications for splinting

The primary indications for the various kinds of splints used in periodontal therapy are as follows:

1) Excessive mobility of one or a group of teeth due to loss of attachment causing patient discomfort while chewing.
2) Mobility of teeth leading to malocclusion.
3) Short-term splints for management of immediate mobility caused by physical injury.
4) Retention of teeth in position subsequent to orthodontic therapy.
5) Prevention of migration of teeth due to primary or secondary occlusal loads.
6) Prevention of migration of teeth due to aggressive periodontal disease.

Lang and Lindhe (2015) presented three specific situations for splinting with a fixed cast restoration:

1) Progressive tooth mobility as a result of gradually increasing periodontal ligament width in teeth with lost alveolar bone height.
2) Increased mobility of a tooth or a group of teeth that affects chewing ability or comfort.
3) Increased segmental bridge mobility despite splinting in a sextant of teeth.

Principles of splinting

The ultimate objective of splinting is to stabilize the afflicted tooth or group of teeth and provide for patient comfort during speech, chewing and at rest. This results in two primary objectives, which are; a) to create an oral environment in which tooth mobility is normal or at least is no longer increasing and b) ensure the patient is able to function comfortably.

The above mentioned goal will be achieved by splinting only if certain basic fundamental principles are applied. These fundamental principles arise from basic physics. Any applied force that may consist of horizontal and vertical occlusal forces produces torque and couple systems with certain centers of rotation. These centers of rotation are what causes progressive loss of attachment. Once the teeth are splinted, the centers of rotation are then redirected, thus providing increased resistance to applied forces. This rationale, Dawson (2006) believes, is the single most important benefit of splinting.

Another important principle as a corollary to the above is the long axis of movement of the teeth in question. If a full complement of teeth exist, they will usually move in a labiolingual direction along a certain long axis of movement. Teeth along an arch will usually have differing long axes of movement. When these teeth are splinted they will be rendered immobile as long as the individual long axes of movement of the teeth are not the same as the long axis of movement of the group of teeth as a single entity. Such is the case in lower anteriors, upper anteriors and any group involving the canines and premolars. In the case of mobile lower second premolars and first molars, the long axis of movement is usually the same and hence a splint will not be as effective as in the other mentioned situations.

Lemmerman (1976) has advocated splinting only if the gingiva is, or can be made, perfectly healthy, and when normal occlusal forces result in mobility, thus making function difficult or impossible. Pollack (1999) has presented cases spanning 26 years and noted that the “inflammatory response around the attachment apparatus must be treated and controlled as part of the diagnosis and treatment of mobility”. One of his conclusions is that periodontal therapy, along with stabilization of severely mobile teeth, has been found to be an effective way to retain teeth in periodontal health, even when as much as 60 to 80% of bone is lost.

Classification of periodontal splints

Periodontal splints can be classified on differing bases. One basis is the kind of material and technique used for creating the splint. Another is the duration of the splint. Both can be combined to have further subdivisions.

Material and technique classification

Non-prosthetic appliances:
- Historical – wire splints, wire layered with composites
- Wire and amalgam splints
- Standalone composite splints
- Fiber reinforced composite splints

Prosthetic appliances:
- Conventional cemented fixed partial prosthesis
- Metal bonded Maryland splints
- Metal free bonded fixed partial prosthesis

Wire splints

Plain wire splints go back a long way in periodontal therapy. The improvements in material sciences have made such splints obsolete. The only application of such splints today is seen in trauma cases when handled by oral surgeons. These splints have generally been carried out with ligature wires. Unfortunately, even though these splints can reduce movement of very severely mobile teeth, the wires tend to slip and loosen and are not conducive to oral hygiene. These splints have been mentioned only for historical purposes and are not recommended by the author.

Stand-alone composite splints

Adhesive composite resin provides successful, durable single tooth restorations. These materials have also frequently been used to bond mobile teeth together. The increase in bond strengths of composite to enamel, as well as dentin, have led clinicians to attempt heroic fusing of very mobile teeth with very poor results. The reason for this is the inability of composite to resist shear forces. Shear stresses crack composite, especially at the tooth-composite interface. Repeated failures have led clinicians to think that composites do not work at all.

Partial fixed prosthesis

A variety of fixed partial prosthesis can serve a dual role, in replacement of missing teeth as well as splinting of compromised abutment teeth. To summarize, it is the author's firm belief that the fixed partial denture has a far greater role to play in periodontal therapy than is curently attributed to it.

Diagnosis and treatment planning

A detailed and comprehensive periodontal examination includes measurement of the mobility of all existing teeth, as well as the direction of mobility. An additional parameter which has to be assessed is the apparent clinical reasons for the mobility. The prognosis of a tooth or teeth may be guarded, and no definitive decision can be made until after initial periodontal therapy. The choice of a fiber reinforced splint is an economical and time saving immediate therapy with very good predictable results.

The reinforced bonded splint

The acceptance and clinical predictability of modern day composite materials and the application of conservative bonding techniques offer a useful methodology for splinting of teeth. Early attempts have been made to embed materials such as wires, pins,

nylon or stainless steel mesh within restorative resins. The inherent problem with these materials has been their inability to chemically integrate with the resin composite. In fact, any such material only serves to further weaken the basic composite structure even though the overall flexural strength of the structure increases. Resin generally fractures at the metal resin interface. Additionally, the volume of composite resin required with such metal core substances significantly increases, which makes the splints more bulky and difficult to maintain.

This has led to the search for an appropriate reinforcement material. The challenge to place a thin but strong composite based splint has been met with the introduction of a high strength, bondable, biocompatible, esthetic and easily manipulated, color neutral fiber that can be embedded in the resin structure. This fiber also bonds chemically with the resin composite. Sufficient research has been undertaken which demonstrated that the fiber reinforcement material provides an increase in flexural strength and the flexural modulus of composite resins.

A variety of reinforcement fibers are available for use with composites for the purpose of splinting:

- Splint It (Jeneric/Petron): Open weave glass fiber ribbon.
- Ribbond Ribbon (Ribbond): Lock-stitched, woven polyethylene ribbon.
- Connect (Kerr): Open weave polyethylene ribbon.
- DVA (Dental Ventures of America): Open tufts of polyethylene fibers.
- Glas-Span (Glas-span): Open weave glass fiber ribbon and rope.
- Fiber-Flex (Biocomp): Tufts of individual Kevlar fibers.
- Fiber-Splint (International Dental Distributors): Open weave glass fiber ribbon.
- Quartz Splint (RTD, France): Quartz glass woven fibers.

The author's choice in material for a glass fiber splint is the "Quartz Splint" made from quartz glass fibers by RTD, France. The salient properties of the material are:

- Made from proprietary quartz fibers.
- Esthetic fibers, as used in RTD fiber post shades.
- High flexural strength.
- Non-metallic, biocompatible composition reactivity.
- Easily flexed and shaped right out of the package.
- Highly compatible, reactive resin matrix.
- Minimally invasive preparation.
- Fibers are pre-impregnated at manufacturing stage.

Clinical technique for the reinforced bonded splint

In a reinforced bonded splint, a single layer of fiber reinforcement is all that is required to form a laminate composite structure. The basic purpose of the splint is to stabilize mobile teeth. The laminate consists of layers of fiber, composite and tooth. As long as the teeth are in close proximity to each other, one layer of fiber between the interproximal contacts is the ideal design to maintain stabilization between the splinted teeth, yet allow for slight physiologic movement of the teeth. In case a microcrack develops in the interproximal composite, the fiber prevents crack propagation. One may even venture to propose that a small crack may act as a stress reliever and help protect the bond between the tooth and the resin.

A larger percentage of reinforced bonded splints are generally placed in the anterior teeth. The most common mode of failure of such a splint is debonding between the resin and the tooth due to shear forces. To counter this stress, the splint should be placed on the tensile side of the dental arch. In the case of

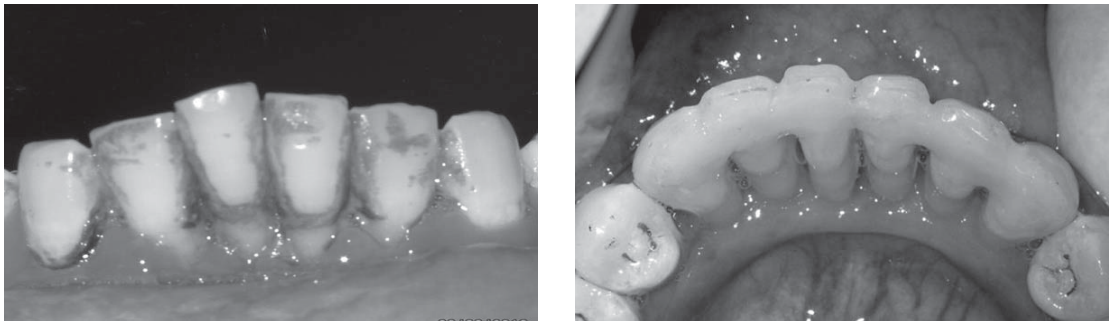

Figure 1. (A) A 28 year old female with advanced periodontal damage and severe mobility of the lower anterior teeth. (B) The lower anterior teeth were splinted with Quartz Splint on the lingual side from canine to canine.

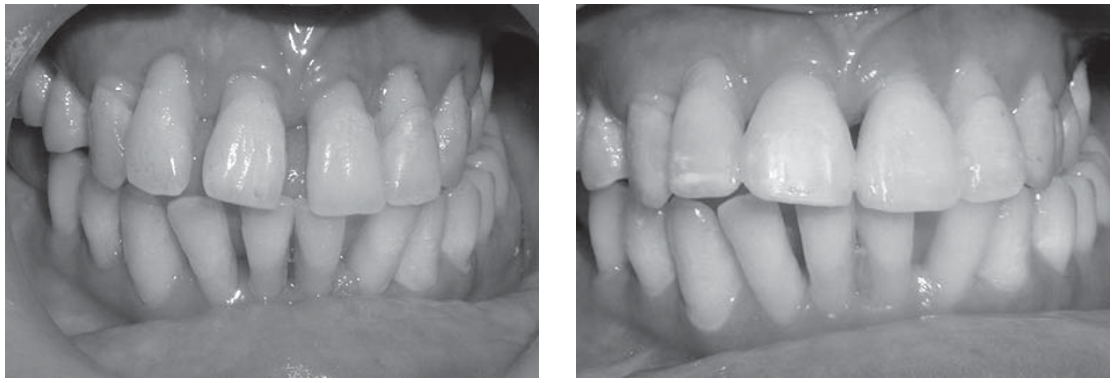

Figure 2. (A) A thirty year old female with mobile maxillary teeth and migration leading to spaces between the anterior teeth. (B) A glass fiber was splinted onto the buccal surface maxillary incisors followed by a direct bonding makeover.

the mandibular anteriors, the tensile side is the lingual. In the maxillary anteriors it is the labial. Hence the splint should be generally placed on the linguals of lower anteriors (Figure 1) and the buccal surface of upper anteriors (Figure 2). An additional advantage is that such placements never interfere with the occlusion of the teeth.

Another excellent application of the bonded splint is post orthodontic tooth retention. Zachrisson (1985) has observed that the greater flexural stiffness of conventional retainers is not a positive attribute.

Armamentarium and manpower

The instruments required, as well as the assistants required for placing a well bonded splint, are quite different than usual. The author, who has placed a large number of bonded splints, is of the firm belief that six-handed dentistry is must for proper placement of mandibular, anterior periodontal splints. The fiber has to be light cured piecemeal while being held in place, while at the same time taking care that uncured fiber areas are well protected and concurrent isolation is being maintained.

One assistant is required for suction and light curing of specific areas of the splint. The other assistant is required to aid in holding the fiber in place while the composite is being applied and then as the fiber is cured in parts. It is necessary to have multiple probes

at hand to hold the fiber interproximally between successive teeth as the light is applied to cure it into place. In addition, the regular armamentarium of bonded dentistry is required, namely acid etch, bonding agent, fiber, flowable composite and regular composite.

An indispensable tool is an instrument that the author has designed. It is called the AA Splint Stabilizer. It is something like a reverse haemostat with two prongs. The instrument can be locked into any one of over ten positions. Each lock position holds the prongs at an increasing fixed distance from each other. The instrument is intended to stabilize the fiber and hold it into position in the interproximal areas just prior to polymerization. The variable distance setting allows the instrument to be used for teeth of almost any size.

The technique

The steps involved in placement of the splint can be listed as follows:

- Identification of the number of teeth to be splinted, i.e. extent of the splint.
- Isolation with cheek retractors or rubber dam.
- Shade selection of the involved teeth.
- Tooth preparation.
- Sizing and trimming the glass fiber for the splint.
- Acid etching of the prepared teeth.
- Application of bond layer.
- Placement of the glass fiber along with flowable/regular composite material.
- Final polymerization of the glass fiber.
- Completing the splint with regular composite material.
- Occlusal adjustment/polishing.

Extent of splint

The extent of the splint is defined by a number of factors. These factors are the number of afflicted teeth, the severity of the mobility, the individual tooth position and the location of the teeth along the arch form. An attempt should be made to include terminal stable teeth in the splint design in order to provide adequate support to the afflicted teeth with compromised bone. A principle to be followed is regarding the long axis of movement of the teeth in question. A given tooth will always display mobility along a certain vertical long axis of movement, along which the movement is essentially in a buccolingual direction. The idea of splinting teeth together is to prevent movement of teeth by fusing multiple teeth with different long axes of movements. In addition, including terminal stable teeth in the splint gives additional support to the overall prosthesis.

Isolation

Placing a reinforced bonded splint requires the same guidelines for isolation as in conventional bonding dentistry. Good isolation and prevention of contamination is of paramount importance. Generally maxillary teeth can be easily isolated for splinting with the aid of cotton rolls, cheek retractors and a high vacuum suction. Lower teeth more often than not, will require isolation with a rubber dam. This is especially true in patients who do not have very good control over their tongue. Even the slightest contamination can lead to ultimate failure of the splint. It is recommended to apply the fiber on the buccal surface of upper teeth which only requires the lips to be kept away from the working area. Placement of the fiber along the lingual of the lower arch is a different scenario and requires as little interference in the working area as possible.

Placement of a rubber dam between each of the first premolars in mildly mobile teeth is a very simple and easily performed task.

The teeth should be marked on the dam with a template and holes punched. Generally no clamps are required when using rubber dam for isolation for periodontal splinting. Setting up the rubber dam takes only two to three minutes but saves operating time and comfort levels of working are greatly magnified. After placing the rubber dam it is a very good idea to place wedges in the interproximal areas which are likely to be present if extensive bone loss has already taken place. The only exception is when a retention splint is being placed after completion of orthodontic therapy.

Tooth preparation

Once the extent of the splint has been ascertained and the terminal teeth identified, tooth preparation is commenced. A groove has to be prepared extending from the distal aspect of one terminal tooth to the distal aspect of the other terminal tooth. The groove begins at the buccal surface about 0.5 to 0.75 mm median to the distoproximal line angle. The groove also ends on the other terminal tooth in the same position. The groove runs through the entire buccal surfaces of all the intermediary teeth. The groove must dip into both the proximal surfaces of all the intermediary teeth as well as the mesioproximal surfaces of the terminal teeth. The groove should ideally be prepared with an air rotor using a thick blunt ended tapering fissure bur.

