
CONTEMPORARY CONCEPTS IN PERIODONTOLOGY & IMPLANT DENTISTRY

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**Edited by
P Mark Bartold
Y Ku**

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Acknowledgements

The 12th International meeting of the Asian Pacific Society of Periodontology (APSP) was successfully held in Seoul, Korea from 22-24 September 2017. Over 530 delegates from 19 countries (Australia, China, Chinese Taipei, Hong Kong SAR China, India, Indonesia, Japan, Korea, Malaysia, Mongolia, Nepal, New Zealand, Philippines, Singapore, Thailand, Tunisia, UAE, USA and Vietnam) attended this APSP meeting with the theme “Contemporary Concepts in Periodontology and Implant Dentistry”. The opening address was given by Prof Yulianti Kemal, President of APSP; Prof Young Ku, Chairperson of the 12th APSP Meeting; and Prof Seong-Ho Choi, President of the Korean Academy of Periodontology. The welcome address was given by Mr Eiichi Shirakawa, Sunstar Group; and Mr Toshio Kakui, Lion Corporation.

The two-day program was very full, with 19 oral presentations delivered by speakers from 15 different countries. In addition, 180 posters were scheduled for presentation. Over the two days, invited keynote speakers and representatives from many countries in the Asian Pacific region presented lectures on a wide range of topics and these are presented in this volume as a record of this meeting.

The poster sessions were very successful. In keeping with the tradition from previous meetings, 8 prizes were awarded for the posters judged to be the best on the day. The abstracts of these posters are included in this volume.

This volume contains an impressive array of contributions from all around the Asian Pacific region and serves as a record of the invited presentations. Each of the chapters covers a unique aspect of current issues in periodontology as we understood them in 2017. As with past APSP Proceedings I am sure this volume will serve as a very important reference source in the years to come.

The APSP wishes to acknowledge the following sponsors for this meeting:

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I want to thank my co-editor Professor Young Ku for his invaluable help in proofreading the 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
April 2018



Attendees at the 12th APSP Meeting

Lower (L to R): Koji Mizutani, Mark Bartold, Shinya Murakami, Young Ku, Yulianti Kemal, Isao Ishikawa, Tara Taiyeb Ali, Huanxin Meng, Asae Hirai

Upper (L to R): Masahide Takedachi, Benjamin Tan, Wang Chenying, Li-Jian Jin, Nguyen Hong Minh, Pintippa Bunyaratavej, Yuniarti Soeroso, Varunee Kerdvongbundit, Bayarchimeg Batbayer, Stanley Lai, Shaili Pradhan, Oyunkhishig Khishigdorj, Seong-Ho Choi, Nannette Vergel DeDios, Warwick Duncan, AR Pradeep, Narongsak Laosrisin, Vivian Virata, Chang Kok Meng, Badiah Baharin

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Chapter 1

A New Era of Regenerative Therapy: REGROTH® (A New FGF-2 Medicine) Changes Regenerative Therapy in Dentistry

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Development of REGROTH®

Periodontal disease is caused by dental plaque (bacterial biofilm) that results in destruction of periodontal tissue through chronic inflammation. Although its cause is clear, periodontal disease still has a high prevalence in many countries and is the primary cause of tooth loss among adults worldwide. Appropriate initial treatment, such as scaling and root planing, reduces periodontal tissue inflammation and halts the progression of periodontal disease. However, simply removing the causative agent of the disease is not sufficient to reliably achieve regeneration of the lost periodontal tissue. To maintain “the quality of life supported by the mouth and the teeth” throughout the life of the patient, the development of a highly predictable technique to regenerate periodontal tissue is urgently needed in the field of periodontics.

Stem cells that can reconstruct targeted tissues and organs are indispensable for regenerative medicine. Specifically, the availability of periodontal tissue stem cells is essential to achieve regeneration of periodontal tissue in the clinic. The results of numerous basic studies show that progenitor cells and undifferentiated mesenchymal stem cells, both of which can be differentiated into

osteoblasts and cementoblasts, are present in the periodontal ligament surrounding the tooth root (Beertsen *et al* 1997, Seo *et al* 2004). A number of clinical studies have been conducted that complement the initial laboratory studies. These clinical studies have led to the development of a variety of periodontal tissue regenerative therapies/agents, including guided tissue regeneration (GTR) and enamel matrix derivative (EMD) methods, both of which have shown some degree of success in clinical use. However, clinicians expect higher safety and stability of outcomes for periodontal tissue regenerative therapy than can be achieved with GTR and EMD therapies. Therefore, the use of growth factors for periodontal treatment has drawn particular attention as a next-generation periodontal tissue regenerative therapy. Since the late 1980s, studies have been conducted worldwide to investigate the outcomes of tissue regeneration during periodontal surgery, using topical application of human recombinant growth factors to activate stem cells existing around damaged periodontal tissue.

REGROTH®, a treatment that utilizes fibroblast growth factor-2 (FGF-2), has been studied for many years and was finally approved for commercial production in September 2016 by the Ministry of Health,

Labor and Welfare of Japan. This product is the world's first and only pharmaceutical that includes growth factors developed for periodontal regeneration (Figure 1).

Efficacy and modes of action

Results of basic research

The active ingredient in REGROTH® is FGF-2. FGF-2 stimulates the proliferation of a wide variety of cell types, including vascular endothelial cells, neuroectodermal system cells, osteoblasts, chondrocytes, vascular smooth muscle cells, and epithelial cells. Additionally, FGF-2 has potential in regenerative medicine as it has potent pro-angiogenic activity and promotes proliferation of undifferentiated mesenchymal cells while maintaining their pluripotency.

In our laboratory, we examined whether FGF-2 promotes regeneration of periodontal tissue using dogs and non-human primates. We experimentally created class II furcation defects and two- or three-walled intrabony periodontal defects. We then filled the defects with a gelatinous carrier that contained 0.1 to 0.4% FGF-2 and conducted histomorphometric evaluation at six and eight weeks after FGF-2 administration (Murakami

et al 2003, Takayama *et al* 2001). We found that topical application of FGF-2 induced periodontal regeneration with statistically significant increases in the volumes of both newly formed alveolar bone and cementum. Sharpey's fibers were restored and the reconstruction of fibrous attachment was confirmed at all treated sites. Moreover, down-growth of the gingival epithelium was suppressed (Murakami *et al* 2003). No abnormal healing, such as ankylosis or root resorption, was detected at the treated sites. Further, promotion of angiogenesis at FGF-2-treated sites was noted. One week after FGF-2 administration, we observed periodontal ligament-derived mesenchymal cells had migrated to, and covered, the root surface facing the bone defects (Nagayasu-Tanaka *et al* 2015). This migration suggests that these cells play important roles in regenerating cementum and fibrous attachments.

Subsequently, we conducted detailed investigations of the effects of FGF-2 on human periodontal ligament (HPDL) cells in vitro (Murakami 2011). These investigations showed that FGF-2 promotes proliferation and migration of HPDL cells. Moreover, it was revealed that FGF-2 stimulates HPDL cells to produce a wide variety of extracellular matrix molecules and vascular endothelial



Figure 1. REGROTH® Dental Kit for periodontal regeneration.

growth factor (VEGF). It appears that, at the site of FGF-2 administration, FGF-2 and VEGF act coordinately to strongly promote angiogenesis. Thus, the actions of FGF-2 can be summarized as follows:

1. FGF-2 promotes the proliferation of periodontal/mesenchymal stem cells remaining around periodontal tissue defects while maintaining their multipotency. Further, FGF-2 increases the density of periodontal tissue stem cells at the wound site.
2. FGF-2 promotes angiogenesis and regulates the production of various extracellular matrix molecules, thereby creating a suitable environment for the regeneration of periodontal tissue and initiating tissue regeneration at the administration site (Figure 2).

Results of clinical trials

In 2001, a nationwide Phase IIA clinical trial (double-blind trial with concurrent control of dose responses, including placebo) was initiated in Japan at 13 dental facilities to investigate the safety and efficacy of FGF-2 in the induction of periodontal regeneration, using a placebo and investigational drugs that contained 0.03, 0.1, or 0.3% FGF-2. This trial found that local administration of 0.3% FGF-2 in two- or three- walled alveolar bone defects induced a statistically significant increase in alveolar bone neogenesis, as detected in standardized radiographs (Kitamura *et al* 2008). Subsequently, a nationwide Phase IIB clinical trial (a dose-response trial) was implemented in 2005 at 25 dental facilities to examine the safety and efficacy of FGF-2, using a placebo and an investigational formulation that contained 0.2, 0.3, or 0.4% FGF-2. As with the Phase IIA clinical

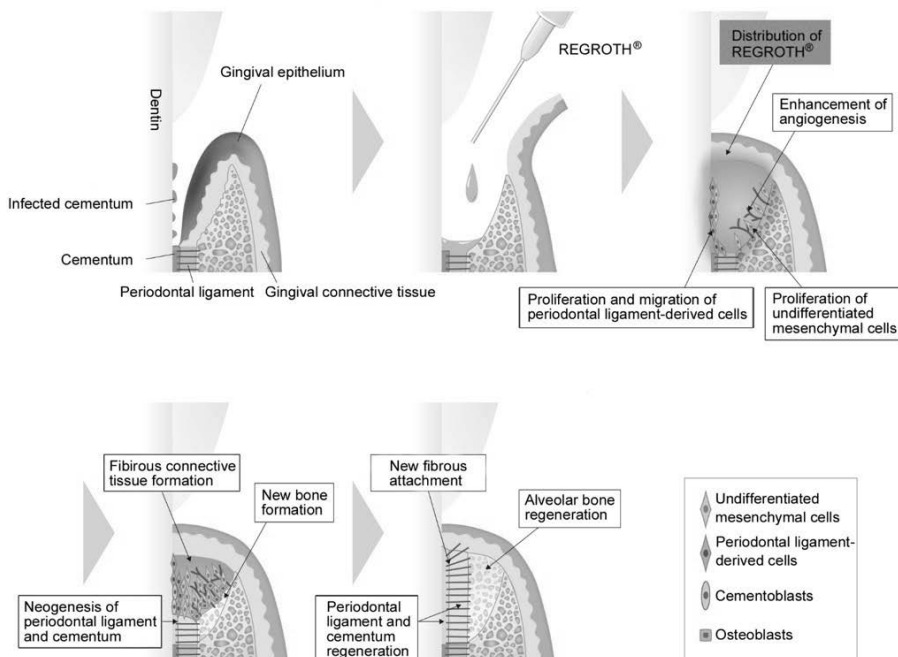


Figure 2. Mode of action of REGROTH®.

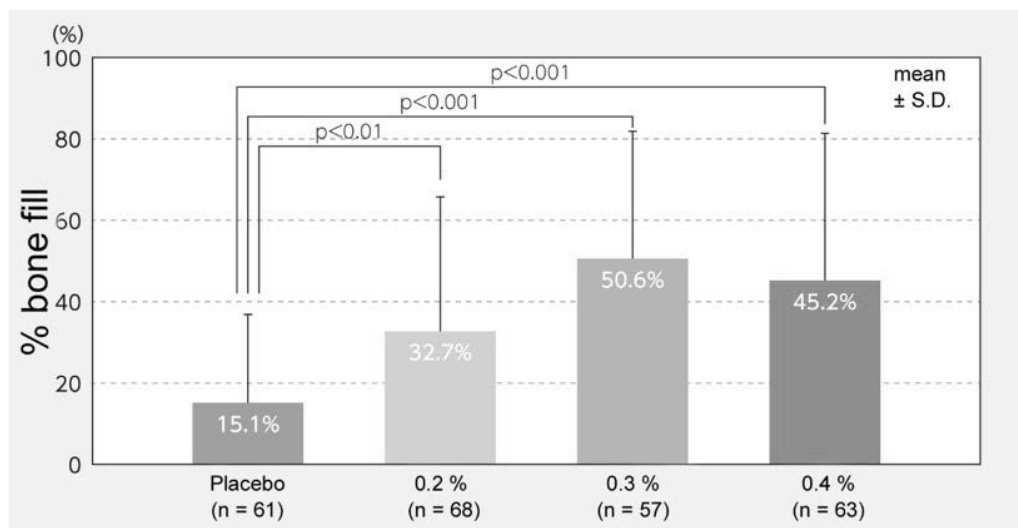


Figure 3. Efficacy endpoints of the Phase IIB clinical trial.

trial, 0.3% FGF-2 was found to induce a statistically significant increase in alveolar bone neogenesis. No statistical differences in efficacy were observed between 0.3 and 0.4% FGF-2 (Kitamura *et al* 2011, Murakami *et al* 2011) (Figure 3). Based on these results, 0.3% FGF-2 was selected as the recommended clinical dose to use for periodontal tissue regeneration. Therefore, a nationwide Phase III clinical trial (a verification study) was undertaken at 23 dental facilities to verify the safety and efficacy of the FGF-2 treatment, using a placebo and an investigational formulation containing 0.3% FGF-2. The study showed that the formulation containing 0.3% FGF-2 induced a statistically significant increase in alveolar bone neogenesis (Murakami *et al* 2011). Additionally, a nationwide non-inferiority trial, comparing EMDs (already in-use) with 0.3% FGF-2, was initiated in 2012 at 15 dental facilities to further investigate the clinical efficacy of the 0.3% FGF-2 treatment. The results showed both non-inferiority and superiority of the 0.3% FGF-2 treatment, compared with EMDs, in clinical efficacy for the induction of periodontal tissue

regeneration, nine months after administration (Kitamura *et al* 2016) (Figure 4). No serious side effects were observed during each of the clinical trial periods.

Usage

As previously stated, five clinical trials, involving approximately 1,000 periodontitis patients, have been conducted throughout Japan since 2001 and the safety and efficacy of 0.3% FGF-2 for periodontal regeneration have been thoroughly demonstrated. In September 2016, the formulation was approved for production under the name of REGROTH® Dental Kit, the Medical Product for Periodontal Regeneration.

Precautions for usage

REGROTH® is a kit product, composed of two cartridges containing lyophilized FGF-2 and a carrier vehicle of 3% hydroxypropylcellulose (HPC). This integrated kit, with separate cartridges for storage and administration, makes it possible to pre-mix the preparations prior to delivery.

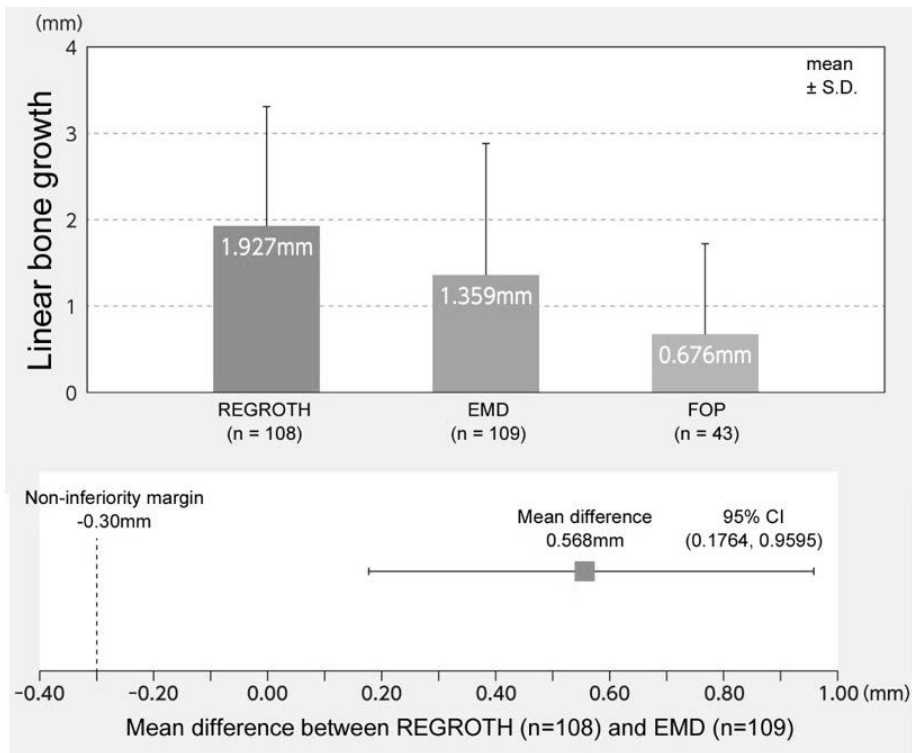


Figure 4. Efficacy endpoints of the Phase III clinical trial.

Furthermore, the kit is designed to prevent users contaminating the contents directly. In addition, the needle used to deliver the formulation has a unique shape with a pointed tip to minimize damage tissue during administration. As the inside of the package is sterilized, users must wear sterile gloves while handling all components of the kit.

The FGF-2-containing lyophilisate must be mixed with solution for each intended application and used within 24 hours after preparation when stored at room temperature. Mixing via syringe slowly, approximately 10 times, easily effectively mixes the lyophilisate and solution, and this process can be completed within minutes. For appropriate application, flap surgery must first be performed in the conventional manner. Irrigation with sterile saline is then performed at the intended site of administration. After the final irrigation, REGROTH® is applied to the bone defect site,

starting from the base of the defect. Care must be taken not to contaminate the defect site with saliva. Immediately after the application of REGROTH®, the site must be covered by flap suture and then the surgery is completed (Figure 5).

Indications, contraindications, and adverse effects

Indications

As stated above, REGROTH® is indicated for alveolar bone defects caused by periodontitis. Its dosage and administration have been verified as the appropriate formulation to fill the site of alveolar bone defects during flap surgery. REGROTH® can be used for periodontitis patients with ≥ 4 mm of periodontal pocket depth and vertical bone defects of ≥ 3 mm. Clinicians should examine

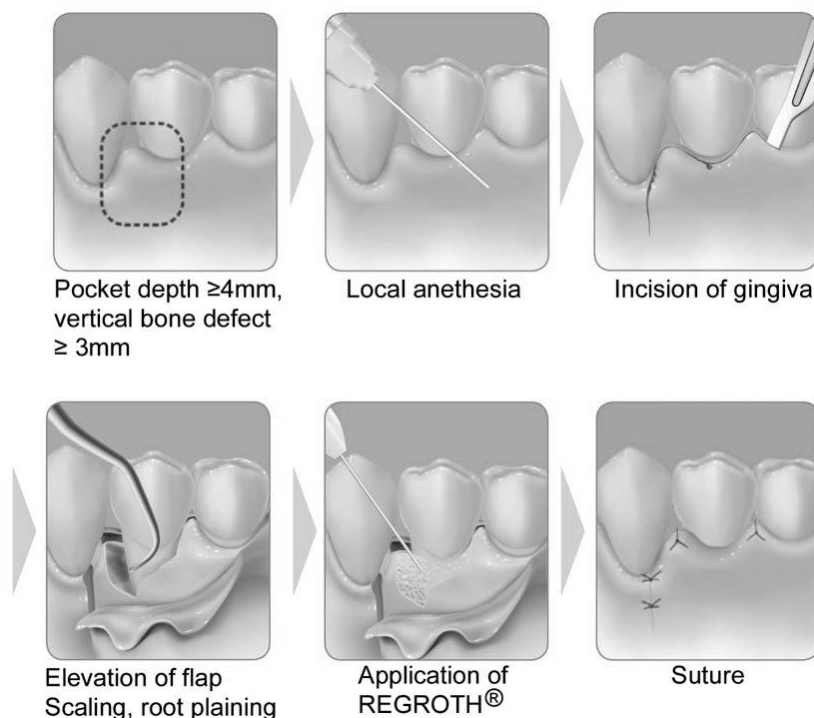


Figure 5. Usage of REGROTH®.

the intended site to determine whether it meets the appropriate criteria for administration before use.

Contraindications

REGROTH® is contraindicated in patients with hypersensitivity to substances included in the formulation, and in patients with a history of oral malignancy. Although no carcinogenicity of FGF-2 has been shown during carefully conducted tests, there is a possibility that application of the drug directly into a site containing tumor cells may promote proliferation and expansion of the tumor. Therefore, an examination of the mouth and an appropriate interview are required before using the formulation.

Future perspectives and possibilities

During treatment of oral disease, a harmonized approach, appreciating both the hard and soft tissues, is required to achieve successful wound healing, regeneration, and restoration outcomes. REGROTH® has been developed with this concept in mind. Interestingly, REGROTH® may be used for other purposes in the field of dentistry besides periodontal treatment.

For example, there is potential for clinical studies to investigate safety and efficacy of therapy with REGROTH® in combination with other drugs/devices. As REGROTH® cannot regenerate new spaces within bone by itself, the treatment of bony defects may be successfully achieved using REGROTH® and adjunctive structural scaffolds such as bone substitutes. In animal studies, the administration of

REGROTH® in combination with β -TCP, a bioabsorbable substitute, demonstrated better periodontal tissue regeneration at the site of one-walled intrabony defects, in comparison with administration of β -TCP alone. Future pre-clinical and clinical studies are needed to develop bone substitutes that can enhance the potential of REGROTH®, and to assess the safety and efficacy of REGROTH® when used with bone substitutes.

Furthermore, REGROTH® may be used for a wide variety of purposes in relation to dental treatment, such as the promotion of osseointegration during treatment with dental implants, as well as application for various plastic surgical procedures such as mucogingival surgery. To examine and expand the potential of REGROTH®, careful and comprehensive clinical studies and trials should be performed to validate its safety and efficacy for these additional uses.

Conclusion

REGROTH®, the world's first pharmaceutical product for periodontal regeneration, has been developed through long-term pre-clinical and clinical studies in Japan. It provides a new treatment option for patients with severe periodontal disease requiring periodontal tissue regenerative treatment. REGROTH® will evolve through comprehensive understanding of the formulation, including its properties and indications as previously stated, appropriate application in clinical use, and collection and examination of a wide variety of feedback. We expect that the REGROTH® formulation will become established as a standard protocol of periodontal therapy.

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Chapter 2

Effect of Combined Therapeutical Methods on Healing of Intra-bony Defects in Regenerative Periodontal Surgery

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Introduction

Periodontitis is an infectious disease that causes destruction of the tissues supporting the teeth. A survey by the Mongolian section of the International Association for Dental Research in 2013 showed that the rate of individuals with tooth loss in Mongolia is increasing as individuals age, from 2.2% in individuals 5 years old to 98% in the 65 to 74 years old age group (Hulan *et al* 2013). The main objective of periodontal therapy is to improve the periodontal health and to preserve the dentition. As part of these objectives, regeneration of the periodontal tissues damaged by periodontal disease has become an important goal. In recent years, studies in periodontal regeneration have focused on biological mediators which have the ability to enhance wound healing and improve clinical benefits of bone replacement grafts (Nevins *et al* 2011). Various biomaterials have been used for periodontal tissue regeneration including autogenous and allogenic bone grafts. However, no single graft material is considered the gold standard for the treatment of intrabony periodontal defects. Periodontal wound healing requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. Biological response modifiers

such as growth factors have been utilized because of their positive effects on such cells. Various preparations of platelet concentrates, rich in biological response modifiers, have been developed to improve soft and hard tissue healing. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate, defined as autologous leukocyte- and platelet-rich fibrin because of its high concentrations of leukocytes and platelets. PRF has been demonstrated to release polypeptide growth factors such as transforming growth factor β 1, platelet-derived growth factor, vascular endothelial growth factor and matrix glycoproteins (such as thrombospondin-1) (Dohan Ehrenfest *et al* 2009, Dohan Ehrenfest *et al* 2010). PRF is superior to other platelet concentrates, such as PRP, due to the easy and inexpensive method of preparation and no requirement for the addition of exogenous compounds such as bovine thrombin and calcium chloride. It is advantageous over autogenous bone grafts which require a second surgical site and procedure (Albanese *et al* 2013). Use of autologous platelet concentrates serves as a safe and appropriate clinical approach. Furthermore, PRF takes longer to be resorbed, which results in a slower and sustained release of growth factors into the wound area. Therefore, PRF has emerged as a promising regenerative material in the field

of periodontics.

Various types of bone graft materials have been used for regenerative periodontal treatment in intra-bony defects. Anorganic bovine bone graft is one material that has been extensively studied and the results from bovine bone research showed good regeneration of periodontal tissue (Albanese *et al* 2013).

The aim of this study was to investigate the effect of a PRF membrane in the presence or absence of anorganic bovine bone graft material on regeneration of infra bony defects in Mongolian patients.

Materials and methods

All patients included in the studies provided signed informed consent. The studies were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients were treated at the Department of Restorative Sciences, School of Dentistry, Mongolian National University of Medical Sciences by the same dentist from March to October 2016. The study design was a randomized, controlled, pilot clinical trial. Patients with no general disease and having generalized chronic periodontal disease participated in the study. The inclusion criteria were non-smokers, aged 20 to 45 years old, periodontal probing depth <5 mm, having a tooth with adequate endodontic treatment or restoration and good level of oral hygiene (plaque control record score <30%) after initial plaque control. Patients were excluded if they were suffering from a systemic disease which could affect the regeneration of periodontal tissue, pregnant or breast-feeding women, smokers, poor oral hygiene, malocclusion and patients on continuous medication. All patients received initial periodontal therapy consisting of oral hygiene instruction, supra and subgingival scaling and root planing. Thus, the data reported

at baseline, represents the clinical situation following initial therapy. Occlusal adjustment was performed if required. Ten patients were selected for this split mouth design study to compare healing of intra bony defects treated by PRF with and without bovine bone graft material (BBGM). The following clinical parameters were measured at baseline and six months after treatment, and compared between groups using the same periodontal probe: bleeding on probing (BOP), pocket depth (PD) and clinical attachment level (CAL). The measurements were made at six sites per tooth. The cemento-enamel junction (CEJ) was used as the reference point.

For radiographic assessments, preoperative and six months postoperative panoramic radiographs were taken. Depth of defect was evaluated using panoramic digital radiography (Morita Veraviewepocs, Japan). The image was analysed using i-Dixel Version 2.1. Radiographic assessments included measuring from the cemento-enamel junction to the bottom of the bone defect (CEJ-BD). Bone fill was determined as the difference in the CEJ-BD distance between preoperative and postoperative assessment time points.

Five patients, not enrolled in the study, each having at least four teeth (single or multirooted) with probing depth ≥ 5 mm on at least one aspect of each tooth, were used for examiner calibration. The examiners evaluated the patients two separate times, 48 hours apart. Calibration was accepted if more than 90% of the recordings could be reproduced within a 1.0 mm difference.

PRF preparation

The PRF preparation was performed according to Choukouran *et al* (2006) using a standardized kit immediately prior to operation. 10 ml of blood was drawn from the antecubital vein of the patient and collected into vacutainer tubes. A portable centrifuge

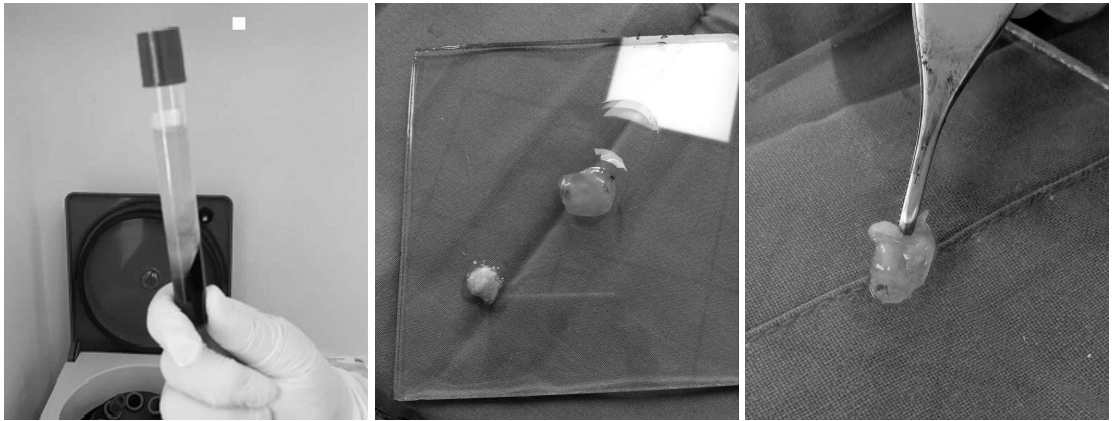


Figure 1. Platelet-rich fibrin (PRF) membrane preparation. (A) PRF after centrifugation; a fibrin clot is obtained in the middle of the tube, which is ready to be used. (B) Bovine bone graft material mixed with PRF. (C) Condensed PRF-membrane.

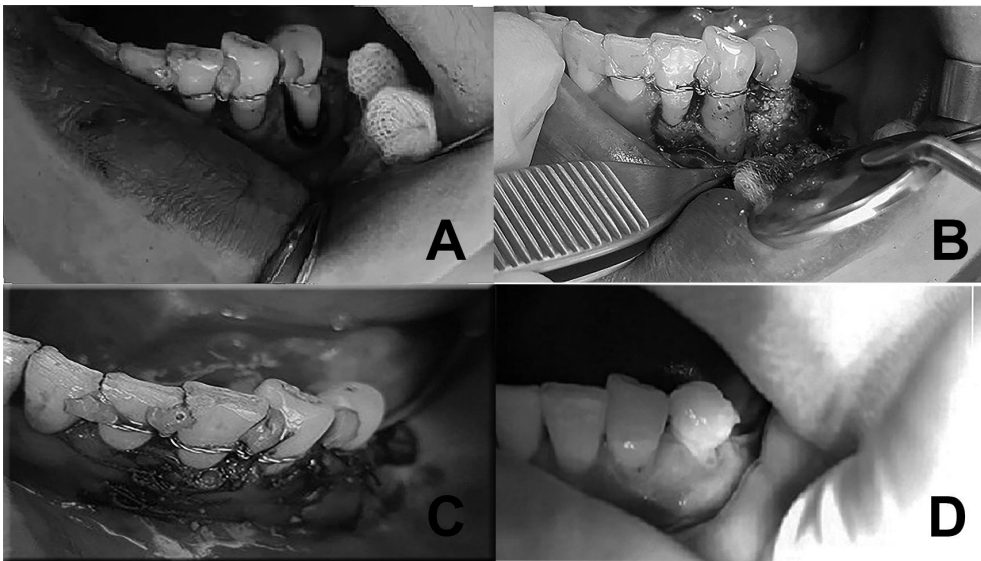


Figure 2. Surgery with PRF+BBGM. (A) Bone loss site before the surgery. (B) Co-location of PRF with BBGM. (C) The flaps were re-positioned coronally. (D) Six months after surgery.

with PRF box (3000rpm, 10 minutes) with eight monovettes, a shaker and a kit with disposable material were used. After spinning the samples, the leucocyte and platelet rich fibrin clot was concentrated in the middle of the tube, whereas the platelet poor plasma (PPP) on top. The PPP was removed so only the PRF pellet remained in the tube (Figure 1). PRF was condensed carefully using a lingual

spatula to about 1 mm thickness and used as a membrane for guided tissue regeneration.

Surgical procedures

Following local anesthesia and intracrevicular incisions, full thickness mucoperiosteal flaps were raised vestibularly and orally. Vertical releasing incisions were

used if necessary for better access to the surgical site or to achieve better flap closure. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed using hand and ultrasonic instruments. No conditioning of root surfaces was performed. In the PRF-BBGM group, bovine bone mineral (BBGM) granules (particle size 0.25 to 1.0 mm, Osstem®) were mixed with the small portion of PRP. The cases were divided into two groups; PRF with bovine bone graft (PRF-BBGM) and PRF without any bone graft material. Care was taken not to overfill the defects. A PRF membrane was trimmed and adapted over the entire defect to cover 2 to 3 mm of the surrounding alveolar bone. No sutures or pins were used for membrane fixation or stabilization. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures (Figure 2).

Postoperative care

All patients received antibiotics for one week (3x250 mg metronidazole/day). Postoperative care consisted of 0.2% chlorhexidine rinses twice a day for two weeks. Sutures were removed 10 days after surgery. Recall appointments were scheduled weekly during the first two months after surgery and once per month for the rest of the observation period. The recall appointments consisted mainly of reinforcement of oral hygiene measures and professional supragingival tooth cleaning.

Statistical analysis

For statistical analysis Stata software was used. For all variables in each group, mean values and standard deviation (SD) were extracted. Paired Student t test was used to compare clinical and radiographic data and p values <0.05 were considered statistically

significant.

Results

A total of 10 patients (four females and six males), aged from 34 to 45 years (mean age 40.2 ± 3.8), were examined in this study. Before treatment, clinical and radiographical measurements were similar for both groups. Post-operative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the study period.

At the initial examination the mean PD (8.2 ± 2.1 and 8.1 ± 2.1 mm) and CAL (6.3 ± 2.2 and 6.8 ± 1.8 mm) measurements were similar in the two groups and no statistically significant differences ($p=0.9$ and $p=0.6$) were found. Both PRF and PRF-BBGM groups showed significant pocket depth reduction ($p<0.005$ for PRF group, $p<0.0005$ for PRF+BBGM group) and CAL gain ($p<0.01$ for PRF group, $p<0.0005$ for PRF+BBGM group) at six months compared with baseline values (Table 2).

The mean PD reduction was 2.7 ± 0.64 mm in the PRF group and 4 ± 0.68 mm in the PRF+BBGM group. The mean CAL gain was 2.8 ± 0.6 mm in the PRF group and 3.62 ± 0.56 mm in the PRF+BBGM group. Significant differences in PD reduction and CAL gain were found between the groups (Table 3). Postsurgical measurements showed greater reduction of pocket depths ($p<0.01$) in PRF+BBGM group compared with PRF group. PD reduction over 3 mm was measured in 10% of the cases treated with PRF and in 80% of PRF+BBGM group. Clinical attachment level was significantly increased in both groups, even more significantly ($p<0.0005$) in PRF+BBGM group. In both groups all sites gained at least 2 mm CAL. CAL gain over 3 mm was measured in 10% of defects in cases treated with PRF and in 50% of PRF+BBGM group.

Parameters	Preoperative	6 months postoperative	P value
BOP	22.2±5.6	14.2±3.9	0.002

Table 1. Pre- and 6 months post-operative mean (±SD) bleeding on probing score.

Parameters (mm)	PRF alone		P value	PRF+BBGM		P value
	Pre-operative	6 months postoperative		Pre-operative	6 months postoperative	
Probing depth	8±2.1	5.3±1.6	<0.005	8.1±2.1	4±1.63	<0.0005
Clinical attachment level	6.3±2.2	3.5±2.1	<0.01	6.8±1.8	3.2±1.6	<0.0005

Table 2. Pre- and post-operative clinical parameters (mean±SD) of groups.

Parameters (mm)	PRF alone		PRF+BBGM		P value
	Mean change	±SD	Mean change	±SD	
Probing depth	2.7	0.64	4	0.68	<0.01
Clinical attachment level	2.8	0.6	3.62	0.56	<0.05

Table 3. Mean change of clinical parameters (mean±SD) of groups.

Parameters (mm)	PRF alone		P value	PRF+BBGM		P value
	Pre-operative	6 months postoperative		Pre-operative	6 months postoperative	
CEJ-BDL	8.5±2.1	6.1±1.8	<0.05	9.1±1.9	6±1.5	<0.005

Table 4. Radiographic parameters in groups.

Parameters (mm)	PRF alone		PRF+BBGM		P value
	Mean change	\pm SD	Mean change	\pm SD	
Bone fill	2.4	0.6	3.1	0.7	<0.05

Table 5. Mean difference of bone fill (mean \pm SD) of groups.

Preoperative measurement showed no significant difference ($p=0.5$) in CEJ-BDL between groups. However, at six months, the mean CEJ-BDL decreased significantly in both groups compared with the baseline data ($p<0.05$ for PRF group, $p<0.005$ for PRF+BBGM group) (Table 4).

The mean bone fill was 2.4 ± 0.6 mm in PRF group and 3.1 ± 0.7 mm in PRF+BBGM group. A significant difference ($p<0.05$) in bone fill was found between the groups (Table 5).

Discussion

Most periodontal regeneration studies investigating PRF have studied PRF as a sole filling material and as an adjunct to other materials (Castro *et al* 2017).

Our present study used PRF as a guided tissue membrane, with and without bovine bone graft material. In both groups the intrabony defects showed significant decreases in clinical parameters of PD reduction and CAL gain compared to baseline values. The BOP values also improved significantly ($p<0.005$) compared to baseline data. Statistically and clinically significant differences in all the investigated parameters were observed between the two treatment groups. The results of this study show that the decrease in PD and gain in CAL were greater when intrabony defects were filled with BBGM. We did not observe any adverse reactions such as abscesses or allergic reactions. These results are in agreement with the results of Lekovic *et al* (2012), who compared PRF with and without bovine porous bone mineral (BPBM)

in the treatment of human intrabony defects. The mean PD in the group treated with PRF only showed a reduction 3.3 mm, while in the PRF+BPBM treated group, the mean PD reduction was 4.4 mm. CAL gain was 2.4 mm versus 3.8 mm in favor of PRF+BPBM group ($p<0.001$). This change was comparable to our study. Mathur *et al* (2015) compared and evaluated PRF and autologous bone grafts in the treatment of intrabony periodontal defects clinically and radiographically. There was significant mean PD reduction and CAL gain in both the PRF treated group (2.67 ± 1.29 mm, 2.53 ± 1.06 mm) and the autologous bone graft treated group (2.4 ± 1.06 mm, 2.67 ± 1.63 mm), but the differences were not significant between groups. Bansal and Bharti (2013) evaluated the effectiveness of PRF as an adjunct to demineralized freeze-dried bone allograft (DFDBA) graft in the treatment of human intra-bony defects and reported that compared to the test group with DFDBA alone exhibited statistically significant changes ($p<0.05$) in PD reduction and CAL gain. They reported that the group receiving PRF+DFDBA exhibited 4 mm of mean PD reduction and 3.4 mm of mean CAL gain as compared with 3.1 mm of PD reduction and 2.3 mm CAL gain in the group receiving DFDBA alone. Similar results were obtained by Agarwal *et al* (2016) with greater PD reduction and CAL gain in PRF+DFDBA ($p<0.05$) compared to single treatments with bone graft alone. The outcomes showed an effective result of PRF in PD and CAL parameters measured, or an improvement of the outcomes in studies where PRF was

combined with other biomaterials.

According to a study by Gupta *et al* (2014), reduction in BOP was observed, from the pre-prophylactic levels to preoperative levels and then up to 12 months postoperatively. In our study the BOP levels were decreased post treatment compared to baseline data, but this change was not statistically significant. Mathur *et al* (2015) also demonstrated no significant difference in mean change in PI, gingival index between the two groups treated with PRF and autologues bone graft. The strict oral hygiene regimen used in this study might be responsible for the positive outcomes obtained in both groups.

The results from this study indicated a greater amount of bone gain in both treatment groups. The bone gain with PRF+BBGM observed in this study is in accordance with the study by Mathur *et al* (2015). They reported significant defect fill in both groups, but mean changes in bone defect fill were noted to be higher in the PRF group (2.93 ± 1.79 mm, 3.94 ± 3.56 mm and 2.01 ± 2.09 mm) compared to autologous bone graft group (2.66 ± 1.84 mm, 1.76 ± 0.83 mm and 1.02 ± 0.58 mm) after six months. Lekovic *et al* (2012) and Agarwal *et al* (2016) also reported significant bone fill in treated groups. Even more significant bone fill was observed in the PRF+BPBM ($p < 0.001$) and PRF+DFDBA groups ($p < 0.05$) compared to PRF alone. Whereas Bansal and Bharti (2015) reported no significant difference in bone fill between the two groups.

It should be noted that in the present study the results indicate there is insufficient data to allow any conclusions to be drawn regarding the efficacy of PRF as a membrane alone or with combination with bone graft in the treatment of intrabony defects. Further studies, with higher numbers of treated defects are required to detect any differences between the treatment groups.

Conclusion

This study demonstrated that treatment with both PRF membrane alone and PRF+BBGM produce significant improvements in clinical and radiographic parameters for intrabony periodontal defects up to a six-month postoperative follow up. Furthermore, the regenerative treatment therapy using PRF with BBGM is more effective than PRF without BBGM.

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Chapter 3

Local Host Modulating Agents in Periodontal Regeneration

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Introduction

Chronic periodontitis (CP) is a dysbiotic inflammatory disease with an adverse impact on systemic health. It occurs as a consequence of the interaction of environmental, genetic, host and microbial factors leading to destruction of tooth supporting tissues in susceptible subjects which results from shift in the balance of preventive and destructive immune mechanisms against microbial pathogens (Hajishengallis 2015, Kinane *et al* 2001). This inflammatory condition may result from localized or generalized colonization of microbial plaque, which speeds up the process of periodontal pocket formation to bone loss, further creating intrabony defects (IBDs). Periodontitis also affects the root trunk of multi-rooted teeth, firstly with tissue destruction and then gradually furcation involvement leading to bone loss (Goodson *et al* 1991, Sternlicht 1963). Treatment of furcation lesions is one of the most demanding tasks in periodontal therapy today. The rate of success experienced in the treatment of furcation lesions is reduced due to incomplete removal of subgingival plaque and calculus in the interradicular area as a result of the complex anatomy of the furcation space (Porciúncula *et al* 2004). Periodontal therapy includes a wide range of

treatment modalities, such as scaling and root planing (SRP), access surgical therapy and surgical regenerative procedures with grafts and biomaterials. Adjuncts to mechanical debridement include the local delivery of antimicrobial/pharmacopotent agents using sustained or controlled-release systems, directly subgingivally targeting specific pathogenic bacteria. These adjuncts offer great promise in the treatment of localized forms of periodontal breakdown to achieve periodontal health and regeneration (Elavarasu *et al* 2012).

Statins, most widely used for lowering the levels of low-density lipoprotein cholesterol, are 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) competitive inhibitors. Patients on statin medications exhibit fewer signs of periodontal inflammatory injury because of statins' diverse, broad-spectrum pleiotropic effects including anti-inflammatory, anti-oxidative, immunomodulatory and antibacterial activities as well as improvement of microvascular function and reperfusion during wound healing processes. Statins stimulate bone formation *in vivo* by inducing the expression of bone anabolic factors, such as vascular endothelial growth factor and bone morphogenetic protein 2 (BMP-2) and promote osteoblast differentiation and mineralization in MC3T3-E1 cells. Periodontal regeneration has been demonstrated using members of

the statin group, such as rosuvastatin (RSV), atorvastatin (ATV) and simvastatin (SMV). Successful gain of alveolar bone volume has been reported in rats, and greater improvement in clinical parameters has been shown when statins are delivered subgingivally as adjuncts to SRP in treating individuals with periodontitis (Goes *et al* 2010, Pradeep *et al* 2010).

Biologic mediators have been used to improve the quantity and quality of the bone. Among these agents, bisphosphonates, which are chemical analogs of pyrophosphate, are known to inhibit osteoclastic bone resorption. Bisphosphonates bind to the hydroxyapatite crystals of bone and prevents their dissolution by interfering with osteoclast function through a variety of direct and indirect mechanisms (Fleisch 1991). Alendronate (ALN) is a second-generation bisphosphonate, which includes aminobisphosphonates with an amino-terminal group. The antiresorptive properties of bisphosphonates change according to their side chains. ALN binds to the crystals of hydroxyapatite in bone and binds preferentially to the bone resorption surface, particularly to those undergoing active osteoclastic resorption (Tennenbaum *et al* 1992). Earlier studies have demonstrated that systemic or topical application of ALN was highly effective at reducing alveolar bone resorption following mucoperiosteal flap surgery (Reddy *et al* 2005, Yaffee *et al* 1995).

