EXPLORING INNOVATIVE FRONTIERS IN PERIODONTAL HEALTH AND IMPLANTOLOGY

Hosted by

Asian Pacific Society of Periodontology

28 - 29 September 2019

Kuala Lumpur, Malaysia

Edited by
P Mark Bartold
Tara B Taiyeb Ali

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Acknowledgments

The 13th International meeting of the Asian Pacific Society of Periodontology (APSP) was successfully held in Kuala Lumpur, Malaysia on September 28-29, 2019. Over 470 delegates from 19 countries (Australia, Cambodia, China, Hong Kong SAR China, India, Indonesia, Japan, Korea, Malaysia, Mongolia, Myanmar, Nepal, New Zealand, Philippines, Saudi Arabia, Singapore, Taiwan, Thailand, and Vietnam) attended this APSP meeting with the theme “Exploring Innovative Frontiers in Periodontal Health and Implantology”. The welcome address and Nations Roll Call was given by Prof Tara Taiyeb Ali, Chairperson of the 13th APSP Meeting, the President's address was made by Prof Young Ku. Welcome addresses were also made by Mr Takeshi Kamigouchi from Sunstar Group; and Mr Fumitomo Noritake from Lion Corporation. Dr. Doreyat Bin Jemun, Principal Director of Oral Health, Malaysia on behalf of the Minister of Health Malaysia, Honorable Dato’ Seri Dr Dzulkifli Ahmad.

The two-day program was very full, with 22 oral presentations delivered by speakers from 14 different countries. The Key Note speakers were Professor Maurizio Tonetti (Hong Kong) and Associate Professor Stephen Chen (Australia). In addition, 140 posters were selected and scheduled for presentation.

The poster sessions were very successful. In keeping with the tradition from previous meetings twelve prizes were awarded for the posters judged to be the best on the day in the categories of clinical research, laboratory research, clinical case reports and systematic / literature reviews. The abstracts of the winning 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 understand them in 2019. As for 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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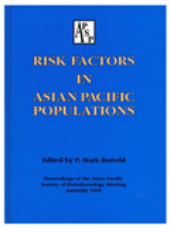
I want to thank my co-editor Professor Tara Taiyeb Ali for her invaluable help in proof reading all of these manuscripts. Finally it is very important to acknowledge that this publication would not have been possible without the untiring efforts of our production editor, Ms Susannah Bartold.

Finally I want to record that this is my last job as Editor of the APSP Proceedings. The first APSP Proceedings was published in 1995 and since then we have published Proceedings for every meeting held by the APSP. It is my sincere hope that this tradition continues. A quarter of a century is a long time and I have seen some wonderful maturity develop in the APSP over these years. I wish my successor every success and hope they find it as enjoyable and rewarding as I have over the past 25 years.

Copies of the cover from each volume published to date are shown below.

Mark Bartold
APSP Editor
March 2020



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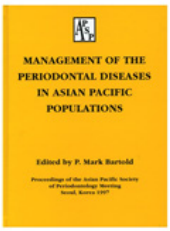

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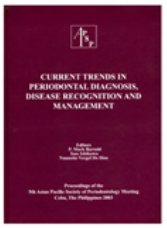

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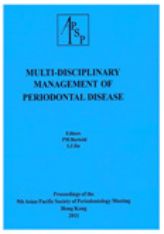

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Exploring Innovative Frontiers in Periodontal Health and Implantology
Edited By: PM Bartold, TB Taiyeb Ali

Chapter 1

Periodontal Ligament Cell Sheet Contributes to the Success of Periodontal Regeneration and to Periodontal Formation on Implant Surface

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[1]Institute of Advanced Biomedical Engineering and Science. Tokyo Women's Medical University
[2]Institute of Dental Research, Osaka Dental University

Introduction

Periodontal ligament (PDL) cells play a key role in the regeneration of periodontal tissues. Some cell populations have been identified from PDL cells that have mesenchymal stem cell (MSC) like characteristics. These cells can differentiate into osteoblasts, adipocytes and chondrocytes. In addition to these characteristics, PDL cells can also differentiate into cementoblasts and form cementum-PDL like structures. Cell sheet engineering is a unique tissue engineering approach that does not disrupt the extracellular matrix components associated with cultured cells.