The groove should be ideally prepared in one smooth stroke without any irregularities on the lateral line angles of the groove. The bur should be placed in the starting position and light pressure applied until the required depth is achieved. The groove should be between 0.5 and 0.75 mm deep. Once the bur has been introduced and the preferred depth achieved, the bur should be moved laterally towards the adjacent tooth. The bur should be held at 90 degrees to the buccal surface at all time with the groove made as parallel as possible to the incisal line angle. This ensures that the bur will move almost 90 degrees when preparing the groove on the proximal walls. The bur tip will make an arc around the crown in a circumference which is always equidistant from the incisal line angle. It is good idea to place the air rotor handpiece in the required position and guide it with the finger of the other hand while preparing the groove from one proximal surface to another. This helps in controlling the depth and direction of the bur while cutting into the tooth surface.

The groove should be placed in the incisal third of the tooth surface when prepping for a maxillary splint. The placement is more or less the same when placing a lingual mandibular splint. On a relative basis, the position of the groove is slightly more apical in the mandibular teeth versus the maxillary teeth. A very important criteria affecting the groove position when there are open interproximal spaces is the location of the contact point or area. This contact point or area will be created when the buildup is being done. If the groove is incorrectly placed it will influence the fiber placement, which in turn will dictate the buildup and location of the contact point/area. The depth of the incisal embrasure which will also be created has also to be envisaged. A very short incisal embrasure is not aesthetically pleasing and hence the groove cannot be placed too close to the incisal line angle.

A minor advantage of a slightly apical position of the mandibular groove is that it allows the operator to utilize the starting bulge of the cingulum which may act as a seat for placement of the fiber.

The next step is to bevel all the margins of the groove. This step is necessary to obtain precise aesthetics since the bevel will help in blending the composite material with the tooth surface to create a natural appearance. The bevel can be done with a medium to thick round headed tapered fissure diamond bur

or its equivalent. The bevel should be 30 to 40 degrees on all the surface margins of the groove. It is a good idea to first bevel either the incisal margin or the apical margin. Once one of the margins has been beveled then the other margin should also be beveled. Then the proximal ends of the groove on each of the involved teeth should also be beveled.

The bur should be held at an angle of about 45 degrees to buccal surface and lightly brushed along the margins. The bevel should extend about 1 to 1.5 mm from the groove along the buccal margins. It is a good idea to view the grooves from a lateral angle to visualize the margins and extent of the bevels. Once the beveling is done the preparation is complete.

Sizing and trimming the glass fiber

The next step is to prepare the glass fiber for adaptation. The fiber has to be longer than the actual arc around which it is to be placed since the fiber has to partially wrap around the proximal surfaces. The extension of the fiber around these surfaces has to be taken into consideration when trimming it. Once the required length has been determined, it is cut with a special scissor and is ready for placement. It is imperative that the fiber not be handled, even with gloved fingers and not contaminated at all. It should be handled gently with clean tweezers and placed on the tooth surface just prior to the actual bonding. The fiber should also not be exposed to too much light and preferably placed under a cover until it is bonded onto the tooth surface. This is because the fiber is pre-impregnated with resin which may start polymerizing on exposure to light. The placement of fiber and its management varies in the different teeth and is described in the following sections.

Scenario A (Lower Anterior Teeth)

In this scenario the fiber is being placed on the lingual surface. On completion of bond polymerization, a small amount flowable composite is taken and placed along the length of the groove. It is advisable to use a viscous flowable composite, as a very fluid material will flow down the tooth. The fiber is then picked up with a tweezer and placed flat on the ledge starting from one end. Here is it imperative to have at least two dental assistants. One assistant would handle the suction and the light curing unit, while the other will assist the dentist in holding the fiber in position. The end of the fiber has to be held against the tooth wall at the distal most aspect of the splint with an explorer. The fiber should be then pressed into the interproximal spaces sequentially with the help of an explorer. Note that the fiber will be splayed out apicocoronally. The other assistant should polymerize the fiber held against the lingual wall of the first tooth in the splint. This exposure to light has to be only for a few seconds. The aim is not to completely polymerize, but rather make the fiber partially retain its new shape. While polymerizing, care has to be taken that no other part of the fiber is exposed to the light. It should be directed only towards the exact tooth for which the polymerization is to be carried out.

The area should only be exposed to light for between five to ten seconds to hold the fiber in place. Once the initial fixation has taken place on the first tooth, the same procedure is repeated for the next tooth. Straight explorers make very good hand instruments to retain the fiber interproximally while it is being polymerized. In this manner the entire fiber is polymerized into place.

Scenario B (Upper Anterior Teeth)

Placement of the fiber and primary polymerization in this scenario is a far easier technique than Scenario A. Since the

fiber has to be placed on the buccal surface, displacement of the fiber does not generally take place and the entire procedure is much easier. The recommended instrument of choice is a composite plastic instrument to hold the fiber in place rather than a straight explorer. Unlike Scenario A, the fiber is not splayed out but rather rolled and packed into the buccal groove.

Scenario C (Posterior Teeth)

As mentioned earlier, the groove is placed occlusally in the posterior teeth. This is generally the easiest of the splints to place. Conversely this is also the least predictable of the three scenarios. The groove is generally along a straight line unless the teeth are malpositioned. As in Scenario B, the fiber is rolled and inserted into the channel after a little flowable composite has been placed. The fiber can be held in place with considerable ease.

One important aspect to take care of while carrying out initial polymerization is that the teeth being splinted be held in a position as close to natural physiological position as possible. Since teeth being splinted are generally very mobile, they tend to get displaced during the initial polymerization.

Etching the tooth surface

The next step is to etch the prepared area for 10 to 15 seconds. It should be noted that enamel may be etched for up to 10 seconds but the dentin should only be etched for about 5 seconds. The base of the groove will generally be in dentin and hence the acid should be first applied along the peripheries of the groove and the beveled area of all the teeth, and then lastly placed within the groove. By the time the acid etch gel is placed in the last of the teeth, the first tooth that has been etched is ready for washing with a gentle stream of water. A high evacuation suction should be placed adjacent to the tooth being washed to directly drain off the acid, thereby preventing an unpleasant taste as well as any untoward interaction of the oral tissues with the acid. The acid generally used is 35% phosphoric acid or any material supplied by the manufacturer of the bonding agent being used.

Application of the bonding agent

The next step is to apply the bonding agent. The current preferred choice is to carry out wet bonding, i.e. apply the bond on a relatively wet tooth surface. The bond should be taken on a bond applicator and lightly applied all over the prepared tooth surface. It should be ascertained that the entire tooth surface is totally covered with the bonding agent. Depending upon the system being used and the manufacturer's instructions, either one between one and three layers of bond have to be applied. The bond is then lightly dried with a light blast of air after waiting for about 15 seconds. This is to allow the solvent, as well as the priming agent which is generally included in fifth generation bonding agents, to evaporate. Once the bonding agent has been dried with a gentle stream of air it should be polymerized with a light cure gun. All areas of the teeth where the bond has been applied have to be polymerized for 20 seconds. After the polymerization the bonded area should have a shiny glassy appearance.

Fiber placement

The next step is to place the fiber in the grooves. Prior to placement of the fiber, small amounts of flowable composite material should be placed in the grooves of the teeth. The overhead dental light should be slightly moved away from the oral cavity and not focused on the area of operation as the overhead light could polymerize the flowable

material. The flowable composite should not polymerize at all and has to be spot cured to hold the fiber in place and absolutely well adapted to the tooth surface of the groove. A very common clinical scenario is the splinting of lower or upper incisors using the canines as the terminal support teeth. This requires the fiber to be adapted and placed around six teeth. Strict maintenance of isolation is a must as the fiber is being adapted and spot cured. These above requirement make it mandatory to have at least two assistants for the operating dentist, i.e. six-handed dentistry. One assistant is required to handle the suction and spot cure the fibers, while the second assistant is required for help in adapting the fiber and protecting the unadapted fiber from being either contaminated or partially polymerized.

The adaptation of the fiber should be initiated from one end of the groove. The fiber is to be picked up with a small tweezer and then placed in the end of the groove. The fiber should be adapted around the tooth in the groove and the other end of the fiber pressed into the end of the groove of the same tooth on the other proximal surface. When this is done the free end of the fiber tends to get picked up and has to be held in place. The fiber can be held in the groove either with the help of the two prongs of a fine tweezer or with two probes. The second assistant should at this point hold the fiber in the next interproximal space. The fiber should then be spot cured onto the first tooth on which the fiber is properly adapted inside the groove. The tooth surface should be polymerized for about five seconds which holding some protective metal surface over the adjacent fiber so that it is not polymerized before being adapted to its requisite tooth. Once the fiber has been spot cured into place the same procedure is to be repeated for the adjacent tooth. This is then further repeated until the entire fiber has been spot cured and polymerized in place. If necessary, a slight amount of flowable composite can always be placed in the groove to adapt the fiber perfectly.

Final polymerization of the fiber

Once the spot polymerization is completed, any free spaces in and around the fiber should be covered with flowable composite. The fiber running from tooth to tooth in the interproximal spaces should be also covered with flowable composite which will totally impregnate the basic fiber. Once this is done the entire splint should be completely polymerized, making sure that all areas of the fiber as well as composite have been exposed to a 20 second curing cycle. This will completely polymerize the fiber and bond it to the tooth surface.

Completing the splint with composite

The next step is select the composite of the right shade and start filling the groove with the composite, making sure that the material spreads on the lateral surfaces of the tooth and completely covers the groove area and blends in with the tooth surface. A nonstick plastic composite instrument is very useful for placing the composite. Specialized hand instruments are available for blending the composite to the tooth surface. The composite material in the right shades should be adapted and the entire preparation filled and blended in with intermittent curing cycles to polymerize the material. The splinted section should then be examined in detail and if any spaces are present they should be filled in and polymerized. Once the entire material has been placed, the next step can be carried out. The operator has to make sure that there is no exposed fiber in the splint at all. All the fiber should be completely layered with composite material. Once this is completed the rubber dam or the cheek retractors and cotton rolls can be removed and the patient

given a break. The entire splint is then washed and cleaned with a water spray. A small hint while removing the cotton rolls is to wet them prior to removing them. The dry cotton tends to stick to the mucosa and may cause a mucosal trauma.

If the splint is not being performed for aesthetic purposes and no interproximal spaces exist the splint should be polished thoroughly (Figure 3).

Occlusal adjustment

The next step is to adjust the occlusion and confirm that the splinted teeth have a group centric position contact and that there are no individual discluding teeth in lateral and protrusive movements. If there is any single tooth in the group of splinted teeth which has a premature contact it has to be adjusted till such time that group function is achieved. It is not always necessary that the tooth in question among the splinted teeth has to be reduced. It may be prudent to adjust the opposing arch tooth in case the tooth is not in balance with occlusal plane of the particular arch. When adjusting the occlusion, an attempt must be made to achieve as close as possible a flat and level occlusal plane for both the arches. If any teeth are adjusted then the adjusted areas should also be subjected to a polishing regimen like the composite material.

Polishing the splint

The next step is to polish the splint. It is a good technique to use standard finishing burs which are used for polishing composite restorations. The same polishing burs should be used to remove excessive composite that may have flowed near the cervical interproximal areas. A suggested sequence of burs is to use size 8 fluted, then 16 fluted, and then 32 fluted flame shaped carbide burs. This is to be followed by a silicone tip of an appropriate shape to finish the splint. Once the splint has been completely polished, a thorough occlusal check has to be carried out for premature contacts. There should not be any single tooth which is overloaded due to a premature contact. Such premature contacts are highly liable to cause stresses and fracture the splint. The entire assembly of splinted teeth should have a balanced occlusal load, thereby distributing masticatory forces evenly between mobile and supporting teeth.

The dentist now has to be a combination artist and bioengineer when using fiber reinforcements. They are no longer just a materials selector but a materials designer. With the advent of fiber splinting, dentistry moves into higher planes of a symbiosis between medical treatment and engineering designs.

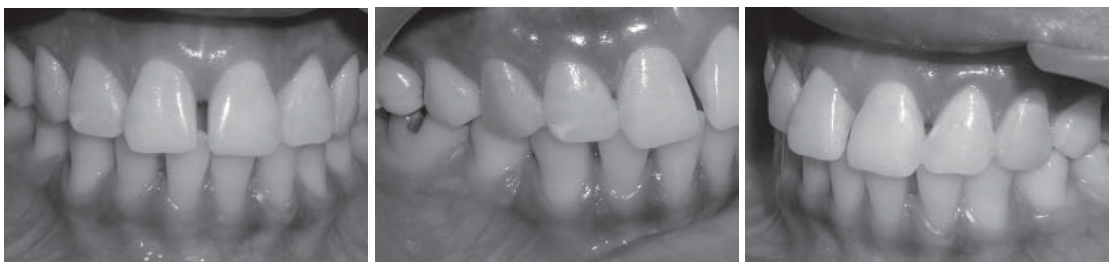

Figure 3. (A) An orthodontically relapsed case with diastemas created between the maxillary incisors. (B) The maxillary canine to central incisor on the right side splinted with glass fiber on the buccal side followed by a direct bonding procedure. (C) The maxillary canine to central incisor on the left side splinted with glass fiber on the buccal side followed by a direct bonding procedure. The patient did not want the diastema between the central incisors to be closed.

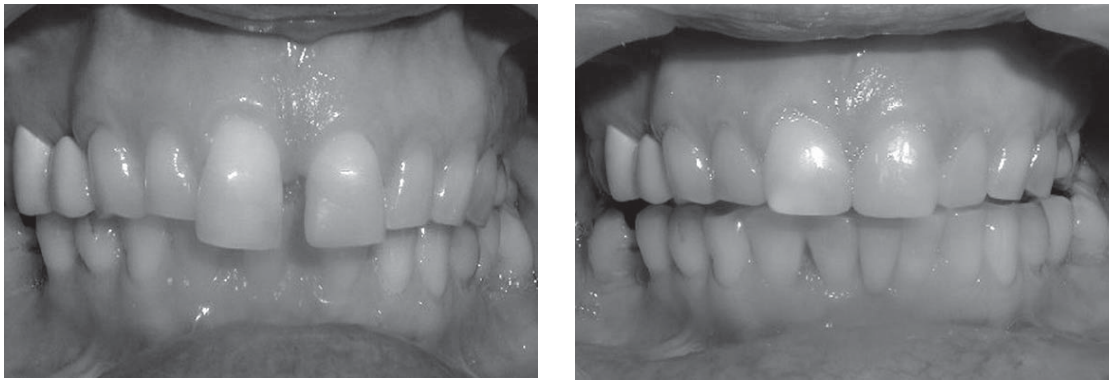

Figure 4. (A) A 65 year old female with migration of the two central incisors and extrusion of the upper right central incisor. (B) A glass fiber splint placed on the central incisors followed by a direct bonding procedure to close the diastema. The fiber was placed on the palatal aspect.