Metformin (MF) is a biguanide that is one of the most common oral hypoglycemic drugs used in the management of type 2 diabetes mellitus. MF lowers both basal and postprandial plasma glucose and works by inhibiting the production of glucose by hepatic cells in the liver. Glucose absorption by intestinal cells is reduced, and glucose uptake and utilization are improved. Besides lowering the blood glucose level, MF could have additional benefits, such as reduction in weight, lowered plasma lipid levels, and the

prevention of some vascular complications (DeFronzo and Goodman 1995, Scarpello and Howlett 2008, Viollet *et al* 2012). Studies conducted to evaluate the *in vivo* and *in vitro* efficacy of MF on bone marrow progenitor cells (BMPC) and their effect on bone regeneration in a parietal lesion model of nondiabetic and streptozotocin-induced diabetic rats showed an increase in enzyme alkaline phosphatase (ALP) activity, deposition of type I collagen, expression of osteocalcin, and calcium deposits in the extracellular matrix of BMPC. *In vivo*, MF administration enhanced the expression of osteoblast-specific transcription factor Runx2/Core-binding factor alpha 1 (Cbfa1), thus stimulating bone lesion regeneration in control and in diabetic rats (Molinuevo *et al* 2010).

Considering the above, various studies were conducted as single-center, randomized controlled clinical trials to investigate the clinical and radiographic effects of 1.2% RSV, 1.2% ATV gel, 1.2% SMV gel, 1% ALN and MF gel when delivered locally as an adjunct to the non-surgical treatment in CP patients with class II furcation defects and IBDs.

Materials and methods

Source of data

For the studies, participants were chosen from the outpatient block of the Department of Periodontology, Government Dental College and Research Institute, Bangalore. All patients were informed about the study and written informed consent was obtained from all the patients. Ethical clearance was obtained from Institutional Ethical Committee and Review.

Patients included were systemically healthy, with moderate periodontitis [probing depth (PD) 5 to 6 mm, clinical attachment level (CAL) 4 to 6 mm] and vertical bone loss ≥ 3 mm on intraoral periapical radiographs or mandibular class II furcation defects in

asymptomatic endodontically vital mandibular molars with radiolucency in furcation area with PD ≥ 5 mm and horizontal PD ≥ 3 mm. Patients with ≥ 20 teeth with neither a history of periodontal therapy in the preceding six months nor any antibiotic therapy were included in the studies. Patients with any known systemic disease, allergic to statins, metformin or alendronate, on systemic statin, metformin or alendronate therapy, alcoholics, tobacco users, pregnant or lactating women were excluded from the studies.

Patient grouping

After selection of the patients by the examiner (ARP), patients were randomly divided into respective groups. The randomization process was made externally by the statistical unit using a computer generated random table, and investigators were neither involved in the randomization process nor aware of the assigned group in all outcome evaluations. Careful instructions on proper oral hygiene measures were given to each patient. For each patient SRP was performed at baseline and patients were treated with respective drugs in respective groups as local drug delivery (LDD) agents. Antibiotics or anti-inflammatory agents were not prescribed after therapy.

Clinical evaluation

Clinical parameters, including modified sulcus bleeding index (mSBI), full-mouth plaque index (PI) score, PD, CAL in CP patients with IBDs and relative vertical CAL (RVCAL), and relative horizontal CAL (RHCAL) in furcation defects were measured at baseline first; then SRP was done and respective gel placement for each patient was done. All the parameters were measured again at six and nine months. In comparative studies between RSV and ATV gels; gels were

redelivered at six months.

Radiographic measurements

Defect depth reduction for each defect was estimated at baseline, six and nine months using an image analyzer. For standardization of radiographs, customized bite blocks and parallel angle technique was used. All radiographs were evaluated by the same masked evaluator (ARP). For radiographic evaluation, all radiographs were scanned at 6,400 dots per inch with a scanner, and the computer-aided software was used to evaluate bone defect.

Primary and secondary outcome measures

The primary outcome of the study included defect depth reduction (in percentage), and the secondary outcomes included CAL, PD, PI, mSBI and RVCAL, RHCAL.

Formulation of 1.2% RSV, 1.2% ATV and 1.2% SMV gel

For preparation of 1.2% RSV gel, a suitable non-toxic, non-allergenic medium of methylcellulose was used. To prepare methylcellulose *in situ* gel the required amount of biocompatible solvent was added to a precisely weighed quantity of methylcellulose. The vial was heated at a temperature of 50°C to 60°C and was agitated using a mechanical shaker to attain a clear solution. A weighed amount of RSV was then added to this mixture and dissolved completely to achieve a homogeneous phase of polymer, solvent, and drug. Thus, the 1.2% RSV *in situ* gel was prepared. 1.2% ATV and 1.2% SMV *in situ* gel were prepared the same way.

Formulation of MF gel

All of the required ingredients of the formulation were weighed accurately. Dry gellan gum powder was dispersed in distilled water maintained at 95°C. The gellan gum was hydrated by stirring the dispersion at 95°C for 20 minutes using a magnetic stirrer (magnetic stirrer 2MLH; REMI, Mumbai, India). The required amount of mannitol was added to the gellan gum with continuous stirring, and the temperature was maintained above 80°C. The required amount of MF was added in a similar fashion. In the final stage of gel preparation, sodium citrate was dissolved in 10 mL distilled water and was added to the mixture with sucralose, citric acid, and preservatives while stirring. The mixture was allowed to cool to room temperature to form a gel.

Formulation of 1% ALN gel

ALN was dissolved in a required amount of distilled water to achieve 1% ALN concentration. A weighed quantity of polymer (2% wt/wt) was taken and added to the distilled water. The mixture was stirred gradually, the polymer was allowed to soak for two hours, and 1% triethanolamine was added to neutralize the polymer solution and to form the gel. The pH was adjusted to 6.8. Finally, the required amounts of methyl paraben (0.1%) and propyl paraben (0.05%) were dissolved in ethanol and added to the gel.

Local drug delivery

0.1 ml RSV/ATV/SMV gel (1.2 mg/mL), 10 μ L prepared ALN gel (10 mg/mL), 10 μ L prepared MF gel (10 mg/mL) was injected into the periodontal pockets (one site per patient) with the help of a syringe with a blunt cannula. Patients were instructed not to chew any hard or sticky foods. They were also advised not to brush near the treated site

or to use any interdental aids for one week's duration. At the six month recall appointment, all parameters (clinical and radiographic) were evaluated again and in comparative studies of RSV and ATV the gels were again delivered to the same sites to the respective group of patients using the same method. Then at nine-month recall (three months from second delivery of the drugs) all parameters were again assessed. During the recall visits, proper oral hygiene instructions were given to all the patients, adverse effects (if any) were noted and supragingival plaque or calculus (if any) was removed.

Statistical analysis

Power analysis calculations were performed before the studies were initiated. Categorical variable (site-specific PI) was expressed as percentage and continuous variable (full-mouth PI, mSBI, PD, RVCAL, RHCAL, and depth reduction) as mean \pm SD. Between the treatment groups, comparison was carried out using the Student t test for a continuous variable following a normal distribution. One-way analysis of variance (ANOVA) and Scheff's Post hoc tests were used to delineate differences in PD, RVAL, RCAL bone defect depth, mSBI and full mouth PI among three groups, repeated measures ANOVA assessed differences in all variables at three examinations, i.e. at baseline, six and nine months. Statistical significance was defined as $P < 0.05$.

Results

A decrease in mSBI score at six months was found, with 1.2% SMV (2.3267 ± 0.8017). The mean decrease in PD from baseline to six months was 4.26 ± 1.59 mm with 1.2% SMV. There was greater decrease in mean IBD (1.41 ± 0.74 mm or 32.54%) with 1.2% SMV compared to placebo (0.09 ± 0.58 mm

or 2.16%).

Mean PD reduction and mean CAL gain were greater with 1.2% ATV group than the placebo group at three, six and nine months. A significantly greater mean percentage of radiographic bone fill was found in the ATV group ($35.49 \pm 5.50\%$) compared to the placebo group ($1.82 \pm 1.32\%$) after nine months.

There was significant improvement in both study groups. At six months there was a greater decrease in mSBI scores with 1.2% RSV (3.71 ± 0.24) compared to placebo (1.48 ± 0.33). The mean decrease in PD from baseline to six months was 4.04 ± 0.34 and mean CAL gain from baseline to six months was 4.2 ± 0.17 with 1.2% RSV. There was a greater decrease in mean IBD (2.23 ± 0.32 mm, 48.58%) compared to placebo (0.46 ± 0.02 mm, 10.02%).

Mean mSBI and PD reductions, CAL gain, and IBD depth reduction with statin (RSV ATV) drugs were significantly greater than with placebo gel LDD. Improvements in these parameters were significantly greater with RSV LDD than ATV or placebo gels at six and nine months.

Greater mean PD reduction and greater mean gain in RVCAL and RHCAL were seen in the RSV group than ATV group at six and nine months. Furthermore, a significantly greater mean percentage of defect depth reduction was found in the RSV group (30.80 ± 8.35 , 41.86 ± 6.76) than ATV group (25.54 ± 8.89 , 34.31 ± 8.04) at six and nine months, respectively.

The mean PD reduction and CAL gain were greater in the ALN group than in the placebo group at two and six months. Furthermore, a significantly greater mean percentage of bone fill was found in the ALN group ($40.4 \pm 11.71\%$) than in the placebo group ($2.5 \pm 1.02\%$).

Mean PD reduction, RVCAL and RHCAL gain were shown to be greater in the ALN group than the placebo group at three, six

and 12 months. Furthermore, a significantly greater mean percentage of bone fill was found in the ALN group ($32.11 \pm 6.18\%$, $32.66 \pm 5.86\%$), compared with the placebo group ($2.71 \pm 0.61\%$, $1.83 \pm 1.51\%$), at six and 12 months, respectively.

Mean PD reduction and mean CAL gain were found to be greater in the MF group than the placebo group at all visits. Furthermore, a significantly greater mean percentage of bone fill was found in the MF group ($26.17 \pm 6.66\%$) than the placebo sites ($3.75 \pm 8.06\%$).

The mean PD reduction, CAL gain, and IBD depth reduction were found to be greater in the MF group than in the placebo group at all visits. The percentage of defect depth reduction was significantly greater in the MF group ($26.8 \pm 5.52\%$) than in the placebo sites ($4.79 \pm 2.30\%$).

Discussion

MF has not only emerged as a first-line drug in the management of overweight and obese patients with diabetes, but experiments have also shown excellent uptake of MF by osteoblast cells when used as a local drug-delivery agent *in vitro* (Shah *et al* 2010, Zhen *et al* 2010). Previous studies have shown that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen. The advantages of local drug delivery, such as high concentrations at the target site with reduced dosage, fewer applications, and high patient acceptability, were considered in the current study as a technique of direct subgingival injection of MF into periodontal pockets of chronic periodontitis patients with intrabony defects (Theilade *et al* 1966).

ALN has been highly effective in reducing alveolar bone resorption following mucoperiosteal flap surgery when given as a systemic or topical application (Reddy *et al*

2005, Tennenbaum *et al* 1992, Yaffee *et al* 1995). Additionally, in one study systemic ALN was used for six months in the treatment of patients with periodontitis and type 2 diabetes and reported significant improvement in the healing response compared with the placebo group (Rocha *et al* 2001). Dental implants coated with hydroxyapatite and ALN resulted in a significant increase in peri-implant bone (Meraw and Reeve 1999). The current study finds improvement in the clinical parameters PI and mSBI compared to baseline at all time intervals. However, there is a significant difference in the full-mouth PI score between the two groups at 12 months. There is also a significant decrease in PD, and significant gains in RVCAL and RHCAL, in the ALN group in the current study, which is similar to the results of previous studies.

Since locally delivered statins were discovered to be potent stimulators of bone formation, the possibility of using these compounds as practical bone anabolic agents has been postulated. There is evidence that statins promote osteoblast differentiation and enhance BMPs in mouse bone marrow stromal cells (Mundy *et al* 1999, Song *et al* 2003, Tobert 1987). SMV increased alkaline phosphatase activity and osteocalcin expression levels in human bone marrow stem cells (Baek *et al* 2005). Systemic statin-treated humans showed an increase in bone mineral density, bone specific alkaline phosphatase activity, and osteocalcin after three months (Tikiz *et al* 2005). However, the effect of statins when administered systemically appears to be a significant mean bone fill in Class II furcation involvement from baseline (1.54%) to six months (25.16%) with SMV suggests a role for SMV in bone formation. This may be because of increased BMP-2 expression during bone regeneration, anti-inflammatory effects and angiogenesis during wound healing (Alam *et al* 2009, Borg *et al* 2000).

ATV can cause enhanced osteoblastic differentiation, increased expression of osteoprotegerin (potent inhibitor of bone resorption) which contributes to its bone sparing effect (Viereck *et al* 2005). Increases in bone level and decreases in tooth mobility was found with the use of ATV (Fajardo *et al* 2010). Similarly, RSV also has osteoblastic properties (Monjo *et al* 2010). Increased reduction in PD, more gain in attachment level and improved bone fill have been found with the use of RSV as LDD agent as well as when used in combination with autologous PRF and porous HA bone graft for treatment of CP patients with mandibular class II furcation defects (Pradeep *et al* 2015, Pradeep *et al* 2016). The clinically significant benefits of RSV compared to ATV might be explained by greater anti-inflammatory action of RSV when compared with ATV due to more effective decrease in CRP levels (Khurana *et al* 2015). RSV is more effective in reducing LDL compared to other statins (Barakat *et al* 2013, Jones *et al* 2003).

Conclusion

Prudent administration of antimicrobial agents following judicious pharmacologic principles will preclude the abuse of chemotherapeutic agents and reduce the potential of developing or selecting drug resistant bacterial strains. Local drug delivery systems with controlled release properties have the potential to be used as a therapeutic component in the management of periodontal diseases. Further study should be directed towards comparison of clinical and microbiologic parameters after the local delivery system in the treatment of chronic adult periodontitis.

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Chapter 4

Platelet Rich Fibrin: Role as a Regenerative Material in Developing Countries

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Introduction

Periodontitis can be defined as an inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms or a group of specific micro-organisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both. These responses can result in a variety of intraosseous defects of various architectures (Newman 2002).

The ultimate goal of periodontal therapy is regeneration of the lost tissues and maintenance of the natural dentition in health and comfortable function. Regeneration has been defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium (AAP 2001). When periodontal disease causes loss of the attachment apparatus, optimal care seeks to regenerate the periodontium to its pre-disease state. Periodontal regeneration involves the formation of alveolar bone, cementum, and a new functional periodontal ligament (Wang 1994). For periodontal regeneration to occur, a number of biologic events, including cell migration, adherence, multiplication, and differentiation, need to occur in a well-orchestrated sequence (Pradeep *et al* 2012).

Periodontal regeneration can be achieved

by various procedures including the use of bone grafts, root surface biomodification, guided tissue regeneration, soft tissue grafts and combinations of these procedures. A material or technique must demonstrate histologically that bone, cementum and a functional periodontal ligament (a new attachment apparatus) can be formed on a previously diseased root surface to be considered a regenerative modality (Zander *et al* 1976).

Although, periodontal regeneration is biologically possible, it is not always clinically predictable. Complete regeneration may not be possible in many situations due to the complexity of the biological events and cells underlying successful periodontal regeneration. Wang *et al* (2006) concluded that factors including primary wound closure, blood supply, defect architecture, space maintenance and wound stability determine the predictability of periodontal regeneration. All these factors play a significant role in determining the amount and extent of achievable regeneration through various grafting modalities.

Successful periodontal reconstruction comprises the regeneration of multiple tissues of the periodontium which regenerate at differential rates. It is a complex biological process in itself which is intricately regulated

between cells, locally acting growth factors and the extracellular matrix components. The key to periodontal regeneration is to stimulate progenitor cells to re-occupy the defect site. Unfortunately, conventional surgical techniques offer only limited potential towards achieving this goal (Gottlow *et al* 1984).

In the early 1980s, a series of experiments were conducted on a procedure to regenerate lost attachment apparatus using a membrane to exclude the epithelium and to provide a space to allowing periodontal cells (which have the potential to regenerate the tissues) to repopulate the wound. Despite satisfactory clinical outcomes, complete regeneration was not always consistently achieved (Nickles *et al* 2009). Later, a novel technique was adopted from the field of regenerative medicine, which involved the morphogenesis of new tissue using three components namely cells, scaffold and signaling molecules in a so-called "tissue engineering approach". This approach was applied to periodontal regeneration, and with further development led to the use of platelet concentrates as a tool for regeneration of periodontal defects (Marx 2004).

As detailed above, periodontal wound healing requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells and osteoblasts. Disruption of the vasculature during wound healing leads to fibrin formation, platelet aggregation, and release of several growth factors into tissues from platelets through molecular signals which are primarily mediated by cytokines and growth factors. There is evidence that the presence of growth factors and cytokines in platelets play a key role in inflammation and wound healing (Giannobile 1996). Platelets also secrete fibrin, fibronectin and vitronectin, which act as a matrix for the connective tissue and as adhesion molecules for more efficient cell migration (Dohan *et al* 2006b). This has led to the idea of using platelets as therapeutic

tools to improve tissue repair particularly in periodontal wound healing.

Platelet-rich fibrin (PRF) described by Choukroun *et al* (2001) is a second-generation platelet concentrate which contains platelets and growth factors in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. PRF clots contain a strong natural fibrin matrix, with concentrates platelets and growth factors (Dohan 2006a). It has a complex architecture of a healing matrix with unique mechanical properties which makes it distinct from other platelet concentrates. PRF enhances wound healing and regeneration and several studies have shown rapid and accelerated wound healing with the use of PRF than without it (Sharma and Pradeep 2011b). PRF is better than other platelet concentrates such as PRP due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous compounds like bovine thrombin and calcium chloride. Autogenous grafts are ideal as regenerative materials; however, a second surgical site and procedure is required, thus PRF can be advantageous over autogenous graft.

PRF has the characteristic of polymerizing naturally and slowly during centrifugation. The thrombin concentrations acting on the collected autologous fibrinogen are almost physiologic because there is no bovine thrombin addition. This aspect is crucial to determine the 3-dimensional organization of a fibrin network. Indeed, during gelling, the fibrin fibrillae can be assembled between them in 2 different biochemical architectures: condensed tetramolecular or bilateral junctions and connected trimolecular or equilateral junctions (Mosesson *et al* 2001).

Thus, PRF has emerged as a promising regenerative material in the field of periodontics especially in developing countries like Nepal where there is limited access to other

regenerative materials because of expense and not being manufactured domestically.

History of platelet concentrates

The use of products from blood to seal wounds and stimulate healing started with the use of fibrin glues, which were first described many years ago and are constituted of concentrated fibrinogen (Matras 1970). Consequently, the use of platelet concentrates to improve healing and to replace fibrin glues, was first described by Whitman *et al* (1997). Platelets contain high quantities of key growth factors, such as PDGF-AB (platelet-derived growth factor AB), TGF β -1 (transforming growth factor β -1) and VEGF (vascular endothelial growth factor), which are able to stimulate cell proliferation, matrix remodeling and angiogenesis. The use of these growth factors to enhance healing is an interesting option, but commercial interests might obscure a lack of true clinical benefits in some cases. Indeed, these concepts have spurred their commercial exploitation with the development of a wide range of preparation protocols, kits and centrifuges. Most of these products were called PRP, the same name as the original transfusion platelet concentrates, which does not allow distinction between the different systems and protocols (Dohan *et al* 2009).

Platelet rich plasma (PRP), a first-generation platelet concentrate, works on the premise that platelets, when sequestered as in PRP, release large quantities of polypeptide growth factors which influence differentiation and proliferation of various cells in periodontal milieu. PRP is composed of 4% RBCs, 1% WBCs and over 95% platelets, a cell type that actively secretes growth factors for initiating wound healing and secreting factors responsible for enhancing cell adhesion, proliferation and migration of various cell types (Jameson 2007, Marx 2004).

PRF was first developed in France by Choukroun *et al* (2001) for specific use in dentistry. This technique requires neither anticoagulant nor bovine thrombin. It is only the centrifuged blood without any addition, which makes it possible to avoid all the restrictions related to blood-derived product reimplantation. This technology requires a table centrifuge and a collection kit. The absence of anticoagulant implies the activation in a few minutes of most platelets of the blood sample in contact with the tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the high part of the tube, before the circulating thrombin transforms it into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top. Platelets are trapped massively in the fibrin meshes (Dohan *et al* 2006a).

Success with the use of PRP led to the development of a material, a second generation platelet concentrate, platelet rich fibrin (PRF) retaining the beneficial aspects of PRP. PRF overcomes the disadvantages of PRP such as manipulation of chemicals, long preparation time and risk of immunogenicity. The clinical potential for bone regeneration with PRP is limited, having a very short release of growth factor profile (Kobayashi *et al* 2016). PRF has been widely used in various treatment procedures (Dohan *et al* 2006a).

In a developing country like Nepal where the prevalence of periodontal disease is very high and with a very low per capita income and limited access to regenerative resources, PRF is a promising form of therapy as it requires inexpensive equipment and armamentarium (Pradhan *et al* 2009, Yee *et al* 2004). A developing country like Nepal lacks resources for manufacture of any kind of regenerative materials and thus relies totally on foreign markets for such materials. The regenerative materials that are available in the

country are all imported increasing the cost. Thus, PRF can be an easily available, cheap and one of the promising forms of therapy in the Nepalese population requiring treatment for periodontal diseases.

How to prepare PRF

Preparation of PRF requires a standard protocol. All available PRP techniques have some points in common. Blood is collected with anticoagulant just before or during surgery and is immediately processed by centrifugation. The time for preparation of the platelet concentrate is variable but is always completed within an hour. A first centrifugation step is designed to separate the blood into three layers, red blood cells (RBCs) are found at the bottom, acellular plasma (PPP, platelet-poor plasma) is in the supernatant and a 'buffy coat' layer appears in between, in which platelets are concentrated. The next steps vary among the numerous protocols but are an attempt to discard both the RBC layer and the PPP to collect only the 'buffy coat' layer. Finally, the obtained platelet concentrate is applied to the surgical site with a syringe, together with thrombin and/or calcium chloride (or similar factors) to trigger platelet activation and fibrin polymerization (Dohan *et al* 2009).

The preparation of PRF is very simple. A blood sample is taken without anticoagulant in 10 ml tube then centrifuged in a table centrifuge at 2700 rpm for 12 minutes. In the absence of anticoagulant, platelet activation and fibrin polymerization are triggered in a natural manner immediately. Fibrinogen is at first concentrated in the upper part of the tube, until the effect of the circulating thrombin transforms it into a fibrin network. The result is a fibrin clot containing the platelets located in the middle of the tube, just between the red blood cell layer at the bottom and acellular plasma at the top (Figure 1).

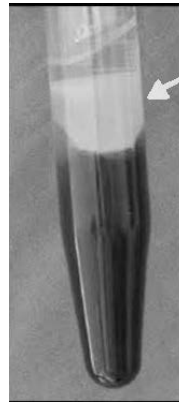


Figure 1. PRF obtained after centrifugation (arrow).

If the duration required to collect blood and launch centrifugation is overly long, failure will occur. The fibrin will polymerize in a diffuse way in the tube and only a small blood clot without consistency will be obtained. It contains cytokines such as IL-1, IL-4, IL-6, and growth factors such as Platelet Derived Growth Factor (PDGF), TGF- β 1 and VEGF (Dohan *et al* 2006b). The combination of fibrins and cytokines within PRF becomes a powerful scaffold with an integrated reservoir of growth factors for tissue regeneration. The fibrin matrix in PRF acts as natural guide for angiogenesis, natural support to immunity and guides the coverage of wounds.

The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. Indeed, without anticoagulant, the blood samples start to coagulate almost immediately upon contact with the tube glass, and it takes a minimum of a few minutes of centrifugation to concentrate fibrinogen in the middle and upper part of the tube. Quick handling is the only way to obtain a clinically usable PRF clot. If the duration required to collect blood and commence centrifugation is too long, failure will occur. The fibrin will polymerize in a diffuse way in the tube and only a small blood clot without consistency will be obtained. By driving out the fluids trapped in the fibrin matrix, practitioners can obtain very resistant autologous fibrin membranes (Dohan *et al*

2006a).

Advantages of PRF over PRP

The time and cost of preparation for PRF are both significantly lower because it does not need any direct activation with additional factors such as bovine thrombin or extrinsic anticoagulants (Choukroun *et al* 2001). Because of its fibrous structure, PRF retains a larger number of cytokines and growth factors in a supportive three-dimensional fibrin scaffold for cell migration (Toffler *et al* 2009). In tissue, PRF dissolves more slowly than PRP, forming a solid fibrin matrix slowly remodeled in the style of a natural blood clot.

Platelets and cytokines are thus effectively retained and released gradually over time (Kobayashi *et al* 2016). The PRF scaffold allows a continuous slow release of growth factors and cytokines over a period of 10 days, in contrast to PRP which has been shown to release most of its growth factors within the first day (Kobayashi *et al* 2016). Therefore, migrating cells near PRF scaffolds are in an environment with fibrin and growth factors throughout their entire growth cycle (Tsay 2004).

There are many advantages of using PRF over the PRP.

1. PRF preparation does not use bovine thrombin or other exogenous activators in the preparation process unlike PRP. The PRF preparation process creates a gel-like matrix that contains high concentrations of non-activated, functional, intact platelets, contained within a fibrin matrix, that release, a relatively constant concentration of growth factors over a period of seven days (Carroll *et al* 2005).
2. It can be squeezed to form a membrane and can be used as fibrin bandage serving as matrix to accelerate the healing of wound edges (Gassling *et al* 2009).
3. The chair side preparation of PRF is

easy and fast and simple processing (Dohan *et al* 2006a). This produces an inexpensive autologous fibrin membrane in approximately 1 minute and hence no cost for membrane and bone graft to the patients.

4. Because of its fibrous structure, PRF retains a larger number of cytokines and growth factors in a supportive three-dimensional fibrin scaffold for cell migration (Toffler 2009).
5. In tissue, PRF dissolves more slowly than PRP, forming a solid fibrin matrix slowly remodeled in the style of a natural blood clot.
6. PRF is not only a platelet concentrate but also an immune node able to stimulate defense mechanisms (David *et al* 2006c).

Thus PRF is a promising, completely autologous leukocyte and platelet concentrate which is being successfully used in various fields of dentistry and medicine. PRF has shown successful results when used as a sole agent in the treatment of periodontal intrabony defects (Agarwal *et al* 2016, Ajwani *et al* 2015).

Types of PRFs

Dohan *et al* (2014) gave the current and most widely accepted classification on platelet concentrates, this classification was an extension of the classification given by Dohan *et al* (2009) and is very simple, and separated the products following at least two key parameters: the presence of a cell content (mostly leukocytes) and the fibrin architecture. This separation allowed defining four main families to regroup the products (Dohan *et al* 2014).

Pure Platelet-Rich Plasma (P-PRP) or Leukocyte-Poor Platelet-Rich Plasma

These products are preparations without

leukocytes and with a low-density fibrin network after activation. All the products of this family can be used as liquid solutions or in an activated gel form thus can be injected (for example in sports medicine) or placed during gelling on a skin wound or suture (similar to the use of fibrin glues).

Leukocyte and Platelet-Rich Plasma (L-PRP)

These products are preparations with leukocytes and with a low-density fibrin network after activation. Like P-PRP, all the products of this family can be used as liquid solutions or in an activated gel form. It can therefore be injected (for example in sports medicine) or placed during gelling on a skin wound or suture (similar to the use of fibrin glues).

Pure Platelet-Rich Fibrin (P-PRF) or Leukocyte-Poor Platelet-Rich Fibrin

These are preparations without leukocytes and with a high-density fibrin network. Per definition, these products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix, they can be handled like a real solid material for other applications. There is only one product in this family, commercially known as Fibrinet PRFM (Platelet- Rich Fibrin Matrix, Cascade Medical, USA).

Leukocyte and Platelet-Rich Fibrin (L-PRF)

These products are preparations with leukocytes and with a high-density fibrin network. These products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix,

they can be handled like a real solid material for other applications.

Some authors are concerned about glass evacuated blood collection tubes with silica particles and have used more biocompatible material titanium for PRF preparation (Takemoto *et al* 2004).

More recently Leukocyte and Platelet-Rich Fibrin (L-PRF) and an injectable formulation of PRF (i-PRF) have been introduced. L-PRF products are preparations with leukocytes and with a high-density fibrin network. These products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix, they can be handled like a real solid material for other applications (Dohan *et al* 2014). Similarly, i-PRF has been developed with the aim of delivering to clinicians a convenient platelet concentrate in liquid formulation. They are prepared using slower and shorter centrifugation speeds and can be either utilized alone or combined easily with various biomaterials (Miron *et al* 2017).

Clinical applications of PRF

PRF is a powerful biomaterial with inherent regenerative capacity and has various clinical applications in periodontology as follows;

1. Intrabony periodontal defects (Ajwani *et al* 2015, Agrawal *et al* 2016).
2. Sinus lift (Tajima 2011, Tajima 2013).
3. Guided bone regeneration and socket preservation (De Risi 2015, Jambhekar 2015).
4. Coverage of gingival recession (coronally advanced or lateral pedicle flap) - acts both as healing and interpositional biomaterial (Eren 2014, Gupta *et al* 2015, Moraschini *et al* 2015).
5. Furcation defect (Bajaj *et al* 2013, Sharma 2011a).
6. Palatal bandage (Kumar 2014).

Limitations of PRF

1. As it is procured from autologous blood in limited quantities, the systemic utilization in general surgery or extensive surgical procedures is limited.
2. Preparation time is relatively short and clinical benefit of PRF depends on speed of handling between blood collection and centrifugation. PRF should be used immediately after preparation as it will shrink.
3. PRF tissue banks are unfeasible. The fibrin matrix contains all the circulating immune cells and all the highly antigenic plasmatic molecules. Thus, PRF membranes are totally specific to the donor and cannot constitute an allogenic graft tissue.

Conclusion

The PRF technique is a simple and inexpensive technique that can be used for the successful regeneration of periodontal tissues. The main advantage is that PRF is from autologous blood thus reducing or eliminating disease transmission through blood and making it one of the cheapest regenerative material and a promising option in biomaterials for periodontal regeneration available to date.

Its advantages include ease of preparation, application, minimal expense, and lack of biochemical modification. It plays a huge role in regeneration during treatment of periodontal diseases in developing country like Nepal where the prevalence of periodontal disease is high and patients are unable to pay for costly regenerative materials.

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Chapter 5

Dental Implants With Periodontal Tissues: Animal Experiments

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Introduction

Osseointegrated dental implants were first introduced by Branemark *et al* (1969) and are now used worldwide to maintain good masticatory function and aesthetics. Today, the osseointegration concept is a well-recognized and established clinical procedure with predictable long-term clinical success.

However, peri-implant complications are reported with high prevalence after five to ten years in use without regular supportive treatment in the mixed dentition (Derks 2016, Koldslund 2010, Lindhe 2008). One of the reasons for these complications has been the characteristics of the implants and the osseointegration process. Host-defense mechanisms against infection may be impaired around implants because they lack a periodontal ligament which possesses a vascular network (Ericsson 1995). Infection around implants can easily extend to the marginal bone. Elimination of causative factors, including bacteria and toxic products, from the implant surface is difficult in daily homecare.

It is proposed that a dental implant with a periodontal ligament will ameliorate such conditions. The periodontal ligament possesses cells with mesenchymal stem cell-like properties. These cells are regarded as useful sources for the construction of periodontal tissues (Giannobile 2010, Seo

2004).

Basic and clinical studies using periodontal ligament cells utilizing “cell sheet engineering” have been carried out for periodontal regeneration (Iwata 2010, Iwata 2015, Tsumanuma 2011).

The cell sheet technique uses a cell culture dish with an intelligent cell culture surface that responds to temperature changes to detach cells (Okano 1993). The application of this technology enables harvesting of cells by reduction of culture temperature without the use of any enzyme. Cell to cell interactions, cell surface proteins, and extracellular matrix proteins are therefore preserved within the sheet. We expected that the cell sheet technology will also be utilized in dental implant treatments.

In this experiment, calcium phosphate (CaP) coating was conducted as an implant surface treatment in addition to blasting and acid etching. CaP was coated by immersion of implants in Hanks' solution. The deposited CaP was stable and protective and was expected to improve cell adhesion onto the titanium surface (Tsutsumi 2009).

In vitro experiments were carried out to enable study of gene expression of cementum related genes in cultured periodontal ligament cells and the adhesion of periodontal ligament cells to the titanium implant surface, with or without treatments.

To form periodontal ligament on implant

surfaces, *in vivo* transplantation experiments in rats and dogs were conducted. The surface treated implants were wrapped with periodontal ligament cell sheet and then inserted into bone sockets prepared in rats or dogs. In the rat experiments, we confirmed the titanium surface induced periodontal formation from the periodontal ligament cell sheet regardless of treatments. In the dog experiments, the surface treated implants, with or without cell sheet, was transplanted into bone to elucidate the effect of the periodontal ligament cell sheet on periodontal ligament formation.

Materials and methods

Animals

Athymic rats (F344/NJcl-rnu/rnu, five males, five weeks old) were used for the experiments studying transplantation of titanium and cell sheets to bone marrow cavity. Beagle dogs (one male, two females, eight months old, mean weight of almost 10 kg) were used for the experiments studying cell attachment to titanium surface and transplantation of titanium and cell sheets to mandibular bone defects. All experimental protocols were approved by the animal welfare committee of the Tokyo Women's Medical University.

Human samples

The institutional review board of the Tokyo Women's Medical University approved the collection of human wisdom teeth which were treated as clinical waste and use of the cells from the teeth for this experiment.

Isolation, culture and stock of human periodontal ligament cells and cell sheet

Human periodontal ligament cells were harvested from the donor teeth which were extracted for a variety of various reasons (Washio 2010). Extracted teeth were rinsed five times with α -Minimum Essential Medium (Life Technologies, Carlsbad, CA, USA) containing 100 U/mL penicillin and 100 μ g/mL streptomycin (Sigma Aldrich, St. Louis, MO) for three minutes each. Periodontal ligament tissues were gently removed from the surface of the mid-third position of the extracted root by scalpel. The tissue was subjected to collagenase/dispase treatment by shaking intensively at 37°C for 45 minutes. The cells were cultured at 37°C with minimum essential medium alpha containing 10% fetal bovine serum and 1% penicillin streptomycin, and medium change or passage using 0.25% Trypsin-1mM EDTA was conducted every three to four days. At passage number three, the cells were cryopreserved with cell freezing medium (CELLBANKER) at -150°C until use.

The cryo-preserved periodontal ligament derived cells were thawed two weeks before the transplantation and were cultured with basic medium to reach the confluent. For production of the cell sheets, the human periodontal ligament derived cells were cultured on 35 mm temperature-responsive culture dish which was coated with poly (N-isopropylamide) on the surface (UpCell) at a density of 4×10^4 cells/dish. The culture medium was changed with basic medium after two days to osteoinductive medium which contained 82 μ g/ml L-ascorbic acid phosphate magnesium salt n-hydrate, 10 nM dexamethasone and 10 mM β -glycerophosphate to basic medium. The medium was changed twice a week and the cells were incubated for almost ten days.

Isolation, culture and stock of dog periodontal ligament cells

Dog periodontal ligament derived cells were harvested from extracted mandibular premolars under the general anesthesia. Before the tooth extraction, the dogs were intramuscularly injected with 0.08 mg/kg medetomidine and 0.3 mg/kg midazolam for anesthetic premedication and then subjected to an intravenous injection of 1 to 2 mg/kg propofol. An endotracheal tube was inserted and anesthesia was maintained with 2 to 4% sevoflurane. Local anesthesia was injected with 2% lidocaine hydrochloride containing epinephrine at a concentration of 1:80000 xylocaine cartridge to prevent bleeding. The method of harvesting cells, culture, and cryopreservation was the same as the method for human periodontal ligament cells as described above. Almost two weeks before the implantation, the frozen dog periodontal ligament cells were thawed and cultured in a basic medium. After subculture, the cells were plated at 4×10^4 cells/UpCell (35 mm dish). The medium was changed to a osteoinductive medium every three or four days. After the additional culture for five to seven days, canine periodontal ligament cell sheet was obtained (Tsumanuma 2011).

Gene expression of cementum markers in cultured human periodontal ligament derived cells

Human periodontal ligament cells (passage six) were cultured into a six-well plate at a density of 3×10^4 cells/well for two days in basic medium, and further cultured with osteoinductive medium for 14 and 21 days. Total RNA was isolated using the QIA shredder and RNeasy Plus Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. For the TaqMan gene expression assay, cDNA was synthesized from 500 ng of

total RNA using the SuperScript VILO cDNA Synthesis Kit (ThermoFisher Scientific). Real-time PCR using StepOnePlus Real-Time PCR system (ThermoFisher Scientific) was performed in triplicate using cementum protein 1 (CEMP1; Hs04185363_s1) probe, bone sialoprotein (BSP; Hs00173720_m1) probe, and b-actin (43263215E) probe (ThermoFisher Scientific). Messenger RNA expression levels relative to b-actin were determined and fold changes were calculated using the values obtained by the Δ CT method (Livak 2001).

Titanium specimen

Commercially pure titanium (grade 2) was used in this study. For the *in vitro* experiments, the titanium foil (10 μ m thickness) was cut into circular shapes with 5 mm diameter. For the experiment studying the transplantation of implants into the bone marrow cavity of rat femur, titanium rods (1 mm in diameter, 2 to 3 mm in length) were used. For the experiments studying transplantation of implants into bone sockets in canine mandibles, a shallow conical shape of titanium with a diameter of 3.5 mm in the upper side and 3 mm in the lower side, and 8 mm in length was used. This configuration is similar in size to narrow diameter implants but does not possess screw threads.

To improve cell attachment, all shapes of titanium specimens in the experimental groups were treated by blasting, acid etching, and CaP coating. For the CaP coating the titanium specimens were placed in the Hanks solution (ThermoFisher Scientific, USA) and then kept immersed at 37°C for 378 hours. The titanium was washed with 70% ethanol and subjected to autoclave treatment before use.

All the titanium specimens were washed with phosphate buffer saline (PBS) or saline solution (Otsuka Pharmaceutical Factory, Inc., Japan) for five minutes twice before use.

Cell attachment to titanium surface

After trypsinization of canine periodontal ligament cells (passage five), the cells were split into 1.5 mL tubes (Eppendorf, Hamburg, Germany) at density of 4×10^5 cells/500 μ L medium and then titanium foil was soaked in the tube. Titanium foil without surface treatments was used as control. The tubes with cells and titanium foil were incubated at 37°C for 20, 40, 60, and 120 minutes. After the incubation, the number of attached cells on titanium foil was calculated by subtraction from 4×10^5 to number of remaining cells in the tube.

Combination of titanium rod / implant and periodontal ligament cell sheets

The cell sheets were obtained by reduction of culture temperature from 37°C to room temperature. The titanium was wrapped with the three-layered cell sheets. The complex of titanium and cell sheets was incubated with medium at 37°C for more than one hour before transplantation and washed with PBS just before use.

Transplantation of titanium rod and cell sheets to bone marrow cavity of rat femur

Under anesthesia with 2 to 3% isoflurane, small incision was made in the leg skin of the rat with a scalpel and the knee joint was exposed by moving the rectus femoris muscle to the outside of the knee with a dental excavator. A bone defect (2 to 3 mm diameter) was made with a dental bur on the intercondylar fossa of the rat femur. After washing the defect with saline solution, the complex of titanium rod with or without surface treatments and cell sheets was inserted into the bone marrow cavity. The moved muscle was repositioned to the knee joint and

the incised skin was sutured. After six weeks of transplantation, the femur was subjected to histological analysis.

Transplantation of the complex of titanium implant and cell sheets into canine mandibular bone and histological preparation

The complex of titanium implant and cultured cell sheet was inserted into dog mandibular bone defects. A bone defect (about 4.5 mm in a diameter and about 10 mm in length) was prepared under anesthesia in the alveolar bone. The defect was formed slightly larger than that of the titanium rod in diameter and length. The complex was inserted into the defect followed by covering with titanium mesh (Bone Plate®, Jeil Medical Corporation, Korea) and firmly suturing the gingival flaps. In control groups, the implant with surface treatment was inserted without cell sheets. Eight to eleven weeks after the transplantation, the dogs were sacrificed by potassium chloride (Terumo, Tokyo, Japan) under anesthetic conditions the same as used for tooth extractions. The inserted titanium and covering tissue was removed together with the bone as a specimen for histological analysis.

Tissue preparation and histological observation and measurement

Histological preparation was conducted at commercial laboratory (Kureha Special Laboratory, Tokyo, Japan). Briefly, the specimen was dehydrated with 70% ethanol and embedded in methylmethacrylate resin for non-decalcified grind section. The sections were stained with Villanueva Goldner method which stained green in hard tissue and stained red in others (cells, fibers, osteoid, etc). The light microscope (Eclipse E800, Nikon, Japan) was used for observation and the

imaging software (NIS-Elements D, Nikon) was used for image capture and histological measurement.

Statistical analysis

All data are expressed as mean \pm standard deviation. The histological measurement and statistical analysis of PCR data were conducted using Student's t-test. The data from experiment of cell attachment to titanium surface was analyzed by one-way repeated analysis of variance followed by Fisher's protected least significant difference post hoc test. A p-value <0.05 was considered statistically significant.

Results

Expression of cementum gene markers in cultured periodontal ligament derived cells
To confirm the ability of cementum

induction in obtained periodontal ligament derived cells, the gene expression of CEMP1 and BSP was observed at day 14 and 21 of culture with osteoinductive medium in human periodontal ligament derived cells. The level of gene expression of CEMP1 in periodontal ligament derived cells was low at day 14. However, the CEMP1 expression level at day 21 was increased up to almost ten-fold compared with day 14. The expression level of BSP was also increased at day 21, which shifted similar to that of CEMP1. Both genes were upregulated by osteoinductive culture in periodontal ligament derived cells.