Development of cell sheet engineering

Okano *et al* (1993) developed a new method to control cell surface adhesion utilizing changes in cell culture temperature and a surface-grafted temperature-responsible polymer named poly N-isopropylacylamide (PIPAAm). At temperatures lower than 32° C, PIPAAm is fully hydrated with an extended chain conformation. However, at temperatures higher than 32° C, PIPAAm is extensively dehydrated and compact. Cells generally adhere to hydrophobic surfaces, but not to hydrophilic surfaces. PIPAAm can be adhered onto tissue culture-grade polystyrene dishes using irradiation with an electron beam. These coated culture dishes allowed intact cells to be cultured and then harvested by exposure to low temperature treatment. These sheets possess intact surfaces and maintain normal cell-cell junctions. Therefore, the temperature-responsive dishes allow cells to maintain substrate adhesivity, growth and secretion activities, even when the culture temperature was changed.

Periodontal application of cell sheet engineering.

The characteristics of human periodontal ligament cell sheets obtained

from temperature responsive culture dishes has been investigated for their potential to assist in regeneration of periodontal tissues. The PDL cells were recovered from the culture dishes as a contiguous sheet accompanied with abundant extracellular matrix, including type 1 collagen, integrin beta 1 and fibronectin. These detached cell sheets shrunk gradually by lateral contraction forces that had been antagonized during the culture by cell contact with the culture dishes. The human PDL cell sheets were then transplanted into a mesial dehiscence model in immunodeficient rats. Histological examination of the transplanted cell sheets of human PDL cells indicated uneventful healing during the experimental period. At 1 week after surgery, transplanted cells were observed to attach onto the dentin surfaces. At 4 weeks after surgery, newly formed immature fibers, which attached obliquely to dentin surfaces, were the predominant feature in all the experimental sites. Such findings were not observed in any control site.

In order to verify the regenerative potential of human PDL cell sheets stimulated with osteogenic differentiation medium, we transplanted cell sheets onto the root surface of defects in athymic rat madibules prepared following the method of King *et al (1997)*. This method is suitable for observing the regenerated cementum and periodontal ligament. The results indicated that most of the experimental group exhibited new cementum and a new attachment of collagen fibers into the cementum layer. No new cementum layer was observed in the control group which was cultured without osteogenic differentiation medium.

Canine studies using PDL cell sheets

Based on the studies referred to above, we studied the use of PDL cell sheets in canine model for periodontal regeneration (Akizuki *et al 2005;* Hasegawa, *et al* 2005; Ishikawa *et al* 2006; Flores *et al* 2008a; Flores *et al* 2008b; Iwata *et al* 2009). Autologous PDL tissues were carefully removed from extracted teeth using a scalpel. After primary culture, PDL cell sheets were prepared using temperature responsive culture dishes. When cells reached confluence, the medium was replaced with culture medium supplemented with 50 microgram/ml of ascorbic acid to enhance the cell sheet. The PDL cells were incubated for another 2 weeks. Then the cell sheet was applied to the periodontal defects. No visible adverse reactions were observed for up to 8 weeks after surgery. Histological observations demonstrated the presence of periodontal tissue regeneration with newly formed alveolar bone, cementum and periodontal ligament formation.

Newly formed periodontal ligament with a rich capillary supply were observed between the alveolar bone and cementum. In the control group which lacked PDL cell sheet, neither alveolar bone nor cementum were found in the defects. Thus we concluded that the PDL cell sheet has a potential to regenerate periodontal tissues.