Literature report

A number of studies have evaluated the effects of fiber reinforcement on composite resins. A report in Clinical Research Associates concluded that fibers improved the flexural strength and flexural modulus of composite resins and that all splints fabricated with these materials were successful after one year. (Clinical Research Associates 1997). Strassler *et al* (2001) reported that of 54 teeth splinted and examined over 12 to 48 months, none exhibited debonding or any caries. All periodontal and orthodontic teeth were intact and only one of eight natural tooth pontics had fractured, and even in this case the fracture was restricted to the composite resin and the ribbon held the tooth in place despite the fracture.

The aesthetic splint

The aesthetic splint is an enhancement of the fiber reinforced bonded splint. This component is added to the basic fiber splint to create a more complete form of splint design. It is defined as "an amalgamation of function, form and aesthetic design replacing all forms of existing splint designs, irrespective of the desired duration of the splint".

This splint is a two component design, with the second component as an optional add-on. The first component of the splint is the same as the regular fiber reinforced splint as explained earlier. The second component is to completely disguise the splint and blend it into the dentition.

The aesthetic component has been basically designed for the management of upper or lower anterior teeth which have open embrasures and gingival recession with mobility ranging from Grade I to Grade III. The different clinical situations where it can be applied are as follows:

- Periodontally compromised teeth with migration (Figure 4).
- Post orthodontic maintenance of teeth in position.
- Migration of anterior teeth with age and increased occlusal forces.
- Anterior alveolar fracture cases.

The steps involved after completing the basic splint are as follows:

- Re-evaluation of the splint.
- Isolation of the splint area.
- Placing retraction cords.
- Acid etching of all the involved teeth.
- Application of bond layer.
- Building up the interproximal spaces.
- Finishing and polishing.

Maintenance of splints

The maintenance of oral hygiene when a splint is in place is a very exacting proposition since access to the teeth and visibility for plaque control is reduced. It requires extra skills on the part of the patient as well as the dentist to maintain good oral hygiene. Effective plaque control and professional caries risk assessment is crucial to the longevity of the splint.

There are certain pre-requisites which have to be considered when placing a splint. To facilitate adequate access for cleaning, a splint must be placed with open gingival embrasures and be properly contoured with no overhanging margins. Extensive emphasis should be placed on the polishing. All surfaces should be absolutely smooth to minimize plaque retention and hence enable long term success for the splint. Posteriorly placed splints require an additional effort by the patient for maintenance of periodontal health.

The selection of plaque control devices in splinted teeth depends upon the type of splint, spaces surrounding the splint and the individual's dexterity in oral hygiene maintenance. Interdental plaque control is the most important factor in splinted teeth. The amount of recession and embrasure area defines the kind of device and effort involved in plaque control. A variety of floss are available but can be used only on the distal surfaces of the end teeth of the splint. Floss cannot be used along the splinted teeth as the interproximal surfaces are fused or bonded. A variety of interdental brushes or single tufted brushes are available for cleaning below the fused contact points. A proper sized brush should be selected by to ensure that no plaque is retained below the composite resin.

Oral irrigation is an important aid for splint patients who are otherwise unable to maintain total plaque control. It must be understood that oral irrigation is only an adjunctive therapy and not a replacement for either regular brushing or interproximal brushes. These devices deliver pulsating jets of water at high pressure which help in flushing out unattached debris prior to brushing. Optionally these devices can also be used to deliver anti-plaque agents along with the water.

Patients with splints should be re-evaluated periodically and professional debridement carried out to ensure complete plaque control. The splint patient should be put on a regular six monthly maintenance schedule. Every recall visit should include a very careful and meticulous supra and subgingival scaling. It is a very good idea to use magnification and extra lighting when working on a fiber-resin splint patient, since it may become very difficult to identify the transition from tooth to composite, especially if a very good aesthetic finish has been achieved. Air polishers and abrasives are to be avoided near splinted teeth. Ultrasonic scalers should be used with great caution when near the resin/tooth interface, as they may lead to inadvertent damage of the splint.

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Chapter 19

Current Approaches to Periodontal Surgical Flaps

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Introduction

Regardless of the different objectives of the various periodontal procedures, such as guided tissue regeneration (GTR) or crown lengthening, there are a few recurring decisions the surgeon has to make regarding flap design.

1) To preserve or remove the dental papilla?
2) How large a flap access is required?
3) To maintain the gingival level, or else apically or coronally reposition it?

Approaches to the dental papilla

In the classical Widman and Modified Widman flap approach (MWF) (Widman 1918), the interdental papilla is removed. This leads to recession following surgery which can be useful in reducing the probing pocket depths. In crown lengthening and apically flap surgeries, we may even carry out judicious gingivectomies to help apically position the flap.

The requirements for achieving predictable success in GTR surgery are of course quite different. Membrane exposure is the most common complication after GTR procedures and leads to an increased risk of infection and reduced success rates (Becker *et al* 1988, DeSanctis *et al* 1996, Mombelli *et al* 1993, Nowzari and Slots 1994). The most common area for these exposures is at the fragile papilla area (Takei *et al* 1985). The inadvertent loss of the dental papilla from a MWF makes good primary closure at the papilla area extremely challenging, which means the membrane is exposed even without soft tissue dehiscence.

The modified papilla preservation flap (MPPF) and the simplified papilla preservation flap (SPPF) were designed to help achieve good primary closure. These flap designs also reduce risk of tissue dehiscence by reducing the tension of the closure at the papilla areas (Cortellini *et al* 1995, Cortellini *et al* 1999).

The modified papilla preservation flap is used when the interdental space is at least 2 mm wide. An initial cut is made on the buccal of the interdental papilla, and the entire papilla is then raised palatally/lingually. This papilla is thus ‘preserved’. In areas where the interdental papilla is less than 2 mm however, there is a high risk of tearing the entire papilla as one tries to raise it towards the palate/lingual. The simplified papilla preservation flap was designed to address this challenge. In this flap design the papilla is cut diagonally dividing it into two triangles, one attached to the lingual and the other buccal. In this way the papilla can be raised with less risk of tearing it, half to the buccal and half towards the lingual.

There is usually a small circumference of inflammation around where the suture passes

through the flap. The suture should therefore not be placed too close to the papilla tip. The tension of the suture should also be controlled so that the suture does not strangulate the papilla, causing necrosis. It has been shown that using a finer suture material, 5-0 to 7-0 as opposed to 3-0, leads to a reduced risk of the suture tearing the flap if overtightened (Burkhardt *et al* 2008).

In the MPPF and SPPF, an internal mattress suture, placed a distance away from the crucial fragile dental papilla area, is used to pull the papilla tips together, so that they may be sutured without tension. Thus, the flap tension is taken up by this internal mattress suture at an area of the flap that is unlikely to dehisce.

Approaches to flap extension

With improved illumination, magnification and introduction of materials like Emdogain, new flap designs were proposed with minimal extensions and access. The limited elevation of the flaps in Minimally Invasive Surgical Technique (MIST) enabled the flap closure be more stable, hence making the blood clot more stable and increasing the prognoses of regeneration (Cortellini and Tonetti 2007). A further modification to the MIST (Modified Minimally Invasive Surgical Technique, or M-MIST) was proposed such that only a buccal triangular flap is elevated, while the papilla and the lingual tissues are left in place (Cortellini and Tonetti 2009).

Interestingly, in a small study of 45 patients, intrabony defects treated with M-MIST alone showed percentage radiographic bone fill of 77±19%, comparable to intrabony defects treated with M-MIST and Emdogain and Emdogain + bone graft combination (Cortellini and Tonetti 2011).

Approaches to managing the post-surgical gingival level

Limiting the surgical flap to within the attached gingiva makes for a more stable closure. This is done by not extending the flap beyond the muco-gingival junction. By keeping the attached gingiva fixed to bone, the chance of recession after surgery is reduced.

The corollary is that should one desire to change the level of the gingiva during the surgery, the flap should be extended beyond the muco-gingival junction, so that it may be mobilised. If the mesio-distal extension of the flap is short, you may also have to place vertical releasing incisions.

Unlike coronally positioning a flap where the flap can be fixed onto the neck of teeth, in order to fix the flap in an apical position, a partial thickness flap may have to be made nearer to the sulcus, leaving the periosteum on bone, in order that the flap may be anchored by it in an apical position (Carnio and Miller 1999, Nabers 1954).

In ridge augmentation procedures, in order to achieve primary closure of the wound, the buccal flap is often coronally positioned. It has been shown that by placing a single vertical releasing incision, the buccal flap may be advanced 1 mm. This increases to 2 mm if two vertical releasing incisions were placed. However, by adding in a periosteal releasing incision, the flap may be advanced by 5.5 mm (Park *et al* 2012).

Conclusion

A considered and well managed periodontal flap can significantly affect the eventual outcome of periodontal surgical therapy. Mastering a variety of flap management techniques, together with knowledge and understanding of the science behind wound healing will enable a surgeon to achieve consistent and predictable outcomes for

patients, with minimal pain and morbidity.

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Chapter 20

Peri-Implant Bone: Preservation or Reconstruction?

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Introduction

Sometimes our patients present for treatment but have insufficient bone to accommodate dental implants. This may arise due to:

1) Bone resorption after tooth extraction, which may be exacerbated by pathological processes such as vertical root fracture, periodontitis or endodontic lesions.
2) Bone aplasia due to hypo-, oligo- or anodontia and/or retained deciduous teeth.
3) Maxillary sinus expansion.
4) Trauma, which may include iatrogenic trauma from an inappropriate extraction technique.

If there is insufficient bone volume to house an implant in the restoratively-ideal position, reconstruction of the alveolar ridge may be required either prior to, or concomitant with, implant placement. Alternatively, it may be preferable to consider an alveolar ridge preservation (ARP) technique at the time of tooth extraction. This paper will provide an overview of the "what, where, why, when and how" of ARP, contrasted with alveolar ridge reconstruction, performed to preserve or create sufficient alveolar volume for dental implants. The paper will also discuss the surgical models in sheep that our research team have designed to examine ARP.

Alveolar Ridge Preservation Technique

ARP has been defined as "a surgical procedure aimed at preventing ridge collapse and preserving ridge dimension after tooth extraction, typically done for purposes of implant site development and involves the use of hard and/or soft tissue biomaterials and/or membranes" (American Academy of Periodontology 2001). ARP may be performed to prevent or minimise post-extraction atrophic bone loss, whereas ridge reconstruction aims to restore bone or tissue loss either before or after implant placement. Both techniques may involve the tooth extraction socket, the edentulous alveolar ridge or the maxillary sinus, and may occur before or during implant placement. Ridge reconstruction may also be required following implant placement, when pathological bone loss occurs following successful osseointegration, for example, as a result of peri-implantitis.

A variety of technologies in different combinations can be employed for ARP, which may include: atraumatic extraction techniques; protection and stabilisation of the blood clot within the tooth socket; bone replacement grafting (BRG) with or without the use of a membrane; other biological technologies; or immediate implant placement. Successful ARP is predicated on a detailed understanding of the complex

process by which bone is formed, remodels and heals, including the coordination and synchronisation of osteoblasts, osteocytes, osteoclasts and endothelial cells, the deposition of bone matrix with an adequate blood supply, the tightly coupled processes of osteoclastic resorption and osteoblastic deposition, and the complex inter-relationships of supporting cells types (macrophages, T-cells, fibroblasts) and cytokines (IL-1, TNF, Wnt family, RANKL/OPG), bone morphogenetic proteins (BMPs), transforming growth factor-β (TGFβ), insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGFA) that drive precursor cells along the osteoblastogenesis/-clastogenesis pathways and link their activities (Bayliss *et al* 2012, Doherty *et al* 2003, Sims and Martin 2014). Recently we have demonstrated similar processes during osseous healing in a sheep periodontal wound model (Baharuddin *et al* 2015).

Post-extraction resorption phenomena are related to the presence of the bundle bone that supports teeth. Bundle bone has been defined by the AAP Glossary as "a type of alveolar bone, so-called because of the "bundle" pattern caused by the continuation of the principal (Sharpey's) fibers into it". An example of bundle bone supporting sheep mandibular premolars is shown in Figure 1 (Duncan 2005). This lamellar structure is rapidly lost after extraction, with most of the reduction in alveolar ridge dimensions occurring in the first six months after extraction (Araújo *et al* 2015, Cardaropoli *et al* 2003, Devlin and Sloan 2002). The remodelling process results in a ridge morphology that is reduced in vertical height and more palatal in relation to the original tooth position (Pietrokovski and Massler 1967). Resorbed alveolar crest defects were classified by Seibert (1983) as Class I (horizontal loss), Class II (vertical loss) and Class III (both horizontal and vertical)

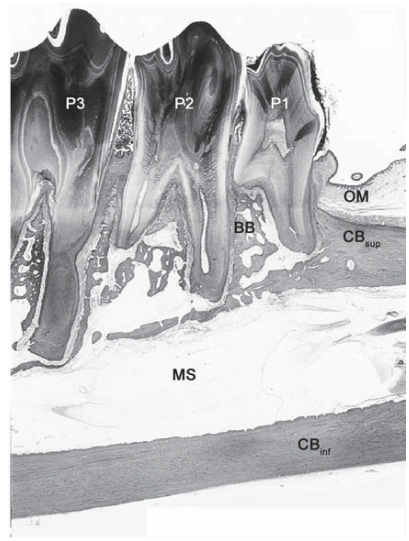

Figure 1. Longitudinal ground section of resin-embedded mandibular premolar teeth in sheep (Duncan 2005). Trabecular bundle bone surrounds and supports the teeth. P1, P2, P3 = 1st, 2nd and 3rd premolars; BB = bundle bone, OM = oral mucosa, MS = marrow space, CBsup = cortical bone (superior border of mandible) CBinf = cortical bone (inferior border of mandible). McNeils tetrachrome and toluidine blue with acid fuschin counterstain.