Attachment of periodontal ligament derived cells to titanium with surface treatment

To investigate the most appropriate titanium surface for cell attachment, dog periodontal ligament derived cells were

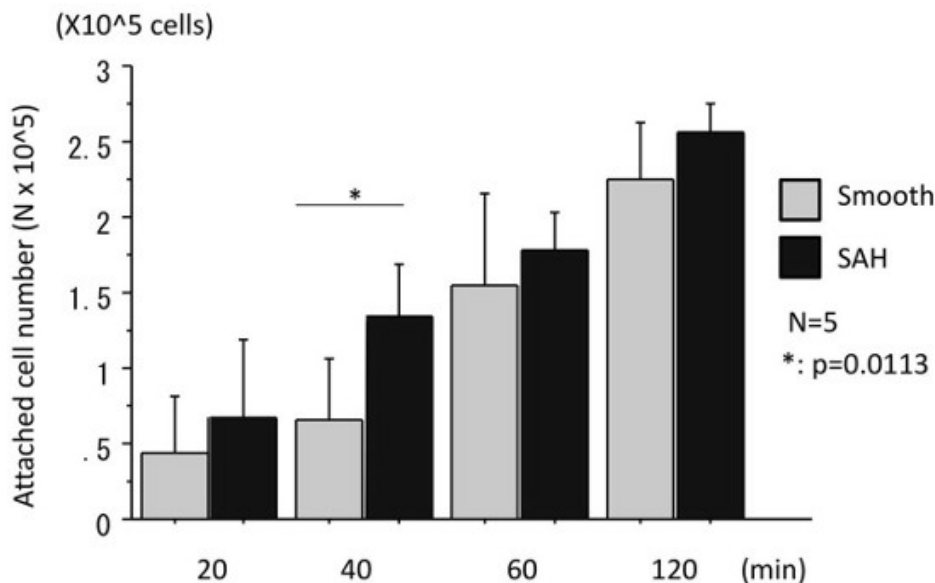


Figure 1. Periodontal ligament derived cells attached quickly to titanium with surface treatment. The number of attaching cells to titanium surface with or without treatments of acid etching, blasting, and CaP coating. Time points shows cultivation period with cells and titanium. CTL shows control group using titanium without surface treatments; EXP shows experimental group using titanium with surface treatments. * shows p-value <0.01

incubated with titanium foil with or without surface treatments. The number of attached cells on both titanium surfaces was increased in a time-dependent manner, and more than half of the spread cells were attached at 120 minutes on both surfaces. It is notable that the time point at which cell attachment started on the treated titanium surfaces was statistically significantly as compared with the control surfaces ($p < 0.01$) (Figure 1).

Transplantation of titanium and human periodontal ligament derived cell sheets in bone marrow cavity of rat femur

To confirm the cell attachment and regeneration of periodontal-like tissues on

the titanium *in vivo*, we transplanted the complex of titanium rod and periodontal ligament derived cell sheets into the bone marrow cavity of rat femurs for six weeks. In the experimental group (complex of titanium rod with surface treatments and periodontal ligament derived cell sheets), some spaces were formed between titanium and new bone as compared with control group (Figure 2). The regenerated periodontal-like tissue contained cementum-like hard tissue and periodontal ligament-like fibrous tissue was observed on the titanium surface inside the space. Osseointegration, which is a commonly observed process in current implant therapies, was observed only partially for the experimental group. In the control

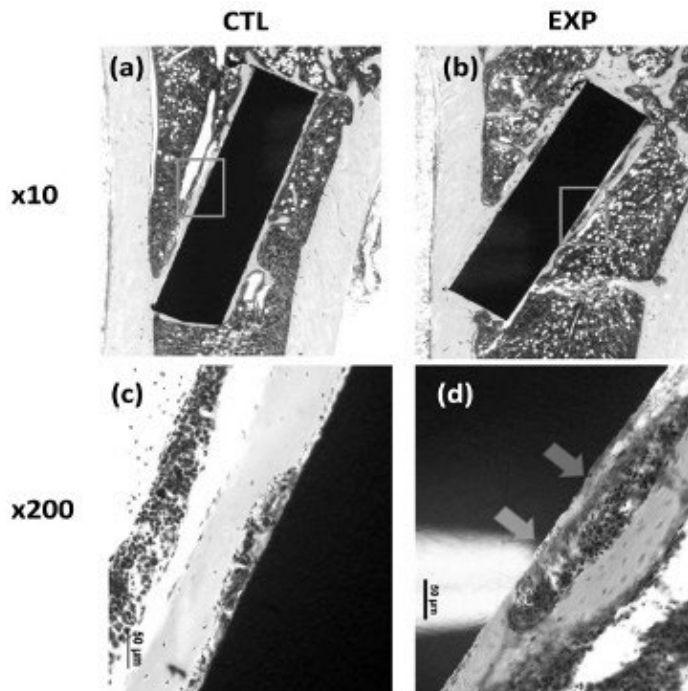


Figure 2. Transplantation of titanium and human PDL derived cell sheets in rat bone marrow cavity model induced periodontal regeneration on the titanium surface. Formation of PDL-like tissue on the titanium surface with treatments was increased than control surface, but these differences were not statistically significant. (A) Histological result of transplantation the complex of titanium without surface treatment and human PDL derived cell sheet for 6 weeks (control). (C) Magnified image of square area of (A). (B) Histological result of transplantation the complex of titanium with surface treatment and human PDL derived cell sheet for 6 weeks (experimental group). (D) Magnified image of square area of (B).

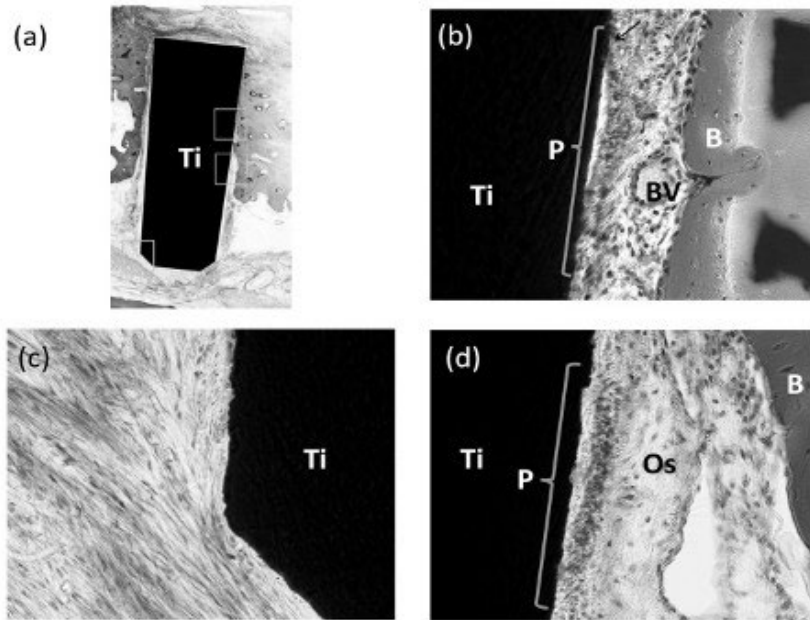


Figure 3. Transplantation of the complex of titanium and cell sheet to the mandibular bone defects in dog. Histology of transplanted tissues and surrounding tissue in experimental group transplanted with titanium with surface treatment and dog PDL derived cell sheet. (A) Total image. (B-D) Magnified images. B, alveolar bone; BV, blood vessel; Os, osteoid-like tissue; P, PDL-like tissue; Ti, titanium.

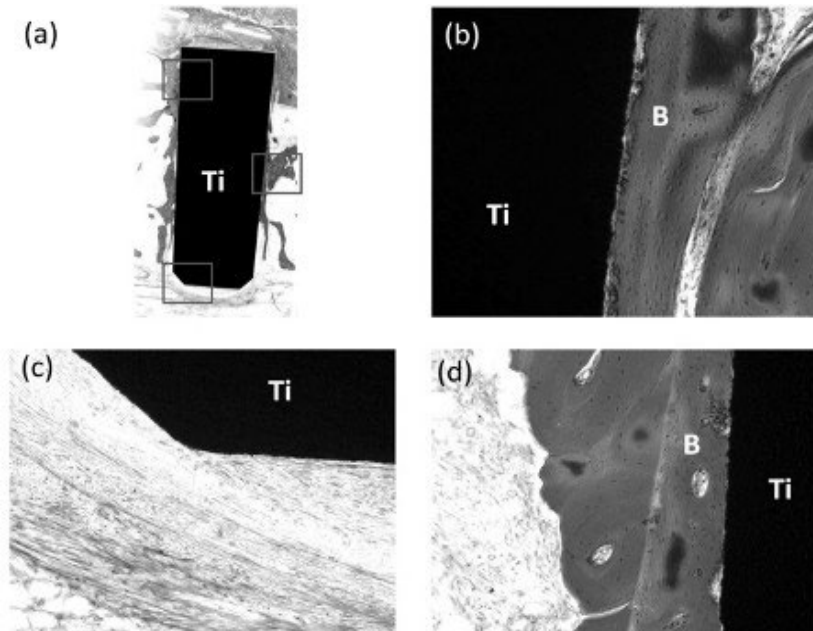


Figure 4. Transplantation of titanium alone to dog mandibular bone defects (control). Histology of transplanted tissues and surrounding tissue in control group, which was transplanted with titanium with surface treatment and without cell sheet. (A) Total image. (B-D) showed magnified images. B, alveolar bone; Ti, titanium.

group, osseointegration was seen on the titanium surface, and some spaces were formed between titanium and new bone, which were filled with bone marrow cells.

The length of attached cell sheet and regenerated periodontal ligament-like tissue around the different treated titanium surfaces was measured. Cell sheets attached more broadly onto the titanium surface with treatments compared with control surfaces. Consequently, periodontal ligament-like tissue formed on titanium surfaces with treatments was increased compared with control surfaces, however these differences were not statistically significant.

Transplantation of complex of titanium and canine periodontal ligament derived cell sheet

For the clinical assessment of this method, adult dogs were used in this study. At the first and second trials, four complexes of titanium implants with surface treatments and periodontal ligament cell sheets were transplanted into bone defects created in the dog mandibles. However, only one implant was successfully retained in fixation with all other implants rejected from the bone defect at eight weeks post-transplantation. To improve the outcomes, transplanted complexes were covered with titanium mesh over the bone before covering with the gingival flap. As a result, all transplanted complexes were successfully retained in fixation for 11 weeks. Finally, three implants with surface treatments wrapped with periodontal ligament cell sheets as experimental group and two implants with surface treatments without cell sheets as control group were successfully retained in fixation inside the bone defect.

In the experimental group, two out of three implants had attached cell sheets around their titanium surface. Histological assessment at 8 or 11 weeks post transplantation, demonstrated

soft tissue was formed between the titanium and alveolar bone (Figure 3). At the area away from bone, fibrous tissue surrounded the titanium. At the area closer to bone, periodontal ligament-like tissue was perpendicularly arranged onto the titanium surface, with blood vessel (BV) and cementum-like calcified tissue (arrow) clearly observed.

In the control group, osseointegration was observed on the titanium surface (Figure 4). The bottom of titanium rod was surrounded with fibrous tissue.

Discussion

The existence of a periodontal ligament around teeth has the advantages of protection against infection and bone resorption from mechanical stress, such as occlusal loading and orthodontic tooth movement, which leads to maintenance of alveolar bone height (Nakajima 2016).

Periodontal ligament derived cells possess the ability to contribute to periodontal regeneration through cementum induction. CEMP-1 has been identified as one of the cementum marker genes expressed by cementoblasts, periodontal ligament cells and other cells located around the vascular network of the periodontal ligament (Alvarez-Perez 2006, Hoz 2012). In this study, CEMP-1 expression was noted in periodontal ligament cells cultured with osteoinductive medium for 21 days. These results suggest that the cell sheets possess the ability to induce cementum after transplantation. Some studies have indicated that CEMP-1 expression in human periodontal ligament cells can be changed by varying culture conditions (Gauthier 2017, Komaki 2012). Thus, further investigations will be necessary to find the most suitable medium condition in order to accelerate cementum formation along with periodontal ligament formation on the titanium implants.

The possibility of new periodontal formation

around implants has been investigated since 1990. The initial observation suggested it was possible to achieve anchorage of dental implants with a periodontal ligament (Buser 1990). Subsequently, many studies have investigated dental implants combined with periodontal tissue (Choi 2000, Jahangiri 2005, Parlar 2005). Most of these studies have involved cell culture methods and animal models using bio-engineering approaches and tissue engineering approaches (Gault 2010, Lee 2017, Lin 2011, Nakajima 2016, Oshima 2014).

The suitable attachment of cells and a titanium surface are necessary for biological implant surfaces. We used “cell sheet engineering” in this study. This technology enabled us to produce tissue from cells without any scaffolds (Okano 1995, Yamato 2001). The efficacy of periodontal ligament cell sheets cultured with osteoinductive medium for periodontal regeneration on dentin has been reported previously.

Recently, it was reported that transplanted periodontal ligament cell sheet with CaP coated polycaprolactone scaffold into a rat periodontal defect model promoted new periodontal attachment formation (Dan 2014).

In this study, we treated the titanium implant surface with CaP in addition to acid-etching and blasting to improve cell attachment. It has been reported previously that CaP coating can accelerate calcification by osteoblasts in a scaffold (Vaquette 2012, Vaquette 2013). This improvement may induce the formation of cementum-like tissue on titanium surfaces. The structure that cementum-like layer anchored periodontal ligament-like fibrous tissue was similar to natural periodontal tissue.

In the rat experiments, the differences in cell sheet covering ratio and periodontal ligament formation ratio between control and experimental groups were not statistically significant. This result may be due to

variability in measurement data. The location and mobility of implant in femur also could have influenced the stability for attachment of cell sheet and periodontal regeneration on the titanium surface.

We also carried out studies in dogs using our developed technology to validate this method for future clinical settings.

To exclude the effect of periodontal formation from surrounding tissue, periodontal ligament cell sheets were not transplanted for the control group. In the experimental group, one sample failed to have the cell sheet fixed onto the implant. This may have been caused by the short adhesive time and mismatch of defect size and implant size. The results suggested that stabilization of cell sheet on titanium surface is important for periodontal formation around titanium implants.

In the future the clinical application of implants with a periodontal ligament is expected as an alternative therapeutic method for replacing missing teeth.

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Chapter 6

Proactive Prevention Is the Key to Global Periodontal Health

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Introduction

Periodontal disease (gingivitis and periodontitis) continues to attract the attention of oral and other healthcare professionals, governments, healthcare payers and providers, social media and the public. This has become increasingly apparent over the last decade, due to the extremely high prevalence and global disease burden, marked effects on oral health, intimate links to systemic diseases, and the resultant huge socio-economic impacts of periodontal disease (Chapple 2014, Jin *et al* 2011, Kassebaum *et al* 2014, Listl *et al* 2015, Marcenes *et al* 2013, Petersen and Ogawa 2012, Pihlstrom *et al* 2005, Tonetti *et al* 2017, Tonetti and Kornman 2013). Furthermore, periodontal disease significantly affects an individual's oral function, general well-being, quality of life and self-esteem. Importantly, it is evident that a majority of the adult populations affected by periodontal disease are not aware of their condition due to the relatively 'silent' nature of the disease, and therefore do not receive the necessary care and treatment to manage this disease. Additionally, self-neglect of oral/periodontal health, and a number of socio-economic factors affecting access to professional care (e.g. unavailability of oral healthcare manpower, lack of oral/periodontal care strategies in public health policies and unaffordability of treatment costs) contribute to a very low level of

periodontal care in many populations (Chan *et al* 2017, Chapple 2014, Jin *et al* 2011, Jin 2015). Accordingly, prevention is advocated as the key to improving global oral health (Lancet 2009). The development of strategies to improve oral health and application of the "Common Risk Factor Approach" are considered to be crucial elements for tackling the serious problems of periodontal disease (FDI World Dental Federation 2013a, FDI World Dental Federation 2013b, Glick *et al* 2016, Jin *et al* 2016, Petersen and Ogawa 2012, Tonetti *et al* 2017).

This paper critically addresses the global burden of periodontal diseases, highlights the key issues of effective promotion of periodontal health, and outlines new opportunities to undertake proactive prevention strategies for the management of periodontal disease via global action and inter-professional teamwork for optimal oral health, general health and wellbeing over an individual's lifespan.

Global burden, challenges and socio-economic impacts of periodontal disease

Interestingly, the Guinness World Records book (2001) ranks periodontal disease as the most common disease affecting humans. More recently, periodontitis has been identified as the most prevalent chronic inflammatory disease in humans (Chapple

2014). Major epidemiological studies have demonstrated that the global prevalence of severe periodontitis is estimated to be 11.2% accounting for some 743 million people globally being affected by this serious oral disease (Kassebaum *et al* 2014). Furthermore, periodontitis has been listed as the sixth most prevalent disease affecting humans among all 291 diseases included in the Global Burden of Disease (GBD) study (Kassebaum *et al* 2014, Marcenes *et al* 2013, Murray *et al* 2012). Indeed, this authoritative epidemiological GBD study demonstrated that the global burden of periodontal disease, as measured by the disability-adjusted life-years, greatly increased by 57.3% from 1990 to 2010. This compares to leading life-threatening diseases such as diabetes mellitus (DM) and cardiovascular disease (CVD) that increased by 69% and 22.6% respectively (Ezzati and Riboli 2012, Jin *et al* 2016, Murray *et al* 2012). Importantly, DM and CVD are two major non-communicable diseases (NCDs) closely linked with periodontal disease through common underlying pathological pathways including infection, inflammation, dysbiosis and shared common risk factors such as smoking and obesity (Jin *et al* 2016, Pihlstrom *et al* 2005, Tonetti and Kornman 2013, Virto *et al* 2017). In addition to DM and CVD, periodontal disease has been shown to be associated with many other systemic diseases and disorders including pulmonary disease, stomach disease, chronic kidney disease, rheumatoid arthritis, cognitive impairment, some types of cancer (e.g. oesophageal and pancreatic cancers), metabolic syndromes, adverse pregnancy outcomes, sleep disorders, and depression (Chan *et al* 2017, Holmstrup *et al* 2017, Jin *et al* 2016, Sanders *et al* 2015, Sundararajan *et al* 2015, Tonetti and Kornman 2013). From a public health perspective, periodontitis is truly a very serious problem, being the leading cause of severe tooth loss in adults worldwide leading to disability

of masticatory and other oral functions, compromised nutrition, general impairment of health and wellbeing, poor quality of life and low self-esteem (Jin *et al* 2016, Shanbhag *et al* 2012, Tonetti *et al* 2017).

Periodontal disease and its resultant consequences also account for a large global socio-economic burden and unacceptable high healthcare costs. For example, around £2.8 (\$4.6) billion has been spent on periodontal care through the UK National Health Service in 2008 (Chapple 2014, PR Newswire 2008). In Malaysia, it has been estimated that the total periodontal care costs would be 32.5 billion Ringgit (~ \$10.5 billion equivalent to 3.83% of the GDP) in 2012, if all periodontitis patients could have been possibly treated (Mohd Dom *et al* 2016). Furthermore, it has been documented that the estimated global indirect cost of productivity losses due to severe periodontitis and tooth loss was as high as \$117 billion in 2010 (periodontitis: \$54 billion; tooth loss: \$63 billion), thereby contributing substantially to the total cost of \$442 billion for management of all oral diseases and oral healthcare (Listl *et al* 2015).

Currently, the epidemic of NCDs is a global health and socio-economic crisis (Beaglehole *et al* 2011). In this regard, periodontal disease, as a major oral NCD, contributes significantly to oral healthcare costs worldwide (Listl *et al* 2015, Tonetti *et al* 2017). The current global healthcare outlook from Deloitte predicts that there will be markedly increased demand and costs, due to the aging population worldwide, rising prevalence of NCDs, high expenses on medical innovations, enhanced patients' awareness and healthcare knowledge, and unpredictable economic status (Deloitte 2017). This report strongly recommended investment in health promotion and disease prevention. Clearly an investment in oral health and periodontal health will be of fundamental importance for tackling the global epidemic and burden of periodontal

disease.

Main barriers, key issues and strategies on proactive prevention of periodontal disease

Globally, periodontal disease has largely been ignored and awareness of periodontal health is low. This may be due to the relatively 'silent' nature of the disease resulting in the disease remaining largely untreated for many years, general self-neglect of periodontal health, limited access to professional care, and a lack of oral/periodontal care strategy in health policies (Chan *et al* 2017, Chapple 2014, Jin 2016). Notwithstanding these problems, a significant number of patients make 'symptom-driven' dental visits, seeking for treatment of the complications and/or consequences of uncontrolled severe periodontitis, such as adjunctive orthodontic treatment of periodontally involved pathologic tooth migration, and replacement of missing teeth with dentures or implant supported prostheses. Unfortunately, dentists often pay less attention to proactively managing the long-term periodontal problems associated with remaining teeth (Jin 2016). A previous report from the American Academy of Periodontology (AAP) reveals that periodontitis is the top cause of tooth loss that drives patient requests for dental implant treatment.

Therefore, low awareness of oral/periodontal health and disease is one of the main challenges facing all dental professionals, policy-makers and the public. Barriers for implementing effective prevention measures of periodontal disease include ignorance of periodontal disease and its consequences, lack of oral/periodontal health promotion strategies, policies and effective care schemes, no or limited access to oral healthcare systems, inadequate educational resources and continuing professional development

programs, limited incorporation of oral/periodontal healthcare strategy into total healthcare regime as well as other mindset and socio-cultural-economic factors (Jin *et al* 2011). A recent study demonstrated that the main reason for dental non-attendance throughout the lifetime of Europeans is the personal perception of dental care/treatment to be 'not necessary' or 'not usual' (Listl *et al* 2014).

Ample scientific evidence shows that oral/periodontal disease and other major NCDs share a number of common risk factors, e.g. smoking and obesity (Jin *et al* 2011, Petersen and Ogawa 2012, Sheiham and Watt 2000, Tonetti *et al* 2017). This has been specifically highlighted and promoted by the United Nations/World Health Organization (WHO) and FDI for tackling all NCDs (FDI 2013a, Petersen and Ogawa 2012, United Nations 2011). As such, the emerging concepts and integrated care strategy of oral/periodontal health and general health through the "Common Risk Factor Approach" have shed new light on proactive prevention of oral/periodontal disease and other NCDs. The Oxford Dictionary defines 'proactive' as 'creating or controlling a situation rather than just responding to it after it has happened' (<https://en.oxforddictionaries.com/definition/proactive>). In general, 'proactive prevention' means 'the control of the external stressor that is a risk factor for disorder' (Catalano and Dooley 1980). Leading dental organizations such as the IADR and FDI have proactively recommended critical strategies and actions for tackling the global burden of oral diseases. The IADR has strategically initiated the formulation of research agendas for reduction of global oral health inequality between and within countries (Sgan-Cohen *et al* 2013, Williams 2011). Meanwhile, the FDI has strongly advocated for improvement in global oral health through proactive education approaches, integrated oral and general

healthcare approaches and expanded roles for dental professionals. These approaches have been promoted through the FDI's Vision 2020 strategy, relevant global policy statements, and the establishment of World Oral Health Day since 2013 with annual themes of 'Healthy Teeth for a Healthy Life' (2013), 'Celebrating Healthy Smiles' (2014), 'Smile for Life!' (2015), 'Healthy mouth. Healthy body' (2016), and 'Live MOUTH SMART' (2017) (FDI 2013a, FDI 2013b, FDI 2017a, Glick *et al* 2012, Jin 2013, Wong and Eiselé 2015).

For health care professionals, enhanced dental-medical collaborations and good teamwork for more cost-effective healthcare delivery need to be developed through co-management schemes as well as two-way referral and patient-centered compressive care approaches for achieving optimal oral/periodontal and general health and healthy aging (Holmstrup *et al* 2017). In daily dental practice, it is well recognized that proactive periodontal care is the foundation of general dental care. To achieve this the following essential issues have been actively addressed:

1. Periodontal screening, risk assessment, diagnosis and prognosis.
2. Formulation of individualized treatment plan and appropriate elaboration of care options.
3. Undertaking well-sequenced treatments via proactive care and multidisciplinary approaches.
4. Long-term regular supportive care (Jin 2016).

Oral health education and routine periodontal care should target plaque biofilms and periodontal/peri-implant inflammation as well as control of environmental and host risk factors (Bartold and Van Dyke 2017, Darveau 2010, Marsh and Devine 2011). Furthermore, proactive promotion of no smoking/smoking cessation and healthy lifestyle is essential. Notably, there are recommended foods that

may reduce inflammation (e.g. good oils, fish, nuts, fruits and tea) (Krans and Kinman 2015). Moreover, accumulating evidence shows close oral-systemic connections that have well defined underlying biological plausibility and that periodontal intervention may bring overall health benefits to patients. It is known that effective periodontal care of patients with chronic periodontitis can improve both glycemic control in Type 2 DM patients and endothelial cell function (Lalla and Papapanou 2011, Teeuw *et al* 2010, Tonetti *et al* 2007). Our recent clinical trial showed that periodontal treatment can significantly reduce immuno-inflammatory response in endothelial progenitor cells from Type 2 DM patients, and this finding contributes to further understanding of underlying mechanisms related to the favorable therapeutic effect on endothelial function (Wang *et al* 2017). Furthermore, a systematic review has demonstrated that effective periodontal care improves patients' oral health-related quality of life (Shanbhag *et al* 2012).

Call for global action and teamwork: An update and perspectives

It is now an exciting time to advocate for periodontal health worldwide, thanks to the new initiatives and opportunities to engage in proactive prevention of periodontal disease via a global action and inter-professional teamwork. It is notable that the first periodontal green paper for stakeholder consultation circulated by European Federation of Periodontology (EFP) has been enthusiastically endorsed or supported by leading international organizations in periodontology, including EFP, AAP, International Academy of Periodontology, Asian Pacific Society of Periodontology and Ibero-Panamerican Society of Periodontics as well as 46 national societies of periodontology from both developed and developing countries

(European Federation of Periodontology 2016, Tonetti *et al* 2017). This is the first global consensus regarding identification of actionable preventive, diagnostic and therapeutic strategies to promote periodontal health and general health, in line with UN/WHO's current policy on prevention and control of NCDs via the "Common Risk Factor Approach". This landmark paper highlights ten key recommendations for proactive prevention of periodontal disease, two practical approaches to periodontal diagnosis for early detection and staging disease severity as well as 14 overarching recommendations and priorities for treatment strategies (Tonetti *et al* 2017).

Coincidentally, the FDI has recently launched the Global Periodontal Health Project that was initiated by the FDI Science Committee and approved by the FDI Council, aiming for 'Achieving global periodontal health' and 'Reducing the global burden of periodontal disease' (FDI 2015). Notably, the World Oral Health Forum during the 2017 FDI World Dental Congress in Madrid, Spain timely set the theme of "Global Periodontal Health: Challenges, priorities and perspectives" (FDI 2017b). Taken together, these new initiatives and global actions will enhance periodontal awareness and a proactive mindset for prevention and optimal oral health for people worldwide. Hence, a new definition of oral health is needed, and this new concept well reflects the multifaceted nature of oral health with three core elements including absence of diseases/conditions, normal physiological function and psycho-social well-being (Glick *et al* 2016, Lee *et al* 2017). This definition acknowledges the value of health promotion and proactive prevention for general well-being and quality of life. The recently proposed measures of optimal oral healthcare consisting of access to dental care, good diagnosis, management of health behaviors, oral treatments, oral prevention

and patient perception), may facilitate global implementation of proactive prevention and oral/periodontal care for achieving the common goal of oral health care professionals (Baådoudi *et al* 2017).

Summary

Periodontal disease remains a major global health burden, and oral/periodontal healthcare is crucial for general wellbeing, quality of life and healthy aging. Oral/periodontal diseases share many risk factors with other NCDs, e.g. tobacco usage, excess sugar consumption, obesity and unhealthy lifestyle. It is time to incorporate proactive prevention and oral/periodontal care into total health care regime integrated with agendas of NCDs, via the "Common Risk Factor Approach". These new initiatives and strategies on oral/periodontal health reshape the future development of oral health care professionals for achieving the ultimate goal of optimal oral/periodontal health and general health, through a global teamwork action.

Acknowledgements

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Chapter 7

Implant Placement in Grafted Sites and Near Vital Structures. Resolving Challenges Using CBCT Imaging: Two Case Reports

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Introduction

More than half a century ago, titanium implants were inserted into the bones of the jaw, and artificial teeth were attached onto them. An adequate amount of bone is needed to support the implant. There are cases where the existing jawbone is not sufficient to receive an implant. Therefore, bone augmentation is done in preparation for implant placement. There are also cases, where vital structures are in close proximity to the intended implant site.

The nasopalatine foramen is also referred to as the incisive foramen (Mraiwa *et al* 2004). There are two lateral canals within the foramen, which are called incisive canals or foramina of Stenson. They transmit the anterior branches of the descending palatine vessels and the nasopalatine nerves (Greenstein *et al* 2006). Occasionally, one to four canals may be present. The nasopalatine foramen is ~4.6 mm wide and located ~7.4 mm posterior to the labial surface of an unresorbed ridge (Mraiwa *et al* 2004). The nasopalatine canal (mean length 8.1 mm) exits the incisive foramen. A large incisive canal may be an obstacle to implant placement in the central incisor region (Greenstein *et al* 2006). When a large canal was present, Artzi

et al (2000) displaced its contents (moved it over without elimination) and placed an implant. In contrast, Rosenquist and Nystrom (1992) enucleated the canal, inserted a bone graft, and subsequently placed an implant. It is also often possible to angle an implant and avoid the canal.

During surgery in the nasopalatine area, some surgeons make an incision around the incisive papilla to avoid cutting the contents of the nasopalatine canal (Sclar 2003).

Careful evaluation is necessary when central incisors are restored with implants. High resorption rates often occurred post extraction (Irinakis 2006, Moya-Villaescusa and Sanchez-Perez 2010). Insertion of implants into the incisive canal may lead to contact of implants with nervous tissue and cause non-osseointegration or lead to sensory alteration (Artzi *et al* 2000, Casado *et al* 2008).

Mardinger *et al* (2008), evaluated the radiologic changes of the measurements of the nasopalatine canal in different resorption phases of the premaxilla alveolus with regard to dental implantation. They showed that in severely resorbed ridges (classes C, D and E, based on Lekholm and Zarb's (1985) classification of the residual bony ridge),

when the palatal canal opening was on the ridge, it occupied a mean of 36.5% (13% to 58%) of the area of the ridge devoted to implant placement in the central position. During osteotomy, when the canal has been penetrated, an augmentation procedure should be performed. The contents of the canal can be removed or displaced prior to placement of the graft (Mardinger *et al* 2008).

Bone loss due to cancer poses a challenge for surgical reconstruction. Although reconstruction of soft tissue defects requires a fasciocutaneous or musculocutaneous flap, composite tissue loss that includes bone should be managed with a flap that contains vascularized bone (Peled *et al* 2005).

The fibula free flap provides a strong long segment of bone and can include a large fasciocutaneous component as well. This versatile flap may be harvested as an osteocutaneous flap or a purely osseous flap (Peled *et al* 2005). The fibula flap provides a successful bone graft with an acceptably low complication rate (Chepeha *et al* 2004). Fibular bone allows to plan osteotomies in relation to the orientation of the bone and its vascular pedicle. Thick cortical bone readily accepts plates and screws for a secure interosseous fixation and osseointegrated implants may be placed in this bone safely (Aydin *et al* 2004).

Immediate implantation may be unsafe for the vascularization of the graft, although other authors have asserted this possibility for both the fibula, and other free bone transfers (Hidalgo 1989, Lyberg and Olstad 1991, Urken 1989). Secondary implantation has the advantage of considering prosthodontic requirements such as implant direction and location.

Irradiation of the vascularized bone graft does not preclude its survival (Jacobsson *et al* 1988, Martin *et al* 1992). Endosseous implants could be placed successfully in an irradiated fibula graft.

In this report, we document two cases; implant placement near the nasopalatine canal with simultaneous bone grafting on the nasopalatine canal and buccal wall, and placement of implants on a fibular osteocutaneous free graft in the mandible that was resected due to squamous cell carcinoma. An implant-based prosthetic restoration has been installed. Successful outcomes are reported for both cases in terms of no mobility, 1 to 3 mm probing depths, no bleeding on probing, and no paresthesia as well.

Materials and methods

Case One

A 61 year old healthy, white male was referred for examination and implant replacement of the edentulous area of tooth 11. The patient lost his tooth from a vertical fracture.

Clinical examination and CT scan

Clinical examination revealed recession on the mesial parts of teeth 12 and 21 of 1.5 mm. There was horizontal and vertical bone loss on 11. The patient has quite a low lip-line, such that when he smiles the cervical part of his maxillary teeth are not seen. CT scans revealed the close proximity of the nasopalatine canal to the intended area of implant placement (Figures 1 and 2). The patient was informed of the possibility of penetrating the canal during osteotomy. An oral surgeon would be present in case the incisive nerve needs to be displaced.

At the time of the surgical procedure, a trapezoidal incision was made with vertical releasing incisions on the maxillary right lateral incisor to the maxillary left central incisor. A full thickness mucoperiosteal flap was reflected, and the nasopalatine canal was located (Figure 3). The nasopalatine

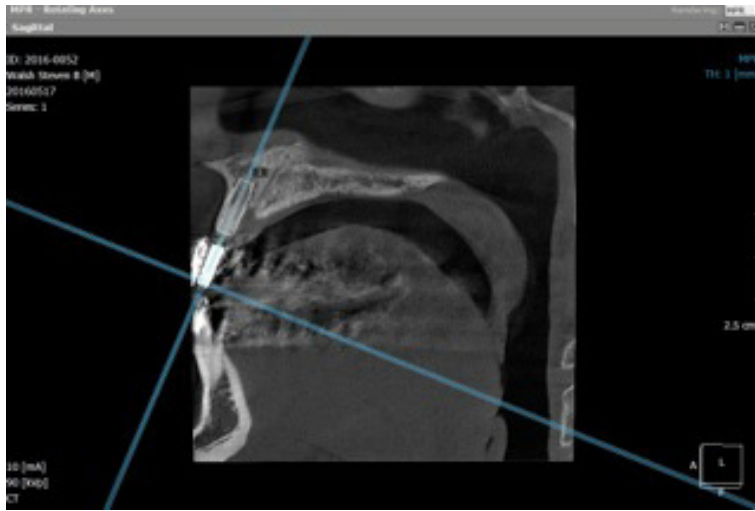


Figure 1. The proximity of the nasopalatine canal with the intended implant placement is seen in the CT scan.

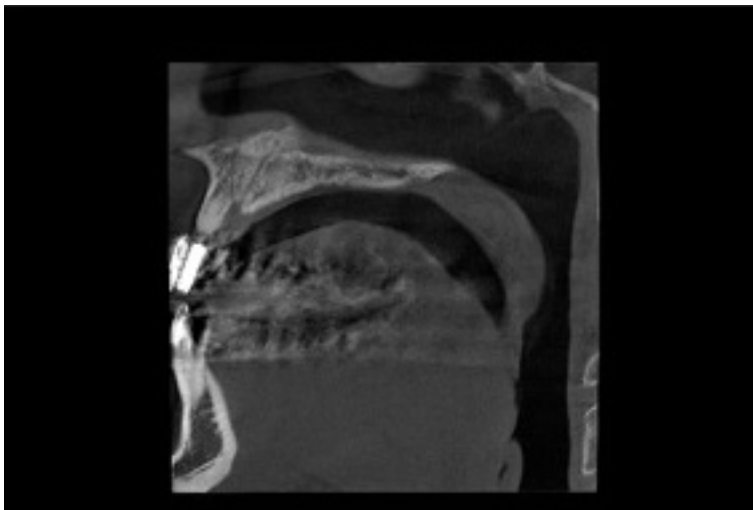


Figure 2. CT scan showing the close proximity of the nasopalatine canal to intended implant placement.

neurovascular bundle was not visible. Osteotomy and insertion of a 3.75x11 mm implant was performed, taking care not to traverse the canal. Bone graft was applied on the nasopalatine canal and on the buccal plate (Figure 4). Initial implant stability was achieved (Figure 5). The flap was replaced and closed using Vicryl sutures and expanded polytetrafluoroethylene (ePTFE) sutures. Postoperative medications included

Clindamycin HCl, Tylenol (as needed) and a chlorhexidine mouthrinse.

The patient was followed-up weekly for three weeks. Sutures were removed after three weeks. There were no symptoms of pain and paresthesia. The patient was recalled for three monthly periodontal maintenance visits. The prosthodontist restored the implant five months after implant surgery (Figures 6 to 8).



Figure 3. A full-thickness mucoperiosteal flap was reflected, and the nasopalatine canal was located.

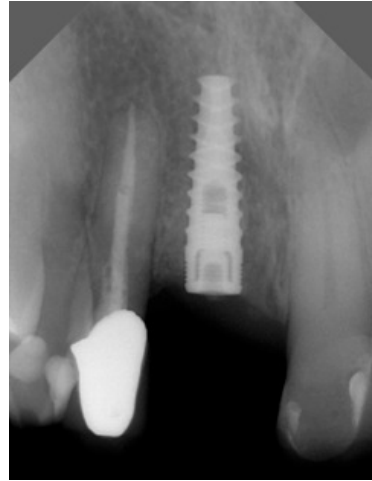


Figure 4. Initial implant stability was achieved.

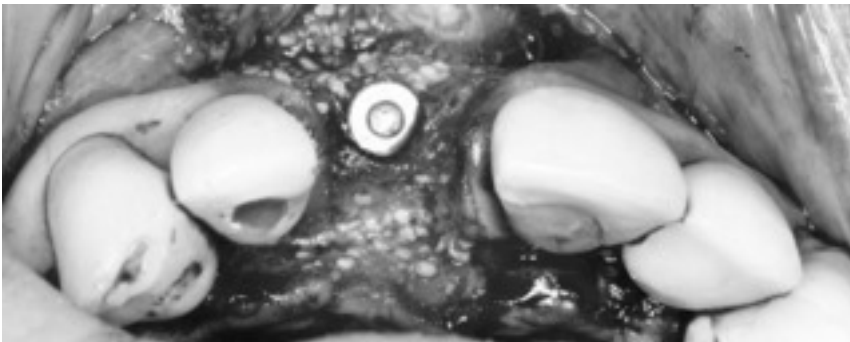


Figure 5. Bone graft was placed on the nasopalatine canal and on the buccal plate.



Figure 6. The patient was recalled after 3 months.



Figure 7. Fabrication of a crown 3 months after implant placement.



Figure 8. Final restoration.

Case Two

A 61 year old healthy, Asian male was referred for evaluation of the possibility of implant placement on a fibular free grafted mandible. The patient had a history of squamous cell carcinoma of the mandible. The grafted mandible was undertaken two years ago.

Clinical examination and CT scan

The patient presented with very thick (9 mm) keratinized soft tissue in the mandible. The patient has a low lip-line. There was sufficient bone height and width. CT scan revealed the location of the mini screws on the inferior border of the mandible. Plotting for the intended three dental implants was made (Figures 9 to 12).

Adaptation of the surgical stent for the osteotomy was a challenge, because of its non-stabilizing nature. A straight incision running along the right mandibular second premolar to the left mandibular second premolar on the mucogingival junction was done. A full-thickness mucoperiosteal flap was reflected. The overlying flap was thinned-out for its adaptability to the healing abutments. Osteotomy was done, and three 2.5x11.5 mm implants were inserted taking care not to traverse the screws of the mandibular plate. Initial implant stability was achieved. The flap

was replaced and closed using Vicryl sutures. Post-operative medication of amoxicillin, an anti-inflammatory drug, and a chlorhexidine mouthrinse were given. The patient was followed-up every week, and sutures were removed on the third week (Figure 13). There were no signs or symptoms of pain or infection. The patient was recalled every month. The final prosthesis was delivered after three months (Figure 14).

Discussion

The use of CBCT scan imaging becomes important in the planning of complex reconstructions. These cases illustrate the challenges of implant placement near vital structures and free fibular graft sites.

The proximity of the nasopalatine nerve presents a challenge to the clinician when placing an implant. Repositioning a nerve is one treatment option. Tozum *et al* (2012) reported that the mean bone length anterior to the canal is 19.17 ± 3.70 mm. Minimum bone length anterior to the canal was 8 mm, and bone width was 7.17 ± 1.49 mm. In some cases, implant placement seems impossible without augmentation.

Placing implants on a grafted edentulous jaw also poses a challenge, especially if the overlying soft tissues are quite thick that includes the corresponding skin, muscle, peroneal arteries and veins. Thus, a CT scan is a valuable tool to evaluate anatomic variations, morphology, and dimensions of incisive canal and incisive foramen (Tozum *et al* 2012).

A close collaboration among the different specialty groups is necessary to obtain good results.

Acknowledgements

I would like to thank Dr Andrea Gonzaga for plotting the CBCT scan and the fabrication

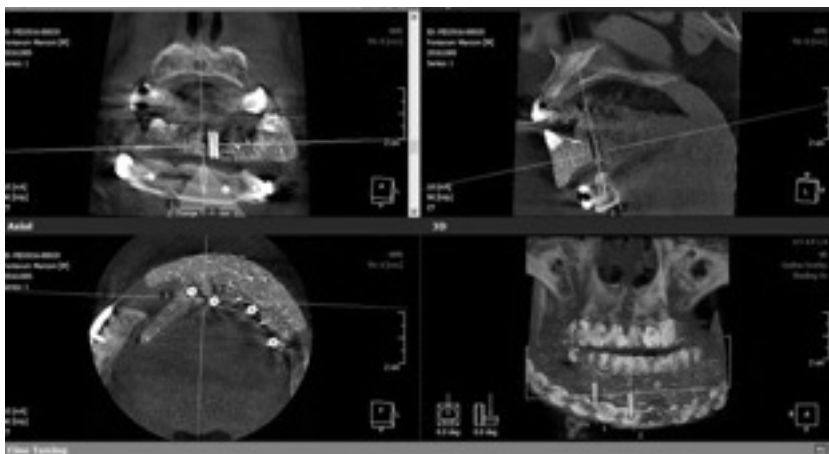


Figure 9. Location of the mini screws on the CT scan.

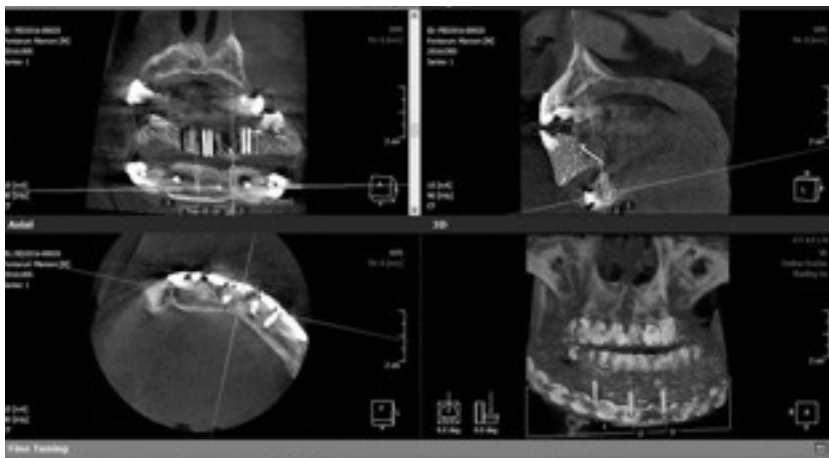


Figure 10. The CT scan reveals the location of the mini screws on the inferior border of the mandible.