(Figure 2). More recently, a classification system for extraction sockets, attributed to Hämmerle and Jung, helped explain how these alveolar ridge defects occur (Rios *et al* 2015). In this system, Class I refers to extraction sockets with intact walls, Class II refers to extraction sockets with a marginal dehiscence or fenestration of the buccal bone wall and Class III defines a tooth socket with a large dehiscence of the buccal bone wall extending near the apex of the socket (Figure 1). In this classification system, Class I sockets would be considered suitable for ARP techniques including immediate implant placement, Class II sockets would require alveolar ridge reconstruction (guided bone regeneration) either prior to or concomitant with implant placement, whereas Class III sites would be best treated by alveolar ridge reconstruction with implant placement as a subsequent

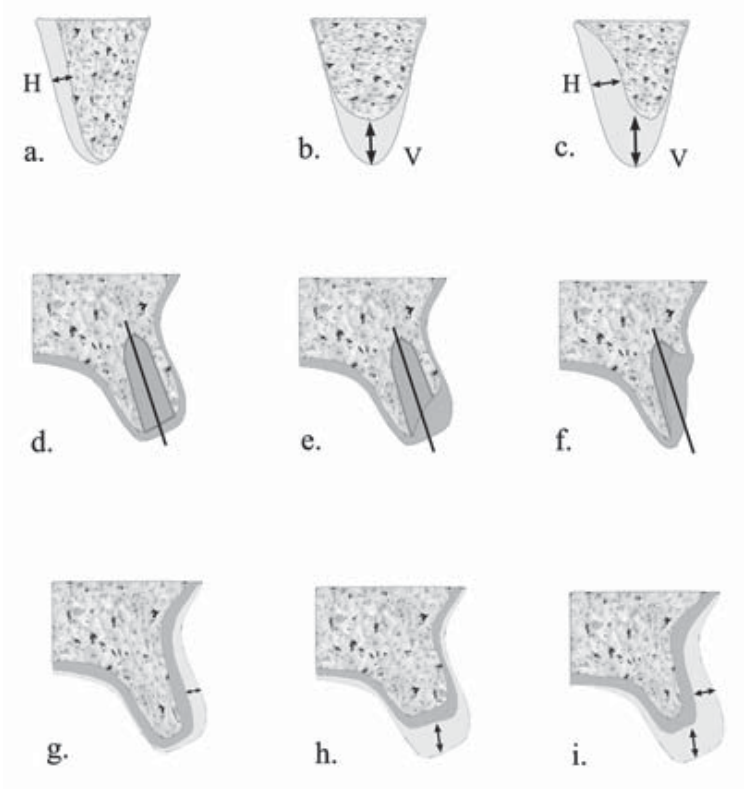

Figure 2. (A - C) Modified from Seibert (1983); diagram of edentulous posterior alveolar ridge showing (A) Class I: horizontal (H), (B) Class III: vertical (V), and (C) Class III: combined horizontal and vertical ridge resorption. (D - F) Modified from Rios *et al* 2015: Hämmerle and Jung classification of tooth sockets showing (D) Class I: extraction socket with intact walls, (E) Class II: extraction socket with marginal dehiscence or fenestration of buccal bone wall and (F) Class III: large dehiscence of buccal bone wall. In this classification system, Class I sockets are suitable for ARP techniques including immediate implant placement, whereas Class II and III require alveolar ridge reconstruction. (G - I) Diagram combining the two classification systems, showing the outcomes for where ARP or Ridge reconstruction techniques have not been used. (G) Horizontal resorption (H), vertical resorption, and (I) combined horizontal and vertical resorption. For (G), GBR and/or onlay bone grafting may be suitable, for (H), short dental implants may be required, and for (I) a split-ridge approach may be necessary.

delayed procedure.

The seminal work in explaining these phenomena was obtained from a dog mandibular premolar model (Cardaropoli *et al* 2003). Using a single dog at each of nine time points, the distal root of the 4th mandibular premolar was removed and mesio-distal histological sections obtained. At days one to three, the socket was filled with blood clot, by day seven a pre-osseous matrix had appeared, by two weeks mineralised woven bone filled the socket. By day 30, 80% of the socket was filled with woven bone, whereas by day 60 the healed ridge had a complete bone bridge across the socket but the percentage of woven bone was reducing; by day 180, 15% of the socket consisted of woven bone, the remainder being replaced with bone marrow. A follow-on study using three dogs per time point at one, two, four and eight weeks and bucco-lingual

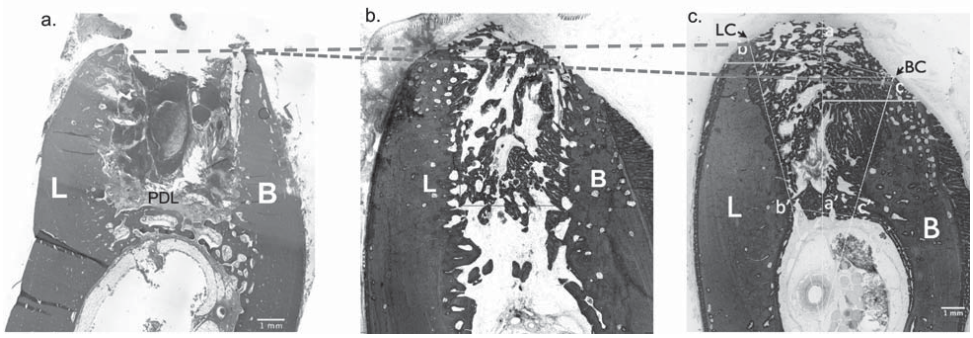

Figure 3. Modified from Liu (2013). Healing of ungrafted premolar extraction socket in sheep. (A) Paraffin-embedded demineralised section at baseline. Socket is occupied by blood clot. L = Lingual, B = Buccal, PDL = residual periodontal ligament. H&E stain. (B) Resin-embedded undemineralised section after 8 weeks. Socket filled with woven bone. McNeil's tetrachrome and toluidine blue stain. (C) Resin-embedded section after 16 weeks. LC = lingual crest, BC = buccal crest, lines a-a' and b-b' delineate original socket. Green dashed line shows loss of buccal bone height over 16 weeks, red dashed line indicates minimal change in lingual crest height. McNeil's tetrachrome and toluidine blue stain.

sections reported maximal bone resorption after eight weeks, occurring in two phases, with bundle bone being resorbed and replaced with woven bone in the initial phase and the thinner buccal plate experiencing greater resorption (Araújo and Lindhe 2005, Huynh-Ba *et al* 2010). Subsequent remodelling involved the outer surface of the alveolar bone with both a horizontal and vertical vector; disuse atrophy, decreased blood supply, and localised inflammation resulting from surgical trauma, pre-existing pathology and/or the extent to which the periosteum was elevated, all played a part in determining the extent of this phase 2 resorption. A key point from the work was the observation that the buccal plate showed markedly greater resorption, as this had implications for aesthetics in anterior maxillary sites in human patients.

Our research group subsequently explored both socket resorption and ARP using BRG in the mandibular premolar site in sheep (Liu *et al* 2015). The three mandibular premolar teeth were extracted in 18 sheep and the sites either grafted with a xenograft (with or without resorbable collagen membrane), a novel resorbable electrospun Ca-P grafting material, or left ungrafted. Healing periods were: baseline (N=2), 8 weeks (N=8) or 16 weeks (N=8). Histometric analysis revealed greater resorption of the buccal plate than the lingual, with woven bone filling the socket after 8 weeks; by 16 weeks this was being transformed into mature trabecular bone, with minimal marrow (Figure 3).

How does the evidence from these animal models relate to the clinical situation?

Tan *et al* (2012) included 12 published papers in their systematic review of dimensional changes following tooth extraction in human patients. They concluded that the most rapid reduction occurred in the first three to six months following extraction, with more gradual changes in dimension thereafter. At six months, horizontal loss was greater than vertical, although this was partially disguised clinically by increases in soft tissue thickness.

Classically, ARP involved atraumatic tooth extraction and filling the socket with grafting materials, followed by elevation of a full-thickness mucoperiosteal flap to achieve

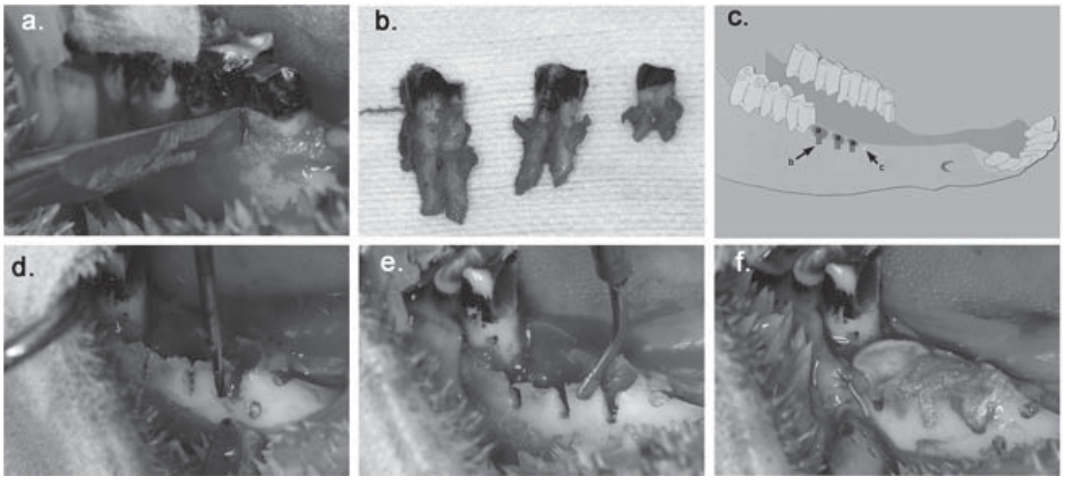

Figure 4. Sheep premolar extraction socket model designed to test ARP for Hämmerle and Jung classification class II defects. (A) Extraction of mandibular premolars P1, P2 & P3. (B) Extracted teeth showing root morphology. (C) Diagram of model; a = tooth sockets, b= standardised slot in buccal wall, c= amalgam marker. (D) Cutting slot in buccal wall. (E) Refining slot using Piezotome. (F) Grafted sites.

primary soft tissue closure. More recently a flapless technique has been described where the papillae and connective tissue attachment to adjacent teeth is preserved; the socket may be grafted and/or have a resorbable membrane tucked under the mucosal margin, but a flap is not elevated and primary tissue closure was not an objective (Landsberg and Bichacho 1994). The flapless technique results in significantly less post-extraction ridge resorption than the more invasive flap approach with similar histological outcomes (Barone *et al* 2014, Barone *et al* 2015).

Many different techniques and materials may be used for bone augmentation prior to or contemporaneous with implant placement, including membrane exclusion, autogenous bone-grafts, xenografts, allografts, growth factors such as bone morphogenetic factors (BMPs) or platelet-rich plasma (PRP), or so-called "adult stem cells" (multi-potential cells, MPC) (Araújo *et al* 2008, Araújo *et al* 2009, Araújo *et al* 2010a, Araújo *et al* 2010b, Baldini *et al* 2011, Haggerty *et al* 2015, Lindhe *et al* 2014, Liu and Kerns 2014, Naik *et al* 2013, Schuckert *et al* 2011, Tognarini *et al* 2008). Each of these different approaches has their merits and draw-backs. Currently a popular technique involves particulate bovine-derived bone, either encapsulated in collagen or retained within the socket using a collagen membrane. Histological evidence however suggests that the xenograft persists within the socket and becomes encapsulated within new bone; some authorities consider that the persistence of xenograft retards complete healing of the site (Lindhe *et al* 2014). For this reason, researchers continue to explore new grafting materials that are completely resorbable whilst also preventing alveolar ridge resorption and promoting new bone growth.

Recently we compared a novel material composed of electrospun cottonwool-like nanocomposite (ECWN) that incorporates amorphous calcium phosphate nanoparticles into a biodegradable synthetic copolymer poly(lactide-co-glycolide), against bovine-derived xenograft, in a sheep premolar extraction model. We found no statistically significant differences between xenograft and ECWN, but after 16 weeks the test sites had

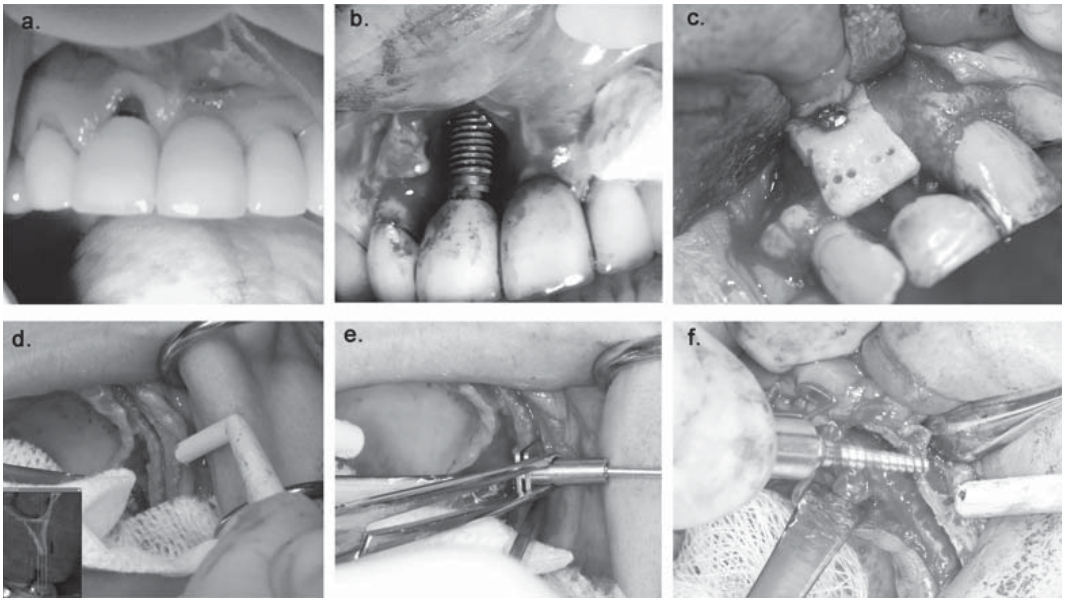

Figure 5. Clinical examples of alternatives to BRG for ARP or ridge reconstruction. (A) Patient treated by immediate placement into a tooth socket, presenting five years after implant placement with continuing buccal recession and bone loss. The patient had no other periodontal lesions. (B) Flap raised to show complete loss of buccal plate nearly to the apex. The implant was removed and the site reconstructed using a block bone graft. (C) Example of a block bone graft placed to restore an anterior site. The incisor had been extracted due to significant an endodontic lesion, with complete loss of buccal plate. (D) Split-ridge treatment for a highly-resorbed edentulous maxillary ridge in a young female patient. Inset shows image from Cone-beam CT cross-section: tall, narrow ridge in relation to tracing overlay of 3.5mm diameter implant. Initial split ridge using Piezotome. (E) Same patient after placing bone spreader (Meisinger Comprehensive Ridge Splitting System®, Salvin Dental Specialities, Charlotte, North Carolina, USA). (F) Same patient prior to placing Meissinger Threaded Spreader. The patient was restored with five narrow implants.

significantly less residual graft material than the xenograft sites (Liu *et al* 2015). We have now further modified our animal model, in order to replicate a Hämmerle and Jung class II socket (Figure 2e) and we are in the process of testing the combination of bovine-derived xenograft and porcine-derived collagen membrane (BioOss® and BioGide®) against a novel equine collagen/biphasic Calcium phosphate combination (Figure 4).

The alternatives to the use of BRG for alveolar ridge preservation or ridge reconstruction in order to preserve or create sufficient bone to accommodate an implant include: immediate implant placement; immediate implant placement with guided bone regeneration; block bone grafting, typically from the chin or ramus region; split ridge procedures; and maxillary sinus grafting (Figure 5). Studies in dog models have shown that immediate implant placement into a tooth extraction socket does not prevent alveolar bone remodelling, with bone remodelling of the buccal plate amounting to approximately 2.5 mm, independent of the implant system used; buccal bone remodelling is significantly more extensive around immediate implants placed in contiguous tooth extraction sites compared with single tooth extraction sites (Al-Shabeed *et al* 2012, Araújo *et al* 2005,

de Sanctis 2009). An analysis of 93 extraction sockets in human patients concluded that "in the majority of extraction sites in the anterior maxilla, thin (≤1 mm) buccal walls were present … in most clinical situations encountered, augmentation procedures are needed to achieve adequate bony contours around the implant" (Huynh-Ba *et al* 2010).

What evidence do we have supporting the use of alveolar ridge preservation in clinical practice?

A number of systematic reviews of ARP have been recently published. Horváth *et al* (2013) concluded that, although ARP does not totally prevent alveolar ridge resorption, some techniques may reduce post-extraction dimensional changes. They noted that intact socket walls and primary flap closure were associated with favourable results, but remarked on the lack of evidence regarding the influence of site location, buccal plate thickness, healing time, antibiotic regime, light smoking, and history of treated periodontitis. They also reported that conflicting histological evidence exists for the benefit of ARP, since de novo hard tissue formation was not a predictable outcome, and some graft materials appeared to interfere with healing. Lack of consistency in the research methodologies meant that no meta-analysis was possible, thus no material or method could be considered superior to another at this stage. Further randomised clinical trials (RCTs) are needed, with ungrafted tooth sockets serving as the control site, and with both clinical (quantitative) and histological (qualitative) assessment included in the outcome measures. The authors concluded that there is some evidence supporting the clinical benefit of ARP in reducing the need for further augmentation at implant placement, but no statement could be made regarding cost-effectiveness, and case selection criteria for performing ARP remain unclear.

Orgeas *et al* (2013) focused their systematic review on the different surgical techniques used for ARP. They found that barrier membranes alone improved wound healing in extraction sites, reducing bone loss by 0.9 mm (vertical) and 2.9 mm (horizontal). They considered that flap elevation and soft tissue primary closure had little effect reducing horizontal and vertical bone loss. They also commented that tooth sockets that do not receive ARP still show normal healing, that the use of xenografts, allografts and/or membranes all add significantly to the cost of tooth extraction and implant placement, and that the cost benefit of ARP remains unproven. They recommended that future RCTs examining the utility of ARP procedures should adhere to the CONSORT statement guidelines in reporting, that a split-mouth design should be used to compare clinical procedures and biomaterials, and that researchers should aim to standardize both surgical procedures and outcome measures.

The systematic review conducted by Avila-Ortiz *et al* (2014) included a meta-analysis and concluded that ARP can prevent post-extraction bone loss in non-molar tooth sockets, both horizontally and vertically, and that flap elevation and the use of a membrane and xenograft or allograft enhanced the outcome of ARP, particularly with respect to midbuccal and midlingual height preservation. They commented that ridge volume loss occurs even after ARP; this procedure may not reliably maintain sufficient bone volume for implant placement in an ideal restorative position, without resorting to further site development.

Our research group has recently completed a Cochrane Collaboration systematic review of ARP for dental implant site development (Atieh *et al* 2015). We included eight RCTs with a total of 233 extraction sites in 184 participants. There was insufficient evidence

to determine clinically significant differences when the various ARP techniques were compared with tooth extraction alone. We found no published trials that evaluated parameters relating to clinical attachment levels, specific aesthetic or prosthodontic outcomes. Our analysis concluded that there is limited evidence that ARP techniques minimise changes in residual ridge height and width six months after extraction, and this evidence is strongest for non-molar four-wall sockets. There is a general agreement that implants can be placed six months after ARP, following a delayed placement protocol. We found no evidence of any differences in implant failure, aesthetic outcomes or any other clinical parameters and no convincing evidence for clinically significant differences between the different grafting materials and barriers. Although there are more published trials suggesting that xenografts are successful for short-term ARP, the quality of evidence is low and the risk of bias high. Further high-quality RCTs are needed to determine the criteria for selection of appropriate cases for ARP, and the ARP technique that provides the most predictable results.

Conclusions and recommendations

There is evidence that tooth extraction results in alveolar ridge resorption. Where dental implant treatment to replace the lost tooth is contemplated, steps should be taken to minimise this resorption. The use of bone replacement grafting and/or membranes for ARP may be appropriate. Alternatively, immediate placement of the implant may be considered. Currently there is insufficient evidence to provide clear guidelines for selection of cases, techniques or materials. Further research is required, initially using animal models to develop new grafting materials, followed by well-designed RCTs that compare these materials against ungrafted tooth sockets in human patients. The following are some recommendations for clinical treatment, which may be modified as new information emerges:

1) Consider immediate implant placement for patients with a thick biotype in non-aesthetic zones and non-infected tooth sockets.
2) Consider delayed immediate implant placement (6 to 8 weeks) for non-infected tooth sockets with intact walls, or patients with thin biotype. Flapless atraumatic extraction should be emphasised. Consider the use of a resorbable membrane to protect the blood clot.
3) Consider BRG+membrane+delayed implant placement for infected sites requiring reconstruction of the socket wall.
4) ARP remains a cheaper and less-invasive option than techniques for reconstruction of the resorbed ridge, although such techniques are effective when residual alveolar ridge volume does not permit implant placement.
5) The search for the ideal ARP biomaterial should continue, with a view towards a material that is both osteoconductive and inductive, resorbable, effective, low-cost and safe.

Acknowledgements

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Chapter 21

Two Adjacent Short Implants Supporting Non-Splinted Crowns in the Posterior Mandible

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Introduction

The posterior region of the jaws usually has the lowest bone heights but higher biting forces, hence conventional dentures may give a "poor fit" and lack stability and retention (Frank *et al* 1998, Hummel *et al* 2002, Misch and Bidez 2005). Implant retained prostheses may be suitable alternatives (Adell *et al* 1990, Andersson 1995, Andersson *et al* 1992, Bränemark *et al* 1985, Schmitt and Zarb 1993, Smith and Zarb 1989). The use of implant prostheses has increased due to their high predictability and success rate. However reduced alveolar bone height and the presence of the inferior alveolar nerve in the posterior mandible limits implant length in this region. Solutions for these issues have been bone augmentation procedures, inferior alveolar nerve transposition and use of short implants (Das Neves *et al* 2006).

Definition of short implants

There has been no consensus in the literature on the definition of short implants. Implants ≤8 mm, completely submerged in bone, as well as implants of length <10 mm have been considered short implants (Friberg *et al* 2000, Johns *et al* 1992). As minimum length for proven predictable success has been determined to be 10 mm, any implant below 10 mm has been referred to as a short implant (Das Neves *et al* 2006, Gentile *et al* 2005, Morand and Irinakis 2007).

Short implants were associated with lower survival rates in earlier studies (Herrmann *et al* 2005, Weng *et al* 2003, Winkler *et al* 2000). However recent studies have shown comparable survival rates for short and long implants (Anitua *et al* 2008, Gentile *et al* 2005, Grant *et al* 2009, Romeo *et al* 2006). The possible reasons that have been proposed are improvements in implant design and surface modification, appropriate surgical procedures, attention to the quality of bone and splinting of implant prostheses.

The use of short implants avoids autogenous bone grafting and associated complications, requires shorter treatment time and is cost effective. In addition, less bone preparation is needed, which provides good access for irrigation and easier placement of implants in terms of angulation and entails lesser morbidity (Das Neves *et al* 2006).

Significance of the present study

It was previously claimed that the best treatment strategy for cases of reduced alveolar bone height was surgical modification by bone grafting techniques, alveolar distraction or inferior alveolar nerve transposition. However, clinicians have created a demand for

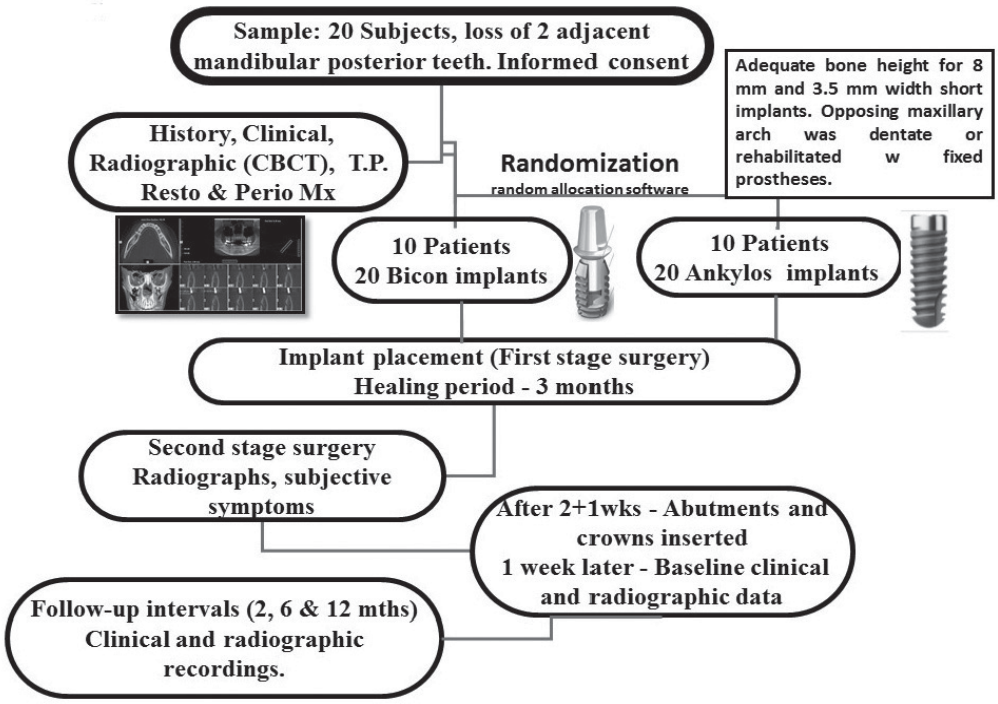

Figure 1. Study flow chart.

shorter implants with comparable successful outcomes to standard length implants. To date, there has been no longitudinal clinical study comparing the outcomes of different short implant systems with a platform switched design for rehabilitation of two adjacent missing teeth in the posterior mandible (Crespi *et al* 2009, Degidi *et al* 2008a).

Aims of the Study

- To investigate and compare the clinical, radiographic and success outcomes of two adjacent short 8 mm dental implants restored with non-splinted ceramo-metal crowns in the posterior mandible, and to compare the outcome between two implant systems during a one year period.
- To evaluate the stability of occlusal parameters of these patients which were balanced using T-Scan III system during a one year period.

Methodology

The study was approved by the Ethical Committee, Faculty of Dentistry, University of Malaya. Strict selection criteria were set. A sample size (20 subjects, 40 implants) was calculated to give a power of 80% (α, two-tailed, set at 0.05) from outcomes reported in previous studies using a computer program [PS Power and Sample Size Calculations, Version 3.0, 2009; Vanderbilt University] (Gentile *et al* 2005, Schulte *et al* 2007).

From the reproducibility results, clinical parameters taken by the examiner were highly reliable and consistent, as the kappa, ICC (intra-class correlation coefficient) and Cronbach's α values were all significantly high. The flow chart of the study is shown in Figure 1.

Baseline (1 week) and review assessments were conducted. Subjective data, for example pain or discomfort, sensory disturbances and/

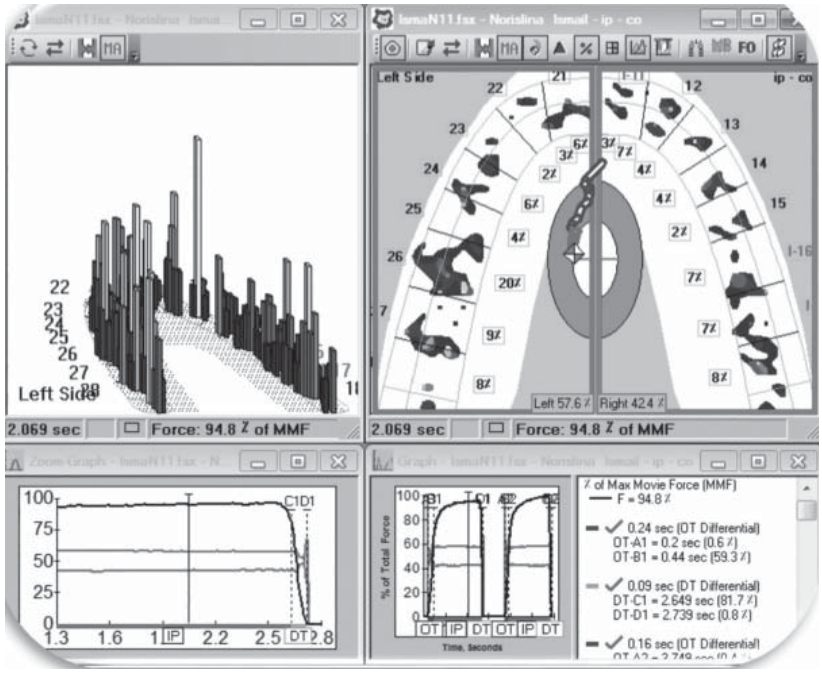

Figure 2. Biting Force Assessment using the T-scan III system® (Tekscan Inc., South Boston, MA, USA).

or crown loosening was noted. Objective implant and peri-implant data, such as plaque index (PI), gingival index (GI), modified bleeding index (mBI), probing pocket depth (PPD), width of keratinized mucosa (WKM), clinical attachment loss (CAL), recession height (RH) and implant stability using Periotest device were recorded (Löe and Silness 1963, Mombelli *et al* 1997, Mombelli and Lang 1994).

Peri-implant bone height was calculated from digital periapical radiographs at baseline, 6 and 12 months. Radiovisiography with Rinn alignment system and customized film holder using self-cured acrylic template was used for taking the radiographs. Dürr Dental DBSWIN 5.0 Imaging Software was used for the radiographic assessments. The crown to implant ratio was determined. Post-operative complications were also recorded.

Biting Force Assessment was performed using the T-scan III system® (Tekscan Inc., South Boston, MA, USA) (Figure 2). The T-scan is a computerized occlusal analysis system that affords the operator a precise way to assess the occlusal forces, direct them axially and uniformly, and distribute them uniformly on the final implant prosthesis and the rest of the teeth. The T-Scan III system consists of a high definition recording sensor. Biting force assessment was performed for all teeth at 2, 6 and 12 months following crown placement.

Based on the clinical and radiographic examinations, the success of implants in our study was assessed according to the Health Scale for Dental Implants as depicted in Table 1 (Misch *et al* 2008).

Results and discussion

Data were not normally distributed for most of the variables when checked by One Sample Kolmogorov–Smirnov test. Medians, 25% and 75% quartiles were reported for the variables that violated the assumption of

Implant Quality Scale Group	Clinical Conditions	Prognosis
I. Success (optimum health)	a) No pain or tenderness upon function b) No mobility c) <2 mm radiographic bone loss from initial surgery d) No exudate history	Very good to excellent.
II. Satisfactory survival	a) No pain on function b) No mobility c) 2-4 mm radiographic bone loss d) No exudate history	Good to very good, depending on the stable condition of the crestal bone.
III. Compromised survival	a) May have sensitivity on function b) No mobility c) Radiographic bone loss >4 mm (less than half of implant body) d) Probing depth >7 mm e) May have exudate history	Good to guarded, depending on the ability to reduce and control stress
IV. Failure (clinical or absolute failure)	Any of following: a) Pain on function b) Mobility c) Radiographic bone loss greater than half length of implant d) Uncontrolled exudates e) No longer in mouth	Failure in all statistical data

Table 1. Health scale for dental implants by ICOI for Implant success (Misch *et al* 2008).

normality and nonparametric tests used in the comparisons between groups at different assessment times. For normally distributed variables, i.e. radiographic data, means±SD were reported and parametric tests were used for the comparisons. For categorical data, proportions and 95% confidence intervals were calculated.