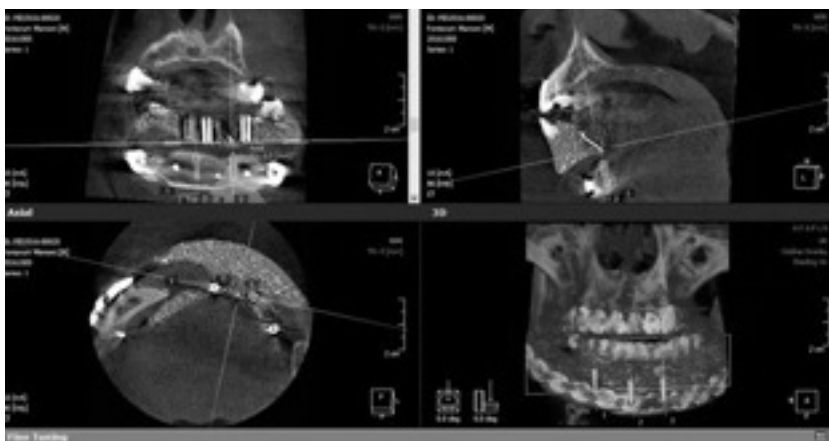


Figure 11. Plotting of the three dental implants was made on the CT scan.



Figure 12. Placement of the three dental implants in the mandible.

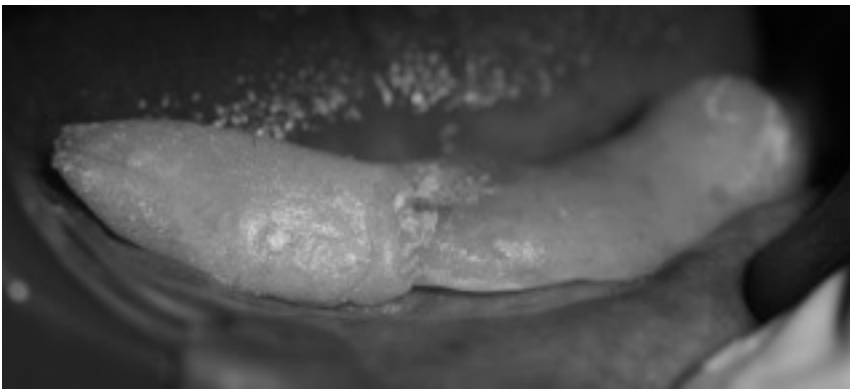


Figure 13. Three weeks post-operative, placement of an acrylic stent to prevent ulceration of the tongue.



Figure 14. Delivery of the final prosthesis three months after dental implant placement.

of the surgical stent.

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Chapter 8

Split-Crest Technique for Alveolar Narrow Ridge: Surgical Considerations

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Introduction

Alveolar bone resorption after tooth extraction is a major challenge for placement of dental implants. The majority of bone resorption takes place within first six months (40% in height and 60% in width), resulting in horizontal bone resorption of 5 to 7 mm. Since 50% of original socket width is lost, proper implant placement is difficult (Wang and Lang 2012).

Several special treatment modalities have been proposed to overcome horizontal bone resorption in order to perform successful implant placement such as split crest technique, osteotome technique, GBR, and onlay grafts.

Amongst these approaches, the split-crest technique, originally described by Simion *et al* (1992), has several advantages including simultaneous implant placement, no need for a second surgical site, pain relief, and shortened overall treatment time. If the alveolar ridge width is 3 mm or greater, but less than 6 mm, the split crest technique may be suitable for successful implant placement. And at least 1 mm of trabecular bone should be present between the buccal and lingual cortical bones.

The split crest technique results in a high implant survival rate (97.4%) with minimal technical complications (6.8%) (Milinkovic and Cordaro 2014). Other studies reported survival rates ranging between 91.7% and 100% with the most common complication

was buccal bone fracture (Bassetti *et al* 2016, Tolstunov and Hicke 2013).

This chapter presents ridge split procedures for horizontal ridge augmentation with immediate implant installation in two cases.

Case One

A healthy 48 year old woman was scheduled to undergo implant reconstruction of the posterior mandibular area. On initial panoramic radiograph a bone defect was noted in the posterior part of the right mandible (Figure 1). A ridge split procedure was planned due to insufficient bone width but adequate height for immediate implant placement in this site. A radiograph revealed the presence of cancellous bone between the buccal and lingual bone plate. A split ridge procedure was carried using an Esset Kit (Osstem, Korea) expansion drill to enable safe expansion (Figure 2). The fracture of the buccal cortical bone occurred during the procedure. The implants sites were prepared according to the manufacturer's manual (Osstem, Korea). Two fixtures were placed and the cover screws were exposed (Figure 3). Routine guided bone regeneration was also used. The fractured bone fragments were broken up using an expansion drill. The minced autogenous bone and a heterogeneous bone allograft (Endobon, Biomet 3i, USA) were mixed, and the gap between the buccal and

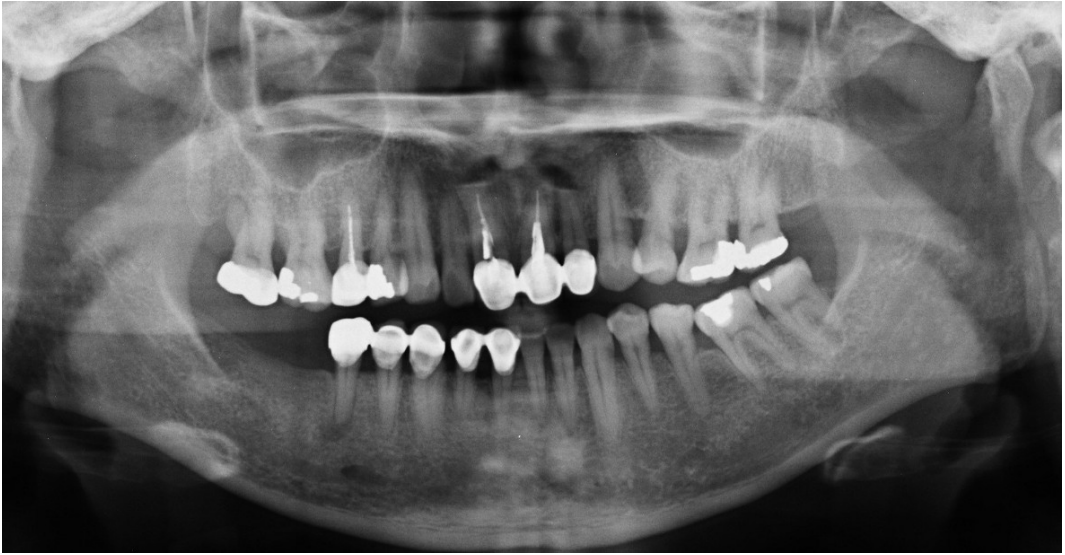


Figure 1. Pre-operative panoramic radiograph of Case One.

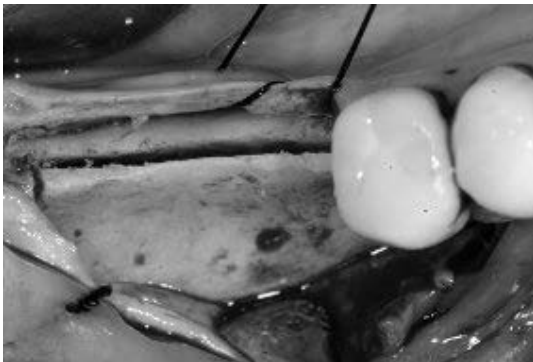


Figure 2. Split ridge procedure.



Figure 3. Implant placement.



Figure 4. Placement of bone grafting materials.

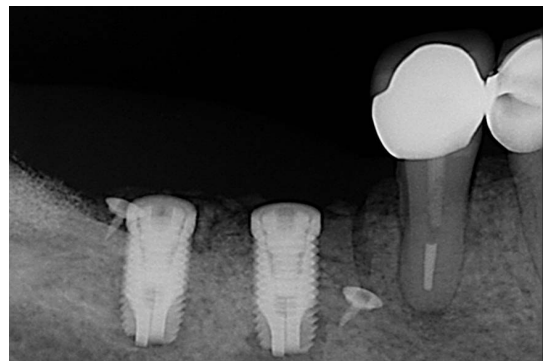


Figure 5. Radiographic view of membrane *in situ*.

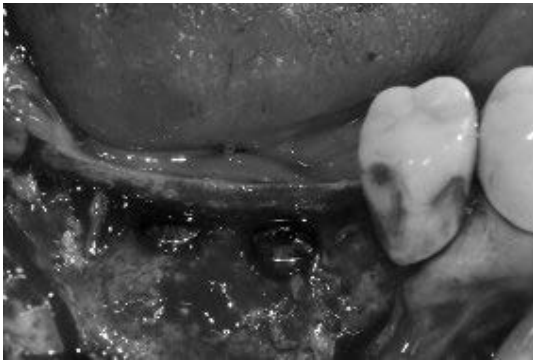


Figure 6. Second stage surgery.



Figure 7. Final prosthesis inserted.

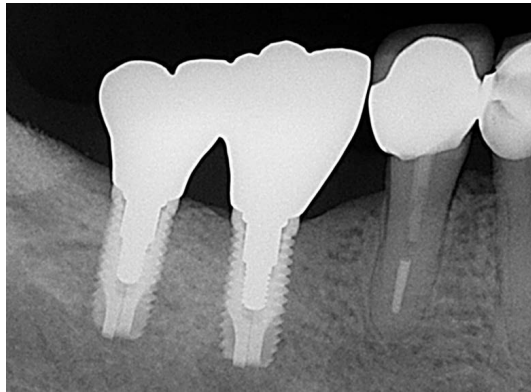


Figure 8. Radiograph of final prosthesis.

lingual plates were covered with absorbable membrane (Geistlich, Switzerland) (Figures 4 and 5). The soft tissues were sutured without tension. The ISQ values (Osstell, Sweden) were measured as 80 for the #46 and 72 for the #47. After six months, second stage surgery was performed (Figure 6). The ISQ values were measured as 76 for #46 and 78 for #47 in secondary surgery. The new bone production was good, and the bone quality was adequate for placement of the implant prosthesis. After ten months, the prosthesis was connected to the implant and no further problems were observed (Figures 7 and 8).

Case Two

A 58 year old male patient presented to our clinic for implant insertion. The radiographs

showed that alveolar bone in the mandibular first molar area was well preserved (Figure 9). However, it was noted that the alveolar bone was resorbed horizontally (Figure 10). A split ridge procedure using ESSET KIT (Osstem, Korea) was performed due to the narrow alveolar ridge and an implant was placed (Dentium, Korea) (Figures 11 and 12). The ISQ value was 77. To obtain primary closure without tension on the surgical site, the superficial fibers of the mylohyoid muscle were separated instead of performing a releasing incision on the buccal flap and the lingual flap was advanced coronally (Figure 13) (Ronda and Stacchi 2011). To increase the buccal width of the #46 implant site, demineralized freeze-dried bone allograft (DO BONE, CG Bio, Korea), deproteinized bovine bone mineral (Cerabone®, Botiss, Germany),



Figure 9. Pre-operative panoramic radiograph of Case Two.

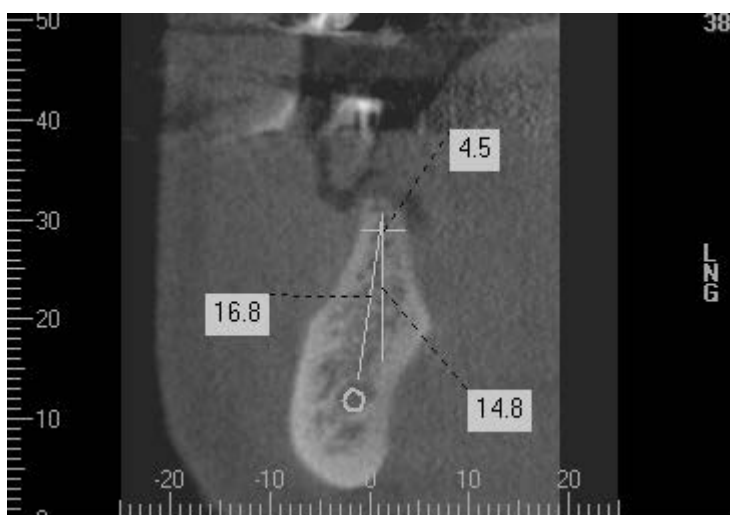


Figure 10. Pre-operative radiographic view of alveolar bone.

and a resorbable collagen membrane (OSSIX® PLUS, Datum Dental, Israel) were used (Figure 14). Primary closure was performed without tension (Figure 15). The implant was placed in an appropriate position and no wound dehiscence occurred (Figures 16 and 17).

Discussion

After extraction of one or more teeth, the

alveolar ridge loses its functional role. This leads to three-dimensional bone resorption and alveolar ridge deficiency. In severely resorbed alveolar bone, various methods such as onlay/inlay graft and guided bone regeneration have been introduced for reconstruction of the inadequate ridge and subsequent immediate implant placement (Atwood 2001). In order for these procedures to be successful, primary stabilization of the implant and proper blood supply to the surgical site are very important.



Figure 11. Split ridge procedure.

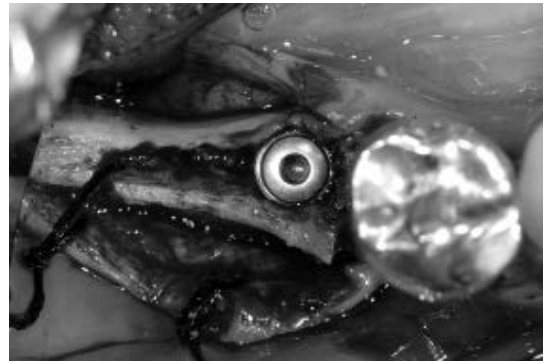


Figure 12. Split ridge procedure.

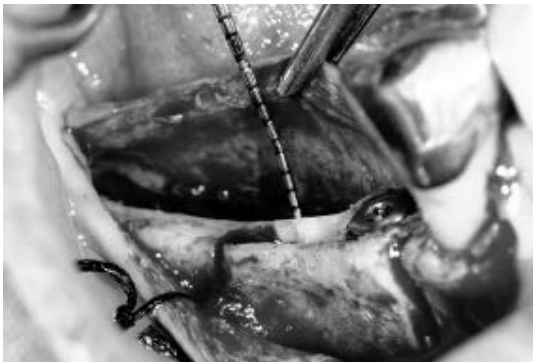


Figure 13. Advancement of lingual flap.



Figure 14. Placement of regenerative materials.

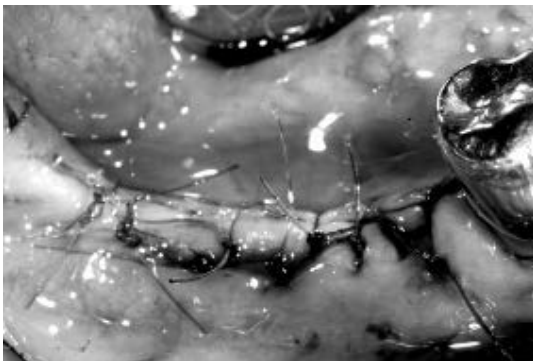


Figure 15. Primary closure.

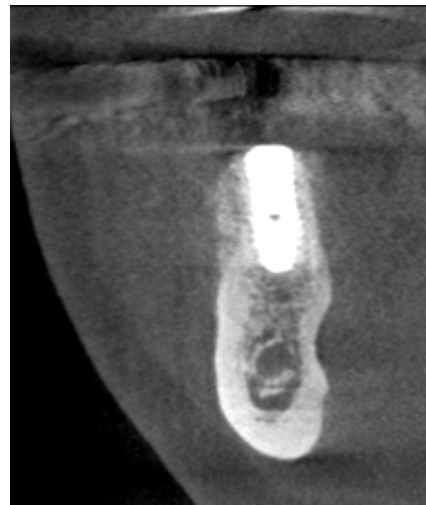


Figure 16. Post-operative radiographic view.

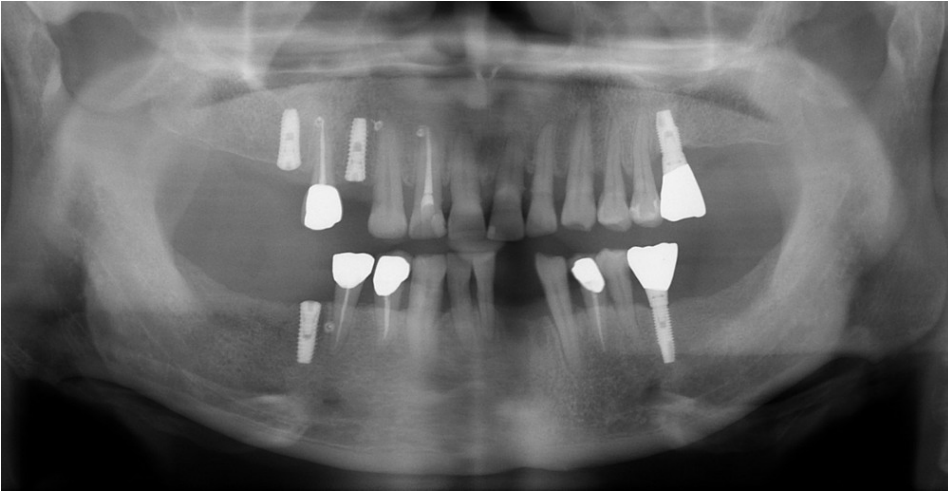


Figure 17. Post-operative radiographic view.

Cortical bone of the mandible is thicker than the maxilla, but the cancellous bone can be reduced. When bone resorption occurs in the mandible, the buccal and lingual cortical bone plates are brought closer to each other, which results in a reduced amount of cancellous bone (Tolstunov 2016). These changes make it more difficult to provide a stable blood supply to any grafted bone and to allow primary stabilization of the implant compared to the maxilla. As a result, it is very difficult to place implants in the posterior mandible at the same time as bone grafting in cases where the alveolar bone is severely contracted (Selcuk *et al* 2004).

In such cases, implant placement can be performed simultaneously using particulate bone grafts and a ridge split technique in a compromised posterior mandible site. This technique involves cutting the alveolar crest of the contracted ridge to expose internal cancellous bone and minimizes the removal of the cortical bone. This enables an adequate blood supply to the grafted bone particles and preserves the cortical plates of the buccal and lingual side to provide the primary stabilization of the implant. This is particularly effective when the residual ridge

width is 3 to 5 mm and there is no vertical bone resorption (Tolstunov 2016). Both patients presented demonstrate that implant placement with bone grafting is possible in the severely resorbed mandible by obtaining sufficient blood supply and stable primary stabilization of the implant at the bone graft site through the ridge split technique.

In simultaneous bone grafting and implant placement an increased ridge makes tension free primary closure of the flap difficult. In these cases, barrier membranes can be used in conjunction with bone grafts, but early exposure of the barrier membrane can adversely affect the grafted bone and implant (Machtei 2001). To prevent this, primary closure of the flap without tension is very important. For the buccal flap, adequate extension through vertical incisions and periosteal releasing incisions is needed. However, the lingual flap cannot be released via vertical incisions or periosteal releasing incisions due to accessibility issues and local anatomical structure.

In the second case report, the lingual flap was elongated through the dissection of the insertion site of the mylohyoid muscle at the base of the flap. This enabled easy extension

of the flap without incision and lowered the risk of injury to the surrounding important anatomical structures.

By gently repositioning the lingual flap coronally and lingually, the periosteum and superficial fibers can be separated from the main body of the muscle with blunt dissection. This is possible because the superficial fibers are firmly attached to the periosteum and are weakly attached to deeper layers of muscle. This procedure exposes muscle tissue and allows sufficient lift upwards for GBR (Ronda and Stacchi 2011). Furthermore, if primary closure of the flap is possible with only the extension of the lingual flap, it is possible to avoid sensory abnormalities caused by damaging the mandibular posterior buccal flap. Therefore, when combined with the ridge split technique, minimizing the periosteal damage of the buccal flap maximize vascularization of the underlying cortical bone plate, results in better bone regeneration (Oppenheimer *et al* 2008).

The ridge split technique used with the coronally advanced lingual flap is a predictable procedure that enables simultaneous bone grafting and immediate implant placement in the resorbed posterior mandible. This allows for good blood supply to the grafted bone particle and provides primary stabilization. In addition, by allowing tension free primary closure of the flap, it is possible to prevent premature exposure of the barrier membrane and minimize periosteal damage, thereby maximizing vascularization of the underlying cortical bone plate. Furthermore, minimization of damage to critical anatomical structures can prevent complications by surgical procedures.

In these case reports, only short follow-up periods were recorded after the final restoration of the prosthesis. Further research is needed to evaluate long-term bone resorption according to gingival biotypes and immediate loading of implant in the molar area.

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Chapter 9

Peri-implant Diseases and Periodontitis: The Insight Focus

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Introduction

Dental implants have become an alternative treatment with high survival rates for replacing the losing teeth in patients who have fully or partially edentulous area (Adell *et al* 1990, Branemark *et al* 1977, Buser *et al* 1991). Peri-implant diseases are a major biological complication which impairs the long-term success (Fransson *et al* 2005, Karoussis *et al* 2003, Pjetursson *et al* 2012, Roos-Jansaker *et al* 2006). Peri-implant diseases are classified into two categories (Zitzmann and Berglundh 2008). In peri-implant mucositis, gingival inflammation was found only around the soft tissue of the dental implant, with no signs of bone loss. In peri-implantitis, gingival inflammation was found around the soft tissue and there was deterioration in the supporting bone of dental implants.

The hypothesis of bacterial infection due to plaque accumulation as the etiologic factor of peri-implant diseases is currently accepted. However, many investigations have shown that peri-implant diseases appear to have a multifactorial origin. Any conditions, which are related to the development of the disease, are considered risk factors (Heitz-Mayfield 2008, Jepsen *et al* 2015, Renvert and Quirynen 2015). Many periodontal related parameters are considered to be local risk factors, which influence the bacterial load and composition at implants, or general risk factors, which

influence individual susceptibility to infection. Examples of local risk factors are oral hygiene status and remaining of deep pockets (Mombelli *et al* 1995, Serino and Ström 2009). General risk factors include a history of periodontal disease, smoking habits, and frequency of maintenance visits (Costa *et al* 2012, Heitz-Mayfield 2008, Pjetursson *et al* 2012, Monje *et al* 2016, Renvert and Persson 2009).

To achieve successful long-term outcomes for implant treatment, peri-implant diseases must be detected and managed earlier for avoiding an implant loss and impaired patients' quality of life. Understanding risk factors and their effect on implant success would help the clinicians prevent implant complications. The aim of this study was to evaluate the association between peri-implant diseases and factors related to periodontal status in order to develop a good treatment protocol, which could lead to long-term and sustainable implant success.

Materials and methods

The study protocol was approved by the ethics committee of the Faculty of Dentistry, Chulalongkorn University. This cross-sectional study enrolled two hundred patients who received endosseous dental implant surgery from the Faculty of Dentistry, Chulalongkorn University between 1996 and

2014. Subjects had at least one dental implant restored with either a fixed or a removable prosthesis, which had been in function for at least one year. Most subjects participated in a maintenance program. All subjects were advised about the objective and process of the study before signing informed consent for participation in this study.

Demographic data and history of implant treatment were recorded, including medical and dental history, history of periodontal treatment, and frequency of maintenance care program. All above data were obtained by history taking, chart review and dental examination. Clinical and radiographic examinations were performed in one visit. Periodontal care at implant sites followed the CIST protocol (Lang *et al* 2004). Finally, all patients received their oral examination report and were then enrolled into the regular maintenance program.

Clinical examination

The clinical evaluation was performed by three examiners assessing the following clinical parameters:

- Modified plaque index (mPII) for all implants (Mombelli *et al* 1987).
- Modified bleeding index (mBII) for all implants (Mombelli *et al* 1987).
- Probing pocket depth in millimeters.
- Modified periodontal screening and record (mPSR) for assessment the present of periodontal status.

Measurement procedure was performed manually using a plastic periodontal probe (12-UNC COLORVUE®; Hu-Friedy, Chicago, USA) for implants and a conventional manual University of North Carolina periodontal probe (UNC-15; Hu-Friedy, Chicago, USA) for natural teeth. Distances were measured to the nearest millimeter.

Radiographic examination

Radiographic examination was undertaken using standardized periapical radiographs. The completed digital radiograph was imported using dental software (Infinitt proprietary software v.2: Infinitt Co., Seoul, Korea) and projected onto a computer screen. The distance from the implant shoulder to the alveolar bone crest was measured in millimeters at the mesial and distal aspects of each implant by one examiner. The most severe bone loss site was selected to represent amount of bone loss of each respective implant.

Case definitions

For implant outcomes, peri-implant health and disease were assessed according to these established case definitions:

- Healthy peri-implant: absence of soft tissue inflammation and bone loss.
- Peri-implant mucositis: presence of soft tissue inflammation without bone loss around an osseointegrated implant (Lang *et al* 2004).
- Peri-implantitis: presence of soft tissue inflammation with bone loss around an osseointegrated implant beyond functional remodeling (Lang *et al* 2004).
- Implant survival: the implant with reconstruction was present at the follow-up examination but its condition is not specified (Lang *et al* 2004).
- Past periodontal status: the diagnosis of chronic periodontitis was categorized based on the American Academy of Periodontology criteria (Armitage 2004). Patients with chronic periodontitis had to present bleeding on probing and pocket depth ≥ 4 mm in at least 30% of the total sites before implant placement.
- Periodontal severity: disease severity was categorized based on the American Academy of Periodontology criteria

(Armitage 2004). Periodontal severity was defined on the amount of clinical attachment loss (CAL) and was designated as mild (1 to 2 mm CAL), moderate (3 to 4 mm CAL), or severe (≥ 5 mm CAL). The disease severity for each subject was represented by sites with the highest severity level.

- Oral hygiene status: oral hygiene was defined as good, fair, and poor (Lertpimonchai *et al* 2017). Poor was a mPLI of >2 and fair was a mPLI ranging from 1 to 2.
- Maintenance status: regular or irregular maintenance status was grouped based on patients' periodic months per recall visit. Patients with >6 months per recall visit were classified as irregular group, while patient with ≤ 6 months per recall visit were classified as regular group.
- Present periodontal status: subjects were classified as a group with mPSR score 4 and a group without mPSR score 4.

Calibration

Prior to the commencement of the study, both intra- and inter-calibration sessions were held for the three examiners on five volunteer subjects who had at least one dental implant restoration. Cohen's Kappa coefficient was applied to standardize data acquisition and the assessment of study variables. The mean intra- and inter-examiner calibration indicated an excellent agreement with Kappa 0.89 and 0.83, respectively.

Intra-examiner calibration of radiographic bone level was analyzed before evaluating the entire implant samples by assessing bone loss of thirty randomly selected implants from the faculty database. A repeat assessment was conducted after one week to evaluate the reproducibility of the method used. An intra-class correlation coefficient of 0.86 was obtained.

Statistical analysis

Commercially available statistical software SPSS version 22.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis. A normality test was used to determine for a normal distribution of study populations. A descriptive analysis was used to determine the prevalence of peri-implant diseases at subject level, the prevalence of parameters related to periodontal status, such as, history of periodontitis and disease severity, current periodontal status, oral hygiene, and maintenance period. The data were presented as frequency, percentage, and range. Chi-square tests were used to evaluate the significance of categorical clinical parameters and analyze the association between these periodontal related variables to peri-implant diseases. Differences were considered significant when a P value <0.05 was attained with the confidence level at 95%.

Results

200 patients with 412 implants were enrolled in this study. The demographic and clinical parameters at the examination time are shown in Table 1. The mean age of the recruited subjects was 57.30 years, ranging from 18 to 79 years. There were 83 (41.5%) males and 117 (58.5%) females. Only 2% of the patients reported to be current smokers. Diabetes was reported by 9% of the patients. Most patients had fair oral hygiene status (77.5%). At the review visit, the average implant placement time and insertion time of final prosthesis were 62.58 and 52.79 months, respectively. The majority of the patients (68%) were irregular attenders for maintenance recall with an average of 12.58 months per recall visit. Regarding the past periodontal status, there were 72 (36%) patients having a history of chronic periodontitis. The prevalence of peri-implant diseases was 60% for peri-implant mucositis

	Characteristic	Number (%)
Gender	Male	83 (41.5%)
	Female	117 (58.5%)
Smoking Habits	Non-smoker	176 (88%)
	Former-smoker	20 (10%)
	Current-smoker	4 (2%)
Diabetes Mellitus	Yes	18 (9%)
	No	182 (91%)
Oral Hygiene Status	Good	35 (17.5%)
	Fair	155 (77.5%)
	Poor	10 (5%)
Maintenance Status	Regular (≤ 6 months/visit)	36 (18%)
	Irregular (> 6 months/visit)	136 (68%)
	Missing data	28 (14%)
Past Periodontal Status	Healthy	2 (1%)
	Gingivitis	126 (63%)
	Treated chronic periodontitis	72 (36%)
Implant Health Status	Healthy	48 (24%)
	Peri-implant mucositis	120 (60%)
	Peri-implantitis	32 (16%)

Table 1. Demographic data and clinical characteristics at subject level.

and 16% for peri-implantitis. Overall implant survival rate was 96% at patient level.

Our study classified implant health status for analysis into two groups; non-peri-implantitis group (healthy and peri-implant mucositis) and peri-implantitis group. The association between implant health status and periodontal variables is presented in Table 2. Data analysis revealed that peri-implant health status was significantly associated with past periodontal status, maintenance status, and present periodontal status.

The prevalence of implant health status in relation to past periodontal status is shown in

Figure 1. We observed significantly higher prevalence of peri-implantitis (25% versus 10.9%) among subjects with a history of chronic periodontitis than those without a history of periodontal disease.

We examined the prevalence of implant health status within a history of periodontal severity. The majority of the 72 patients having a history of chronic periodontitis were diagnosed as having severe chronic periodontitis (55.6%). The highest prevalence of peri-implantitis was observed in subjects having a history of severe chronic periodontitis (35%). The association between the implant

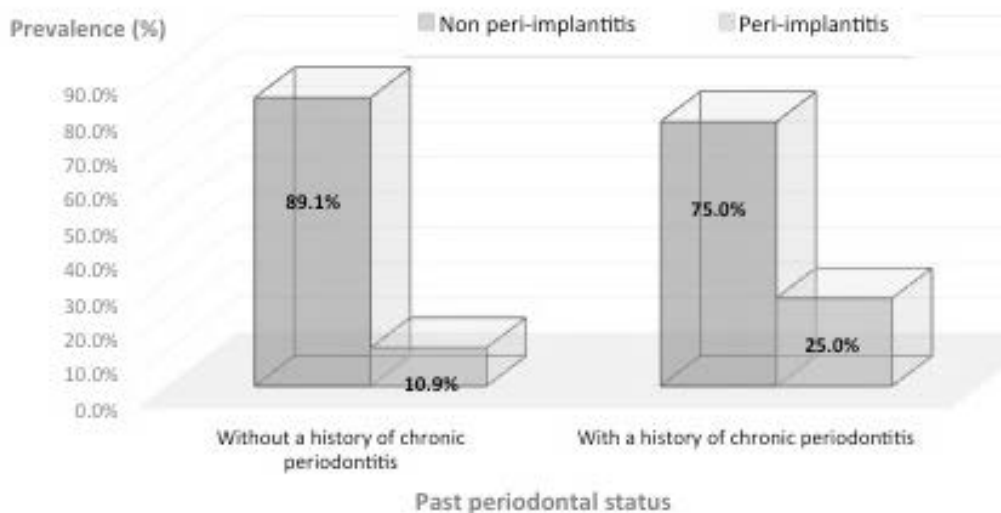


Figure 1. Distribution of implant health status related to past periodontal status.

Periodontal variables	Total N (%)	Non-peri-implantitis N (%)	Peri-implantitis N (%)	P-value†
Past periodontal status	200 (100%)	114 (67.9%)		0.009*
Without a history of chronic periodontitis	128 (64%)	54 (32.1%)	14 (43.7%)	
With a history of chronic periodontitis	72 (36%)		18 (56.3%)	
Oral hygiene status	200 (100%)	33 (19.6%)		0.894
Good	35 (17.5%)	127 (75.6%)	2 (6.3%)	
Fair	155 (77.5%)	8 (4.8%)	28 (87.4%)	
Poor	10 (5 %)		2 (6.3%)	
Maintenance status‡	172 (100%)	34 (23.6%)		0.04*
Regular group	36 (20.9%)	110 (76.4%)	2 (7.1%)	
Irregular group	136 (79.1%)		26 (92.9%)	
Present periodontal status§	197 (100%)	160 (96.4%)		0.03*
Without mPSR score 4 presented	187 (94.9%)	6 (3.6%)	27 (87.1%)	
With PSR score 4 presented	10 (5.1%)			

Table 2. Association between peri-implant health status and independent variables.

† Chi-square test. ‡ Missing 28 data. § Excluded 3 fully edentulous cases. * Statistical significant: $p < 0.05$.

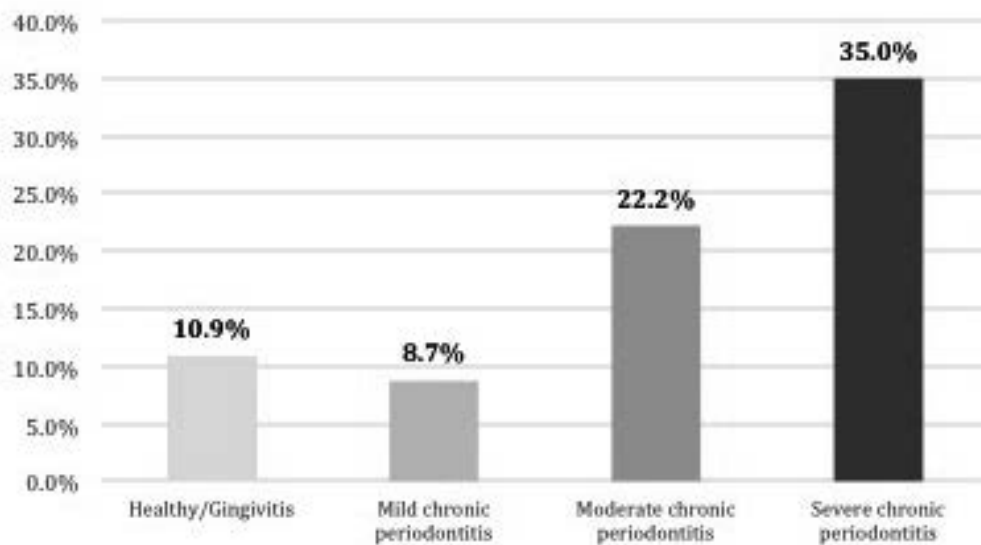


Figure 2. Prevalence of peri-implantitis in relation to a history of periodontal severity.

health status and a history of periodontal severity was statistically significant (Figure 2).

Discussion

The aim of this study was to evaluate the association between peri-implant diseases and factors related to periodontal status. In general, there has been a high variability among studies regarding case definition of peri-implant diseases. Some studies defined peri-implant diseases with combined bleeding on probing and probing pocket depth parameters (Mombelli *et al* 1995). Our study used established case definitions as defined by Lang *et al* (2004). Peri-implant mucositis was defined by bleeding on probing on at least one aspect of the implant, whereas peri-implantitis identified a result of radiographic bone changes beyond functional remodeling around implants addition to inflammation. These selected criteria had a critical impact on data interpretation. The prevalence of peri-implantitis from our cross-sectional analysis

was 16% at subject level, which appeared to be close to 22% estimated weight mean of peri-implantitis from previous meta-analysis (Derks and Tomasi 2015). Nevertheless, when compared to 43% of peri-implant mucositis from a recent meta-analysis, 60% of patients diagnosed with peri-implant mucositis in our study was considerably higher (Derks and Tomasi 2015). These different outcomes might be explained as a consequence of various case definitions used, and measurement errors which were associated with clinical parameters such as probing pocket depth and bleeding on probing. Moreover, the influence of the accessibility for probing, the angle or force applied to the probe when penetrating into different degrees of inflamed peri-implant tissue could explain the higher prevalence of peri-implant diseases in our study.

There have been numerous studies and systematic reviews indicating that peri-implant diseases are affected by past periodontal history with a positive correlation. In addition, several clinical studies have reported a different prevalence of peri-

implantitis between patients with and without a history of periodontitis (Karoussis *et al* 2003, Roos-Jansåker *et al* 2006, Schou *et al* 2006). Our study has shown a strong influence of a history of chronic periodontitis as a risk indicator for peri-implantitis. We found that patients with a history of chronic periodontitis presented significantly higher prevalence of peri-implantitis (25%) than those without a history of chronic periodontitis (10.9%). Likewise, previous studies have demonstrated significantly greater incidence of peri-implantitis from patients with a history of chronic periodontitis than patients without such a history, 28.6 % and 5.8%, respectively (Karoussis *et al* 2003). Similarly, another study also demonstrated that patients treated for periodontitis prior to implant therapy had a significantly higher prevalence of peri-implantitis (37%) compared with periodontally healthy patients (17%) (Choi-Yan Lee *et al* 2012).

Not only is the influence of periodontal condition prior to implant therapy important, but the severity of a previous history of periodontal disease should also be considered. In this study, there was a high prevalence of peri-implantitis subjects who had a history of severe chronic periodontitis (35%). This finding was similar to previous study which demonstrated more pronounced prevalence of peri-implant bone loss in subjects with a history of moderate and severe periodontitis (Roccuzzo *et al* 2010). Together these findings confirm that patients with a history of severe forms of chronic periodontitis are susceptible for the higher risk of peri-implantitis.

The prevalence of peri-implant health status in relation to oral hygiene was investigated. Although our study did not find any association, one study found that higher total plaque scores was statistically associated with peri-implant diseases with dose dependent effect (Ferreira *et al* 2006). Subjects with poor oral hygiene were more

prone to develop peri-implantitis with OR of 2.9. These different results could be explained by the dissimilarity of research methodology and subjects' characteristic. In the present study, patient oral hygiene status was assessed by mPLI score, whereas the other studies used various criteria of plaque index. Furthermore, our study had only 5% of patients categorized into poor oral hygiene group. This small proportion of poor oral hygiene patients might jeopardize the statistic outcomes between peri-implant diseases and oral hygiene status.

There is considerable evidence demonstrating that lack of peri-implant supportive therapy is a risk factor of peri-implant diseases. For example one study has reported that absence of preventive maintenance is significantly associated with peri-implantitis (Costa *et al* 2012). Similarly, another study reported that peri-implant maintenance therapy (PIMT) interval is significantly associated with peri-implant diseases at both the patient and implant levels (Monje *et al* 2016). From this study it was concluded that PIMT can potentially prevent biologic complications and must be factored into a patient's risk profiling. Furthermore, they suggested a minimum recall PIMT interval of five to six months. As a consequence, in our studies we used regular and irregular maintenance groups with six months/visit as a cutting-off point. Our results also confirmed the significant association between peri-implantitis and irregular maintenance status with most peri-implantitis subjects (92.9%) having an irregular maintenance recall schedule.

Although there are many studies that have investigated the association between peri-implant diseases and past periodontal status, fewer studies have investigated patients' current periodontal condition. The presence of residual periodontal pockets around the remaining dentition can be sources of subsequent bacterial colonization of the

installed implants. One study has demonstrated that the residual periodontitis subgroup, which had at least one pocket >6 mm at the follow up examination, presented significantly greater mean probing pocket depth, mean bone loss, and prevalence of peri-implantitis than the non-periodontitis subgroup (Cho-Yan Lee *et al* 2012). To evaluate the influence of the current periodontal status in this present study, mPSR score was used as the clinical parameter. Subjects were classified as a group with mPSR score 4 (>6 mm depth periodontal pocket present at the review visit) and a group without mPSR score 4 (no >6 mm depth periodontal pocket). As for other studies, our study found a significant association between peri-implantitis and present periodontal status.

This study had some limitations of its research methodology. The demographic data and history of implant treatment were obtained from a secondary data source, such as treatment records, which have the risk of being incomplete. Moreover, this was a cross-sectional analysis that verified only an association between peri-implantitis and various putative risk indicators. Future investigation using a longitudinal study approach will be required to clarify the true risk factors.

Conclusion

Patients with a history of periodontitis were significantly associated with increased incidence of peri-implantitis, especially those patients with a history of severe periodontitis. Therefore, these patients should be treated and well maintained for their periodontal disease before implant placement and afterwards so that the successful long-term outcomes of implant treatment can be achieved.

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Chapter 10

Development of a Novel Animal Model for Testing Antimicrobial Agents Against Periodontitis and Peri-implantitis

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Introduction

Periodontitis (gum disease) is an inflammatory disease caused by bacterial infection of the supporting tissue around the teeth. It affects one in three New Zealand adults, causes loss of teeth and has been linked to serious conditions including cardiovascular disease, stroke and pre-term birth (Genco and Borgnakke 2013, Haisman *et al* 2010, Jatrana *et al* 2009). Missing teeth may be completely replaced using titanium dental implant screws and crowns; this highly effective treatment is becoming very common, but is expensive and not without risk (Esposito *et al* 2014, Levin and Haloerin-Sternfeld 2013, Setzer and Kim 2014). Peri-implant mucositis is an inflammatory lesion, confined to the soft tissue around implants, while peri-implantitis affects the supporting bone and causes painful disfiguring infections, resulting in the loosening of the implant and eventually causing implant loss or removal (Figuerio *et al* 2014). Peri-implantitis is found in up to 43% of individuals with implants, and peri-implant mucositis in up to 50% (Atieh *et al* 2013, Lindhe and Meyle 2008, Mombelli *et al* 2012). The causative bacteria are thought to be the same as those responsible for periodontitis

and tooth loss (Cho-Yan Lee *et al* 2012).

Current multimodal treatment strategies for both periodontitis and peri-implantitis, such as the Cumulative Interceptive Supportive Therapy protocol, involve physical disruption of biofilms and chemotherapy with disinfectants and antibiotics (Lang *et al* 2004). These have limited success and may be only capable of slowing the disease process at best (Heitz-Mayfield 2008, Kotsovilis *et al* 2008, Mombelli and Lang 1998, Mombelli 2002, Norowski and Bumgardner 2009).

Development of new strategies for treatment of peri-implantitis and periodontitis rely upon appropriate preclinical animal models for testing before they can be analyzed in human patients. Various animal models exist. In the main these depend upon the induction of a bacterial-induced, immuno-inflammatory lesion around teeth and/or dental implants, generally combined with the presence of a foreign body such as a silk ligature ligated around the teeth or implants. The degree of destruction and defect morphology may be variable, and some models compensate for this by surgically creating an acute defect (although these tend to show spontaneous repair), or by combining an acute surgically-created defect with ligature-induced chronic

inflammation (for reviews see Kantarci *et al* 2015, Pellegrini *et al* 2009, Schwarz *et al* 2015, Struillou *et al* 2010). Large animal models have the advantage that implants of similar dimensions to those used in humans may be examined, and that the teeth and alveolus also have anatomical dimensions more closely comparable to the human periodontium.