Demographic and implant data

Results for twenty healthy patients (6 males and 14 females) are presented with complete demographic and implant data in Table 2.

The clinical and radiographic implant outcomes of the two study groups appear to be comparable and also consistent with those found in previous clinical studies on short implants (Annibali *et al* 2012, Grant *et al* 2009, Griffin and Cheung 2004, Venuleo *et al* 2008) (Figures 3-9).

Healthy tissue and good oral hygiene were denoted by stable peri-implant clinical parameters, low bleeding tendencies and low probing depths (2 mm). These results concurred with those reported in implant-supported single tooth replacements, which might be attributed to patient compliance, ease of oral hygiene around a crown and oral hygiene reinforcement during follow-up (Bornstein *et al* 2005, Gibbard and Zarb 2002). The Periotest values indicated satisfactory osseointegration and the overall decrease in Periotest values over time indicated positive development of implant stability. The significant difference in Periotest values between the two implant groups could

Variable	Group	Bicon patient/ implant n (%)	Ankylos patient/ implant n (%)	Total	p* value
Gender	Male Female	1(10) 9(90)	5(50) 5(50)	6 14	0.014*
Race	Malay Chinese	6(60) 4(40)	3(30) 7(70)	9 11	0.111
Age group (years)	25-44 45-70	5(50) 5(50)	6(60) 4(40)	11 9	0.751
Bone quality (Hounsfield units)	D1 D2 D3 D4	0(0) 8(40) 12(60) 0(0)	2(10) 3(15) 11(55) 4(20)	2 11 23 4	0.040* Min Exp count = 1
Implant location	Premolar Molar	2(10) 18(90)	0(0) 20(100)	2 38	0.065
Abutment diameter (mm)	3.5-4.9 5-6.5	6(30) 14(70)	7(35) 13(65)	13 27	0.999
Abutment angulation (˚)	0 7.5 10 15	17(85) 0(0) 3(15) 0(0)	15(75) 1(5) 0(0) 4(20)	32 1 3 4	0.043* Min Exp count = 0.50 less than 5
Implant side (numbers)	Right Left	6(31.6) 14(68.4)	10(50) 10(50)	16 24	0.333
Crown/implant ratio (groups)	≤ 11.1-2	4(20) 16(80)	1(5) 19(95)	5 35	0.15
Crown/implant ratio** (Mean value)	Mean ± SD	1.35 ± 0.21	1.36 ± 0.34	40	0.85**

Table 2. Demographic data for Ankylos and Bicon patients (implants) and comparison between them. Values are expressed as patient or implants number and percentage.
*indicates significant difference between implant groups ($p \leq 0.05$) based on chi-square test
** indicates significant difference between implant groups ($p \leq 0.05$) based on independent samples t- test.

be attributed to differences in implant design and surface.

The favorable one year bone changes concurred with reported marginal bone outcomes of other short implant studies (Döring *et al* 2004, Urdaneta *et al* 2011, Venuleo *et al* 2008) (Table 3). It could be explained by several factors, such as surface characteristics of implants, lack of the external micro-gap, occlusal analysis and adjustment, and the shape of the abutment neck.

All implants fell into the category of success (optimum health) on the implant health scale, providing evidence for the efficacy of short implants (Table 4). The high success rate, bone increase and minimal post-loading complications of the implants support the non-splinting concept. The crown to implant ratio (C/I) was comparable for the two implant systems and was within the acceptable ratio (up to 4.95) that was unrelated to crestal bone loss or failure rate (Anitua *et al* 2015,

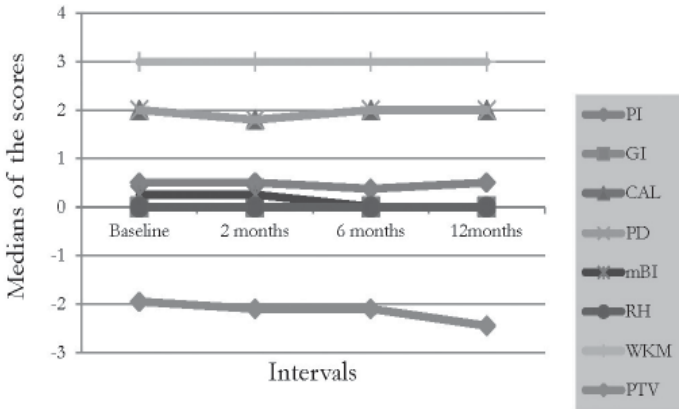

Figure 3. (A) Peri-implant clinical parameter changes over measurement times for Ankylos implants. Significant change over time intervals for GI, WKM & PTV means ($p<0.05$) by Friedman test.

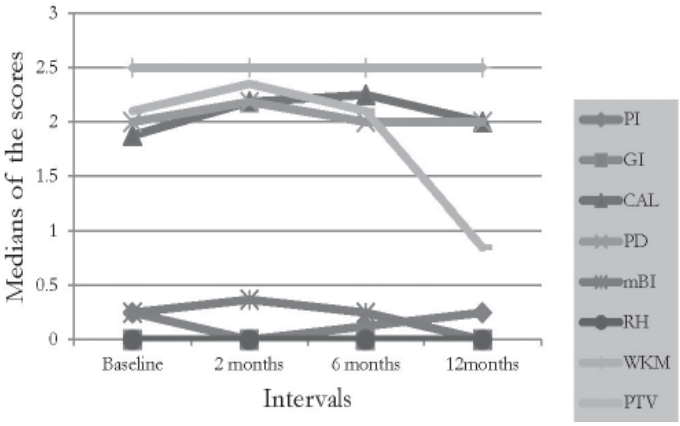

Figure 3. (B) Peri-implant clinical parameter changes over measurement times for Bicon implants. Significant change only in Periotest value means ($p<0.05$) by Friedman test. PTVs decreased over time.

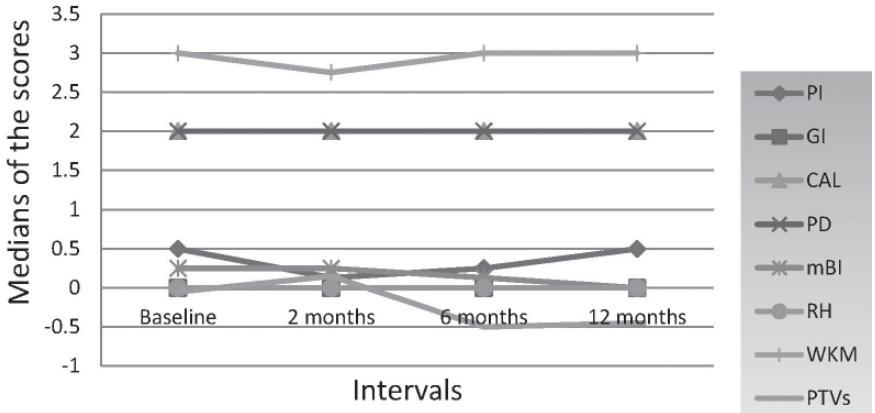

Figure 4. Peri-implant clinical parameters at all time intervals for all implants.
Significant decrease in GI at 6-mths interval only * Change in PTVs was significant at 12 mths in comparison to all previous intervals *$p \leq 0.01$ (Friedman test)

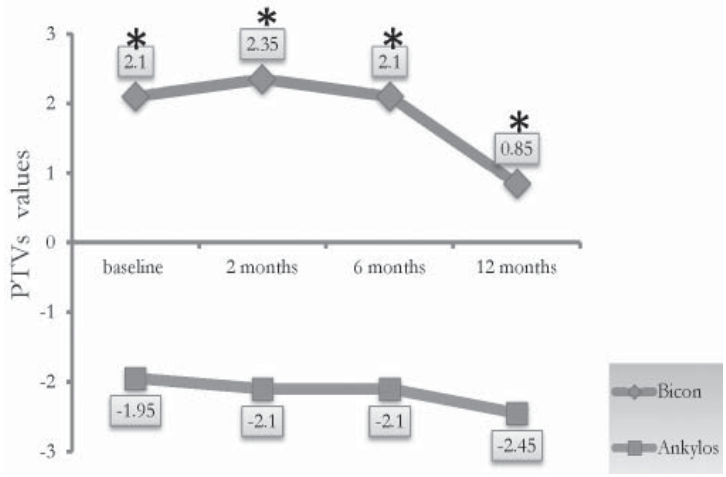

Figure 5. Change in Periotest values over measurement times for both implant systems.
* indicates significant difference between implant groups ($p \leq 0.05$).

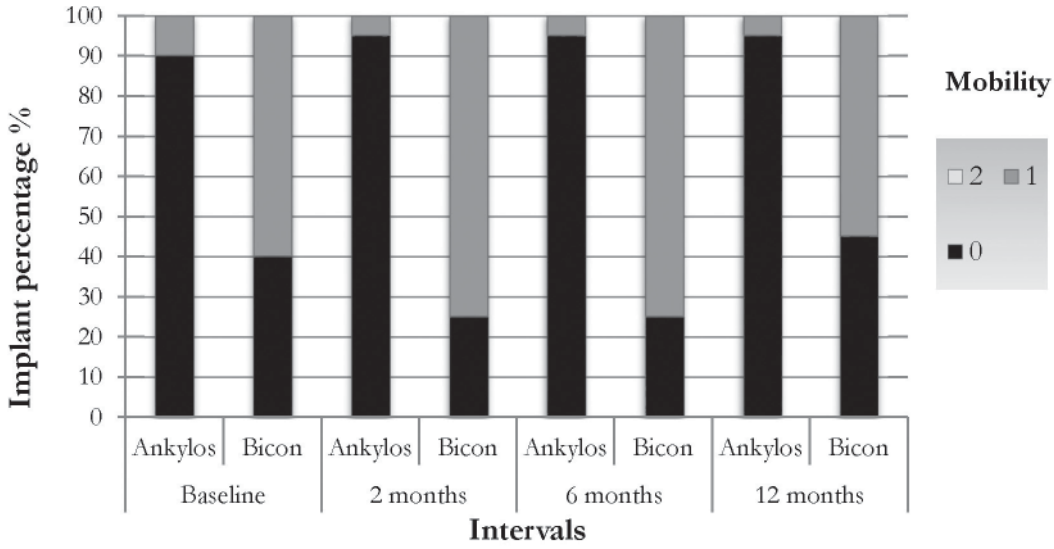

Figure 6. Mobility scores for Ankylos and Bicon implants over various time intervals.
Score 0, PTVs −08 to 0: good osseointegration
Score 1, PTVs +1 to +9: a clinical examination is required
Score 2, PTVs +10 to +50: osseointegration is insufficient
No significant difference within groups over time was detected, based on Cochran test.
Significant difference between two implant groups, based on Chi-square test ($p \leq 0.05$).

Radiographic Parameter	Implant system	Baseline Mean ± SD	6 months Mean ± SD	12 months Mean ± SD	p value
Bone Level	Ankylos	2.038 ± 0.62	2.117 ± 0.47	2.00 ± 0.42	0.27*
	Bicon	2.727 ± 1.14	2.655 ± 1.14	2.607 ± 1.18	0.28*
	P value**	0.02**	0.06	0.04**	
	All implants	2.383 ± 0.97	2.386 ± 0.90	2.305 ± 0.93	0.22
Bone Change	Ankylos		-0.08 ± 0.38	0.035 ± 0.33	0.03#
	Bicon		0.073 ± 0.37	0.120 ± 0.40	0.30#
	P value**		0.209	0.467	
	All implants		-0.003 ± 0.38	0.078 ± 0.36	0.02#

Table 3. Differences in bone level means and mean changes (mm) ± SD between Ankylos and Bicon implants and the differences for all implants (both groups combined) at the various time intervals.
At the end of 1 year, bone gain for ALL implants was 0.08 mm
* p≤0.05 based on repeated measures ANOVA; ** p≤0.05 based on independent samples t- test; # p≤0.05 n paired sample t-test.

Time interval (mths)	Implant system	#at start	Successful	Satisfactory	Compro-mised	Failure	Implant under risk	Success survival rate (%)
0-6	Ankylos	20	20	0	0	0	0	100
	Bicon	20	20	0	0	0	0	100
6-12	Ankylos	20	20	0	0	0	0	100
	Bicon	20	20	0	0	0	0	100

Table 4. Life table analysis; Implant success/survival rates based on ICOI consensus criteria.
Values are expressed as implant number in each category.
All implants (100%) were in the successful category at the 1 year interval without any dropout cases.

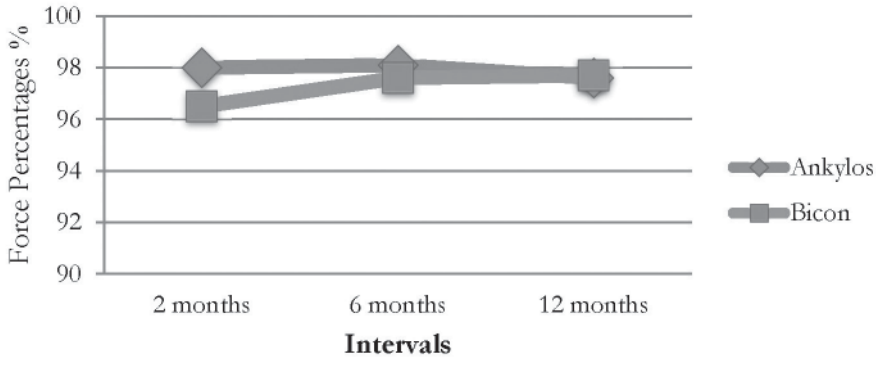

Figure 7. Maximum force (%) recorded at various intervals for both implant groups.
Maximum force = relative force of pressure at maximum intercuspal position.
No statistically significant differences were recorded at any intervals within (based on Friedman test) and between (Mann-Whitney test) both implant groups.

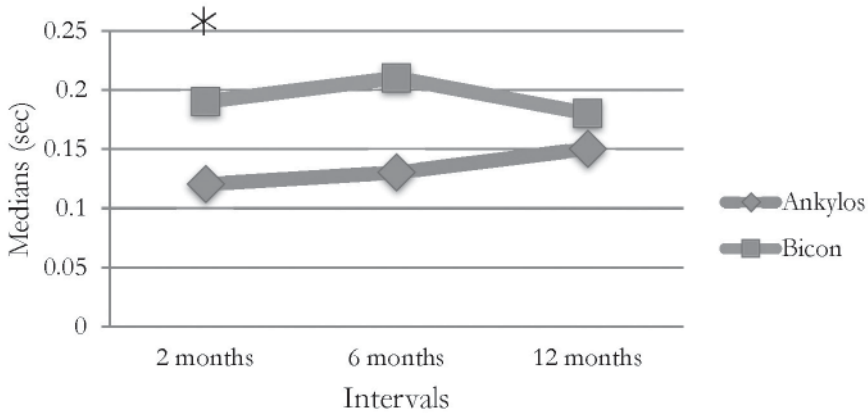

Figure 8. Occlusion time (seconds) recorded at various intervals for both implant groups.
Occlusion time = time from first contact to maximum force in maximal intercuspal position.
No significant differences in the occlusion time except at 2 months where significantly shorter occlusion time was recorded in Ankylos group (p=0.03) based on Mann-Whitney test (comparison bet groups).