Our Faculty has a long history of research using domestic sheep as an animal model (for reviews see Duncan 2014, Duncan 2016). The development pathway has included naturally-occurring periodontitis in sheep, the development of acute surgical, and surgical plus chronic ligated defect models, in the furcation of the second mandibular premolar in sheep for guided tissue regeneration research, and two-stage and one-stage delayed implant placement into healed mandibular ridges in sheep, with and without periodontitis (Danesh-Meyer *et al* 1997, Duncan *et al* 2000, Duncan *et al* 2001, Duncan *et al* 2003, Duncan 2005, Duncan *et al* 2015, Frisken *et al* 1989, Laws *et al* 1988, Mohammed *et al* 1998, Siddiqi *et al* 2017, Whelan *et al* 1997). To date we have yet to develop a model for peri-implantitis in sheep.

Ideally the disease model should follow the pattern for such models established by Lang *et al* (1993) and subsequently Isidor (1997) in Macaque monkeys and by Berglundh *et al* (1992) and Lindhe *et al* (1992) in dogs, that is, a split mouth design where implants are installed into healed premolar extraction sockets on one side of the jaw, and then both the implants and the contralateral premolar teeth are ligated for varying lengths of time to establish disease. These seminal studies established the similarities and differences between periodontitis and peri-implantitis, and these models have been used by others to study regenerative surgical techniques for peri-implantitis (Berglundh *et al* 2011, Schou *et al* 2003a, Schou *et al* 2003b). It has also

been noted that maintaining the progression of ligature-induced models requires repeated interventions and may not be an accurate model for naturally-occurring disease; Martins *et al* (2014) commented that “the ideal canine peri-implantitis induction model would be a naturally occurring peri-implantitis induction without the action of any ligature”. The Korean research team of Kim *et al* (2012) approached this problem by placing implants in a supra-alveolar position for three weeks and then fully or re-inserting the implants for continued healing.

It would also be desirable to have an animal model for implant healing following one-stage placement as an immediate procedure into tooth extraction sockets. Vlamink *et al* (2008) attempted to establish a submerged, immediate placement protocol in the mandibular premolar extraction sockets of seven sheep, however 19 of the 22 implants failed or were clinically mobile after 26 weeks healing, giving an overall success rate of just 13.6%. To date, no other studies of immediate implant placement into sheep tooth sockets have been reported.

The aim of this pilot project was to develop a novel sheep model with a split-mouth design, consisting of a combined acute surgical-chronic ligature-induced periodontitis around teeth and (on the contralateral side) peri-implantitis around implants. Two kinds of dental implants were examined, one with a blasted surface for the apical portion and machined coronal surface, the other with an oxidized surface. As a secondary aim, we also conducted a pilot experiment to place implants immediately as a single-stage procedure into fresh extraction sockets in the sheep.

Methodology

24 female Romney cross ewes aged three to four years were used in this study. The study was performed with approval from the Otago

University Animal Ethics Committee, AEC # 23-15. All surgical work was carried out under sterile conditions in a full operating theatre at Invermay Agricultural Research Centre.

Surgical site

We chose to use the mandibular premolar sites for these experiments. Previously we have shown that the anatomical arrangement of mandibular incisors in sheep is quite different to humans, having a shallow alveolar housing and deep collagenous lingual pad, whereas the premolar site is more analogous to the human situation (Duncan 2003a, Duncan 2003b) (Figure 1).

Anesthesia and tooth extraction

Details of the techniques for general anesthesia, tooth extraction, implant placement and histological preparation have been previously reported (Duncan 2005, Duncan *et al* 2008, Duncan *et al* 2015, Duncan *et al* 2016, Liu *et al* 2016, Sharma *et al* 2016). Images of all surgical phases are shown in Figures 2 to 4. Briefly, first, second and third mandibular teeth on the left hand lower jaw were extracted under general anesthetic and the sites allowed to heal for three months (Figure 2).

Implant placement

After three months, the sheep were again anaesthetized. Surgical flaps were elevated on the left side and one Southern Implants MSC tapered 13 x Ø 4.0 mm implants (external hex) were placed into the more posterior site; these implants have a blasted surface with a machined (smooth) coronal portion which is designed to be easier to clean in the presence of peri-implant infection. One Nobel system implant was placed into the anterior site on the left side. A variety of diameters 3.75 to

5 mm and lengths 8.5 to 15 mm were used, all with parallel sided configuration and TiUnite surfaces, ranging from 3.75 mm (n=8 implants) to 5 mm (n=7) to 5mm (n=5) in diameter and with diameters of 8.5 mm (n=1), 10 mm (n=5), 11.5 mm (n=3), 13 mm (n=5) or 15 mm (n=6). Both the anterior and posterior implants were placed using a two-stage protocol, with the implant buried to allow full healing for ten weeks (Figure 3). Cover screws were placed and the sites sutured.

Implant stage 2 and defect creation around teeth and implants

After a further ten weeks integration, the coronal portion of both implants on the left side of the mandible were exposed. Appropriate trephine burs were used to create a 5 mm deep trough around the shoulder of each implant. Cover screws were removed, a trans-gingival healing abutment was placed, and a 3-0 silk ligature tied around the implant threads, positioned within the created surgical intra-bony defect. The surgical site was closed with resorbable sutures (Figure 4).

Simultaneously, full-thickness flaps were raised around the first and second mandibular premolars on the right side of the lower jaw. A trough was created around the teeth on buccal and lingual surfaces and extending interproximally, using a round bur and a round Piezoelectric tip. A 3-0 silk ligature tied around the implant threads, positioned into the created surgical intra-bony defect. The surgical site was closed with resorbable sutures (Figure 4). All sites were irrigated with *P. gingivalis* pure culture at baseline. All sites were radiographed and were sampled microbiologically using curettes prior to initiation of disease.

Immediate implants

An additional four sheep received

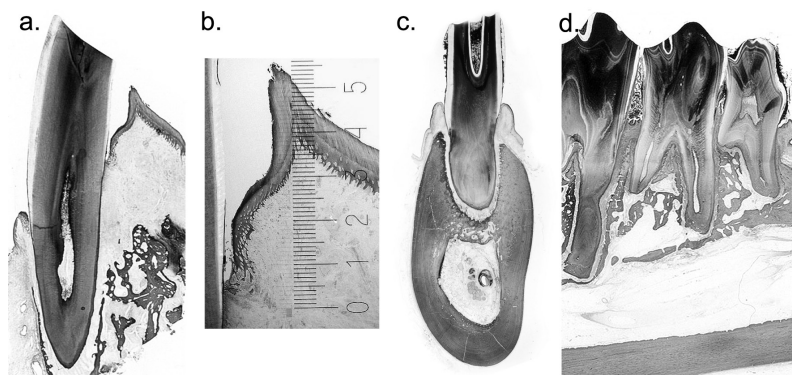


Figure 1. Normal dimensions of the incisor and premolar teeth in sheep. (A) Sheep mandibular incisor (sheep do not have maxillary incisors). The lingual pad is on the right. (B) magnified view of the naturally-occurring pocket on the lingual, above the lingual pad. Approximately 5 mm in probing depth. (C) Buccolingual section through the second mandibular premolar in sheep. Wide periodontal ligament and extension of enamel to the alveolar crest on the buccal. (D) Mesio-distal (para-sagittal) view of the three premolar teeth in sheep, from first premolar (P1, right) to third premolar (P3, left). The loosely-trabecular alveolar bone surrounds the teeth above a large marrow space.

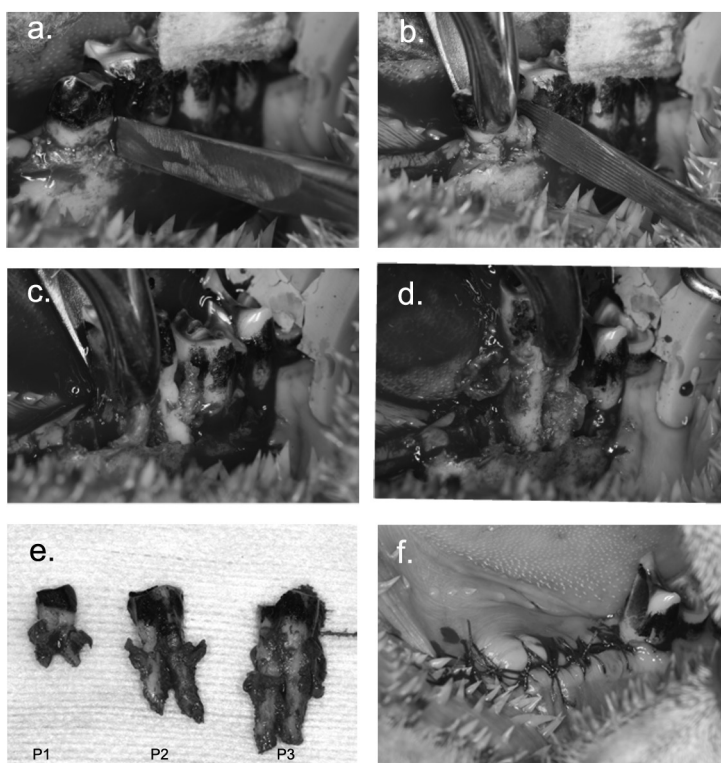


Figure 2. Sheep mandibular premolar tooth extractions. (A) Elevation of first premolar. (B) Elevation of second premolar. (C) Forceps delivery second premolar. (D) Forceps delivery third premolar. (E) Extracted first premolar P1, second premolar P2, third premolar P3. (F) Sutured site.

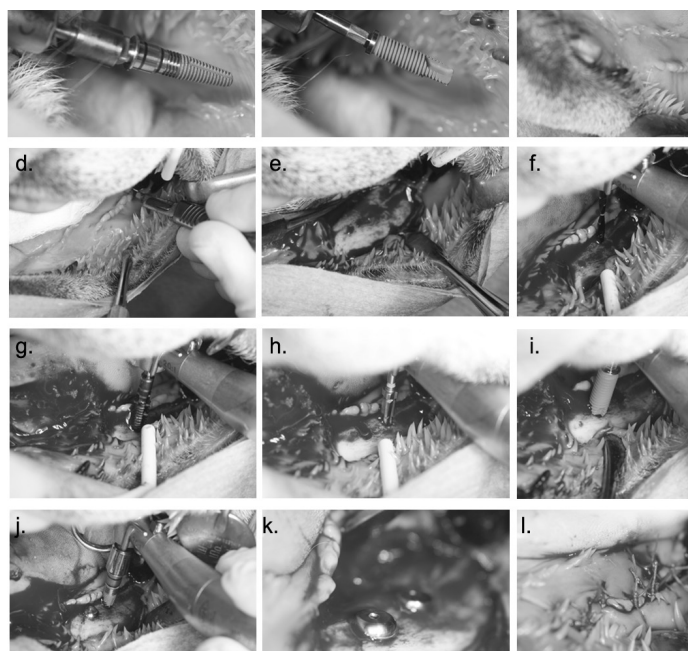


Figure 3. Implant surgery. (A) Southern tapered MSC implant. (B) Nobel TiUnite parallel-sided implant (C) Healed tooth extraction site. (D) Flap incision. (E) Flap raised. (F) Preparing sites with 2 mm twist drill. (G) Tapping anterior sites.(H) Tapered bur for posterior sites (I) Placing Nobel implant in anterior site. (J) Placing Southern implant in posterior site. (K) Cover screws. (L) Sutured site.

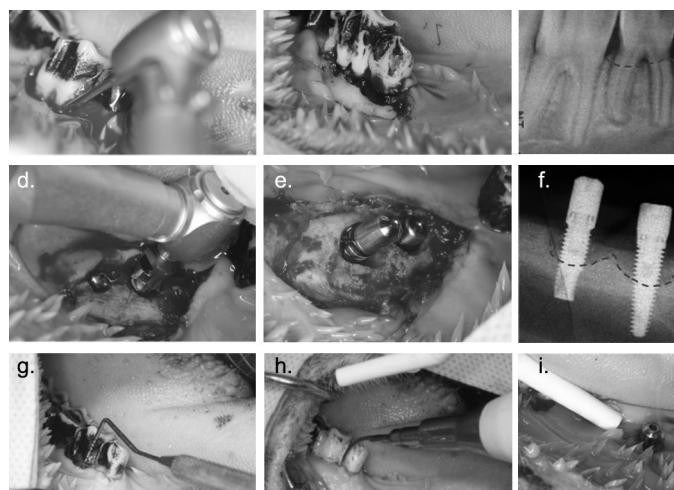


Figure 4. Creating and treating ligature-induced disease in the sheep mandibular model. (A) A round bur was used to create a trough around the lower right premolars. (B) Ligatures were tied around premolars. (C) Clinical radiograph of premolars. Dashed red line indicates surgically-created lesion (D) A trephine bur was used to create a trough around implants. (E) Ligatures were tied around implants. (F) Clinical radiograph of implants. Dashed red line indicates lesion. (G) Measuring pocket depth and attachment levels around teeth. (H) Scaling teeth after ligature removal. (I) Scaling implants after ligature removal.

immediate implants placed into fresh tooth extraction sites on the left side only. These animals did not have disease created around either the teeth (on the right side) or implants (Figure 5). Each sheep received a Nobel Replace Tapered implant with internal trilobe connection and TiUnite surface into the anterior site (all 4.3 mm diameter, either 10 mm (n=2) or 13 mm (n=2) long). All posterior sites received one Southern Implants MSC tapered 13 x Ø 4.0 mm implants (external hex). All implants were immediately restored with healing abutments.

Ligature removal and scaling

After seven weeks of ligature-induced disease, the 20 delayed-implant animals had the ligature removed under general

anaesthetic. The teeth and implants had clinical measurements recorded (periodontal or peri-implant probing) using a standard periodontal probe with an 0.5 mm ball end and Williams markings. Six sites were measured around each tooth (mesiobuccal and mesiolingual, midbuccal and midlingual, distobuccal and distolingual). Four sites were recorded around implants (mesial, buccal, lingual, distal). Gingival or mucosal recession and pocket depths were recorded separately and combined arithmetically to determine attachment levels. All sites were radiographed. Microbial flora was sampled from around the implants, the premolar teeth and the anterior incisors in all sheep using a periodontal curette.

All diseased premolar and implant sites were then scaled and root planed using an

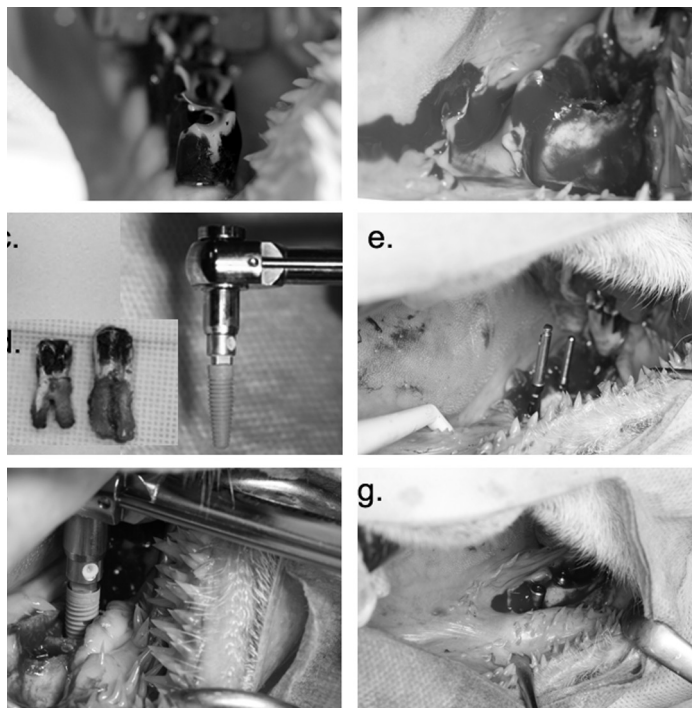


Figure 5. No-disease control group (N=4 sheep), immediate implants placed into tooth extraction sockets. (A) Mandibular premolars. (B) Premolars extracted. (C) Tapered Nobel Trilobe implants with TiUnite surface on hand ratchet torque driver. (D) Inset: extracted premolars showing relationship between root length and implant. (E) Initial preparation using 2 mm and 3 mm twist drills. (F) Placing immediate implants into tooth sockets. (G) Placed implants with healing abutments as immediate one-stage protocol.

ultrasonic scaler and hand instruments. A novel chemotherapeutic gel formulation was applied using a syringe and blunt cannula to half of the animals, however this will not be reported further here. For this discussion, the two groups (with and without chemotherapeutic gel) have been combined into a single group of four animals (eight implants total) per time point.

Euthanasia

Four sheep were euthanized after one, two, four, eight and 16 weeks. Peri-implant clinical measurements, radiography and microbial sampling were repeated for the four animals at each time point prior to euthanasia. The sheep were then cannulated through the carotid arteries and exsanguinated from the jugular vein with simultaneous perfusion with formalin.

Descriptive histology

The implants were separated into individual samples. The premolar teeth were trimmed to a block containing the two mandibular premolars. All tissues were then dehydrated, embedded in methacrylate resin, sectioned, glued to plastic slides, ground and polished to a final thickness of 90 to 130 μm and stained with MacNeils Tetrachrome and Toluidine blue. In some cases, sections were obtained in both bucco-lingual and mesio-distal orientation, but for most the orientation was bucco-lingual. Some slides were counterstained with acid red. The morphology of the lesion and degree of inflammation was described for the most central section from each specimen.

Histomorphometric analysis

Sections were viewed using an Olympus Vanox-T microscope (Olympus Australia Pty

Ltd, Australia) at 20X magnification. Digital images were captured using a Diagnostic Instruments SPOT RT Color camera (SciTech Pty Ltd, Australia). Analysis was performed by a separate examiner (AS) who was not involved in the surgery and who was blinded with respect to treatment group. The two most central sections from each implant were chosen for analysis and both sides of each implant were evaluated. After the best three consecutive threads from each implant were identified, the bone-to-implant contact (BIC) within each thread was measured in calibrated images using Image J software (National Institutes of Health, Bethesda, USA) (Gottlander and Albrektsson 1991). BIC was expressed as a percentage of the total perimeter of each thread. Three blind %BIC estimates were produced for each section and results were compared and averaged. Ten sections were randomly chosen and analyzed by a second experienced reader (WD) in order to ensure reliability of the data. The distance

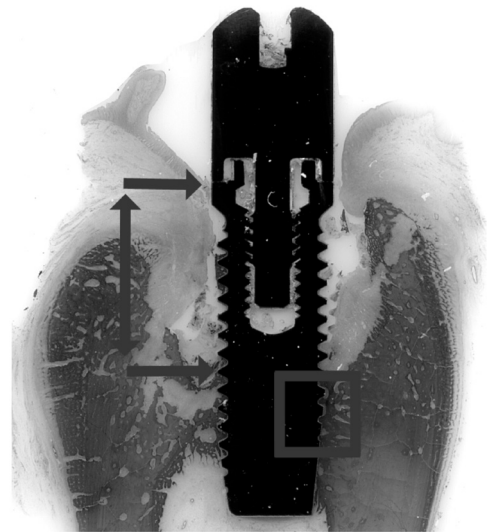


Figure 6. Histomorphometric measurements. Measurement from shoulder of implant to first bone-to-implant contact (S-fBIC) (red arrows) and percent bone-implant contact (%BIC) for the best three consecutive threads (red box).

from the implant shoulder to first bone-implant contact (S-fBIC) was also measured bilaterally on each section and averaged for each implant (Figure 6).

Microbiology

Plaque samples from a premolar and implant region were sampled using a periodontal curette and placed into 1 mL of sterile PBS buffer prior to ligature placement, after disease induction and at euthanasia. Plaque samples were stored at -20°C until analysis. The defrosted tubes were centrifuged for one minute, mixed with 20 mL InstaGene™ matrix, incubated at 56°C for 15 to 30 minutes, vortexed at high speed for ten seconds, and subsequently incubated at 100°C for eight minutes. The samples were then vortexed at high speed for ten seconds and centrifuged at 10,000 to 12,000 rpm for two to three minutes. The supernatant from each sample, now containing extracted DNA, was collected and placed in 126 separate, sterile Eppendorf tubes. These samples were sent on dry ice to New Zealand Genomics Laboratory (NZGL) at Massey University. Bioanalysis PCR and high throughput Illumina sequencing was performed using a 16S universal primer by NZGL.

Statistics

Statistical analyses were performed using SPSS PASW Statistics 18 software. Implant survival and clinical measurements were compared using parametric paired t-tests and ANOVA. As the histomorphometric data showed non-normal distribution, the non-parametric Mann Whitney U test was used to determine differences in %BIC and S-fBIC between groups. Pearson's coefficient test was employed to compare the %BIC estimations between different examiners.

Results

Animal survival

Two animals died during the tooth extraction phase due to causes unrelated to the experimental protocol; these animals were replaced. None of the animals died during the course of the experimental treatment period and no animal lost $>10\%$ bodyweight.

Delayed implants and tooth survival

No teeth were lost during the study period. Seven out of 40 implants with peri-implantitis (17.5%) failed at ligature removal. A further four implants failed during the active treatment period; the overall survival rate for implants with ligature-induced peri-implantitis was 72.5%. Implant failures by implant type and position, for the different time points, are shown in Table 1; baseline in this table is defined as following ten weeks of integration and seven weeks of disease induction. Implant survival rate by implant type is shown in Table 2. Overall, 20% of the Southern implants failed, and 35% of the Nobel implants failed; this difference was statistically significant (paired t-test, $p=0.02$).

Immediate implant survival

Four sheep received two immediate implants each; a total of eight implants were placed. No sheep died during the 16 weeks integration period. One Nobel implant was lost and two had marked peri-implant bone loss apical to the implant shoulder (Figure 7). One Southern implant had no bone-to-implant contact and was considered a failing implant; the other three were successfully integrated with no evidence of peri-implant bone loss (Figure 7). Nobel versus Southern Implants success rate was 25 versus 75% but this did not differ significantly ($p=0.2$); an additional

	Baseline	1 week	2 weeks	4 weeks	8 weeks	16 weeks	Total
Nobel, anterior (N=20)	4	0	0	1	0	2	7
Southern, posterior (N=20)	3	0	0	0	0	1	4

Table 1. Implant failures by treatment group and by implant position.

	Implant survival rate %
Nobel implants (Implant 1) N=20	65
Southern implants (Implant 2) N=20	80
Overall (all implants) N=40	72.5

Table 2. Implant survival rate by implant type and treatment group.

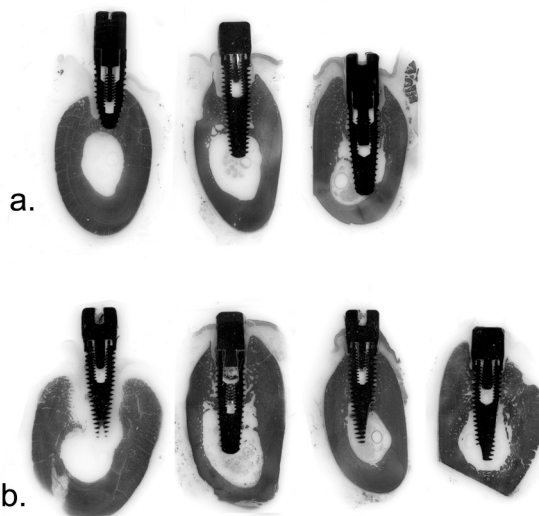


Figure 7. Immediate implants in the sheep model. (A) Nobel implants. Two show bone loss apical to the implant shoulder. (B) Southern Implants. One shows complete failure of integration; the remainder are well integrated with no bone loss apical to the shoulder.

four sheep with a similar pattern of survival would be required to demonstrate a statistical difference.

Clinical measurements

Periodontal probing data for each of the two premolars were combined to give

a single mean figure for all six sites, for the two premolars combined. Peri-implant probing data for each of the two implants were combined to give a single mean figure for all four sites, reported separately for implant 1 and implant 2 and then combined for the two implants together. This procedure was repeated for recession and for the calculated

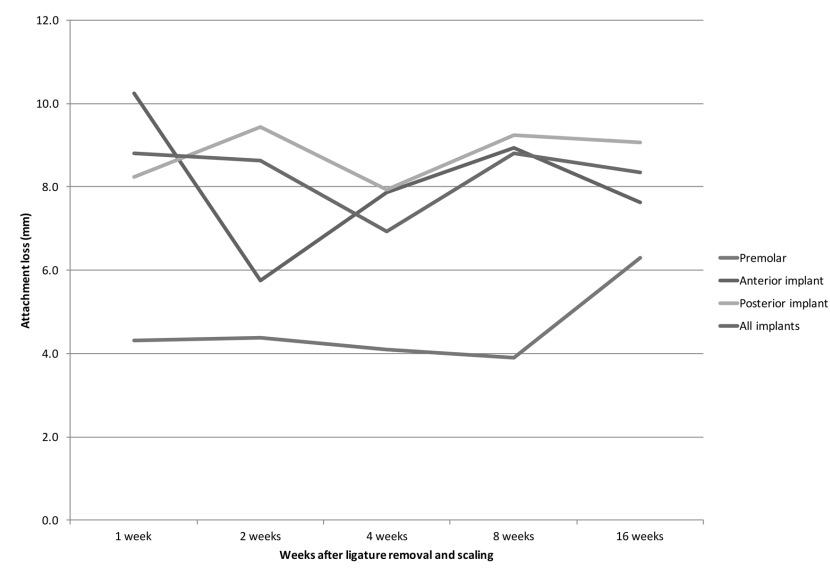


Figure 8. Changes in pocket depth after ligature removal and scaling for premolar teeth, anterior and posterior delayed-placement implants and all implants combined.

attachment level.

Data were averaged for all 10 sheep in the test and the control group, to give a mean figure for each parameter at baseline. Data for the four animals at each time point (one, two, four, eight and 16 weeks) were then averaged to give a mean for each time point. The changes in pocket depths for premolars and for the delayed-placement implants are shown in Figure 8. This illustrates the relative severity of disease around the implants and the relatively mild disease around the teeth. Pocket depths around implants improved more over time, however this was impacted by implant losses, and the small numbers of implants at each time point. Pockets around teeth showed little change after treatment. The changes in attachment level over time are shown in Figure 9, for implants versus teeth; a similar pattern is apparent.

Microbiology

The high throughput sequencing gave an indication of the various bacterial genera

present within the samples. However, due to the small number of test animals in the study leading to high sample variance, no statistically relevant differences in genera abundance between samples could be determined. *Prevotella*, *Bacteroides*, *Porphyromonas*, and *Tannerella* were consistently detected at higher levels in diseased sheep samples compared to baseline samples (implant site $p < 0.01$), which is consistent with literature reports related to periodontitis and peri-implantitis (Paster *et al* 2001). The remaining genera identified in the samples, *Fusobacteria*, Firmicutes, Actinobacteria, and SR1, are Gram-positive anaerobes that are known to prominently feature in periodontal infection sites (Slots 1979).

Radiography

Radiographs were taken of all specimens prior to surgery, after creation of the lesions and at euthanasia. Figure 10 shows postmortem radiographs (mesiodistal orientation) for test and control implants after one week, as well

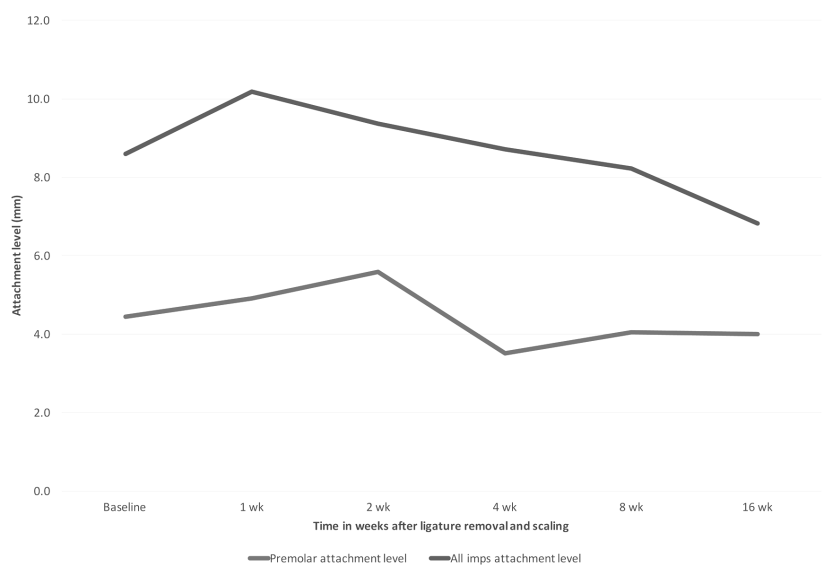


Figure 9. Changes in attachment level over time, after ligature removal and scaling for premolar teeth versus all implants combined.

as micro-radiographic images (bucco-lingual) obtained for the histological thick-sections after sectioning and prior to mounting onto slides for grinding and polishing. The extent of the surgically-created peri-implant lesion is indicated by the red line.

Histology

Representative sections from only the one week and the 16 week groups are shown below. The results from the two, four and eight week sheep showed progressive changes intermediate to that seen at one week and 16 weeks.

Premolars with periodontitis after one week

After one week, specimens demonstrated a surgical defect on the buccal surface, with apical relocation of the alveolar margin. Some also showed notching of the root surface. The wide periodontal ligament that are characteristic of sheep teeth can be seen.

The gingiva was closely adapted to the teeth in the region of the cemento-enamel junction; the enamel extends subgingivally in this species. Compared to the lingual surfaces, the buccal gingiva exhibited apical downgrowth overlying a mild to moderate inflammatory reaction (Figure 11).

Implants with peri-implantitis after one week

Both the Nobel and Southern implants exhibited an acutely-inflamed intra-bony surgical defect extending up to 50% of the length of the implant body, with the apical portion remaining integrated into bone and extending into the marrow space. Beneath the apical part of the surgically-created defect the bone was infiltrated by an acute inflammatory focus. Inflamed epithelium lined the defect, along with what appeared to be bony sequestrae (Figure 11).

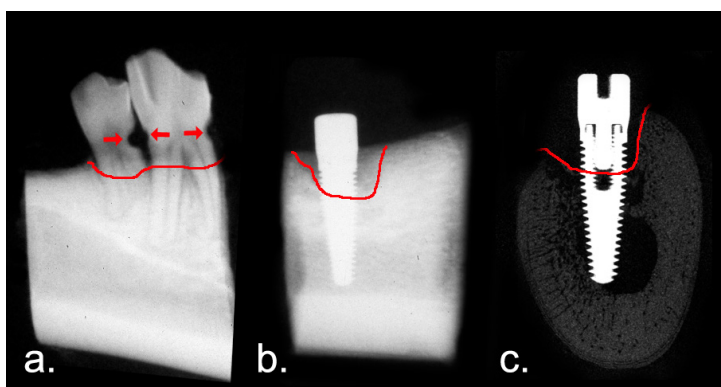


Figure 10. Postmortem radiographs (a & b) and histological radiomicrograph (C) for one week delayed-placement ovine implant. (A) Premolar teeth; arrows indicate notching in teeth during creation of defect; line shows extent of defect. (B) Southern Implant mesio-distal view. (C) Southern Implant bucco-lingual view; line shows extent of defect.

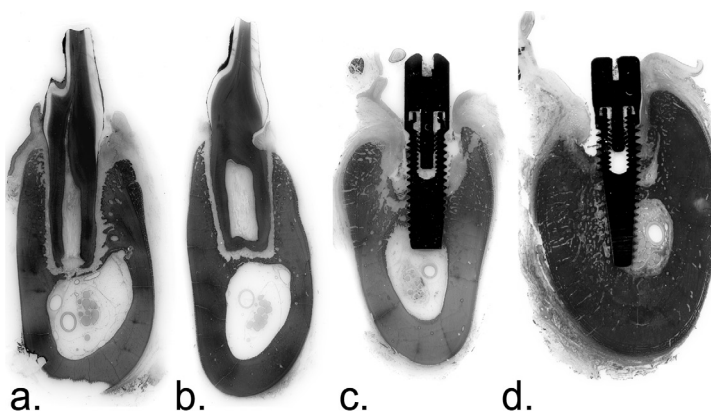


Figure 11. Histological specimens, one-week premolars with periodontitis and implants with peri-implantitis. (A) Periodontitis on buccal (right) surface with inflammation. (B) Periodontitis with mild inflammatory reaction with notch on buccal surface of premolar. (C) Nobel implant with highly-inflamed surgically-created defect; apical portio still osseointegrated. (D) Southern Implants tapered implant with highly-inflamed surgically-created defect; apical portion still osseointegrated. Stained with MacNeils tetachrome and toluidine blue, acid red counterstain.

Premolars with periodontitis after 16 weeks

Persisting periodontal pockets on the buccal surface of the premolars were apparent, with little evidence of inflammation. In some cases the pocket epithelium extended into a notch on the buccal surface. Formation of calculus on the tooth surface within the pocket was seen. The buccal alveolar margin was at a more apical position, but reparative

bone formation was observed in the site of the surgical defect. The surviving Nobel and Southern implants showed evidence of continued apical migration of the chronic inflammatory defect with suppuration, food debris and bony sequester filling the wide intra-bony defects. The inflammatory lesion was in direct contact with the alveolar bone along with exuberant attempts at osseous repair (Figure 12).

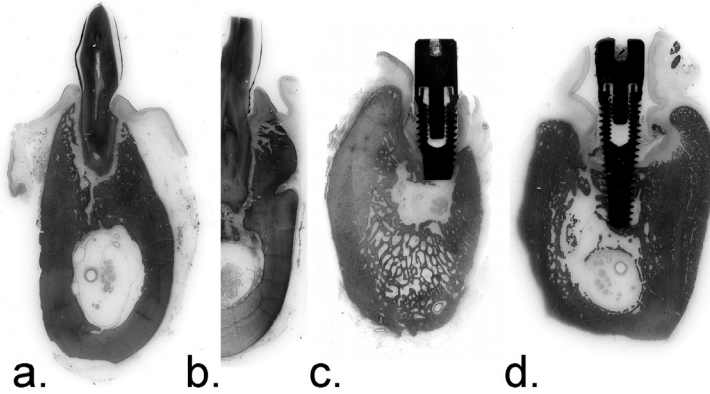


Figure 12. Premolars with periodontitis and implants with peri-implantitis after 16 weeks. (A) Second premolar with notched buccal surface. Mature pocket epithelium with little inflammation fills the notch. (B) Buccal half only of a second premolar. Mature pocket epithelium lines a persisting pocket; calculus can be seen on the root surface. Evidence of reparative bone healing can be seen on both specimens. (C) Nobel implant and (D) Southern Implants tapered implant. Both show progressive chronically-inflamed peri-implantitis lesions with suppuration, bone sequester, apical migration of the investing bone, lined with pocket epithelium. The inflammatory lesion in (D) extends into the alveolar bone.

Histomorphometric measurements

The %BIC was relative low for the immediately-placed implants after 16 weeks osseointegration (52% and 56% for Southern and Nobel) (Figure 13). For delayed-placement implants, the %BIC dropped at two weeks after induction of peri-implant disease to between 31 to 33%, and then rebounded to lie between 50 to 60% BIC at all time points thereafter. There were no discernible differences between the two types of implants for %BIC. For the measurement of S-fBIC, this was markedly lower for the immediately-placed Southern implants compared with the Nobel implants (2.6 vs 5.1 mm) (Figure 14). After induction of disease, S-fBIC increased to between 5.4 and 6.4 mm at one week, 5.3 to 5.9 mm at 4 weeks, and peaked at 7.3 to 7.7 mm at 8 weeks before dropping to between 6.0 to 6.9 mm at 16 weeks. The numbers were too few to allow valid statistical comparison, however with the exception of the immediately-placed implants, there was little to distinguish the two implant types.

Discussion

This is the first study to report simultaneous ligature-induced periodontitis and peri-implantitis in a sheep animal model, and the first to report successful osseointegration of implants placed as an immediate one-stage procedure into fresh posterior extraction sockets in sheep. Disease was successfully induced around teeth and implants, and this was reflected in the increase in proportions of periodontopathogens detected after lesion creation.

The failure rate for the delayed-placement disease-induced implants was high, with a survival rate of 72.5%. No teeth were lost from the contralateral side during the same time period. The survival rate for the immediately-placed implants was 87.5%, however this included a failed implant and several with incomplete healing and peri-implant bone loss; the success rate for the immediate implants was thus 50%. This was higher for the Southern implants which had a tapered configuration and machined coronal portion

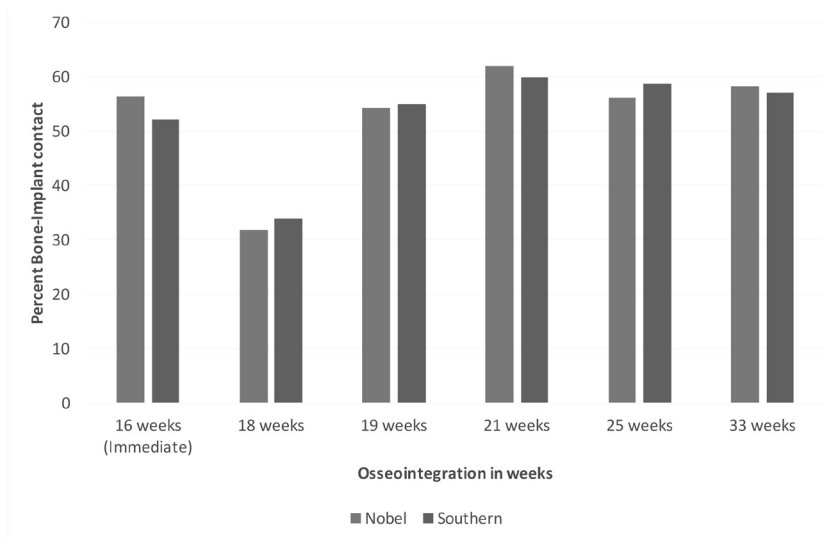


Figure 13. Average percent bone-implant contact by implant type.

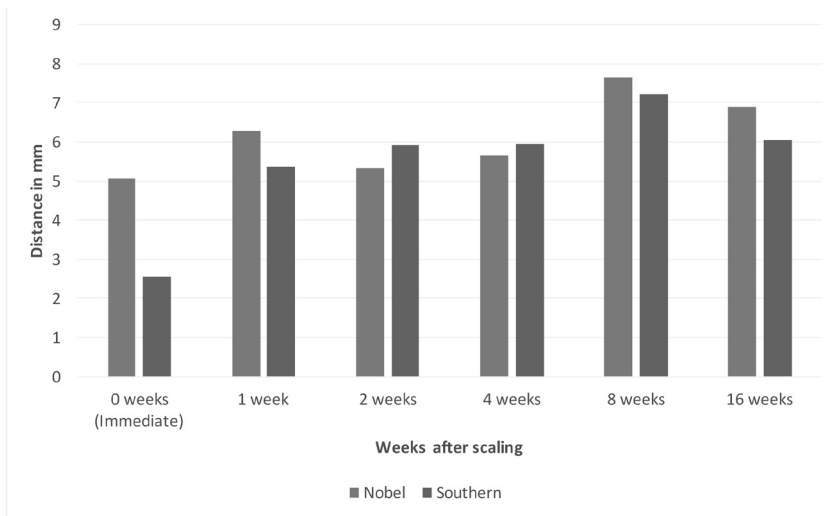


Figure 14. Average distance from implant shoulder to first bone-implant contact, by implant type.

(75% success) compared with the Nobel implants with a parallel-sided configuration and anodic oxidised TiUnite surface (25%).

The probing depths around premolar teeth were relatively shallow, and showed little discernible change over time after scaling, although histological evidence suggests that the tissue became less inflamed and there were signs of osseous healing. Recession became more apparent around the teeth after

16 weeks. The pocket depths around implants were about twice as deep as around teeth, and worsened initially over time, before showing some improvement; this is most likely because the most severely-involved implants were progressively exfoliated. This pilot study was designed to compare an antimicrobial treatment against scaling alone (details of which are not included in this paper), and thus there were no implants which had disease

created and then did not receive any scaling. Future studies comparing untreated versus treated peri-implantitis in this model should be explored.

A small subgroup received immediately-placed implants that were restored with healing abutments. The Nobel TiUnite-surface implants had twice the vertical distance from implant shoulder to first bone-implant contact after 16 weeks, reflecting the histologically-observed peri-implant bone loss. This may have been due to the anterior positioning of the Nobel implants where buccal bone may have been thinner, however even those implants placed deep into the tooth sockets still showed failure of the bone to bridge the gap between socket wall and implant. The Southern implants performed better in this respect. These Southern MSC implants have been designed to be placed into “at risk” patients with a history of periodontitis, which the sheep animal model represents; thus, this pilot study provides some support for this approach to implant treatment in periodontitis patients. Others have also reported that implants with the TiUnite surface demonstrate a larger inflammatory infiltrate and more rapid progression of ligature-induced peri-implantitis in the dog model compared with blasted and machined surface implants (Albouy *et al* 2011, Albouy *et al* 2012). In the current study, both the immediately-placed implants and the delayed-placement implants in disease-induced animals showed comparatively poor bone-implant contact, which was particularly noticeable in animals shortly after creating disease. Neither implant type showed a superior outcome for %BIC.

Little comparative data exists for implants placed into intra-orally in sheep. Two other groups have published work where implants have been placed in the inferior border of the sheep mandible from an extra-oral approach. Implants in this unloaded, extra-oral submerged model were not subjected to

the same biomechanical and microbiological challenges as intra-oral implants, however the percent bone-implant data is informative, as the implants are placed into the same dense mandibular bone. Values were reported by Trisi *et al* (2011) ranging from 15 to 39% after one week, to 45 to 50% at six weeks. Jimbo *et al* (2014a, 2014b) reported similar values in the range of 15 to 34% at three weeks and 20 to 57% at six weeks. For longer healing periods, values of 34 to 36% have been reported at eight weeks and of 46 to 59% at 12 weeks (Consolo *et al* 2013, Trisi *et al* 2015).

In a delayed-placement intra-oral model in the goat maxilla, Caulier *et al* (1998) reported survival rates of 82% at three months and 67% at six months for submerged unloaded implants, with an overall survival rate of 74%; %BIC ranged from 11 to 15% for machined titanium and 30 to 35% for plasma-sprayed calcium-phosphate coatings, at three and six months respectively. Loaded implants with healing abutments in the same model had a survival rate of 81% at 10 months, but only 37% of machined titanium (mTi) implants survived, compared with 85% of CaP-coated implants (Caulier *et al* 1997). The %BIC ranged from 26.5% (mTi) to 69% (CaP) and the distance from alveolar crest to first bone-implant contact ranged from 7.0 ± 4.0 mm (mTi) to 9.6 ± 2.2 mm (CaP) after 10 months healing (Caulier *et al* 1997).

Our research groups previous data for intra-oral implants in the sheep posterior mandible has shown a range of values for survival or success and for %BIC (Table 3). In three experiments involving 132 implants with eight different configurations and surfaces placed into 57 sheep, the overall survival rate after 12 weeks healing was 90.2% (Duncan 2005, 2006). For unloaded, submerged implants after 12 weeks, this ranged from 75% survival and 15% BIC for machined surfaces, to 81% survival and 84 to 88% BIC for machined and blasted implants, to 100% survival and 85%

BIC for plasma-sprayed CaP. For indirectly-loaded one-stage implants, survival ranged from 50% for machined surfaces to 87.5% for Straumann SLA surfaces, and %BIC for the same implants from 29 to 73%. Subsequent experiments revealed blasted, anodised (TiUnite) and blasted etched (Osseotite) surfaces achieving 60, 30 and 20% survival and 34, 66 and 64% BIC respectively after three months indirectly loaded using a one-stage approach, and 30, 60 and 30% survival with 23, 53 and 52% BIC after six months using a two-stage loaded protocol (Eggerath *et al* 2006, Fitzgibbon *et al* 2006, Kim *et al* 2006). A subsequent experiment using novel calcium-phosphate-anodized implants achieved 82.5% survival with %BIC of 58 (blasted) to 84% (anodized) using a one-stage immediately-loaded approach after one month's healing and 97% survival with %BIC ranging from 46 (blasted) to 83% (anodized) using a two-stage approach (Duncan *et al* 2015a, Duncan *et al* 2015b). Using a one-piece, one-stage protocol and comparing blasted titanium (Ti) implants to machined-surface zirconia (Zr) implants after 12 weeks, Siddiqi *et al* (2017) reported survival of only 40 (Zr) to 70% (Ti) but %BIC was still 60 (Ti) to 72% (Zr).

Overall, for 300 implants placed into 121 animals, implant survival rates ranged from 30 to 100% and %BIC ranged from 16 to 85%. For short-term healing in the sheep model, most modern implants can be expected to achieve survival rates >80% and %BIC >70%. In the current experiment, both the immediate implants and the delayed implants with induced disease showed poorer responses, indicative of the higher risks posed by immediate placement and by plaque-induced peri-implant inflammation.

Conclusions

This pilot study represents a first attempt

to create a split-mouth, surgical and ligature-induced chronic inflammatory model of periodontal and peri-implant disease in sheep. It was also a first attempt to place immediate implants into tooth sockets in sheep. Insufficient animals were used at each time point for complete statistical analysis, however the results from this initial study are sufficiently encouraging to warrant further studies using larger numbers of animals. Some trends were detected that suggest that implant surface and configuration may affect outcomes of treatment involving immediate implant placement or delayed placement into patients at risk of periodontitis. The results also suggest that the disease created around the implants may have been too severe in an animal model where the treatment provided did not involve surgical repair and grafting (which is recommended for severe peri-implant disease in humans). At the same time, the ligature-induced periodontal disease may have been insufficiently severe and thus susceptible to the robust healing capability of this animal species, which would tend to mask any difference between treated and control animals. Future experimentation should involve a much shallower defect around the implants and a deeper defect around the teeth.

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Chapter 11

Saving Compromised Implants

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Introduction

Dental implant treatment for replacement of missing teeth has brought considerable benefits for both patients and clinicians. In healthy patients, the success rate of implants over 15 years of follow-up has been recorded as 91.5%, and marginal bone levels can be maintained with limited bone loss (Dierens *et al* 2013). However, mechanical and biologic complications related to implants are steady increasing, which is of concern to dentists who require good implant success. Peri-implant disease is a biologic complication that has emerged as a public health issue in recent years. However, there is little understanding of this clinical problem, and a clear consensus on the precise etiology and treatment has not been secured.

Definition

The term peri-implant disease, described as an infectious disease by Mombelli, was introduced in 1988 to describe an inflammatory reaction in the tissues surrounding implants. It was categorized into peri-implant mucositis and peri-implantitis. In 1994, Albrektsson and Isidor defined peri-implant mucositis as a reversible inflammatory reaction of the peri-implant soft tissue, and peri-implantitis

as an inflammatory process resulting in the loss of supporting bone. Recently, Heitz-Mayfield (2016) proposed composite criteria for peri-implantitis including; deep pocket (>5 mm) formation with bleeding on probing or suppuration concomitant with well-defined peri-implant marginal bone loss. The most important sign of inflammation around implants is bleeding on probing. From the 2014 European Federation of Periodontology Workshop, bleeding on probing has been recognized as the main clinical sign and a predictor for diagnosing the onset and progression for both periodontitis and peri-implantitis.

Prevalence

Globally, periodontitis is the 6th most common disease with 50% of the world's population estimated to suffer from this condition, and 11% of these possibly having aggressive periodontitis. Concerning peri-implant disease, 10% of implants and 20% of patients are likely to develop this disease 5 to 10 years after implant placement (Mombelli *et al* 2012). A recent review indicated that peri-implant disease has a prevalence of between 1 to 47% at the implant level (Monje *et al* 2016). A meta-analysis has reported a mean prevalence of 43% for peri-implant

mucositis and 22% for peri-implantitis. A recent systematic review, based on a European consensus conference, revealed that the prevalence of peri-implant mucositis and peri-implantitis ranges from 19 to 65% (Derks and Tomasi 2015). Overall there appears to be considerable variability on the incidence of the conditions. The cause for such heterogeneity might be due to the varying diagnostic criteria and standards for implant success in studies.

Unlike natural teeth, each implant system has its own implant-bone level relationship. Therefore, in order to determine physiologic and pathologic bone loss, the initial radiographic information at the time of placement is necessary but not always available. Also, radiographic reference points vary according to the implant system being used, as does the pattern of marginal bone loss, which cause problems for establishing a universal diagnostic standard. Nevertheless, the reporting of a relatively high prevalence of peri-implant disease necessitates further evaluation for treatment planning, treatment methods, and long-term success.

The future prevalence of peri-implant complications can be predicted to increase, as the prevalence of peri-implant disease of present surpasses that of 20 years ago. The length of the maintenance period for implants can be considered a risk factor. Implant placement protocols have changed vastly compared from the strict protocol described and utilized 40 years ago. Development of newer materials and technology has introduced advanced procedures with interesting new protocols, which facilitate easier decision-making. Clinicians need to consider whether these may contribute to the increasing prevalence of peri-implantitis.

Etiology

Three theories have been proposed for the etiology of peri-implantitis, which are

bacterial infection, occlusal overload, and/or compromised healing (Koka and Zarb 2012). Although the exact cause of peri-implantitis has not been established, it is generally understood to be an infectious disease, in which a bacterial biofilm drives the causative mechanisms. Bleeding on probing (BOP) and suppuration are present as is in periodontitis and periodontal pathogens are found to be associated with failed implants. Removal of the biofilm results in reduction of inflammation (Persson and Renvert 2014). The mechanism of biofilm formation on natural teeth surface is well established and biofilm formation on implant surfaces is reported to occur in a similar manner. Initial bacterial colonizers attach to the implant surface using adhesion molecules, subsequently secreted extracellular substances and secondary colonizers comprising the pathologic bacteria attach on top to form the mature of biofilm. Biofilm formation on implant surfaces is partially dependent on the characteristics of the implant surface such as wettability/hydrophobicity, and surface free energy (SFE).

Even if the bacterial biofilm provides the causative mechanism, the primary pathogens or peri-implantitis specific pathogens still remain to be identified. Nonetheless, peri-implantitis is considered to be a mixed-microbial anaerobic infection. There is data that suggests chronic periodontitis and peri-implantitis share similar pathogenic flora, but the composition in peri-implantitis is different and appears more complex than in periodontitis (Maruyama *et al* 2014). Periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, and *T. denticola*) have been associated with both healthy implants and those affected by peri-implantitis (Casado *et al* 2011). When compared to periodontitis, there appear to be significantly greater numbers of pathogens such as *P. gingivalis*, *T. forsythia*, *P. intermedia*, or *T. denticola* in peri-implantitis

(Albertini *et al* 2015). It is possible that periodontal pockets associated with natural tooth may act as a reservoir of pathogens that induce peri-implantitis since the microbiota of periodontitis and peri-implantitis display significant similarities (Botero *et al* 2005). This is further supported by the finding that microbiota on the implant of the fully edentulous patient demonstrate different profile compared to those of the partially edentulous patients (Zambon and Haraszthy 1995). Despite this it is also noteworthy that species not associated with periodontitis were detected such as *Staphylococcus aureus*, *Enterobacteriaceae*, *Candida albicans* and *Pseudomonas aeruginosa* (Salvi *et al* 2008). Therefore, it might be plausible that the microbiota of peri-implantitis present distinct differences to that seen for periodontitis (Renvert and Quirynen 2015).

Risk factors

As gingivitis is to periodontitis, peri-implant mucositis can be presumed to be the precursor of peri-implantitis (Costa *et al* 2012). The reduced prevalence of peri-implantitis in patients receiving regular supportive therapy supports this presumption. Although implants may seem to be comparable to natural teeth in certain ways, the stark differences in histologic appearance of the attachment of the soft and hard tissues to implants compared to teeth cannot be ignored. However, to date, there is no evidence demonstrating any difference in the mechanism of the inflammatory reaction around implants and teeth. The risk factors associated with the induction of peri-implant mucositis and peri-implantitis can be categorized into general and local factors. History of previous periodontitis is one of the general risk factors that contribute to peri-implantitis and implant failure (Daubert *et al* 2015). Interestingly, implants placed in sites in which the cause of extraction was

periodontitis revealed greater risk for peri-implantitis (Schou *et al* 2006). Systemic disease can also be a risk factor. As for periodontitis, diabetes and smoking can lead to increased risk for peri-implantitis (Daubert *et al* 2015, Rinke *et al* 2011). There has been suggestion that cardiovascular disease such as hypertension may influence the development of peri-implantitis, but further research is required to verify this.

Peri-implantitis is an infectious disease, thus an environment for accumulation of bacterial biofilm can provide a local risk factor. Remaining deep periodontal pockets can act as a reservoir for the accumulation of bacterial pathogens, which can colonize the implant surface (Aoki *et al* 2012). Implants that have poor accessibility for oral hygiene have a high risk for developing peri-implantitis. According to the literature, 48% of implants with peri-implantitis had no accessibility for oral hygiene (Serino and Strom 2009). Therefore, prosthesis designs that allow good access for oral hygiene measures are important for long term success. Rough surface implants are reported to be more susceptible to peri-implantitis than the smooth (Teughels *et al* 2006). Furthermore, the implant surface characteristics also influence the treatment outcomes of peri-implantitis. For example, peri-implantitis treatment success rate of TPS implants (14.3%) has been shown to be statistically significantly lower than for SLA surfaces (58.3%) (Roccuzzo *et al* 2017). Lack of keratinized tissue is another factor that may impede plaque control; therefore, securing and adequate amount of keratinized attached tissue may be beneficial for long term maintenance (Lin *et al* 2013).

The positioning of an implant at the time of placement may also act as a risk factor. Labially placed implants outside of the bony housing and the associated dehiscence may lead to the development of peri-implantitis. If soft tissue is lost labially, esthetic problems may develop.

Implants placed too near to natural teeth without consideration of physiologic bone resorption may also contribute to increased risk of peri-implantitis as well as compromise of the periodontal tissues of the adjacent tooth. In the case of multiple implant placement, disharmony of the marginal bone level can interfere with plaque control, which may also result in the development of peri-implantitis.

Excess cement materials

Excess cement materials remaining in the implant sulcus after crown cementation seem to act as a trigger factor for peri-implantitis provoking an inflammatory response. Remaining cementum in the sulcus not only in itself induces a foreign body reaction, but also provides the structural obstruction for buildup of bacterial biofilm. Excess cement has been reported to be associated with 81% of peri-implantitis cases. Removal of the excess cement resulted in resolution of inflammation in 74% of implants (Korsch *et al* 2014)

Treatment

The goal of treatment of peri-implantitis is to resolve the inflammatory reaction as judged by reduction in BOP or suppuration and to stop the bone loss progressing (Sanz and Chapple 2012). Recently published composite criterion for successful treatment of peri-implantitis suggests that the target outcomes should be pockets of less than 5 mm with the absence of BOP and no further bone loss at the 12 month review (Heitz-Mayfield and Mombelli 2014). Apart from these treatment goals, an ideal treatment should aim for re-osseointegration of exposed implant surface with soft and hard tissues lost due to peri-implantitis.

Treatment of the failing implant involves explantation of the implant followed by reconstruction of the lost hard and soft tissues, and placement of a new implant. However, the

lack of clear etiology and effective treatment method call for reconsideration. Another option is to treat the peri-implantitis to try to retain the implant. However, effective decontamination measures and the methods to encourage re-osseointegration are yet to be determined. Furthermore, it is difficult to predict that the new implant placed in a previous site affected by peri-implantitis will be disease free. As discussed earlier, implants placed in the site of previous periodontal disease are predisposed to greater risk of developing peri-implantitis.

Based on the theory that bacterial biofilm is the fundamental cause of peri-implantitis, it seems reasonable to use treatment methods similar to those used for periodontitis. However, the treatment outcomes for these two diseases may be different. Nonsurgical therapy can be applied to mucositis and peri-implantitis but the results are unpredictable. According to the literature, surgical therapy which allows better access to the implant surface than nonsurgical results in more predictable clinical outcomes. BOP after non-surgical treatment has been reported to range between 19 to 84% of the sites whereas for surgical treatment the BOP levels ranged between 13 to 53%.

Different approaches for the treatment of peri-implant mucositis have been attempted such as the use of curettes, air polisher, ultrasonic scaling laser, photodynamic treatment, and adjunctive systemic and locally delivered antimicrobial agents, but no single ideal method could be identified. Repeated self-irrigation of the inflamed area has been reported to be effective for enhancing the treatment outcome (Renvert and Quirynen 2015). However, unresolved inflammation after nonsurgical treatment at re-evaluation stage often signals the need for surgical approach.

The outcome of peri-implantitis therapy may differ according to the surgical technique.

Even though the criteria for success vary in the literature, the success rate is below expectation when aforementioned treatment goals are considered. Since the bacterial biofilm provides the causative mechanism for peri-implantitis, one study incorporated the use of antimicrobials to their surgical protocol. In this five year clinical study, 27 patients received surgical treatment and maintenance therapy with CHX irrigation at each visit. The results showed that deep pocket depth and bleeding on probing remained in 39% of the patients (Serino *et al* 2015). Another study demonstrated the efficacy of anti-infective surgical treatment as a method to treat peri-implantitis as an infectious disease. It was found that only 19% of the patients had residual pockets with BOP after 12 months. We must take into consideration that success of peri-implantitis treatment is not only dictated by the method of treatment but also the surface characteristics of the implant. In 2017, it was reported in a seven year clinical trial that implants with SLA surface showed 58.3% success rate, whereas the TPS surface showed 14.3%.

Surface decontamination

Since peri-implantitis is an infectious disease caused by a bacterial biofilm, the major focus of treatment should be on meticulous removal of biofilm by implant surface decontamination, and provision of an environment that prevents the recolonization of bacteria during the healing phase (Renvert and Polyzois 2015). Various methods of treatment have been introduced such as ultrasonic scaler, curette, laser, air abrasive, photodynamic therapy, laser, cotton swabbing with chlorhexidine or saline and locally delivered antibiotics. Implantoplasty, a concept of "root planing for implants", has also been attempted. However, no one therapy has been shown to be better compared to the rest.

Therefore, a combined approach of different therapies with adjunctive antibacterial treatment is advised, and has been reported to significantly improve the treatment outcome. Studies have also shown adjunctive antibiotics use with periodontal therapy may enhance the efficacy of treatment. Tetracycline is one antibiotic that can be used as an adjunct for periodontal treatment. It is known to be an effective antimicrobial agent for the control of periodontal pathogens including *Prevotella intermedia/nigrescens*, *Fusobacterium*, *Bacteroides forsythus*, *Campylobacter rectus*, *A. actinomycetemcomitans*, *P. gingivalis* and *Eikenella corrodens*. Minocycline is a broad spectrum tetracycline antibiotic, which can be locally administered during surgery. It has been shown that the adjunctive use of minocycline in surgery can significantly enhance the resolution of inflammation. Repeated administration of minocycline ointment into pockets deeper than 6 mm at surgery and maintenance period of up to six months, has resulted in significant improvement of clinical indices including BOP and PD compared to the control. The number of sites showing PD reduction of more than 3 mm was notably greater in the group administered local minocycline. This was attributed to the prevention of bacterial recolonization during the initial healing period by the repeated application of minocycline. Therefore, it would be reasonable to expect the adjunctive use of local antibiotic administration to be effective for treatment of peri-implantitis.

In 2016, a preclinical trial showed that disease resolution can be achieved without the use of adjunctive systemic and local antimicrobial therapy (Albouy *et al* 2011). However, in another study in which the treatment outcome was evaluated after 12 months in 31 patients, it was found that adjunctive systemic antimicrobial treatment can be effective, as in 48% of patients,

suppuration and BOP disappeared. This study however, did not report findings related to bone level changes. In a similar study, systemic antibiotics combined with surgical therapy resulted in resolution of BOP and probing depth of less than 5 mm in 47% of patients after one year (Heitz-Mayfield *et al* 2012). In terms of the bone level changes, 92% of implants showed stable or increased bone level after one year.

The treatment outcome may decline with longer follow up periods. Recently, one study reported a gradually decreasing success rate of surgical treatment protocol with adjunctive anti-infective agents over long term follow-up periods (Heitz-Mayfield *et al* 2016). The results revealed that 79% of patients satisfied the successful treatment criteria after one year. The success rate declined to 75% after three years evaluation and 63% after five years. They concluded that the final success rate of surgical protocol at five years was 63 to 80% considering the dropped-outs during the follow up. Another study reported similar results on success rate (Canullo *et al* 2015). According to the success criteria suggested by Heitz-Mayfield, the success rate of surgical therapy was reported to be 43 in 71 cases (61%). The other 28 implants (39%) had residual pocket formation, nine of those showed clinical attachment loss over five years. It is not feasible to perform surgical therapy at every visit over a long-term follow up, therefore, proactive antimicrobial therapy must be administered to increase or maintain success over long term.

Supportive peri-implant therapy

The outcome for treatment of peri-implantitis depends on surgical therapy followed by appropriate supportive peri-implant therapy. Therefore, regular monitoring using the periodontal probe as well as radiographic assessments for detection of

marginal bone level changes is crucial.

Supportive peri-implant therapy for the prevention of peri-implantitis comprises non-surgical therapy. Although nonsurgical therapy can be unpredictable for the treatment of peri-implantitis, the formation of bacterial biofilm may induce mucositis, which is the prerequisite for the development of peri-implantitis. Therefore, control of biofilm accumulation can be a preventive measure for peri-implantitis. However, unlike management of natural teeth, a mechanical approach alone around implants can be insufficient due to the poor accessibility of the implant surface. Thus, adjunctive antibacterial application is advised. Planning of three to six month regular recall and re-evaluation is important. According to a recent study, prevalence of peri-implantitis was related to the interval of recall visits. Therefore, short check-up intervals can be advised for patients in the high risk category for developing peri-implantitis.

Finally, treatment of peri-implantitis should include the following protocol; pretreatment phase, surgical access, adjunctive anti-infective protocol and comprehensive maintenance protocol. In the pretreatment phase, general and local risk factors should be evaluated and factors that impede plaque control should be removed. Nonsurgical treatment with adjunctive antibiotics can be performed; however, the necessity of surgical treatment should also be assessed. It is advised that a full thickness mucoperiosteal flap is raised to secure access to the contaminated implant surface, and mechanical and chemical decontamination methods should be performed to obtain a clean implant surface.

It has been reported that the prevalence of peri-implantitis may be related to the implant system. Therefore, it is important to choose a system that has long term evidence for marginal bone stability.

Saving compromised natural teeth and compromised implants

Implants were once regarded as panacea to replace compromised teeth and even healthy natural teeth. However, recent reports have revealed mechanical and biological complications that are threats for long term success. Unfortunately, the etiology of such biological complications is still unknown, let alone the correct remedy. Removal of a failing implant and placement of a new one will not necessarily prevent the complication from occurring again. There is need for determination of correct and successful treatment protocols for the management of peri-implantitis. There are various risks related to replacing compromised teeth with implants, therefore, careful decision making is necessary prior to extraction. Furthermore, the pros and cons of implants must be discussed in detail with the patient, and most importantly, the clinician must try their best to save compromised teeth.

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Chapter 12

Clinical Strategies for Implant Treatment in Patients with Periodontitis

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Introduction

Along with the development of oral rehabilitation of partially or totally edentulous patients with dental implants, implant placement in patients with a history of treated periodontitis became more widely used, with implant survival rates reportedly up to 90% over a period of three to 16 years (Heitz-Mayfield 2008, Heitz-Mayfield and Huynh-Ba 2009). However, a number of previous studies have demonstrated that a previous history of periodontitis is a critical determinant of increased risk for the development of peri-implantitis (Karoussis *et al* 2003, Pesce *et al* 2014, Renvert and Persson 2009, Simonis *et al* 2010, Sousa *et al* 2016). There is a trend that the incidence of peri-implantitis is higher and the long-term success rate is lower in patients treated for periodontitis. Moreover, two recent studies have demonstrated that residual periodontal pockets are a risk indicator for development of peri-implantitis in patients treated for periodontitis (Lee *et al* 2012, Pjetursson *et al* 2012). The authors stressed that the lack of maintenance of periodontal health, rather than a previous history of periodontitis, is the critical determinant for increased risk of peri-implantitis. Substantial scientific evidence has shown that a lack of regular periodontal and implant maintenance

treatment was associated with peri-implant diseases (Hultin *et al* 2007, Monje *et al* 2016, Roccuzzo *et al* 2010, Roccuzzo *et al* 2012, Roccuzzo *et al* 2014). A systematic review aimed at assessing the impact of maintenance therapy on the incidence of peri-implant diseases concluded that minimum maintenance therapy intervals should be five to six months (Monje *et al* 2016). However, studies investigating appropriate intervention recall timing to minimize the risk of peri-implant diseases in patients with different periodontal conditions are limited.

Moreover, it is noteworthy that the previous studies have been based mainly on people in developed countries. However, periodontal conditions in the Chinese population are obviously different due to the variety of ethnic origins and socioeconomic conditions. According to the results of the Third National Oral Health Epidemiological Investigation in China, the prevalence of periodontitis is 38.9% in 35 to 44 year old residents and 71.3% in 65 to 74 year old residents (Qi 2008). In addition, the periodontal treatment rate and maintenance rate is low. It is of concern that following periodontal therapy and implant therapy in China, periodontal and peri-implant maintenance is neglected. The placement of dental implants to replace missing teeth has become increasingly popular

in China. However, clinical outcomes of implant therapy in periodontally compromised patients (PCP) is unpredictable. To the best of our knowledge, there have been a lack of longitudinal studies evaluating the clinical outcomes of implant treatment in Chinese patients with different periodontal conditions and frequency of maintenance therapy. Evidence for the outcome and risk factors of implant restoration complications in Chinese patients treated for severe periodontitis is also rare. Therefore, our group have carried out a series of retrospective cohort studies to:

1. Evaluate implant survival rate, peri-implant clinical and radiographic parameters in patients with tooth loss due to non-periodontitis reasons, chronic periodontitis and aggressive periodontitis.
2. Analyze the relationship between periodontal conditions at different phases and peri-implantitis.
3. Evaluate risk factors for peri-implantitis in periodontally compromised patients.
4. Investigate the influence of initial periodontal status and maintenance therapy frequency on the outcome of implant therapy of Chinese patients with different periodontal conditions.
5. Assess outcomes of early-loading short implants (length 6 mm) with splinted-fixed dental prosthesis in the posterior region.
6. Detect dynamic colonization of implant and adjacent teeth by putative periodontal pathogens in periodontitis patients.

Clinical studies of implant therapy in patients with different periodontitis

Part 1. Clinical and radiographic study of Locking-Taper implants in patients treated for periodontitis

Objectives: To evaluate the survival rate, peri-implant clinical and radiographic parameters of locking-taper implants in

patients having lost their teeth due to non-periodontitis reasons, chronic periodontitis and aggressive periodontitis, and analyze risk factors of peri-implantitis.

Material and Methods: 145 subjects had 315 Locking-Taper implants (Bicon) placed and were followed up for one to five years. The subjects and implants were classified into three groups, tooth loss due to non-periodontitis (NP) reasons, chronic periodontitis (CP) and aggressive periodontitis (AgP). NP included 44 subjects with 100 implants, CP 70 subjects with 132 implants, and AgP 31 subjects with 83 implants. Periodontal parameters were recorded before subgingival scaling and root planning (T0), at the end of active periodontal therapy (T1), and at the time of the last recall (T2). Immediately after installation of the final restoration and at the time of the last recall (T2), peri-implant probing parameters were recorded and radiographs obtained. The degree of association between peri-implantitis and several potential risk factors including periodontal conditions at T0, T1, and T2 was investigated using Pearson chi-square test and logistic regression analysis.

Results: After active periodontal therapy, mean probing depths (PD) in CP and AgP were reduced from 4.05 mm, 5.20 mm at T0 to 3.07 mm, 2.96 mm at T1 ($p<0.001$, $p<0.001$), percentages of $PD>6$ mm were reduced from 33.2%, 58.5% at T0 to 14.4%, 10.5% at T1 ($p<0.001$, $p<0.001$). Between T1 and T2 the periodontal parameters remained stable ($p>0.05$). Cumulative survival rates of implants in NP, CP, and AgP was 100%, 97.6%, and 100% for one to five year follow-ups with no statistically significant differences found. At T2, the mean implant PD was 2.78 mm, 2.96 mm and 2.97 mm in NP, CP, and AgP respectively, with NP significantly lower than the other two groups ($p=0.006$, $p=0.01$). The percentage of implant sites with $PD>6$ mm was 3.7% in CP and 4.8% in AgP, both significantly higher than 1.17% in NP

($p=0.003$, $p<0.001$). 8.4% of implant sites showed PD at least 2 mm deeper than those at prosthesis installation were found in CP group, significantly higher than NP (4.3%) ($p=0.003$). Mesial and distal marginal bone change per year was -0.02 mm. The percentage of bone loss <0.5 mm was 91.1% in total. Mesial marginal bone loss per year in AgP was significantly higher than in NP ($p=0.033$). 13.0% implants in NP demonstrated bone gain >0.5 mm, significantly more than both CP and AgP ($p<0.001$). The prevalence of peri-implantitis in periodontitis patients was 19.0% ($N=100$) at patient level and 11.2% ($N=214$) at implant level. Patients with peri-implantitis at T2 presented a significantly higher mean PD, mean BI, percentage of PD >6 mm in residual teeth at T2, compared with those without peri-implantitis (3.42 ± 0.47 vs 2.93 ± 0.47 , 2.29 ± 0.46 vs 1.81 ± 0.67 , 19.2 ± 10.0 vs 9.0 ± 8.6 , $p<0.01$).

Conclusions: Periodontal conditions of patients with chronic and aggressive periodontitis were significantly improved after active periodontal therapy and remained stable during one to five years. The short-term survival rates of Locking-Taper implants in patients treated for CP and AgP was no less than those who lost their teeth for non-periodontitis reasons, and marginal bone was totally stable during one to five years. More implants inserted in NP tended to show marginal bone gain after loading, compared with those in the CP and AgP groups. However, more sites with increasing peri-implant PD were found in CP and AgP patients, compared with NP, and bone loss per year was higher around implants inserted in AgP patients than NP patients. The prevalence of peri-implantitis in patients treated for periodontitis was significantly associated with periodontal conditions before and after implant installation but not before periodontal treatment.

Part 2. A retrospective cohort study of peri-implant conditions in Chinese patients with different periodontal conditions and maintenance frequency

Objectives: To compare the periodontal and peri-implant conditions of Chinese patients with a history of moderate or severe periodontitis and periodontally healthy patients (PHP) and to evaluate the influence of maintenance therapy frequency on the outcome of implant therapy.

Methods: 140 partially edentulous patients who had received SLA implants with a minimum one year follow-up period after implant loading were recruited. A total of 227 implants were included. Based on the initial periodontal examination, the participants were divided into three groups: PHP, moderate periodontally compromised patients (PCP) and severe PCP. The three groups were further subdivided into two groups based on the maintenance therapy after restoration of dental implants: “MF (maintenance frequency) >1 per year” group with a mean maintenance therapy interval of less than one year and “MF <1 per year” group with a mean maintenance therapy interval of more than one year. Clinical examinations were performed around the implants and natural teeth at follow-up. The following clinical parameters of implants were assessed: implant survival/loss, peri-implant probing depth (PDi), peri-implant bleeding index (BLi), implant plaque index (PLIi), peri-implant bleeding on probing (BOPi), implant bone loss (BLi). Comparisons of the peri-implant conditions were performed between the patients with different periodontal conditions.

Results: Implant survival rate was 100% for all three groups. No inter-group statistically significant difference in BLi was observed, but the mean deepest PDi and mean BLi in severe PCP were significantly higher than PHP and moderate PCP ($P<0.05$). At the site level, the

severe PCP had more sites with PDi >5 mm and BOPi+ compared with PHP and moderate PCP ($P < 0.001$). The difference in prevalence of PDi >5 mm with BOPi+ on the site, implant and patient levels between MF >1 per year and MF <1 per year sub-groups of the severe PCP was statistically significant ($P < 0.05$).

Conclusions: No difference in the short-term implant survival rate and peri-implant marginal bone loss were found among the three groups. However, patients with a history of severe periodontitis are at greater risk of peri-implant disease (PDi >5 mm & BOPi+) compared with periodontally healthy patients and moderate periodontally compromised patients. In particular, severely periodontally compromised patients who had a poor adherence to maintenance therapy have a high incidence of biological complications.

Part 3. A prospective, multicenter study assessing early loading with short length implants in the posterior maxilla and mandible: A three year follow-up study

Objective: The aim of this multi-center study was to prospectively assess clinical and radiographic outcomes of 6 mm implants placed in the posterior jaws, in a Chinese population, using an early-loading protocol.

Methods: A total of 45 subjects (77.8% with chronic periodontitis) were enrolled at three study sites, standardized professional periodontal treatments were undertaken before placement of the implants. In total, 95 implants (diameter 4 mm, length 6 mm; OsseoSpeed TM 4.0 S; DENTSPLY Implants, Sweden) were placed, using a one stage surgery procedure and loaded with a screw-retained, splinted, ceramic fixed prosthesis six weeks later. Clinical and radiographic examinations were performed preoperatively, post-surgery, at loading, and 6, 12, 36 months after prosthesis placement.

Results: The data from the one year follow up has been published (Han *et al* 2016). Four implants failed before loading; all other implants showed favorable clinical and radiographic findings throughout the observation period. The results from a three year follow up study showed that three year survival and success rate was 95.8% and mean marginal bone level changes were minimal (0.10 ± 0.49 mm). 62.0% implants experienced no bone loss and bone gain occurred around 22.5% of the implants. The mean PD change was 0.6 mm and PD change less than 2 mm was found in 91.6% of the implants. During the study period, an average of 38.7% of the implant sites had plaque and 41.9% of the implants had bleeding on probing.

Conclusion: Three year data indicates that the use of 6 mm long implants is a predictable treatment in posterior regions. Considering the high prevalence of periodontitis, relatively poor oral hygiene in the Chinese population and only short implants engaged in the bone, proper periodontal treatment before implant installation is mandatory, and strict follow-up maintenance is a prerequisite for long-term success.

Part 4. Dynamic colonization of implant and adjacent teeth by putative periodontal pathogens in periodontitis patients

Objectives: To analyze the changes in six periodontal microorganisms in the peri-implant sulcus of implants and adjacent teeth of periodontitis patients.

Methods: 24 partially edentulous patients with 60 implants and 62 adjacent teeth were included in this study and divided into three groups: healthy control group (HC, N=5), aggressive periodontitis group (AgP, N=5) and chronic periodontitis (CP, N=14). After thorough periodontal therapy, subgingival dental plaque and submucosal implant plaque

samples were collected onto filter paper from the mesiobuccal side and distobuccal side of the implants and adjacent teeth at the following five time-points: before implant placement (T1); before stage two operation (T2); one month after prosthetic restoration (T3); one year after implant loading (T4) and two years after implant loading (T5). The DNA of bacteria were extracted from all samples, and processed using Polymerase Chain Reaction (PCR) amplification to detect *Fusobacterium nucleatum* (*Fn*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Treponema denticola* (*Td*), *Tannerella forsythia* (*Tf*), and *Aggregatibacter actinomycetemcomitans* (*Aa*).

Results

The results showed that the survival rate of implants was 100% (60/60). Only *Fn* and *Aa* were found in 1.61% adjacent natural teeth at T1 and *Fn* and *Tf* at T2. At T3, *Tf* was detected in 1.67% implants and *Fn* & *Tf* were found in 6.45% & 1.61% adjacent teeth. At T4, the respective detection rates of *Fn*, *Pg*, *Pi*, *Tf*, *Td* were 24.19%, 15%, 13.33%, 8.33% and 8.33% in the implants and 20.97%, 14.52%, 4.84%, 3.23% and 4.84% in the adjacent teeth. At this time point *Aa* was not found in implants or natural teeth. At T5, the respective detection rate of *Fn*, *Pg*, *Pi*, *Tf*, *Td*, and *Aa* was 38.33%, 28.33%, 20%, 23.33%, 18.33%, and 3.23% in the implants and 20.97%, 14.52%, 4.84%, 3.23%, 4.84% in the adjacent teeth. The detection rate of these bacteria increased from T4 to T5 but the difference was not significant ($p=0.142$). The detection rate of *Fn* in AgP group significantly increased from T4 to T5 ($p=0.014$) while *Pi* and *Td* were largely found in CP group in comparison with AgP group. The clinical parameters including PD at the sample collection site, PD at the deepest implant pocket site, soft tissue thickness during implant placement, PD >6

mm percentage in natural teeth sites were all significantly raised for the implants with a positive microbial detection. When AgP and CP groups were subcategorized according to whether microorganisms were detected, the positive detection subgroup demonstrated a poorer clinical condition compared with the negative detection subgroup and HC group. The results of Binary Logistic regression analysis further indicate that implant average PD ≥ 4 mm is a risk factor (OR=4.78) for the presence of periodontal microbes around implants after one year loading (T4). Implants with an average PD >4 mm, maintenance frequency no more than once a year, and percentage PD >6 mm higher than 10% of full-mouth sites are risk factors for positive microbial findings (OR=6.22 5.23 3.41) after two years loading (T5).

Conclusion

Periodontal pathogenic microorganisms were hardly found around implants and adjacent teeth from T1 to T3 following thoroughly standardized periodontal and implant treatment. However, *Fn* began to appear starting from T4 and increased in T5. The implants with positive pathogen detection displayed worse clinical condition but the adjacent teeth remained stable, which indicated the approximal natural teeth may possess stronger resistance to microbial influence. The implant average PD and maintenance frequency are two important factors that require the most attention. The recommended maintenance interval is no more than six months.

Recommendations for implant treatment in patients with periodontitis

From the above studies, a clinical strategy for implant treatment in patients with periodontitis has been developed as follows:

1. The most important stage is to provide proper oral hygiene instruction to patients who are rehabilitated with a dental implant, and carry out thorough initial periodontal therapy to remove plaque biofilm and provide infection control. Some patients may need further surgical treatment if unstable deeper pockets remain.
2. Reassess the compromised condition and ensure the implant treatment has been based on a sound periodontal condition. This means that there should be no PD >6 mm pockets around natural teeth. It is recommended that PD >6 mm in more than 10% of full-mouth sites is risk factor for microbial colonization.
3. To assist with good plaque control, high quality prosthetic constructions that allow accessibility for oral hygiene around implants is very important.
4. Providing ongoing supportive periodontal and peri-implant therapy will enhance good outcomes for implant treatment. Regular visits to the therapist for periodontal control and maintenance in a well-organized scheme should be based on individual tooth/implant-site risk assessment methods but at least 6 monthly.

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Chapter 13

A Conservative Approach to Implant Surgery

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Introduction

Many patients are busy, fearful of dental visits and cost conscious. Large complex bone grafting during stage one implant surgery can lead to increased incidence of post-operative swelling and pain.

Implant surgery complexity, morbidity and cost may be minimized if:

1. Bone is preserved as far as possible at the time of tooth extraction.
2. Ridge expansion techniques are used instead of only drilling during osteotomy of the implant site.
3. Narrower diameter implants are used.

Ridge preservation

Tooth extractions should be done in an atraumatic fashion. When the buccal plate is intact, a good conservative option is to immediately replace the extracted tooth with a dental implant, or to wait four to six weeks for soft tissue healing for an early placement of the implant.

Sclar (1999) presented a conservative flapless socket preservation procedure which he termed the Bio-Col technique. For this procedure the extraction socket is filled with an anorganic bovine bone substitute and then covered with a collagen plug. This procedure can be effectively used when the buccal plate is intact after tooth extraction. Implant stage

one surgery can be carried out 6 months later. However, as stated earlier, when the buccal plate is intact, it is more rational to leave the socket to heal by itself. Performing socket preservation surgery when the buccal plate is intact lengthens rather than shortens the treatment. The practicality of Sclar's Bio-Col procedure however, is that it is performed flapless with very little patient morbidity.

Elian *et al* (2007) presented a simplified approach to socket preservation. This is a technique that can be used when there is buccal bone dehiscence, but soft tissue is still present. A collagen membrane is trimmed into an ice-cream cone shape, round at the coronal and a long triangle shape at the apical. After the socket has been thoroughly but gently curetted, this trimmed membrane is placed inside the socket to separate the buccal soft tissue from the bone graft (Figure 2). The bone graft will press against the membrane to hold it in place (Figure 2). The membrane should extend over the opening of the socket and then underneath the palatal flap by 2 to 3 mm (Figure 3). The membrane can be held down by suturing it to the palatal tissue or tucking beneath the palatal flap and held by a cross mattress suture (Figure 4). Like Sclar's procedure, the membrane is left exposed, and the whole procedure done in a flapless fashion.

In a study population of 35 patients, using the ice-cream cone technique, and where the buccal dehiscence did not exceed 50% of the

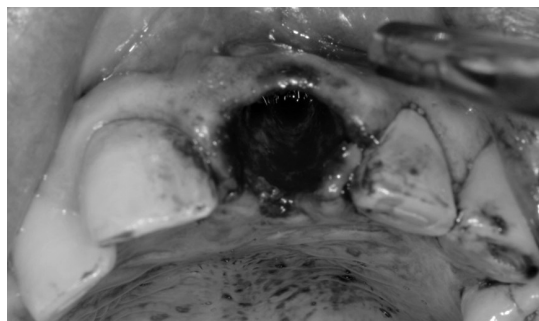


Figure 1. After extraction, 50% buccal dehiscence was noted.

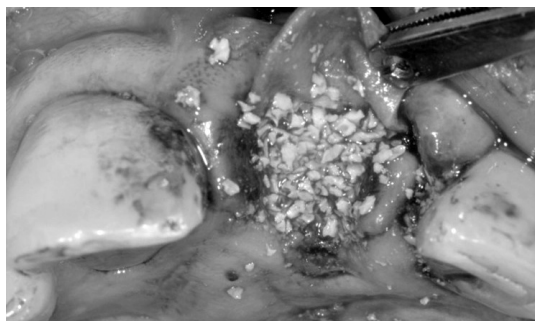


Figure 2. An ice-cream cone shaped membrane was placed within the socket to keep the bone graft separate from the buccal mucosa.

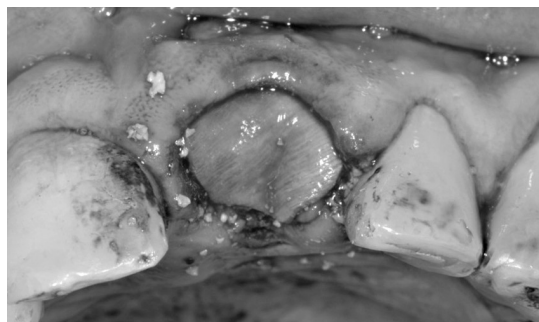


Figure 3. The membrane extends over the opening of the socket and then underneath the palatal flap by 2 to 3 mm.

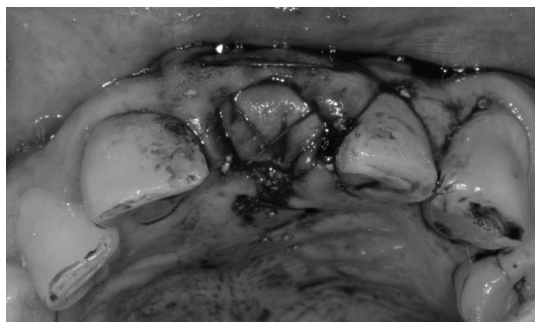


Figure 4. The membrane is held down by mattress sutures.

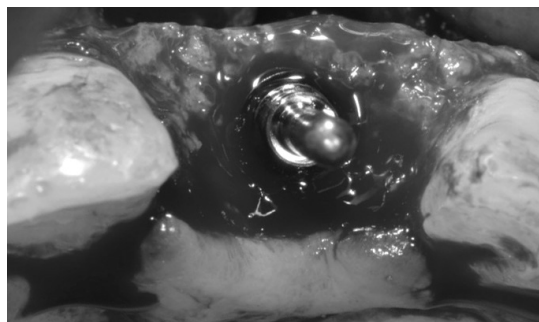


Figure 5. Ridge at stage 1 surgery six months later was sufficient for placement of a dental implant.

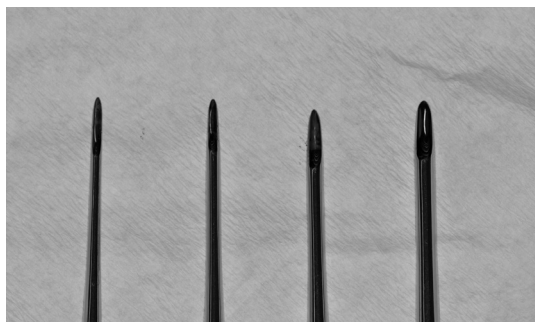


Figure 6. The Nentwig bone spreaders are flat spear shaped instruments that comes in widths of 2.2 mm, 2.8 mm, 3.4 mm and 4.0 mm.

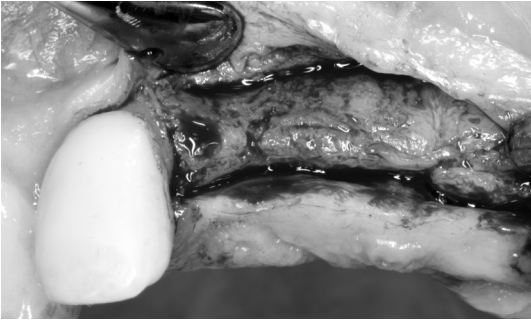


Figure 7. This 4 mm wide ridge width was deemed too narrow for a 4.3 mm implant and patient was advised that a GBR may be necessary.



Figure 8. Osteotomy initiated with 2 mm pilot drill.

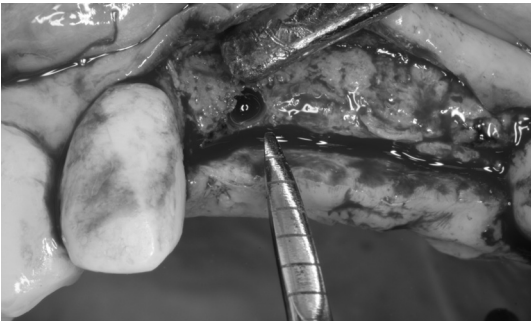


Figure 9. Osteotomy was continued with the Nentwig bone spreaders.