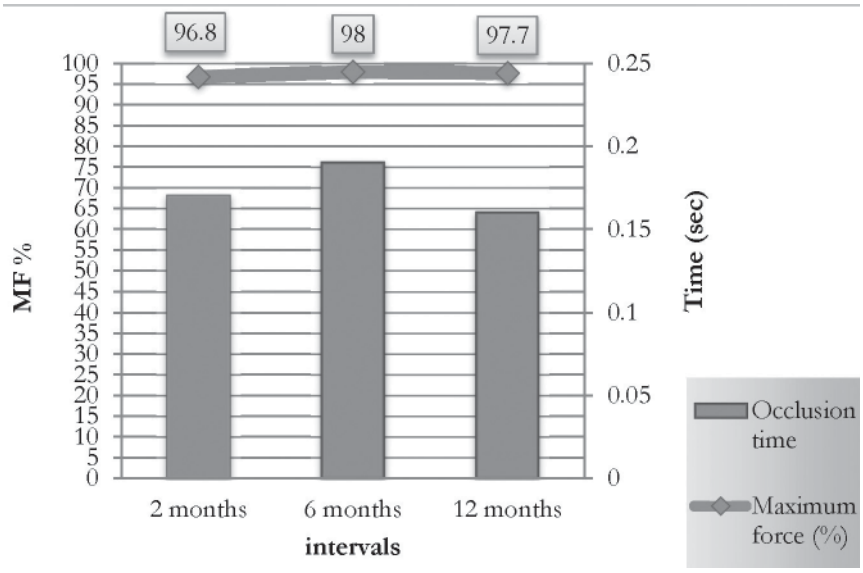

Figure 9. Maximum force (%) and occlusion time (sec) for both Ankylos and Bicon implant groups combined (All) at various intervals.
No significant difference in the occlusal parameters at any intervals indicating parameters remained stable during the one year post-loading period (Friedman test).

Birdi *et al* 2010, Blanes *et al* 2007, Rokni *et al* 2005, Schulte *et al* 2007, Tawil *et al* 2006).

Conclusions

- Clinical and radiographic peri-implant tissue responses were favorable for both Ankylos and Bicon systems with an average peri-implant marginal bone increase of 0.1 mm after one year.
- Occlusal force distribution was stable and in equilibrium after initial adjustments and during the entire study period.
- The use of 8 mm short implants in the partially edentulous posterior mandible appears to be highly predictable over a one year period with a 100% success rate.
- Crown to implant ratio of 1.4±0.28 for ALL cases was within an acceptable range (Anitua *et al* 2013, Birdi *et al* 2010, Blanes *et al* 2007, Rokni *et al* 2005, Schulte *et al* 2007, Tawil *et al* 2006).

Acknowledgement

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Conflict of interest

No conflict of interest in the use of the implant systems is declared.

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Current Issues in Periodontics
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Chapter 22

Periodontal Competency for the Long-Term Success of Dental Implants

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Introduction

Dental implant therapy is widely accepted as the first choice for rehabilitation of edentulous sites. Since the landmark Toronto conference in 1982, we have witnessed great advancements in surgical and prosthetic techniques in implantology. However, it has been stated that biological and biomechanical factors might adversely affect the long-term success of placed implants.

Among the biological factors, the optimal volume of bone housing around the fixture and presence of attached and/or keratinized tissues around the implant are believed to be very important. Augmented bone can be obtained via guided bone regeneration (Brunel *et al* 2001, Fiorellini and Nevins 2003, Hämmerle and Karring 1998, Mardas *et al* 2010, Simon *et al* 2000). To attain an adequate width of keratinized tissue, many periodontal plastic surgical procedures such as the apically positioned flap, free gingival graft and subepithelial connective tissue graft have been carried out (Barone *et al* 2008, Bartee 2001, Kim *et al* 2013, Schrott *et al* 2009, Wennström *et al* 1994). Careful consideration should be given to managing the flap when implant therapy is planned in periodontally compromised extraction sites with limited soft and hard tissues.

Two clinical cases are presented to illustrate surgical techniques for dental implants, originally introduced in periodontal therapy. Guided bone regeneration, widening the width of attached gingival with graft and vertical and/or horizontal augmentation will be discussed. These clinical cases were completed at the Seoul National University Dental Hospital over the last five to ten years by one periodontist.

Case 1

A 57 year old systemically healthy female presented to our clinic with a wire retained temporary crown on a lower incisor (Figure 1A). The remaining alveolar bone was compromised both vertically and horizontally (Figure 1B-C). After fixture installation, all threads were exposed on the buccal aspect and initial stability of the implant was limited (Figure 1D).

Deproteinized bovine bone mineral (Bio-Oss®) was grafted around the exposed implant surface and a nonabsorbable TefGen membrane was used to cover the graft (Figure 1E). After seven months it was noted that part of the healing cap was exposed. At this time a second surgery was performed and newly formed tissue was found covering the previously exposed implant threads. A healing abutment was connected to the implant fixture (Figure 1F-H). Clinical photographs taken

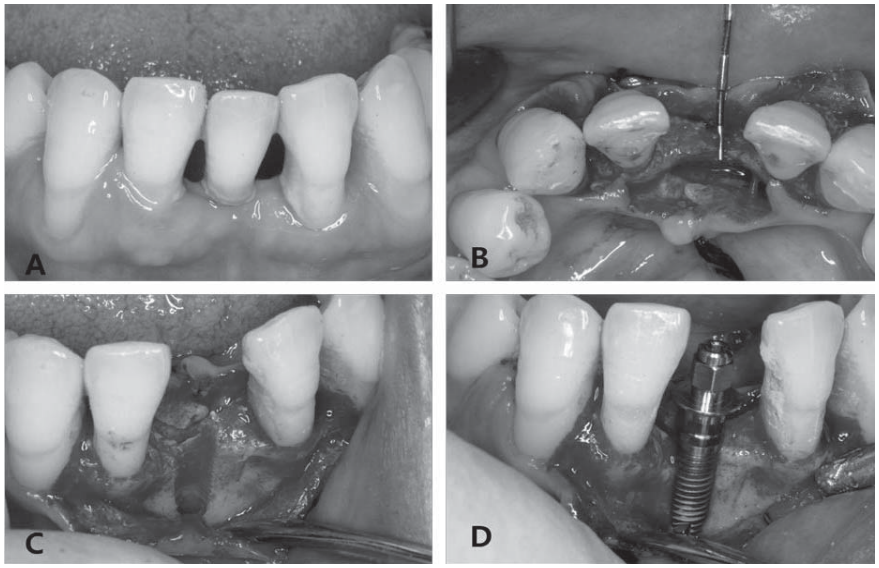

Figure 1A-D. Narrow diameter implant placed in dehiscence type defect on lower anterior. (A) Temporary resin crown was fabricated over the tooth extracted site. (B) Available bone width was extremely limited. (C) Dehiscence type defect was created after drilling. (D) Whole length of buccal thread exposed.

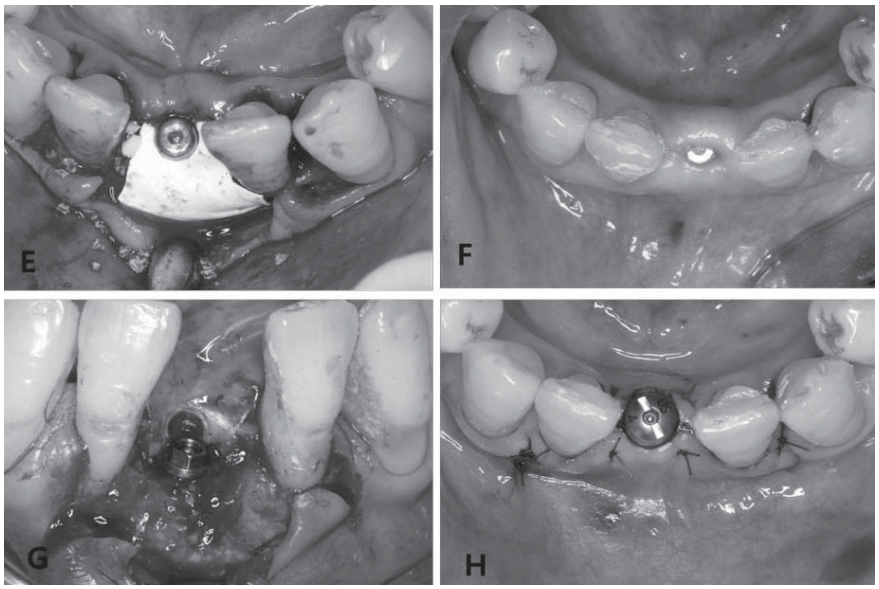

Figure 1E-H. Narrow diameter implant placed in dehiscence type defect on lower anterior. (E) TefGen membrane applied over the grafted bone mineral particles. (F) Cover screw exposed at second surgery. (G) Newly formed bone-like tissue can be seen over the exposed buccal thread. (H) Healing abutment was connected and sutures placed.

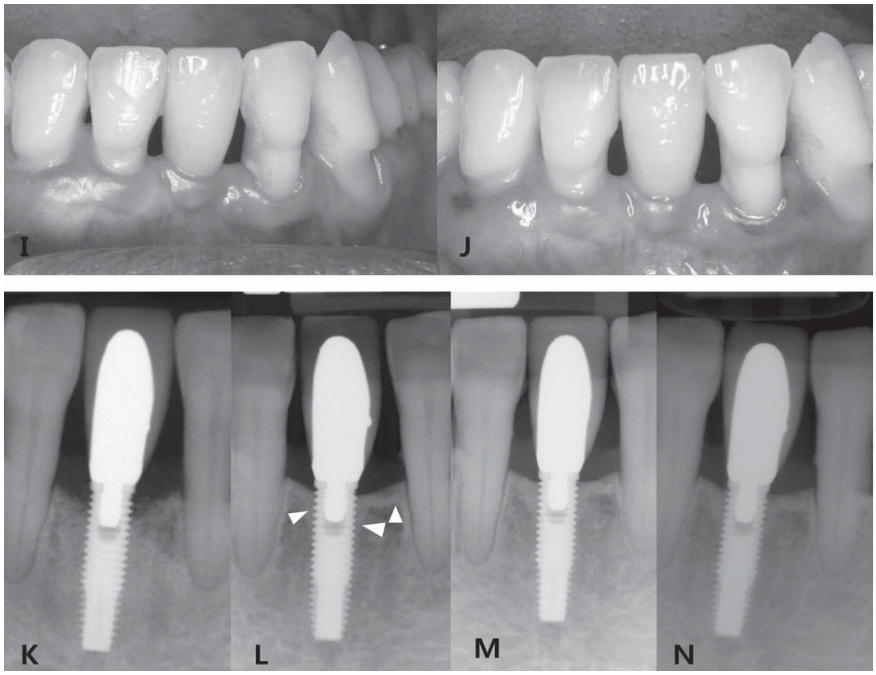

Figure 1I-N. Narrow diameter implant placed in dehiscence type defect on lower anterior. (I-J) Clinical photos of dental implant after nine and eleven years, respectively. (K) Periapical radiographic view of implant one year after the crown restoration. (L-N) Newly formed lamina dura was well maintained over six, eight and ten years, respectively, after placement.

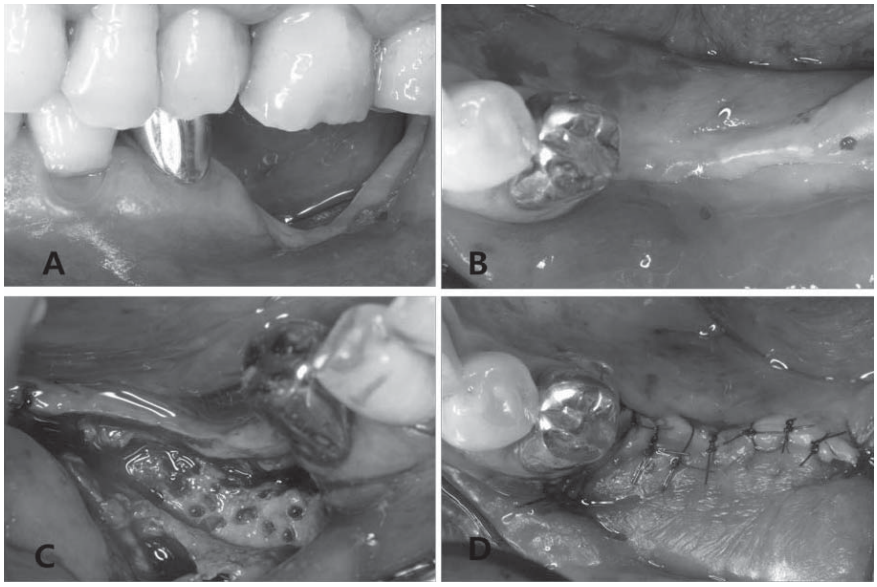

Figure 2A-D. Implant placed in periodontally compromised extraction socket and keratinized masticatory palatal mucosa was grafted around the implant. (A) Clinical photo of buccal aspect before operation. (B) Clinical photo of occlusal aspect before operation. (C) Decortication was done at site preparation. (D) Primary closure was performed.

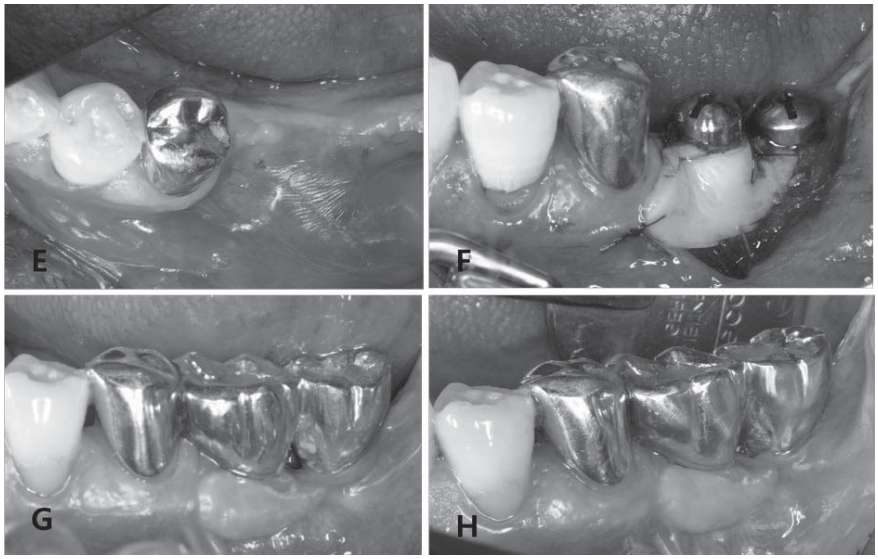

Figure 2E-H. Implant placed in periodontally compromised extraction socket and keratinized masticatory palatal mucosa was grafted around the implant. (E) At second surgery, limited keratinized gingival can be seen in buccal side. (F) Donor tissue was tightly sutured over the recipient site. (G-H) Grafted tissue was well maintained for two and five years, respectively, after the surgery.