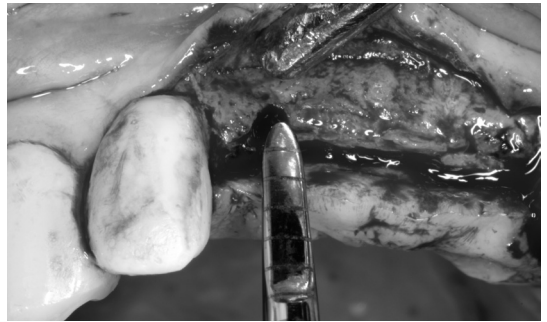


Figure 10. Bone spreader was inserted with the flat part facing buccally.

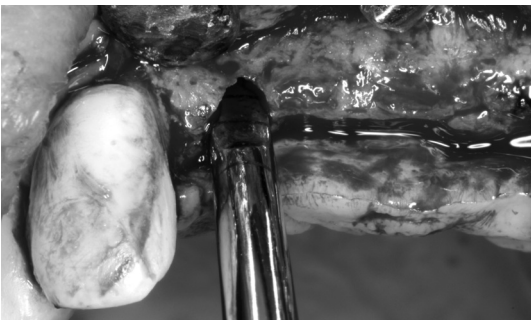


Figure 11. Bone spreader rotated 90° clockwise, back to original position and then 90° anti-clockwise.



Figure 12. Round osteotomy was pre-formed.

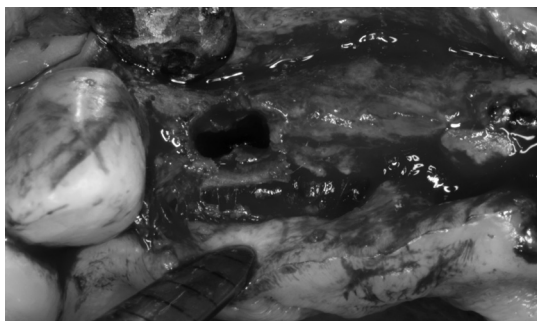


Figure 13. Ridge width widened before using the 4.3 mm diameter tapered drill to reduce the amount of bone the drill will cut away.

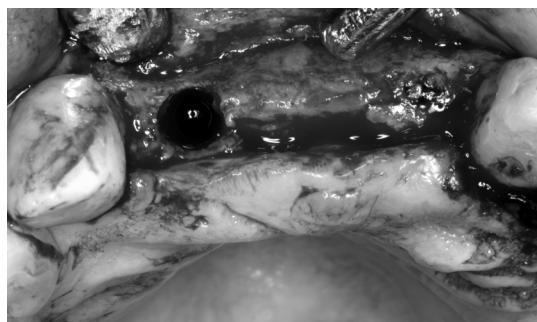


Figure 15. Buccal bone dehiscence was avoided by bone spreaders in conjunction with implant drills.

socket, Eskow and Mealey (2014) found that 33 out of 35 sites allowed for the placement of implants in the correct restorative positions. Two out of 35 sites (5.7%) did not have sufficient bone to place an implant. Of the 33 sites that allowed implants to be placed, nine sites (27%) require further guided bone regeneration procedure.

Ridge expansion during stage 1 implant surgery

Bone spreaders and expanders expand and compress bone in alveolar ridges. This technique can be used in both the maxilla and the mandible, although the maxillary bone tends to be softer and more malleable. Cancellous bone should be present and alveolar ridge width should be at least 3 mm.

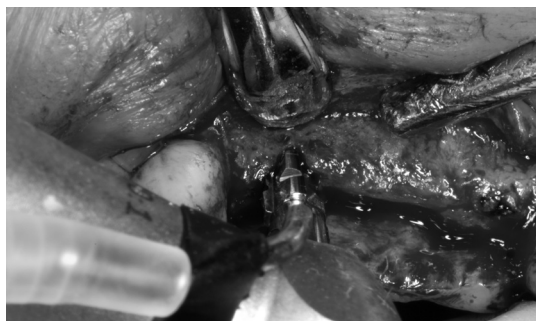


Figure 14. Osteotomy with 4.3 mm tapered drill.

In this technique, bone spreaders are used after the initial pilot (1.3 to 2 mm) osteotomy preparation is done, before proceeding on with the wider twist drills. The Nentwig bone spreader (Ustomed Instrumente) is a flat-spear shaped instrument. It comes in a set of four instruments with widths of 2.2 mm, 2.8 mm, 3.4 mm and 4.0 mm (Figure 6). After inserting the bone spreader into the pilot osteotomy, it is rotated on its axis 90° clockwise, back to its original position and then 90° anti-clockwise, thereby forming a circular shaped osteotomy (Figures 8 to 12). Select the bone spreader up to one size smaller than the final twist drill for the implant diameter one plans to use, and finish the osteotomy with the final twist drill (Figures 13 to 15). Up to 3 mm of ridge expansion can usually be achieved.

If a 4 to 4.3 mm implant is planned on a 4 mm ridge, it is likely that a bone graft procedure is necessary. With the use of bone spreaders, the ridge could be expanded to 6.5 mm, thereby removing the need for a bone graft.

Narrower diameter implants

Conventionally, standard diameter implants (3.75 to 4.1 mm) have been recommended to restore central incisors, canines, and premolars; and wide diameter implants for molars; especially when the patient presents with heavy occlusal wear patterns (Klein *et*

al 2014).

Narrow diameter implants (3.0 to 3.5 mm) have been recommended to restore mandibular incisors and maxillary lateral incisors. There are concerns that should they be used in load bearing areas, implant fixtures may fracture and there may also be failure of osseointegration due to reduction in the implant-bone contact surface area (Quek *et al* 2006). Prosthetically, restoring a narrow diameter implant into a molar restoration without proper considerations may result in an over-contoured emergence profile, and be challenging to maintain oral hygiene (Graves *et al* 1994).

Renouard and Nisand (2006), examined the impact of implant length and diameter on implant survival rates. They defined short implants as those between 6 to 8 mm in length, narrow implants as those between 3 to 3.4 mm in diameter, and wide implants as those 4.5 mm or more in diameter. In this review of data taken from Medline of 53 papers that fit his criteria, he found that the survival rate of 6 to 8 mm length implants was comparable to longer implants, and also that implant diameter and survival rates have no relationship.

A recent retrospective clinical study reported that Branemark Mark II, Mark III and NobelSpeedy 3.3 mm diameter implants had a 95.1% survival rate in edentulous and deficient posterior ridges, comparable to that of wide diameter implants (Malo *et al* 2011). The mean peri-implant bone loss was 1.16 mm, 1.53 mm, and 1.74 mm at one, five and ten years follow up. Another study on 12,737 Ankylos (Dentsply) implants showed that there was no significant difference in the cumulative survival rate (CSR) of 3.5 mm diameter implants and 4.5 mm diameter implants after an average of 60.7 months (Krebs *et al* 2015).

Papadimitriou *et al* (2014) undertook virtual surgical planning of 1760 implants using the existing CT scans of 200 patients

and found that the use of 3.3 mm diameter implants increased the odds ratio for ridge augmentation being unnecessary by 2.2 (95% confidence interval) relative to the 4.1 mm diameter implants. This will significantly reduce costs for patients as well as post-surgical morbidity. The clinician also benefits from less chair time and because the surgery is straightforward, chances of surgical complications occurring are reduced.

With improvements in the strength of the implant body materials and improvements in abutment designs, it can be expected that there will be a move towards using narrower diameter implants.

Conclusion

This paper describes a simple strategy to reduce the need for complicated implant stage 1 surgery with use of a flapless socket preservation surgical design, ridge expansion technique and the use of narrower diameter implants.

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Chapter 14

Clinical Experiences of Trauma from Occlusion: Risks, Signs and Therapy

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Introduction

Trauma from occlusion is defined as injury to the periodontal tissues resulting from occlusal forces that exceed the adaptive capacity of the tissue (Newman 2015). This issue has been of concern since 1917 when Stillman first introduced the concept of excessive occlusal forces being the primary cause of periodontal disease, and stressing occlusal therapy as a mandatory procedure to control it. In 1933 Orban and Weinman used histologic observation of human autopsy materials to conclude that occlusal forces did not have a major effect on periodontal deterioration. During the 1950s and 1960s, animal research revealed almost the same results as those presented by Orban and Weinman. During the 1960s until the 1970s, Glickman and colleagues performed a series of animal model and human autopsy studies and concluded that the term of “co-destructive” refers to the effects of trauma from occlusion. This meant that the occlusal forces may lead to apical and bony defects and, together with gingival inflammation, are two separate pathological processes causing vertical bone loss (Harrel 2003).

In human autopsy studies, Waurhaug (1979) found that there was an association of bone loss with the downgrowth of plaque,

but that there was no relationship between occlusal forces and vertical bone loss. More recently, Harrel (2003) noted that the weakness in prior occlusal studies using human autopsy material was a lack of knowledge regarding the patients’ occlusal relationship during life, and therefore any conclusions could not be considered as definitive.

Two extensive animal studies were published evaluating the effect of plaque and excessive occlusal forces in animal models. Polson *et al* (1974) used squirrel monkeys, and Lindhe *et al* (1974, 1976) used beagle dogs. They both found that excessive occlusal forces alone without plaque cause a loss of bone density and mobility of the tooth, but would not cause attachment loss. When the excessive forces were removed, the loss of bone density was reversible. In the presence of both excessive occlusal forces and plaque, more bone density was lost. In the beagles, there was evidence of attachment loss when plaque and excessive forces were both present, but the same result was not found in squirrel monkeys. These studies ultimately established that bacterial plaque is the initiating factor and main cause for progression of periodontal disease. Harrel (2003) stated that the application of the information obtained from the animal models shows little or no tendency toward periodontal deterioration

under natural conditions. As a result, research on the periodontal deterioration that occurs in human must be approached with caution.

The 1999 International Workshop for the Classification of Periodontal Diseases and Conditions concluded that there was no clear evidence that occlusal forces were a factor in plaque-induced gingival disease or connective tissue loss. On the other hand, studies on human subjects in the 1990s until the 2000s concluded that occlusal discrepancies appear to be a significant risk factor that contributes to more rapid periodontal deterioration, and that treatment of the occlusion, together with other periodontal treatments, positively affects the results of such treatment (Harrel 2003). Shaddox and Walker (2010) stated that occlusal trauma was one of the local factors that can limit successful periodontal treatment and that occlusal therapy needs to be addressed in conjunction with procedures to resolve inflammatory lesions. A recent study on maxillary and mandibular first molar teeth found there is a correlation between the crown-to-root ratio and trauma from occlusion, which is characterized by gingival recession, loss of attachment, tooth mobility, and thickening of the lamina dura (Anggraini *et al* 2017). This study showed that a disproportionate crown-to-root ratio is one dental condition that leads to a risk of trauma from occlusion.

Clinical experience in the Periodontal Clinic

Faculty of Dentistry Universitas Indonesia has shown that teeth with severe periodontal deterioration and mobility are associated with trauma from occlusion. Therapy for traumatic occlusion, together with other periodontal treatments, showed good outcomes. This clinical experience revealed similar results to previous human studies demonstrating that occlusal discrepancies appear to be a significant risk factor contributing to more rapid periodontal deterioration, and that treatment of the occlusion, together with other periodontal treatments, positively affects the results.

Dental conditions associated with risk of trauma from occlusion

Clinical experience in the Periodontal Clinic Faculty of Dentistry Universitas Indonesia showed that the dental conditions associated with risk of trauma from occlusion are as follows:

1. Occlusal interference/occlusal discrepancies of the upper and lower teeth in centric relation, closure arc, or protrusive and lateral excursions of articulation/functional closure. In posterior teeth, occlusal interference of one tooth may be associated with trauma from occlusion. However, for anterior teeth, occlusal interference of one or two teeth may be



Figure 1. Occlusal interference associated with trauma from occlusion.

associated with trauma from occlusion (Figure 1). It seems that most of the trauma from occlusion in anterior teeth is associated with occlusal interference.

2. A disproportionate crown-to-root ratio (Figure 2). Clinical experience found severe periodontal deterioration in some of teeth with disproportionate crown-to-root ratio. Anggraini *et al* (2017) found that in the maxillary and mandibular first molar teeth, there is a correlation between the crown-to-root ratio and trauma from occlusion. The disproportionate crown-to-root ratio is sometimes generalized and this can appear as generalized aggressive periodontitis (Figure 3).
3. Edge-to-edge or cusp-to-cusp occlusal relationship. Some patients with this type of occlusal relationships exhibit severe bone deterioration that also appears as generalized aggressive periodontitis (Figure 4). However, this has a different cause to that of generalized periodontal destruction seen with disproportionate crown-to-root ratio cases. In certain cases, the condition of disproportionate crown-to-root ratio together with the edge to edge occlusal relationship can occur on the same tooth so that both dental conditions contribute to the risk of trauma from occlusion (Figure 5).
4. Parafunctional habits such as bruxism (Figure 6).
5. Teeth that have no contact with other teeth also have a risk to trauma from occlusion.

Clinical and radiographic signs of trauma from occlusion

The clinical and radiographic signs of trauma from occlusion vary in severity that ranges from mild to severe periodontitis. In mild traumatic lesions, radiographic signs show increased width of the periodontal ligament space and, after some time, there

is increased density of the surrounding bone caused by new bone formation in response to increased occlusal forces (Newman 2015). Disruption of the lamina dura may also occur. More severe traumatic lesions demonstrate increased tooth mobility and deep infra-bony pockets that may create a periodontal abscess with or without pulp necrosis that requires endodontic treatment (Figures 7 and 8). Kundapur *et al* (2009), found that tooth mobility, which is a feature of trauma from occlusion, has a positive association with gingival recession. However, there is no statistically significant relationship between the presence of a positive fremitus, a sign of trauma from occlusion, and wear facets with gingival recession.

Clinical experience revealed that teeth with occlusal trauma may show gingival recession, tooth migration, tooth fracture, root resorption, abfraction, gingival cleft, fibrous gingival enlargement that can be diagnosed as fibromatous epulis, or ring-shape gingival margin enlargement diagnosed as McCall's Festoon (Figure 9). Kanas and Kanas (2011) stated that excessive occlusal forces can result in dystrophic changes in the periodontal ligament, alveolar bone, and pulp, leading to periapical inflammation, external root resorption, and bone sclerosis that is shown as radiopaque thickening of the apical alveolar bone. Zavala *et al* (2017) found a relationship between the variables of traumatic occlusion and abfraction. On the other hand, Nascimento *et al* (2016) stated that abfraction lesions have a multifactorial etiology such that the combination of different etiological factors will result in the initiation and further development of differences in their clinical appearance. Thus, identification and management of potential etiological factors are crucial for proper diagnosis and treatment planning.

Other cases of trauma from occlusion have shown generalized periodontal



Figure 2. Trauma from occlusion associated with disproportionate crown-to-root ratio.



Figure 3. Generalized trauma from occlusion caused by generalized disproportionate crown-to-root ratio.

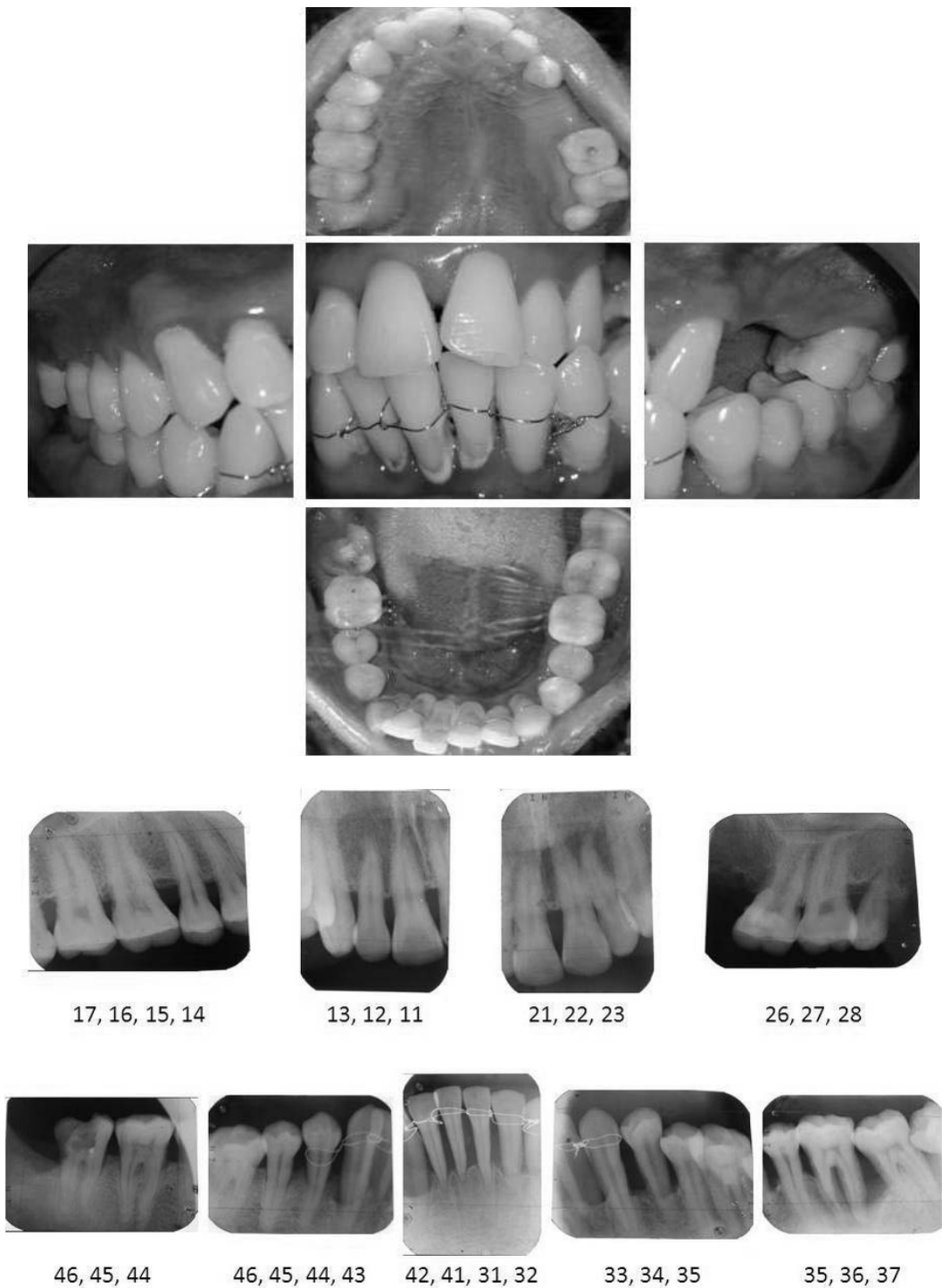


Figure 4. Intraoral and radiographic views of male patient, 48 years old, with generalized edge-to-edge occlusal relationship associated with trauma from occlusion.

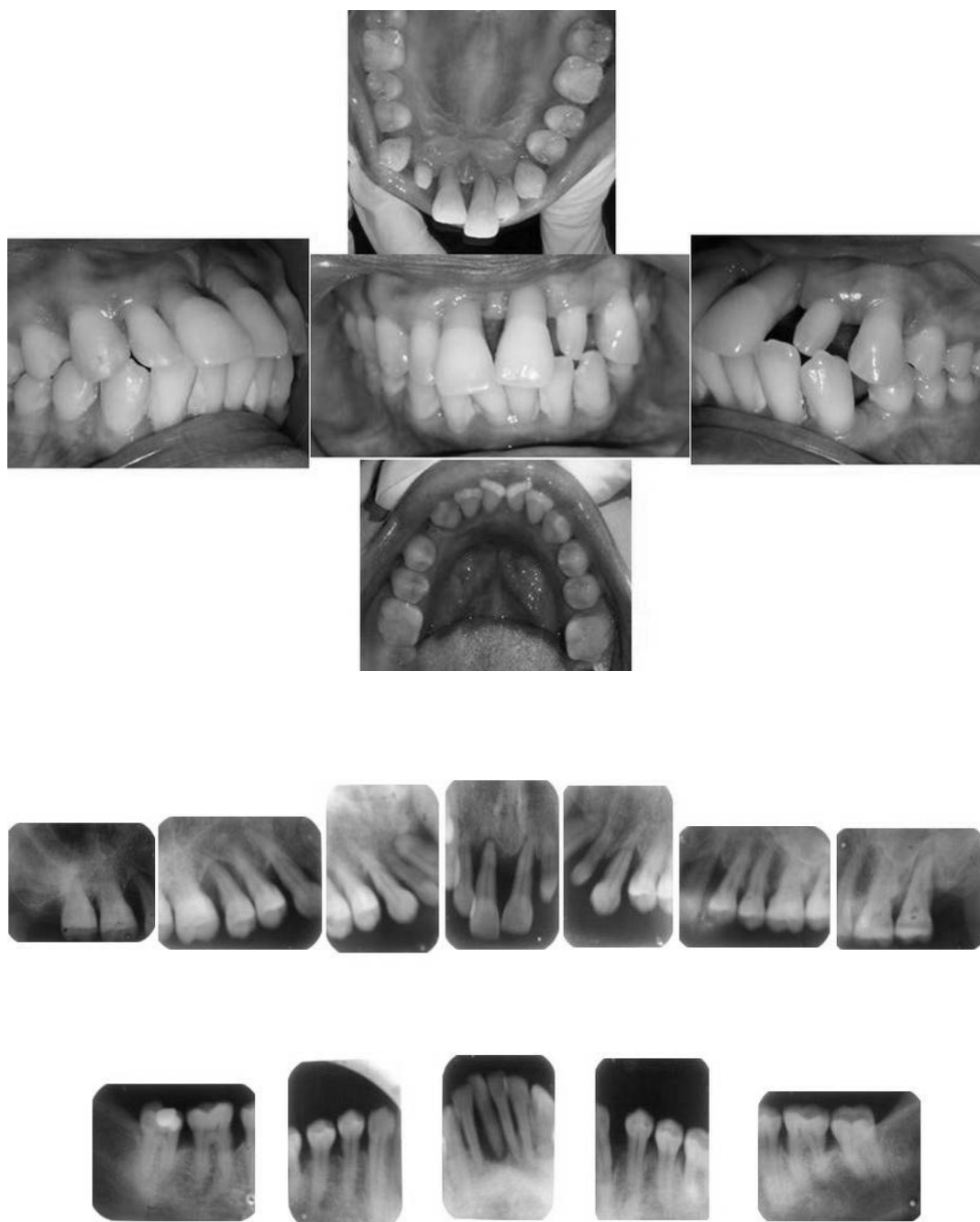


Figure 5. Intraoral and radiographic views of female patient, 47 years old, with generalized edge-to-edge occlusal relationship, together with disproportionate crown-to-root ratio of maxillary premolars, and occlusal interference of anterior teeth, associated with trauma from occlusion.



Figure 6. Intraoral and radiographic views of trauma from occlusion associated with bruxism.



Figure 7. Radiograph of infrabony pocket of tooth with trauma from occlusion.

deterioration associated with generalized edge-to-edge inter-occlusal relationship or generalized disproportionate crown-to-root ratio. These forms of trauma from occlusion are usually diagnosed as generalized aggressive periodontitis instead of generalized severe chronic periodontitis. There are differences between those two generalized forms of periodontitis. For example, in

generalized chronic periodontitis associated with generalized trauma from occlusion, even though some teeth usually mobile, the number of teeth missing, and amount of tooth migration is less than that seen in aggressive periodontitis (Figure 10). In addition, the severity of the disease in aggressive periodontitis is greater in younger patients. These features seem indicative of the



Figure 8. Radiograph of tooth 25 with trauma from occlusion and pulp necrosis requiring root canal treatment.



Figure 9. Fibromatous epulis on tooth with trauma from occlusion.



Figure 10. Intraoral and radiographic view of patient, female, 46 years old, diagnosed with generalized aggressive periodontitis.

differences between the generalized chronic periodontitis and generalized aggressive periodontitis as stated by Armitage *et al* (2010). Studies have also shown that the mechanisms and regulations of bone loss associated with chronic or aggressive periodontitis appear to be biochemically similar, but there are differences in the speed of bone loss (Bartold *et al* 2010). Comparisons of the microbiology of these forms of periodontitis, both appear to be associated with certain pathogens listed by the 1996 World Workshop in Periodontics, including *P. gingivalis*, *T. forsythia*, *C. Rectus*, *Eubacterium sp.*, *P. micra*, and *Treponema sp.* However, localized aggressive periodontitis appears to be associated with *A. Actinomycetemcomitans* (Armitage 2010). There are preliminary studies suggesting that individuals with generalized aggressive periodontitis have higher subgingival levels of *Selenomonas sp.* (Favery *et al* 2008) and *T. lecitithinolyticum* (Riep *et al* 2009) compared to patients with chronic periodontitis. With regards to the immunopathology of both diseases, Ford (2010) stated that at present it is not possible to identify real differences in the immunopathology of the two diseases. Stabholz *et al* (2010) summarized that the identified risk factors for periodontal diseases are very similar for both periodontitis. Monteiro *et al* (1996) found that patients in the generalized aggressive periodontitis group presented with significantly increased depression and loneliness compared with the patients in the chronic periodontitis and control groups. Armitage and Cullinan (2010) concluded that age of onset and family history are important for either diagnosis or classification of the two diseases. Deas and Mealey (2010) stated that a better understanding of the true nature of patients currently identified as having aggressive periodontitis may lead to more effective treatment approaches.

Clinical experience shows that there

are differences between the periodontitis associated with trauma from occlusions compared to periodontitis associated with other causes, i.e. dental plaque, calculus, food retention, food impaction. In periodontitis associated with trauma from occlusion the amount of periodontal deterioration is more than that seen in periodontitis associated with other causes. This seems to be true for both the localized or generalized forms of periodontitis.

Diagnosis of trauma from occlusion

Trauma from occlusion is usually diagnosed in patients complaining of: pain in certain tooth (especially when biting), tooth mobility, oedema of the gingivae, gingival enlargement, or tooth migration.

Extraoral examination should be performed to determine the presence of asymmetrical face; temporomandibular disorder by evaluation the ability of patient to open their mouth maximally; the direction, sound, pain, and deviation of the midline during opening and closing of the jaw; pain in palpation of temporomandibular joint, and tension or spasm of head and neck muscles (Devitt 2015).

Intraoral examination of all teeth is performed for the presence of clinical signs of trauma from occlusion, i.e. dental abscess, gingival enlargement, gingival recession, gingival cleft, periodontal pocket, loss of attachment, tooth mobility, tooth migration, abfraction, and tooth fracture. Examination of teeth with periodontal deterioration is performed to determine the presence of any conditions associated with increased risk for trauma from occlusion, i.e. interference during centric relation and articulation; edge-to-edge or cusp-to-cusp occlusal relationship; occlusal abrasion; and the contact relationship between teeth.

Radiographic examination of teeth with

clinical signs of trauma from occlusion is performed to evaluate the presence and severity of bone deterioration, periapical and furcation lesion; and the anatomical form of the tooth to evaluate the possibility of disproportionate crown-to-root ratio that are associated with the risk of trauma from occlusion.

Diagnosis based on these examinations may be of mild to severe chronic periodontitis due to plaque and calculus, that in certain teeth is aggravated by trauma from occlusion; or as aggressive periodontitis that in certain teeth is aggravated by trauma from occlusion.

Therapy for trauma from occlusion

The principal therapy for trauma from occlusion is elimination of periodontal inflammation and adjustment of occlusion to eliminate the occlusal forces. The procedures consist of initial therapy (emergency therapy, scaling and root planing, occlusal adjustment, and splinting) and surgical therapy (curettage, gingivectomy/gingivoplasty, flap operation, regenerative therapy) as needed.

Occlusal adjustment procedures vary depending on the condition affecting the risk of trauma from the occlusion. Selective grinding is one of the occlusal adjustment procedures that eliminates the occlusal trauma associated with occlusal interference or disproportionate crown-to-root ratio. The procedures of the occlusal adjustment are as follows:

1. If the trauma from occlusion is associated with occlusal interference of the upper and lower teeth in centric, grinding should be performed on the upper tooth. The occlusal interference should be detected visually during clinical examination procedures, and the spot to be ground can be marked by using articulating paper during the movement of the mandible to the occlusal interference position.

This procedure prevents the tooth from being placed into open bite, which may cause tooth extrusion and relapse of the occlusal interference after adjustment. In a situation where the lower tooth is mobile and indicated to be splinted, then the lower tooth can be ground, dismissing the occlusal interference and reducing the load simultaneously. The splint may prevent tooth extrusion and relapsing of the occlusal interference. The principle of these procedures is similar to Harrel (2003) who concluded that occlusal therapy to minimize the occlusal interference together with other forms of periodontal treatment positively affects the progress and treatment of periodontal deterioration.

2. If the trauma from occlusion is associated with a disproportionate crown-to-root ratio of the premolar and molar teeth, the occlusal forces can be eliminated by grinding the bucco-occlusal and linguo-occlusal surface of the tooth, by about one sixth of the total bucco-lingual width of each occlusal surface. This method is based on the rule that the wider the occlusal surface, the more pressure there is on the periodontal tissue.
3. If the trauma from occlusion is associated with a cusp-to-cusp or edge-to-edge occlusal relationship, orthodontic treatment is needed to change the occlusal relationships.
4. If the trauma from occlusion is associated with a parafunctional habit such as bruxism, a bruxism splint is needed to minimize the occlusal pressure.
5. If the trauma from occlusion is associated with a single tooth, or not from contact with another tooth, the occlusal adjustment is associated with prosthetic or restorative procedures after treatment of the periodontal inflammation.
6. If the trauma from occlusion is associated with orthodontic problems, i.e. edge-

to-edge interocclusal relationship, the occlusal adjustment is associated with orthodontic procedures after treatment of the periodontal inflammation.

To eliminate inflammation, some cases show good healing after scaling, root planing, occlusal adjustment, and sometimes splinting or curettage. However, in cases with pocket depths greater than 6 mm, flap surgery, with or without bone grafting, is required following initial therapy. In fibroma cases, gingivectomy and gingivoplasty are needed, sometimes along with flap surgery depending on the depth of the pockets. Good results can be achieved after periodontal treatment with or without surgery, showing improvement in the depth of the pockets, loss of attachment, mobility, and bone regeneration. In endodontic-periodontic cases, when pain or periodontal problems are not resolved after endodontic and periodontal treatment, the possibility that the traumatic occlusion was not eliminated may need to be considered. In such cases, after occlusal adjustments, the outcome of the periodontal therapy should be favorable.

Conclusion

Recent research conducted on human subjects and clinical experience indicates that excessive occlusal forces appear to be a significant risk factor contributing to more rapid periodontal deterioration. Attention to the dental conditions contributing to the risks of trauma from occlusion including occlusal interference, disproportionate crown-to-root ratio, edge-to-edge occlusal relationships, parafunctional habits, and tooth which has no contact with other tooth may need to be a part of routine procedures in dental treatment to provide successful outcomes.

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Chapter 15

Quantitative Analysis of Classical and New Putative Periodontal Pathogens in Subgingival Biofilm

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Introduction

Periodontitis is a common, progressive disease of the supporting tissues of the teeth that is widespread among majority of populations (WHO 1978). Periodontal diseases refer to the inflammatory processes that occur in the supporting tissues surrounding the teeth in response to bacterial accumulation (dental biofilm) (Loesche and Grossman 2001). The tissue damage is mediated by destructive host immune responses believed to be orchestrated by a team of periodontal pathogens in subgingival biofilm (Darveau 2010).

The oral cavity is not sterile. 25,000 bacteria species have been detected in the human oral cavity and about 1,000 bacterial species identified by modern molecular biological techniques in the dental biofilm (Zaura *et al* 2009). According to Socransky *et al* (1998) species associated with periodontal disease belong to the so-called red and orange complexes. Bacteria of the other complexes did not show any association with periodontitis and seemed to be compatible with periodontal health. By the late 1990s, *Porphyromonas gingivalis*, *Tannerella forsythia* (previously *Bacteroides forsythus*) and *Treponema denticola*, the so-called red

complex, became well-established periodontal pathogens (Socransky *et al* 1998). Other recognized pathogens, but of less importance were members of the so-called orange complex. Since then, these species have been recognized as classical periodontal pathogens, and their association with periodontitis has been documented in populations from different parts of the world using different techniques (Rylev and Kilian 2008).

However, the last decade or so has witnessed a paradigm shift towards a metagenomic approach to studying microbial composition of dental biofilm, employing open-ended molecular techniques (Paster *et al* 2001). This has not only doubled the richness of subgingival biofilm, but also identified a number of new putative periodontal pathogens: *Treponema*, oral Synergistetes, oral TM7 and *Filifactor alocis* (Downes *et al* 2009, Jumas-Bilak *et al* 2007, Vartoukian *et al* 2007, Vartoukian *et al* 2012). There is currently growing interest in exploring these new putative pathogens and their role in periodontitis. However, so far, there have been no attempts to assess their association with the classical pathogens in subgingival biofilm and how this could relate to periodontal health status.

Objectives

The objectives of the current study were to explore associations among classical and new putative pathogens in subgingival biofilm and to assess their relative importance in chronic periodontitis.

Calculation of the sample size was based on 80% power, a confidence level of 95%, and a target odds ratio of two using OpenEpi, an online statistical resource available from <http://www.openepi.com/v37/SampleSize/SSCC.htm> (Sullivan *et al* 2009).

Ethical approval was obtained for this study (Ethical No. DF OB1416/0067(P)) from the Ethics Committee, Dental Faculty, University Malaya. Each patient was given an information sheet explaining the nature of the study and explanation was also given verbally. Written consent was then obtained from each patient.

Methodology

The design of this study was a case control study whereby the comparison between the case and control groups with similar risk or causative factors were studied.

Study subjects were adults, 30 to 60 years old, who had a minimum of 20 teeth, out of which 40 were chronic periodontitis patients and 40 healthy controls. A questionnaire was completed, periodontal examination performed, and pooled subgingival biofilm samples were obtained from the subjects.

Cases included had periodontal pocket depths ≥ 5 mm on four teeth (at least one tooth per quadrant) with a Community Periodontal Index (CPI) score ≥ 3.5 (3.5-5.5 band on the WHO probe partly visible/invisible). Controls had pocket depths ≤ 3 mm at four teeth (at least one tooth in each quadrant) with a CPI score ≤ 2 . The subjects did not have any relevant history of systemic condition or disease and had not received periodontal or antibiotic

therapy within the previous three months (Amano *et al* 1999).

Supragingival biofilm was removed by hand curette from the sites. Subgingival biofilm was obtained from each subject using sterile paper points, taken from the deepest four pockets, one in each quadrant, and pooled in a tube containing TE buffer (Invitrogen, USA). Biofilm samples were taken from non-bleeding pockets ≤ 3 mm in non-periodontitis subjects (control) and pooled in a tube containing TE buffer. Samples were stored at -80°C until the PCR processing stage (within three months). All laboratory work was performed at a Molecular Research Laboratory.

DNA was extracted from the samples using Purelink Genomic DNA Kit (Invitrogen, USA) according to manufacturer's instructions. Taqman q-PCR technology (Holland *et al* 1991) was used to detect and quantify all the pathogens as well as total bacteria in the samples using previously validated primers and Taqman probes on ABI 7000 real-time PCR system (Nagashima *et al* 2005). Primers, probes, and positive controls for the study species were designed in collaboration with Primer Design, UK, as ready to use optimized kits.

The species investigated (quantified) were *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Parvimonas micra*, *Filifactor alocis*, TM7 species and oral Synergistetes (Table 1).

For the oral Synergistetes - primer/probe set covered *Fretibacterium fastidiosum*, *Fretibacterium sp.* oral taxons 358, 359, 360, 361, 362, 452, 453; *Jonquetella anthropi*, *Pyramidobacter piscolens*. For the oral TM7s - primer/probe set covered TM7 oral taxons 347, 348, 350, 351, 355, 356 (I025), 437.

Taqman q-PCR assays were used to determine the absolute and relative counts of *P. gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Parvimonas micra*, *Filifactor*

Test species	Sequences 5'-3'	Target gene	Product size	Ref
Total bacteria	F-primer: AAACCTCAAAGGAATTGACGGGG R-primer: TTGCGCTCGTTGCGGGACT Probe: FAM-CTGTCGTCAGCTCGTGTCTGTA-BHQ	16S rRNA	205 bp	(Al-Hebshi <i>et al</i> 2010)
<i>P. gingivalis</i>	F-primer: ACGAATCAAAGTGGCTAAGTT R-primer: TTAGTCGCATTTTCGGCTGAT Probe: FAM-CCTGCTGTTCTCCATTATAAACCATACGG-BHQ	fimA	85 bp	
<i>T. forsythia</i>	F-primer: GATAGGCTTAACACATGCAAGTC R-primer: GTTGCGGCGAGGTACATAC Probe: FAM-TTACTCACCCGTGCGCCGTCG-BHQ	16S rRNA	99 bp	
<i>T. denticola</i>	F-primer: GGGCGGCTTGAAATAATRATG R-primer: CTCCTTACCGTTCGACTTG Probe: FAM-CAGCGTTCGTTCTGAGCCAGGATCA-BHQ	16S rRNA	92 bp	
<i>P. micra</i>	F-primer: TGAGCAAACCTACCTTACACAG R-primer: GCCCTTCTTACACCGATAAATC Probe: FAM-ACCGCATGAGACCACAGAAT CGCA-BHQ	16S rRNA	112 bp	
Oral Synergistetes	F-primer: GGAGTACGGTCGCAAGATTG R-primer: GTAAGGTTCTTCGGTTGCAATC Probe: FAM-ACAAGCGGTGGAGCACGTGTTAAT-BHQ	16S rRNA	98bp	Current study
<i>Filifactor alocis</i>	F-primer: ACCCTCAAAGTTGCCAAAATTATTAT R-primer: TACTCCCTTCTTCTGTTAAATCT Probe: FAM-TCGCTCTTTTGCCGCCCTCTCTTGC-BHQ	16S rRNA	101 bp	
Oral TM7s	F-primer: GCTCGTGTCTGAGATGTTT R-primer: ATCCCCCTCTTCCCTCCCCG Probe: FAMTAAGTCCATCAACGAGCGCAACCCCTT-BHQ	16S rRNA	107 bp	

Table 1. Sequences of the primers and probes used in the quantitative PCR assays.

alocis, oral Synergistetes, and oral TM7s. Microbial associations were assessed using cluster analysis.

Statistical analysis

Microbial data included log transformed absolute counts and relative counts (% total bacteria) of each of tested species /phylotypes. Complementary log-log and negative-negative log functions were used for absolute and relative counts respectively. Data was non-normally distributed (Kolmogorov-Smirnov statistics), so were summarized as medians and interquartile ranges. Detection rates were reported as percentages (prevalence).

Microbial associations

Pearson correlation and squared Euclidean distance for each pair of species/phylotypes based on relative counts were calculated. Resultant similarity coefficients rescaled to 0 to 100% were used for clustering using average linkage.

Associations between microbial parameters and periodontitis

Differences in absolute and relative counts between the two groups was done using Mann-Whitney test with Bonferroni's adjustment for multiple comparisons. This was further analyzed using Ordinal regression (OLR) [adjustment for confounders e.g. age, sex, lifestyle habits, mean PI]. Receiving operator characteristic (ROC) curve analysis of microbial counts was utilized to assess their usefulness as markers of periodontitis. Logistic regression was used for Prediction Analysis of chronic periodontitis [case vs control] and periodontal indices. SPSS version 20 was used.

Results

As shown in Table 2, cases were significantly older, had significantly more smokers and significantly higher mean PI. All assays achieved excellent linearity ($R^2 > 99.6\%$) and a detection limit of 5 to 10 copies/ reaction (Figure 1).

Variable	Controls (n=40)	Cases (n=40)	P*
Age, mean±SD	32.57±1.92	41.45±7.29	0.001
% Males	45.9	54.1	NS
% Cigarette smokers	32.4	67.6	0.012
% Water pipe smokers	22.2	77.8	NS
% Qat Chewers	50	50	NS
Plaque index, mean±SD	0.40±0.37	1.75±0.40	0.001
Mean CPI score, mean±SD	0.11±0.27	2.73±0.38	0.001
Mean CAL score, mean±SD	0.10±0.19	1.13±0.70	0.001

Table 2. Clinical characteristics of the study groups.

* P-value: Chi square test for categorical variables and Mann-Whitney test for scale variables. NS: not significant.

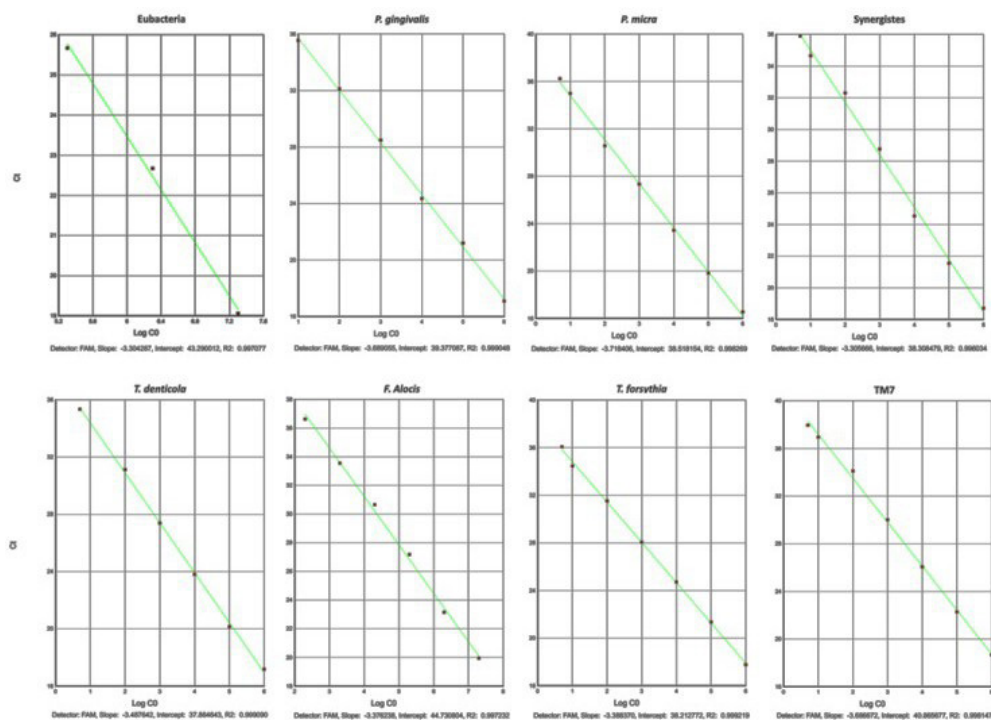


Figure 1. q-PCR assays.

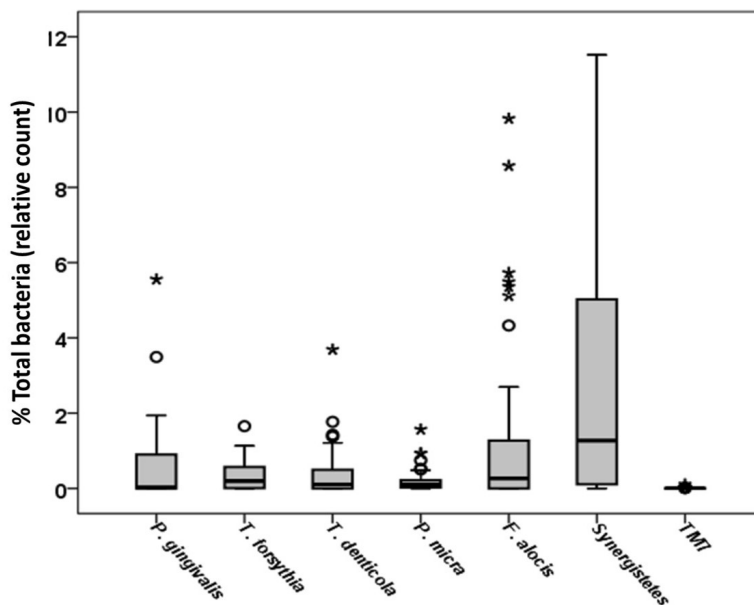


Figure 2. Counts of tested taxa. Box plot showing overall median and interquartile range of log relative counts of tested taxa in subgingival biofilm. The error bars represent data within 1.5 interquartile range above Q3 and below Q1. Circles and stars are outliers.

The detection rate was 100% for all species/ phylotypes except *P. gingivalis*, *T. denticola* and oral TM7s that were detected in 71.2, 91.2 and 95% of the samples, respectively. Median log total bacterial count was 9.17 copies/ sample. For individual species/phylotypes, the median log and relative counts ranged 4.38-7.18 DNA copies/sample and 0.0016-1.44% respectively, being lowest for oral TM7s and highest for oral Synergistetes (Figure 2). All species/phylotypes were detected at relative count of <1% in 75% of the samples except oral Synergistetes and *F. alocis*. Oral Synergistetes exceeded 1% in 52% of the samples and 5% in 25%, reaching 11.5% in outliers. *F. alocis* was detected at >1% in 30% of samples, >2% in 15% approaching 10% in outliers.

Figure 3 is a dendrogram showing microbial clustering in subgingival biofilm. Pearson correlation coefficient was calculated for every pair of the tested taxa; the resultant similarities were then rescaled to 0-100% and used for clustering using average linkage.

As expected, a red-complex including the three classical members, *P. gingivalis*, *T. forsythia* and *T. denticola* was formed. However, oral Synergistetes linked with *T. forsythia* at a rescaled similarity of 100%, joining the complex at a shorter distance than

P. gingivalis and *T. denticola*. Oral TM7s joined the orange complex member *P. micra* at a rescaled similarity of ~60%. The same clusters formed using squared Euclidean distance, although the rescaled similarities were smaller.

All taxa were detected at significantly higher levels in the cases than in the controls, except for TM7 that did not withstand correction for multiple comparisons ($p \leq 0.0063$) (Table 3). However, using ordinal regression, *P. gingivalis* and *F. alocis* failed to maintain a significant association with periodontitis after adjustment for confounders. Age accounted for much of the variation between the cases and controls for these two species.

Similar findings were seen for the relative counts (Table 4). Based on significance level and effect estimate, oral Synergistetes showed the strongest association with periodontitis.

Better discrimination between health and disease was achieved by microbial log absolute counts rather than relative counts (Figure 4). The log counts of oral Synergistetes were the best marker of periodontitis followed by those of *T. forsythia*, *P. micra* and *T. denticola* (area under the curve >0.80). The identified log count cut off values for these were 7.2, 6.4, 6.13 and 6.0, respectively.

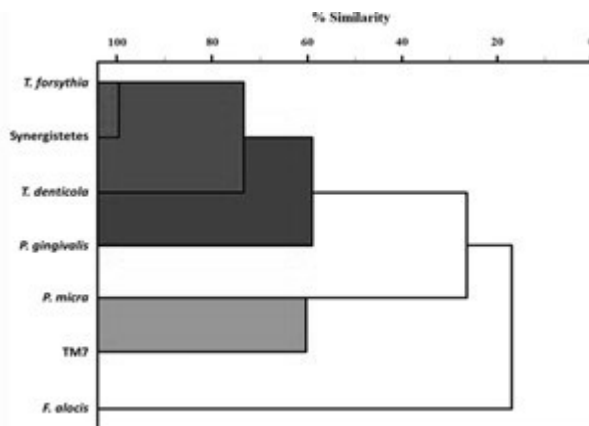


Figure 3. Dendrogram showing microbial clustering in subgingival biofilms.

Species	Controls (n=40)	Cases (n=40)	P1 [¶]	P2 [§]	B*
Total bacteria	9.01 (8.70-9.30)	9.20 (9.04-9.24)	NS	NS	-
<i>P. gingivalis</i>	4.50 (3.23-6.37)	6.88 (3.07-7.48)	< 0.001	NS	-
<i>T. forsythia</i>	5.33 (4.44-6.23)	6.98 (6.55-7.16)	< 0.001	< 0.001	1.80
<i>T. denticola</i>	4.96 (2.74-6.01)	6.79 (6.19-7.19)	< 0.001	0.002	1.60
<i>P. micra</i>	5.51 (5.02-6.09)	6.58 (6.20-6.77)	< 0.001	< 0.001	1.64
<i>F. alocis</i>	5.17 (3.70-6.86)	7.15 (6.53-7.56)	< 0.001	NS	-
Oral Synergistetes	6.16 (5.45-6.99)	7.93 (7.54-8.07)	< 0.001	< 0.001	1.97
Oral TM7	4.07 (3.52-4.56)	4.62 (4.07-5.09)	NS	0.003	0.72

Table 3. Median (interquartile range) log (absolute) counts of the test species/ phylotypes in subgingival plaque from cases and controls. NS: not significant. [¶] P value by Mann-Whitney test; significance at 0.0063 adjusting for multiple comparisons. [§] P value by ordinal logistic regression adjusting for demographic variables, oral habits and mean plaque index; significance at 0.0063 adjusting for multiple comparisons. Pearson and Deviance statistics not significant (model well-fitted). Ordinal logistic regression rather than linear regression was used because the dependent variable was non-normally distributed. * Effect estimate based on regression analysis; reported for significant differences only.

Species	Controls (n=40)	Cases (n=40)	P1 [¶]	P2 [§]	B*
<i>P. gingivalis</i>	6.7E-03 (1.1E-04-4.7E-01)	3.8E-01 (1.2E-04-1.3E+00)	0.001	NS	-
<i>T. forsythia</i>	1.4E-02 (3.1E-03-2.8E-01)	3.8E-01 (1.9E-01-7.6E-01)	< 0.001	< 0.001	1.17
<i>T. denticola</i>	1.0E-02 (6.2E-05-2.6E-01)	2.4E-01 (8.7E-02-6.5E-01)	< 0.001	< 0.001	1.28
<i>P. micra</i>	3.4E-02 (1.1E-02-1.1E-01)	1.3E-01 (1.0E-01-3.5E-01)	< 0.001	< 0.001	1.22
<i>F. alocis</i>	1.9E-02 (3.1E-04-9.2E-01)	5.2E-01 (2.6E-01-1.5E+00)	0.005	NS	-
Oral Synergistetes	1.9E-01 (2.8E-02-1.1E+00)	4.1E+00 (1.7E+00-6.2E+00)	< 0.001	< 0.001	1.47
Oral TM7	9.9E-04 (2.4E-04-4.7E-03)	2.0E-03 (4.5E-04-5.8E-03)	NS	NS	-

Table 4. Median (interquartile range) relative counts (% total bacteria) of the test species/ phylotypes in subgingival plaque from cases and controls. NS: not significant. [¶] P value by Mann-Whitney test; significance at 0.007 adjusting for multiple comparisons. [§] P value by ordinal logistic regression adjusting for demographic variables and lifestyle habits and mean plaque index; significance at 0.007 adjusting for multiple comparisons. Model assumptions are fulfilled: the dependent variable is ordinal; Pearson and Deviance statistics not significant (model well-fitted). * Effect estimate based on regression analysis; reported for significant differences only.

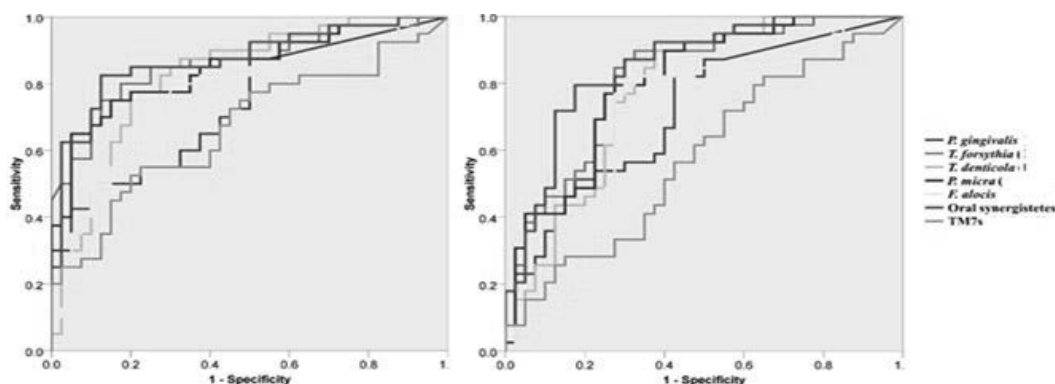


Figure 4. Receiving Operator Characteristic (ROC) curve analysis.

The resultant sensitivities and specificities were 82.5 and 82.5% respectively for oral Synergistetes; 80 and 77.5% for *T. forsythia*; 77.5 and 77.5% for *P. micra*; and 77.5 and 75% for *T. denticola*.

Only age and mean plaque index were identified as predictors of periodontitis (Model 1); however, when excluded on the assumption of being an intermediate factor, gender and the relative counts of oral Synergistetes and *P. micra* were shown as additional predictors (Model 2). The odds ratio for the latter was extremely large (975) (Table 5).

In the predictive OLR model, the relative counts of *P. micra* along with age and mean plaque index were identified as predictors of both mean CPI and clinical attachment loss (Table 6). Qat chewing was an additional predictor of clinical attachment loss.

Discussion

The study subjects were recruited as part of a larger epidemiological study in which periodontal health status was assessed using CPI. Shortcomings were overcome by including CAL measurements, although full mouth assessments would have been preferable in assessing prevalence and extent of periodontitis.

Absolute counts were reported in DNA

copies rather than bacterial cell numbers which does not influence the validity of comparisons between subjects. For *P. gingivalis*, the assay was based on *fimA* gene, which is species-specific. Counts of each taxa were normalized to total bacterial counts (relative quantification) to adjust for variations in sampling and thus to improve reliability of comparisons between samples (Lyons *et al* 2000, Kuboniwa *et al* 2004). Due to design difficulties, the TM7s primers/probe set covered only seven of the 12 clones in the HOMD, however clone I025 was included as it has been particularly linked to periodontitis.

Microbial association with periodontitis

New putative pathogens, such as oral Synergistetes, oral TM7s and *F. alocis*, have been associated with the etiology of chronic periodontitis (Brinig *et al* 2003, Schlafer *et al* 2010, Vartoukian *et al* 2009, You *et al* 2013). The present observations are in agreement with Nonnenmacher *et al* (2001) who reported gram positive microorganisms such as *P. micra* and Eubacterium species have also recently been implicated in chronic periodontitis. *P. micra* (as a representative of the orange complex) was recently shown to have strong association with chronic periodontitis in a sample from the same

	Predictor	P	Odds ratio	Pseudo R2 §
Model 1: *	Age	0.064	1.41	0.707
	Mean plaque index	0.011	2.6E+4	
Model 2: *	Age	< 0.001	1.92	0.608
	Gender **	0.022	17.9	
	<i>P. micra</i> relative count	0.009	975	
	Synergistetes relative count	0.059	1.48	

Table 5. Independent predictors of periodontitis-multiple logistic regression model. Disease status (case/control) as dependent variable and age, gender, lifestyle habits, mean plaque index and relative counts as independent variables. § According to Cox and Snell (1989). * Mean plaque index was included in model 1 and excluded in model 2 with the assumption of being an intermediate factor. ** Males compared to females.

	Predictor	P	Effect estimate	Pseudo R2 §
Mean CPI	Age	< 0.001	0.22	0.832
	Mean plaque index	< 0.001	2.23	
	<i>P. micra</i> relative count	< 0.001	2.32	
Mean CAL	Age	< 0.001	0.22	0.843
	Mean plaque index	< 0.001	2.35	
	<i>P. micra</i> relative count	< 0.001	2.14	
	Qat chewing status	< 0.001	1.48	

Table 6. Independent predictors of mean CPI and CAL - Multiple Ordinal Logistic Regression (OLR) model. Mean CPI or CAL as dependent variable and age, gender, oral habits, mean plaque index and relative counts as independent variables; model assumptions are fulfilled: the dependent variable is ordinal; Pearson and Deviance statistics not significant (model well-fitted). Ordinal logistic regression rather than linear regression was used because the dependent variable was non-normally distributed.

§According to Cox and Snell.

ethnicity (Al-hebshi *et al* 2014). A number of other recent studies have also identified it as a major pathogen in periodontitis (Belstrøm *et al* 2014, Colombo *et al* 2009).

F. alocis has been found to possess virulence factors making it capable of inducing periodontal destruction (Aruni *et al* 2011, Moffatt *et al* 2011). As an exception,

in the present study, oral TM7s did not show significant association with disease, and in a previous study were shown to only be associated with mild periodontitis (Brinig *et al* 2003). In a previous study on a sample from the same population, *P. gingivalis* also did not show an association with periodontitis, suggesting that this species may be less

important in populations from South Arabia (Al-hebshi *et al* 2014). In fact, subgingival microbial profile associated with periodontitis seems to vary by geographical location, e.g. the relative abundance of major periodontal pathogens in subgingival biofilm have been shown to significantly differ among chronic periodontitis populations from different countries (Haffajee *et al* 2004). Different strains of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* with variable virulence have been identified in different parts of the world. This is probably related to host tropism (adaptation of specific bacterial strains to certain host genetic lineage).

Oral Synergistetes, *T. forsythia*, *P. micra*, and *T. denticola* maintained highly significant differences, withstanding both adjustment for confounders and correction for multiple comparisons. Oral Synergistetes showed the highest effect estimate in the OLR (Ordinal Logistic Regression) analysis and the best sensitivity and specificity in ROC curve analysis, suggesting that they may be playing a more important role in the disease than the classical pathogens. These findings substantiate the rapidly growing evidence for their strong association with periodontitis.

In fact, certain Synergistetes, such as *Fretibacterium fastidiosum* and OTU 4.2, have already been implicated in the etiology of periodontal disease, and may soon occupy the forefront as putative periodontal pathogens (Vartoukian *et al* 2009, You *et al* 2013). It is likely that this cluster (A) is involved in the interaction with the red complex described here.

Age and mean plaque index were found to be the only predictors of periodontitis; however, when mean plaque index was removed, *P. micra* and oral Synergistetes appeared as predictors of the disease. In the ordinal regression model, the relative counts of *P. micra* were found to be predictors of CPI and CAL independent of age and mean plaque

index. *P. gingivalis* showed weak association with periodontitis in the current study, and one plausible explanation is that its role was taken over by other pathogens in a subset of the patients.

The findings also demonstrate that all putative pathogens are present in low abundance at healthy sites and that an increase in their proportions to a certain “threshold” is required for triggering periodontitis, which is compatible with the ecological plaque hypothesis (Marsh 2004). What ecological factors drive the pathogenic microbial shifts in subgingival biofilm are not yet elucidated but an increased supragingival plaque mass (high plaque index) can be one such factor. In fact, mean plaque index in the current study masked the effect of the tested putative pathogens in the logistic regression analysis suggesting it may well be an intermediate factor.

Associations among classical and new putative pathogens

The current study is probably the first attempt to explore associations among classical and new putative pathogens in subgingival plaque and to compare directly their relative importance to chronic periodontitis.

To our knowledge, this is the first time oral Synergistetes have been reported to strongly cluster with the red complex. In fact, they were the first to join the cluster by linking to *T. forsythia* at 100 rescaled similarity, indicating that they may be more important members of the red complex than *P. gingivalis* and *T. denticola*; the latter two joined the complex at distances comparable to those originally reported by Socransky *et al* (1998).

TM7s linked with *P. micra* at a rescaled similarity of 60%, suggesting they may be members of the orange complex, but this needs to be explored further by including other complex members. *F. alocis* did not

show association with any of the tested taxa. In fact, *F. alocis* has been recently shown to be inhibited by *P. gingivalis*, and this could explain its failure to cluster with the red complex (Wang *et al* 2013).

Conclusions

Oral Synergistetes are presented here as new members of the red complex, with relative importance to periodontitis exceeding that of the classical members. Further work to identify which specific Synergistetes are involved is warranted. Only *P. micra* presented as a predictor of periodontal health parameters which, along with findings from other recent studies, suggests that the role of this bacterium in periodontitis should be revisited.

Acknowledgements

The work was entirely funded by Institute of Research Management and Monitoring (IPPP) No. PG101-2012B, University Malaya, Malaysia. All experiments were carried out at the Molecular Research Laboratory, University of Science and Technology, Yemen. Appreciation is expressed to the research team particularly Abdulrahman Alalimi (PhD student, UM), Associate Professor Nezar Alhebshi and Abeer Aldubaibi, technician in Yemen laboratory.

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Poster Presentations

The following is a record of the posters awarded prizes at the
12th Meeting of the Asian Pacific Society of Periodontology

Case Report - 1st Prize

A case of periodontal tissue regeneration therapy for endodontic periodontal disease with fused mandibular incisors

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Introduction: The endodontic-periodontal lesion is caused by infection from different parts of the root canal and gingival sulcus. Generally, it is considered that the prognosis of teeth suffering from severe endodontic-periodontal disease is poor.

Objectives: This case report describes periodontal tissue regeneration therapy performed on teeth suffering from endodontic-periodontal disease with fused mandibular incisors, and the improvements in periodontal tissue.

Case management: A 47 year old male presented with clinical and radiographic evidence of an endodontic-periodontal lesion around the mandibular right central incisor and lateral incisor. Vertical and horizontal bone loss was observed on the radiograph and the site had 6 mm or more of periodontal pocket depth at the first visit. After the initial periodontal therapy, we performed periodontal tissue regeneration with enamel matrix proteins at the site. After re-evaluation, the final restoration was placed, and the patient entered the maintenance phase.

Conclusion: The periodontal attachment status was well maintained for more than two years post-operatively. It was indicated that periodontal tissue regeneration therapy based on appropriate diagnosis progresses well for severe teeth with endodontic-periodontal disease.

Case Report - 2nd Prize

Periodontitis and systemic lupus erythematosus

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Introduction: Systemic disease is a risk factor in periodontal disease. In contrast, severe generalized periodontal disease may also contribute to the development of systemic disease and has an adverse effect in controlling the systemic disease. The majority of systemic diseases manifest in the oral cavity, including Systemic Lupus Erythematosus (SLE), an autoimmune chronic systemic disease. To date, the etiology of SLE is still not clear, but if diagnosed early and adequate therapy is given the prognosis can be good. Several epidemiological studies have reported a greater incidence in Asian and African-American women. In 2010 in the rheumatic polyclinic at Hasan Sadikin Hospital, Bandung there were 291 patients with SLE, being 10.5% of total patients seeking treatment. Treatments for SLE patients with periodontitis are still not understood by the general public.

Objectives: This case report will discuss the abnormalities that occur in the periodontal tissue of SLE patients and how to treat them.

Case management: A 55 year old female patient with history of SLE attended the practice with the complaint of bleeding gums and dry mouth. On clinical examination, there was gingival enlargement in the anterior region of dextra maxilla, hyperemia and oedema, and pocket depth of 5 mm. Case management was with oral hygiene instruction and scaling.

Conclusion: A good periodontal treatment can improve oral hygiene and reduce gingival inflammation, with a resulting increase in wholebody immunity.

Basic Research Category - 1st Prize

Optimization of a chronic periodontitis mouse model

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Introduction: Japan has a rapid aging society and it is expected that this trend will continue in the future. Periodontitis is an inflammatory disease caused by periodontal disease bacteria in which alveolar bone is destroyed with an increase in inflammation and chronicity.

Objectives: In experimental periodontitis mouse models, a method of ligaturing teeth with sutures or transferring periodontal pathogenic bacteria is often adopted. In periodontitis, alveolar bone resorption due to chronic inflammation is the main symptom of the disease state. Conventional periodontitis models have room for improvement as a model of chronic inflammation in the method of inducing inflammation/observation period. In this study, alveolar bone resorption was induced by a method with little mechanical irritation, and the progress of periodontitis in young and old mice was compared. In addition, the validity as a model of chronic bone resorption was evaluated.

Methods: LPS from *Porphyromonas gingivalis* was administered 12 times (twice per week) on the palatal gingiva of eight or 24 week old C57BL/6J mice. LPS was injected by micro-syringe (33 G) at a dose of 20 µg per shot. Peripheral blood was collected at one and four weeks after the final administration, femur, spleen and maxilla were also collected. Micro CT scan and tissue slices were prepared.

Results: Injection of LPS by micro-syringe did not result in laceration of the gingiva. As seen by micro CT, bone resorption by LPS was greater in older mice than in young mice. Comparison between one and four weeks after the final administration resulted in bone resorption in older mice progressing further after four weeks than in young mice.

Conclusion: LPS administration with a micro-syringe caused alveolar bone resorption in both young and old mice. In older mice, lingering bone resorption even after LPS administration was shown.

Basic Research Category - 2nd Prize

Effects of brain-derived neurotrophic factor as an adjunct to nonsurgical periodontal treatment on ligature-induced periodontitis in dogs

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Introduction: We have revealed that application of brain-derived neurotrophic factor (BDNF)/high molecular weight hyaluronic acid (HMW-HA) complex with flap surgery enhanced periodontal tissue regeneration in moderate periodontal defects in dogs and monkeys. Moreover, BDNF exerted apoptotic and cytostatic effects in gingival epithelial cells in vitro. These characteristics of BDNF may realize non-surgical regenerative therapy for small periodontal defect.

Objectives: In this study, we investigated the effect of scaling and root planning (SRP) with local application of BDNF/HMW-HA complex on the changes in clinical parameters and histology of periodontal tissue in dogs with ligature-induced periodontitis.

Methods: One week after scaling, the clinical parameters of gingival index (GI), clinical attachment level (CAL), periodontal pocket depth (PPD) and bleeding on probing (BOP) of beagle dogs (10 to 14 kg, 12 to 20 months old) were recorded by standardized methods. These parameters were measured at four sites per tooth: mesio-buccal, disto-buccal, mesio-lingual and disto-lingual. Thereafter, following sulcular incisions, mucoperiosteal flaps were raised and 3-0 silk ligatures were tied around the cervical region of mandibular second, third and fourth premolars (P2, P3 and P4) to induce experimental periodontitis. The flaps were then repositioned coronally and sutured by the interrupted suture method with 4-0 silk sutures. The ligatures were maintained for five weeks. One week after ligature removal, clinical parameters were recorded to evaluate periodontal status. Then the dogs were divided into four groups: no-treatment, SRP alone, SRP followed by local application of HMW-HA and SRP followed by local application of recombinant human BDNF (500 µg/ml)/HMW-HA complex. HMW-HA or BDNF/HMW-HA complex were topically applied to the periodontal pocket using syringe. Two weeks after the treatment, clinical parameters were recorded and anesthetized animals were perfused for histological analysis (hematoxylin & eosin staining and azan staining).

Results: The BDNF/HMW-HA group showed significant improvement of clinical parameters compared to those of the other groups in dogs. Histological analysis of the BDNF/HMW-HA group indicated suppression of apical migration of epithelial tissue and milder infiltration of inflammatory cells than it was observed in the other three groups. Furthermore, new cementum and alveolar bone were generated and collagen fibers were inserted into them in the BDNF/HMW-HA group.

Conclusion: BDNF as an adjunct to non-surgical periodontal treatment has potential to reduce excess inflammation and to promote periodontal tissue regeneration.

Basic Research Category - 2nd Prize

Lithium-containing silicate bioceramics stimulated cementogenic and osteogenic differentiation of periodontal ligament cells via activation of Wnt/ β -catenin and ERK signaling pathway

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Introduction: The ultimate goal of treatment of periodontal disease is not only to control inflammation and to prevent further development of the lesion, but also to achieve regeneration of the destroyed periodontal tissue (including periodontal ligament, alveolar bone and cementum). As the main cells of the periodontal ligament, periodontal ligament cells (PDLs) are the basis of periodontal tissue repair and regeneration. In this study we synthesized novel lithium-containing silicate (LCS) bioceramics and examined the cementogenic/osteogenic differentiation of PDLs after cultured in different concentrations of LCS bioceramics extracts in DMEM medium. Meanwhile, we further studied the involvement of the Wnt/ β -catenin and ERK signaling pathway during this process.

Objectives: To examine the role of LCS bioceramics in the cementogenic/osteogenic differentiation of PDLs and the signaling pathway during this process.

Methods: In this study we synthesized novel lithium-containing silicate (LCS) bioceramics and examined the cementogenic/osteogenic differentiation of PDLs after cultured in different concentrations of LCS bioceramics extracts in DMEM medium. Meanwhile, we further studied the involvement of the Wnt/ β -catenin and ERK signaling pathway during this process.

Results: Our results found that a certain concentrations of LCS extracts could significantly stimulate cementogenic/osteogenic differentiation, including alkaline phosphatase (ALP) activity, Calcium deposition and periodontal regeneration-related gene expression (ALP, OPN, OCN, BSP, CAP, VEGF, colland CEMP1) and protein expression (ALP, OPN, colland RUNX2) of PDLs. Moreover, after we added the ICG-001(a Wnt/ β -catenin signaling inhibitor) and SCH772984 (a ERK signaling inhibitor) respectively, the periodontal regeneration-related gene/ protein expressions were distinctly inhibited, which indicated that two signaling pathway may play an important role in cementogenesis/osteogenesis of PDLs induced by LCS bioceramics.

Conclusion: The study demonstrated that LCS bioceramics are a promising bioactive material for periodontal regeneration, and decent scaffolds to transport PDLs for periodontal defect repair, consequently to offer a new treatment strategy for the treatment of periodontitis.

Basic Research Category - 3rd Prize

Roles of age, gender, and level of OHIS to gingivitis in elementary students

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Introduction: Gingivitis is an inflammation of the gingiva, with clinical symptoms of hyperemia, oedema, gingival contour changes, and gingival bleeding.

Objectives: The aim of the study is to examine the correlation between age, gender, and OHIS with the incidence of gingivitis in Province of Banten, especially in Renged 1 and Renged 2 Elementary School, Tangerang Regency.

Methods: The study was an observational study, descriptive-analytic with cross-sectional approach. 316 students aged 8 to 12 years old were involved in the study (grades 2 to 6 of elementary school). They were examined intraorally using Oral Hygiene Index Simplified (OHI-S) as described by Greene and Vermillion (1964), and the Gingival Index by Loe and Silness (1963).

Results: Chi-square test reveals that there is significant correlation between OHIS and gingivitis ($p = 0.000$), but there were no significant correlation between age and gingivitis ($p = 0.594$) and gender with gingivitis ($p = 0.146$).

Conclusion: It can be concluded that gingivitis has a significant correlation with OHIS, but does not have a significant correlation with age and gender. With the increase of age, the incidence rate of gingivitis decreased, which is expected because knowledge of oral hygiene is increased. Gingivitis in female students was higher than males, with the majority exhibiting mild gingivitis, due to hormonal factors in females aged 8 to 12 years old who are approaching the phase of puberty. Students aged 8 to 12 years old with bad OHIS have a risk of gingivitis 4.97 times greater than students who have a good OHIS.

Basic Research Category - 3rd Prize

Effectiveness of RGD application in periodontal regenerative cell sheet therapy: Study in *Macaca nemestrina*

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Introduction: Tissue engineering in the form of cell sheets cultivated from stem cells has been developed to overcome the regenerative limitations in one wall bone defects. In a previous study, cell sheet application with a chitosan carrier gave a good result in treating bone defects. A continuing study was done in *Macaca nemestrina* by adding RGD (arginine-glycin-aspartic acid) on the chitosan as a carrier for cell sheet application. RGD allows a favorable environment for stem cells and enhances reattachment and regeneration of cell. Periostin, one of the protein that regulates coordination and interaction of periodontal regeneration and tissue repair, could be used as a marker in early bone regeneration.

Objectives: To evaluate the effectiveness of RGD modified chitosan on cell sheet application in treatment of one wall bone defects.

Methods: To obtain cell sheets, *Macaca* PDL was cultured and applied on chitosan as a control group. RGD modified chitosan group was applied with cell sheet from the same cultured cell. A one wall bone defect was created on lateral incisors of two *M. nemestrina* (n=8) and two groups of treatment were applied on defect. GCF was collected from each tooth every week for a month and stored in a -80°C refrigerator. Samples were centrifuged for ten minutes at 12,000 g and prepared for protein assay procedure using ELISA Human POSTN Kit (Elabscience). Results were analyzed with Mann-Whitney statistic test on SPSS.

Results: There were no statistically significance differences of periostin level between group chitosan cell sheet and RGD-modified chitosan cell sheet statistically at first, third and fourth weeks (Mann-Whitney; $p > 0,05$). By week two there were statistically significance differences in periostin level between two groups (Mann-Whitney; $p < 0,05$; $p = 0,049$).

Conclusion: RGD-modified chitosan could enhance cell sheet attachment into the tooth thus increased the capability of cells to differentiate and proliferate. Periostin might be secreted the most on second week stage of healing.

Basic Research Category - 3rd Prize

Elucidation of the role of NR4A1 in CsA induced gingival overgrowth

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Introduction: Drug induced gingival overgrowth (DiGO) is a side effect principally associated with anticonvulsant (e.g. phenytoin), various calcium channel blockers, or immunosuppressant (e.g. CsA: cyclosporine A) medications. We aimed to clarify the mechanism of DiGO and succeeded in establishing CsA induced gingival overgrowth mice model. It has been recently reported that the lack of active orphan nuclear receptor NR4A1 leads to persistent activation of TGF- β signaling and tissue fibrosis, and NR4A1 agonist inhibit skin, lung, liver, and kidney fibrosis in mice. We hypothesized that NR4A1 is involved in DiGO and is a potential target for treatment DiGO.

Objectives: To elucidate the role of NR4A1 in CsA induced gingival overgrowth mice model.

Methods: The maxillary second molars of C57BL/6j mice were ligated with 5-0 silk threads for one to five weeks. A number of these one week ligatured mice were administrated CsA (50 mg/kg/day). Gingival width measurements and histological evaluation assessed gingival overgrowth. The expressions of Tgfb, Nr4a1, Pai1 and Col1 mRNA in the mice gingival tissue were analyzed by RT-PCR.

Results: The combination of ligature and CsA administration induced significant gingival overgrowth. Ligature increased Tgfb and Nr4a1 mRNA expressions, but did not induce gingival overgrowth. CsA inhibited Nr4a1 mRNA expression, and maintained Col1 mRNA expression.

Conclusion: These results suggest that the reduced NR4A1 expression by CsA inhibited TGF- β signaling, thereby increased collagen expression.

Clinical Research Category - 1st Prize

Influence of CYP1A1 rs1048943 variants and blood lipids interactions on severity of generalized aggressive periodontitis in Chinese Han population

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Introduction: CYP1A1, as an important subfamily of cytochrome P450 enzymes, has been reported to be associated with periodontitis and influence generalized aggressive periodontitis (GAgP) patients' response to non-surgical treatment. Dyslipidemia may increase the risk of periodontitis and may also be correlated with CYP1A1.

Objectives: To evaluate the influence of interactions between single nucleotide polymorphism (SNP) of CYP1A1 rs1048943 and blood lipids on severity of GAgP.

Methods: A cross-sectional study of 233 patients with GAgP was conducted at Peking University Hospital of Stomatology. Clinical parameters such as probing depth (PD), attachment loss (AL) and bleeding index (BI) were measured. Serum total cholesterol (TC), triacylglycerol (TG), high and low density lipoprotein (HDL and LDL) were measured. SNPs of CYP1A1 rs1048943 were genotyped by the time of flight mass spectrometry. Multiple linear regression models were used to estimate the interactions between SNP of CYP1A1 rs1048943 and blood lipids on severity of GAgP.

Results: After adjusting for potential confounders, CYP1A1 gene SNP rs1048943 was associated with increased PD for GAgP in GG genotype ($\beta=0.89$, 95%CI: 0.35, 1.44) and in GA genotype ($\beta=0.43$, 95%CI: 0.15, 0.72) compared with AA Homozygotes. And increased AL in GG genotype ($\beta=1.31$, 95%CI: 0.62, 2.06) and GA genotype ($\beta=0.57$, 95%CI: 0.19, 0.94). Interactions were found between CYP1A1 gene SNP rs1048943 and HDL for PD (Pinteraction=0.0001) and AL (Pinteraction=0.0002) and between rs1048943 and LDL for BI (Pinteraction= 0.0238) in patients with GAgP.

Conclusion: CYP1A1 gene SNP rs1048943 G allele was associated with increased PD and AL for GAgP. The gene variants interact with blood lipids to modulate the severity of GAgP. This gene-lipid interaction may contribute to new strategies for the prevention and treatment of GAgP in the future, and the mechanism of the interactions need to be further studied.

Clinical Research Category - 2nd Prize

Gingival crevicular fluid cytokines during the induction of experimental gingivitis in humans.

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Introduction: An experimental gingivitis model provides a useful and controlled method of investigating the beginning of the inflammatory process. Cytokines are responsible for the first responses to plaque accumulation before any clinical signs of inflammation appear.

Objectives: The goal of this study was to evaluate the changes in the levels of eight cytokines in gingival crevicular fluid during a two week induction phase in experimental gingivitis in humans.

Methods: Experimental gingivitis was induced in 20 healthy subjects who stopped cleaning their mandibular teeth for two weeks. GCF samples from 12 mesiobuccal sides on the mandibular teeth were taken at baseline and at one and two weeks of plaque accumulation. The percentage of sites with clearly visible plaque accumulation were assessed at every visit and the percentage of bleeding sites were measured at baseline and week two. Eight cytokines (IL-2, IL-4, IL-10, IL-17, IL-6, IL-1 β , IL-8 and TNF- α) from GCF samples were analyzed using a multiplex bead array assay (Luminex Performance Assay).

Results: Clinical parameters and GCF volumes showed significant increases in all subjects and between time points. Only two out of eight cytokines (IL-8 and IL-1 β) were detectable within the reliable range of assay. Gingivitis induction in this study was associated with a significant decrease in IL-8 levels within the subjects, between baseline and week 1, and between baseline and week two. There were also significant relationships between IL-8 levels & the percentage bleeding and plaque scores. The pattern of IL-1 β changes varied between subjects and there was no statistically significant shift in IL-1 β levels in this study.

Conclusion: IL-8 is involved in the earliest cytokine changes in experimental gingivitis in humans. The multiplex bead array assay provided a high-throughput platform for simultaneous detection of analytes in GCF.

Clinical Research Category - 2nd Prize

Analysis of the association between alveolar bone resorption and atherosclerosis risk

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Introduction: Periodontitis is considered to be linked with atherosclerotic vascular diseases and other metabolic diseases. Observation studies have demonstrated an association between periodontal disease and atherosclerotic vascular disease, independent of known confounders. However, little is known of a causative relationship of these.

Objectives: In the present study, we investigated the relationship between alveolar bone resorption, a characteristic feature of periodontal disease, and future change of risk markers for atherosclerotic vascular diseases in subjects who receive yearly comprehensive health examinations in a community-based population.

Methods: The study population was recruited from comprehensive health examinations at Nagano Health Promotion Center, Japan, from 2004 to 2015. Randomly selected 533 subjects (male: 383, female: 150; age, mean \pm SD: 50.2 \pm 10.6 years) had full mouth panoramic x-rays at both baseline and 5 years. The subjects were divided into two groups based on amount of bone resorption; mild (<30%) and moderate/ severe (\geq 30%). Information on age, gender, smoking status, snacks, alcohol intake, metabolic syndrome risk factors, and baseline alveolar bone resorption scores were obtained and tested as potential confounders in the statistical models. The amount of risk factors for atherosclerotic vascular diseases were evaluated between at baseline and over five years. The level of significance for predictor variables was set at $p < 0.05$.

Results: Atherosclerotic risk markers (i.e. high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)/HDL-C ratio, and triglyceride) in subjects who have moderate/severe alveolar bone resorption at baseline demonstrated were significantly higher risk compared to those with mild alveolar bone resorption ($p < 0.05$). Five year changes of those markers in subjects with moderate/severe bone resorption demonstrated significant change towards higher risk compared to those with mild bone resorption at baseline ($p < 0.05$).

Conclusion: These epidemiologic data suggested that alveolar bone resorption relates to future risk of metabolic disease.

Clinical Research Category - 3rd Prize

Autologous fibroblast injections following a “papilla priming” procedure: A novel technique in papilla regeneration

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Introduction: One of the most difficult and elusive goals for the periodontist in the reconstructive, regenerative and aesthetic aspect of periodontal therapy is the regeneration of the lost interdental papilla. Tissue engineering and minimally invasive procedures may allow us to overcome the limitations of traditional therapy and resolve interdental papillary loss in novel ways.

Objectives: The aim of this study was to assess the efficacy of autologous fibroblast injections following a minimally invasive papilla priming procedure to augment interdental papillary insufficiency.

Methods: Patients with interdental papillary insufficiency were enrolled in this study. Papillary loss was measured by a periodontal probe from the base of the contact area to the tip of the interproximal papilla. Sites having <4 mm of interdental papillary loss were selected. These sites were randomly grouped into test and control sites. A novel “papilla priming procedure” was done in all sites; following which test sites received autologous fibroblast injections, whereas control sites received normal saline injections. The percentage change in papillary height of the sites was evaluated from baseline to the 3-month visit. A visual analog scale (VAS) was also used by the examiner and subject to assess the change from baseline to three months.

Results: The difference in papillary height from baseline to three months approached statistical significance in test sites as compared to the control sites; suggested that autologous fibroblast injection improved interdental papillary height. The difference in the VAS scores was significant in test sites from baseline to three months in contrast to control sites.

Conclusion: This early-phase study using autologous cell transplantation of fibroblasts following a papilla priming procedure and analysis of the VAS assessments, suggested that this minimally invasive treatment was efficacious for treating papillary insufficiency.

Clinical Research Category - 3rd Prize

Analysis of formyl peptide receptor 1 protein level as an indicator of neutrophil chemotaxis dysfunction in aggressive periodontitis

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Introduction: A decrease in neutrophil chemotaxis function may cause increased susceptibility to aggressive periodontitis (AP). Neutrophil chemotaxis is affected by formyl peptide receptor 1 (FPR1), which, when activated, will respond to bacterial chemotactic peptide formyl methionyl leucyl phenylalanine (FMLP). FPR1 protein level is decreased in response to a wide number of inflammatory stimuli in AP patients.

Objectives: This study aimed to assess the alteration of FPR1 protein value in AP patients and if FPR1 protein levels could be used as an indicator of neutrophil chemotaxis dysfunction in AP.

Methods: This was a case-control study with 20 AP patients and 20 control subjects. Three milliliters of peripheral blood were drawn and analyzed for FPR1 protein level with ELISA. The data was statistically analyzed by Mann-Whitney test ($p > 0,05$).

Results: The mean value of FPR1 protein level in AP group was 0.353 pg/mL (0.11 to 1.18 pg/mL) and the mean value of FPR1 protein level in control group was 0.296 pg/mL (0.05 to 0.88 pg/mL). P value 0.787 > 0.05 suggested that there is no significant difference in FPR1 protein levels in both groups.

Conclusion: The present study suggests that FPR1 protein level is not significantly altered in AP patients and could not be used as an indicator of neutrophil chemotaxis dysfunction.

Clinical Research Category - 3rd Prize

Oral health burden in Hong Kong local population: A radiographic investigation to determine the specific age group for accelerated periodontal destruction

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Introduction: Periodontal disease is one of the two major oral diseases at high prevalence rates that affect human beings. The severe form of this disease can affect in a range from 5 to 20% and remains a major global oral health burden. An oral health survey conducted in 2011 by the Hong Kong Government showed that about 11.3% of adults aged 35 to 44 years old suffered from periodontal tissue attachment loss more than 5 mm. A recent global study indicated that the peak in incidence of periodontal disease is about 38 years old. Therefore, the access to professional preventive and diagnostic treatment during these key years or even earlier is very crucial in order to provide better outcomes and healthy aging. All of these should first start by having a proper diagnosis via a full mouth comprehensive periodontal evaluation and periodontal probing must be the key component of every dental visit.

Objectives:

1. To investigate the past history of periodontal disease (periodontal tissue destruction in radiographic examination) in different age groups in a selected Hong Kong subjects aged from 20 to 44 years old.
2. To investigate the accelerated periodontal disease progression among them.

Methods: This was a retrospective study using the patient's panoramic radiographs taken from October 2015 to October 2016. Hong Kong Chinese people aged from 20 to 44 years old having a panoramic radiograph taken during consultation with at least one tooth present in the radiograph were included. The subjects were identified through the hospital database (Salud System). A patient list was generated, and the subjects were stratified into five groups according to their age. The five groups are 20 to 24, 25 to 29, 30 to 34, 34 to 39 and 39 to 44 years old. Afterwards a random list was generated and the first 20 eligible subjects in each group were included for investigation. Bone loss was measured from the radiographs using Schei ruler to record down the percentage of loss of each tooth mesial and distal. The percentage of bone loss in each age group was analyzed according to the two model: (1) the percentage of sites with more than 20% of bone loss, and (2) the percentage of sites with more than 30% of bone loss. The models were fitted into a three-level logistic mixed effect regression (Patient x Tooth x Site) for statistical analysis. The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 17-004).

Results: A total of 100 panoramic radiographs were studied (20 for each age group). Both models showed that a patient with a higher age would be more likely to have tooth

sites with more than 20% bone loss (model 1) and more than 30% bone loss (model 2). There is a much higher chance to develop more than 20% and 30% bone loss at a tooth site for age 35 to 39 (OR >9, $p < 0.001$) and age 40-44 (OR >29, $p < 0.001$). As a multilevel model was fitted for possible clustering among observation, from the intraclass correlation, different teeth in the same patient are mildly associated ($ICC \approx 0.35$) and different sites at the same tooth are strongly associated ($ICC \approx 0.50$).

Conclusion: The current local study agreed with the literature which indicated that the peak in incidence of periodontal disease is about 38 years old. From the results shown, it is reasonable to recommend patients receive a detail diagnostic (full mouth comprehensive periodontal evaluation) and preventive care at an earlier age of 30 before the development of bone loss occurs. The stakeholders involved in policy making for oral health should also be aware of the results from this clinical research, in order to provide better health outcomes and healthy aging in their population.