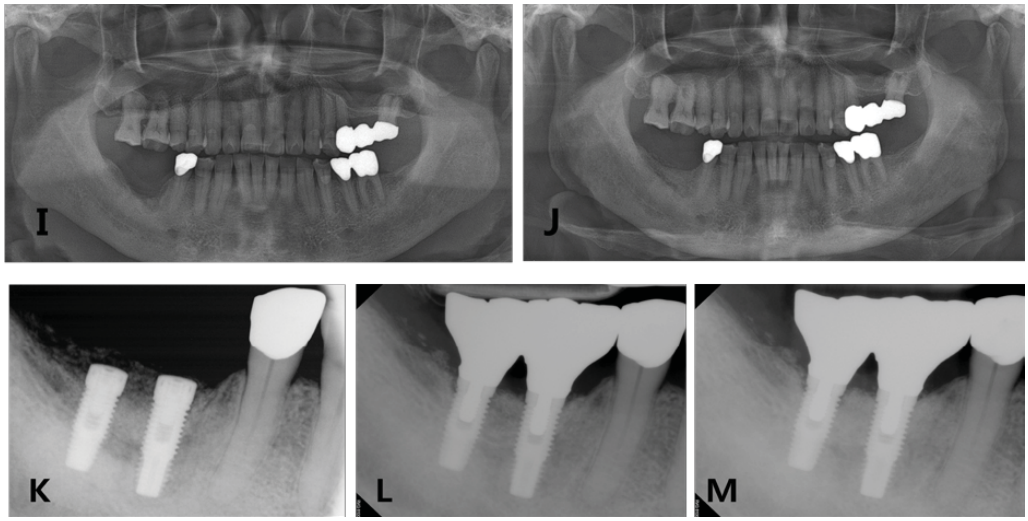

Figure 2I-M. Implant placed in periodontally compromised extraction socket and keratinized masticatory palatal mucosa was grafted around the implant. (I-J) Panoramic radiographs were taken before and after the site preparation. (K) Periapical radiographic view of dental implants after first surgery. (L-M) Alveolar bone level was well maintained and grafted bone was homogenized with the pristine bone two and five years after placement, respectively.

nine and eleven years after implant installation (Figure 1I-J) demonstrated that the dental implant installed in the lower anterior site was maintained in health condition without any biological and mechanical problems.

Periapical radiographs showed the coronal part of alveolar bone around the fixture had an irregular border (Figure 1K) one year after insertion of the prosthesis. Prominent lamina dura was seen after six years (Figure 1L) and the level of alveolar bone was not changed after eight and ten years (Figure 1M-N).

Case 2

A 69 year old male patient was referred from a local clinic for implant insertion in quadrant four. He had no particular past medical history. Both right first and second mandibular molars had been extracted six months previously and the extraction sockets were completely closed. Severe soft tissue atrophy with limited vestibular depth was noted (Figure 2A-B). After decortication, a ridge augmentation procedure was carried out using deproteinized bovine bone mineral (OCS-B®, Nibec, Korea) and a collagen membrane (BioGide®) (Figure 2C-D). Tobramycin was administered intramuscularly and Augmentin antibiotics were prescribed for seven days.

After seven months, two external type fixtures were installed and bone augmentation was carried out to cover the exposed second to third threads of the fixture. A second surgery was carried out three months later. After connecting the healing abutments, masticatory palatal keratinized mucosa was grafted over the prepared recipient site to widen the keratinized gingiva (Figure 2E-H). Sufficient keratinized gingiva was preserved after two and five years (Figure 2G-H).

Radiographic examinations revealed a severe alveolar bone defect at the first visit (Figure 2I). As detailed above, site preparation was carried out and resulted in a radiolucent demarcation line being visible on the radiographs between the pristine bone and grafted bone (Figure 2J). A post-operative radiograph was taken after the first surgery and grafted bone particle could be seen over the coronal thread (Figure 2K). Periapical radiographs were taken two and five years after the first surgery (Figure 2L-M). The grafted particles had become visibly homogenous with the pristine bone and the alveolar bone level around the platform of the fixtures was relatively well maintained over time.

Discussion

The quantity and quality of alveolar bone is not always sufficient for placement of dental implants, especially when a tooth has been extracted due to advanced periodontal disease. Soft tissues also may not always be adequate to maintain implants in a healthy state. In order to place implant fixtures in compromised sites, it is always necessary to carry out a careful examination and diagnosis for such challenging cases. Advanced periodontal surgical procedures such as guided bone regeneration, apically repositioned flaps and free gingival grafts are often needed to overcome these unfavorable situations.

In lower anterior edentulous sites, available alveolar bone is often very limited and it may not be possible to install an implant even with a narrow diameter. In such cases, some threads of the implant are inevitably exposed outside the bone housing. In these cases, guided bone regeneration procedures should be considered provided the fixture has initial stability. Even if the cover screw is not fully covered with gingiva, the newly formed bone like tissue can be seen after several months and routine prosthetic procedures can be undertaken. It is interesting that the grafted bone undergoes bone remodeling and can develop into cortical

bone which can be identified radiographically over time.

In extraction sockets where the tooth was extracted due to advanced periodontitis, the remaining alveolar bone for dental implant placement is often extremely deficient. Anatomical structures (e.g. inferior alveolar nerve) may also compromise the treatment plan and another surgical procedure such as site preparation may be required. During the procedure, the flap should be tightly closed for primary closure which inevitably reduces the width of attached gingiva. Keratinized palatal masticatory mucosa can be grafted over the recipient site and the graft can be maintained over several years.

Taken together, it can be concluded that periodontal competency is needed for the long-term success of dental implants, especially when the implant therapy is planned in the extraction socket where tooth was removed due to advanced periodontitis.

Conclusion

Various surgical techniques used in conventional periodontal therapy should also be applied in dental implant treatments for long-term success. Therefore, periodontal competency might be regarded as an essential factor for the success of dental implants.

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Poster Presentations

The following is a record of the posters awarded prizes at the 11th Meeting of the Asian Pacific Society of Periodontology

Research Category - 1st Prize

In vitro study of Er,Cr:YSGG laser on attachment of PDL fibroblasts to extracted root surface

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Introduction: The attachment of periodontal progenitor cells to the root surface is an essential process for periodontium regeneration. The use Er:Cr:YSGG laser for root surface modification may be possible.

Objectives: This study aimed to investigate the *in vitro* effects of two frequency settings of Er:Cr:YSGG laser in conjunction with ultrasonic root debridement on the attachment of cultured human periodontal ligament fibroblasts to periodontal diseased root surfaces.

Methods: Periodontal hopeless extracted teeth and healthy premolars extracted for orthodontic reasons were collected. Root specimens were prepared and divided into five groups: untreated healthy (Normal), untreated periodontal (Perio), periodontal surfaces treated by ultrasonic debridement only (Ultra), by ultrasonic debridement followed by 30Hz Er,Cr:YSGG (Laser30), and followed by 50Hz irradiation (Laser50) group. 25 specimens in each group were incubated with human periodontal ligament fibroblasts then attached cells were compared by cell viability assay after 5 days. An additional 6 specimens in each group were incubated for SEM cell morphological investigation after being cultured for 24 hours and 3 days.

Results: Among treated groups, Laser50 group presented highest attached cells which was significantly higher than Ultra ($p < 0.05$) and Perio group ($p < 0.01$). The number and cell characteristic of attached cells in Laser50 group was most comparable to Normal group.

Conclusion: Adjunctive use of Er,Cr:YSGG laser, in particular, the 50Hz setting seems to improve root surface biocompatibility, thus accommodating cellular attachment.

Research Category - 2nd Prize

Roles of Periodontopathic Bacteria in Canine Mitral Regurgitation

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Introduction: Periodontitis in companion animals is an almost identical disease to that in humans in terms of disease course and clinical presentation. However, the relationship between periodontal pathogens and cardiovascular diseases in dogs is unknown.

Objective: To investigate the presence of DNA from periodontopathic bacteria in heart specimens in dogs with mitral regurgitation.

Methods: A total 81 dogs with mitral regurgitation were referred to the Nihon University Animal Medical Center and 193 specimens from heart samples were harvested during cardiac surgery for mitral valve replacement. The distribution of an animal periodontopathic organism *Porphyromonas gulae* (*P. gulae*), in addition to seven human periodontopathic bacteria, were examined by polymerase chain reaction (PCR) with species-specific sets of primers.Trypsin-like enzyme activity, pocket depth and bleeding on probing on the designated tooth were measured as clinical parameters.

Results: Polymerase chain reaction analysis of heart samples identified periodontopathic bacteria in 46 out of 81 study animals. Among eight periodontopathic bacteria studied, four species (*P. gulae*, *Tannerella forsythia, Fusobacterium nucleatum*, *Campylobacter rectus*) were detected in dog heart specimens. *P. gulae* was the most frequently detected species. Those four bacterial species were highly detected in oral specimens from the same subjects. More than 70% of the subjects possessed trypsin-like activity in their oral cavity. Mean pocket depth in subjects with heart specimens with periodontopathic bacteria showed significant high scores compared to the negative subjects.

Conclusion: These results suggest that periodontopathic bacteria are related to bacteremia and may contribute to the development of cardiovascular diseases in canines.

Research Category - 3rd Prize

The Roles of *Porphyromonas Gingivalis* Proteolytic Activity in Production of Volatile Sulfur Compounds

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Introduction: *Porphyromonas gingivalis*, an etiological bacterium of periodontal disease, has been reported to produce significantly high volatile sulfur compounds (VSCs), such as H_2S and CH_3SH. Arg-gingipains (RgpA and RgpB) and Lys-gingipain (Kgp) are cysteine proteinases that are known to be responsible for the virulence of this microorganism.

Objective: To examine the roles of gingipains in the production of VSCs in oral cavity.

Methods: Paraffin-stimulated saliva samples were collected from adult volunteers. The VSCs levels produced by whole saliva were measured using portable gas chromatography (OralChroma™). Detection of salivary bacteria was performed by PCR assay. The role of *P. gingivalis* gingipains in production of VSCs were examined by adding an anti-gingipain egg yolk antibody (IgY-GP) to whole saliva and *P. gingivalis* culture. The level of VSCs production by *P. gingivalis* from human serum culture was compared using gingipain deficient mutants (KDP129: *kgp*$^-$, KDP133: *rgpA*$^-$ and *rgpB*$^-$, and KDP136: *rgpA*$^-$, *rgpB*$^-$ and *kgp*$^-$).

Results: H_2S and CH_3SH levels were significantly increased in all subjects after 3 hours incubation. *P. gingivalis* was detected in salivary specimens. Adding the IgY-GP to whole saliva and *P. gingivalis* culture inhibited H_2S and CH_3SH production. Both KDP129 and KDP136, the Kgp-deficient mutants, significantly reduced CH_3SH levels in human serum compared to wild-type strain.

Conclusion: These results suggest that *P. gingivalis* significantly produced VSCs. Trypsin-like enzyme of *P. gingivalis* plays an important role in VSCs production and Kgp may contribute to the degradation of substance protein and the production of VSCs in oral cavity.

Case Report/Literature Study Category - 1st Prize

Hemisection of Molar with Questionable Prognosis: A Case Report

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Introduction: Severe vertical bone loss with mobility and periodontal abscess on molars is often treated with extraction of the tooth. Hemisection is an alternative which maintains both tooth and periodontal tissue. Hemisection refers to the sectioning of a mandibular molar into two halves followed by removal of the diseased root and its coronal portion, most commonly performed on mandibular molars with class II or III furcation involvement.

Objectives: To emphasize the importance of endodontic and periodontics treatment when dealing with endo-perio lesions and to demonstrate the considerable healing potential of the periodontal aspect.

Case: A 55-year-old male patient reported to the Department of Periodontology, Padjadjaran University, Bandung, Indonesia, with a chief complaint of tooth mobility in the lower right posterior sextant. On intraoral examination, deep pocket depth and abscess in a vital mandibular first molar was found. Grade II mobility and grade III furcation involvement was reported. Radiographic examination showed severe vertical bone loss on distal root region of the affected tooth.

Case Management: Initial periodontal treatment on all regions and endodontic treatment of the mandibular first molar was performed. Removal of the distal root was followed by bone graft and PRF membrane placement to the affected area. The final restoration was performed using crown and bridge.

Conclusion: Endodontic treatment followed by hemisection was beneficial in preserving molars with periodontally questionable prognosis.

Case Report/Literature Study Category - 2nd Prize

The Role of Platelet Rich Fibrin (PRF) in Periodontal Regeneration of Intrabony Defect

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Introduction: Regeneration of lost structures has become the primary therapeutic goal in periodontics. The objective of periodontal regenerative therapy is replacement of the bone, cementum, and periodontal ligament on previously diseased root surfaces. Growth factors such as platelet-derived growth factors exert potent effects on wound healing, including regeneration of periodontium. Platelet rich fibrin (PRF) provides a concentrate of such growth factors, accelerating the wound healing process.

Objectives: This literature review serves as an introduction to the PRF concept and its potential clinical application in periodontal intrabony defect regeneration.

Literature review: Regeneration of deep intrabony defects remains a complicated procedure, due to slow and difficult integration of the grafted material into the physiological architecture. The regenerative and bone healing of platelets has garnered much attention over the last few years. PRF is a healing biomaterial that consists of platelets, leucocytes, and growth factors. Platelets release growth factors which have been shown to stimulate bone growth and repair. The PRF also aims to improve the handling of particulate grafts, facilitate graft placement and stability, improve the rate and quality of the vascular ingrowths, increase bone regeneration, enchance soft tissue healing and exert mitogenic effects on critical cells.

Conclusion: PRF is a very promising option in biomaterials for periodontal regeneration, it is also a simple and inexpensive technique. But larger, longer termed longitudinal studies with strong sampling techniques are needed to definitively answer questions about the benefits of PRF.

Case Report/Literature Study Category - 3rd Prize

Asthmatic Control in Periodontitis Patients Through Neurogenic Switching Mechanism

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Introduction: The theory that oral infection could induce systemic disease has been known since the 20th century. Today the dental profession has acknowledged the existance of systemic diseases influenced by dental infections, such as cardiovascular disease, diabetes, and pregnancy complications, but there is still not sufficient literature discussing the relationship between asthma and periodontal disease.

Objectives: This literature review aims to illustrate the process of asthma which is triggered by periodontal disease through neurogenic switching mechanism.

Literature review: Neurogenic switching is an interplay interaction of neurogenic and immunogenic inflammation, where an inflammation in one site of the body can lead to inflammation at a distant location via mast cell-nerve interaction. This theory suggested that asthma etiology is not just due to atopy and immunogenic mechanism, but also because of nerve fiber stimulation. Lipopolysaccharides, the endotoxin from *Porphyromonas gingivalis*, a bacteria causing periodontal disease, is able to induce the neurogenic switching mechanism which eventually triggers asthma symptoms. Reduction of dental plaque has been proven to improve clinical symptoms, lung function, FEV1 reversibility and immunological markers in asthmatic patients.

Conclusion: Periodontal disease could elicit the presence of asthma symptoms through neurogenic switching mechanism, and dental plaque control should be part of asthma patient management.