Translational studies using PDL cell sheets

An optimal protocol for extraction, expansion and characterization of human PDL cells has been examined for use in clinical trials (Iwata *et al* 2010; Iwata *et al* 2018). Human PDL cells are best extracted from human PDL using with collagenase/ dispase rather than trypsin/EDTA. These

cells exhibit good osteogenic potential both *in vitro* and *in vivo*. A protocol for the successful cultivation of human PDL cell sheet has been proposed for clinical application. The validation of the safety and efficacy of human PDL cell sheet has also been studied for use in clinical trials. Human PDL tissues from three donors were enzymatically digested and the obtained cells were cultured media containing autologous serum in a cell processing center (CPC). The safety and efficacy of human PDL cell sheets were evaluated both *in vitro* and *in vivo*. *In vitro* studies showed that the human PDL cell sheets had high alkaline activity and periostin expression and no contamination with microorganisms. *In vivo* studies revealed that human PDL cell sheets, implanted with dentin blocks, formed cementum and PDL-like tissues in immunodeficient mice. These human PDL cells presented no evidence of malignant transformation. Thus human PDL cell sheets fabricated in CPCs appear to be safe products and possess the potential regenerate periodontal tissues.

Human trials using PDL cell sheets

In these studies, autologous PDL cells from patients were used and cell sheets were fabricated using temperature responsive culture dishes. Subsequently, the safety and efficacy of autologous PDL cell sheets were evaluated in a clinical setting. A single-arm and single-institute clinical study was performed for ten periodontal patients. Wisdom teeth were extracted from the patients diagnosed with chronic periodontitis, ranging in age 33 to 63 years and periodontal tissue were scraped for cell sources. Three-layered PDL cell sheets were constructed using temperature responsive culture dishes and transplanted in an autologous fashion following standard flap surgery. Bone defects were filled with beta-tricalcium phosphate granules. Various clinical parameters were recorded at baseline, 3 months and 6 months. Cone-beam computed tomography was performed at baseline and 6 months. Additionally, mid-long term follow-up were performed with 7 patients agreements. Results showed that this method was safe and no serious adverse events occurred. All the findings including reduction of periodontal probing depth, clinical attachment gain and increase of radiographic bone high were improved in all 10 cases at 6 months after the transplantation. The results of this study have validated the safety and efficacy of autologous PDL cell sheet for use in treating severe periodontal defects. Stability of the clinical outcomes was good when observed over long term follow up. However, we should consider that autologous cells can only be used from the original donor and this can result in considerable medical costs. Therefore, we are now planning to establish an allogenic cell bank of PDL cell derived MCSs.

Application of PDL cell sheet engineering for functional implant therapy.

Currently dental implant therapy is considered as one of the most important procedures in dentistry. In particular, osseointegrated implants have been used worldwide as a reliable and consistent procedure to replace missing teeth. However recent surveys reveal that peri-implantitis is a major and growing major problem in implantology.

Peri-implantitis may occur in 28 -56 per cent of subjects after 5-10 years of use. It has been suggested that peri-implant soft tissue may have an impaired defense

capacity against exogenous irritation due to lack of periodontal supra-crestal periodontal ligaments. Accordingly the possibility of inducing new ligament formation around implants has been investigated. Buser *et al* (1990) accidentally induced periodontal ligament formation around titanium implants 12 months after implantation in close approximation to natural teeth in monkeys. Since then many investigators have tried to establish a methodology to induce periodontal ligament around titanium dental implants. However, to date a clear and rigid methodology has not been established.

We have been studying ways to produce cementum and PDL fibers on implant surfaces using cell sheet engineering (Washio *et al* 2010; Washio *et al* 2018; Iwasaki *et al* 2019). We used PDL-derived cells, which contain multi-potential stem cells, as the cell source and cultured them on an implant material constituted of commercially pure titanium treated with acid etching, blasting and a calcium phosphate (CaP) coating to improve cell attachment. Implantation of these constructs into canine mandibular bone has been developed as an autologous model. We have confirmed that PDL-derived cells cultured with osteo-inductive medium had the ability to induce cementum formation. The attachment of PDL cells onto the titanium surface with three surface treatments was accelerated compared with that using a smooth titanium surface, at 40 min after starting incubation. In the canine model, histological observation indicated that formation of cementum like and PDL-like tissue was induced on the titanium surface with surface treatments and that the PDL-like tissue was perpendicularly oriented between the titanium surface with cementum-like tissue and the bone, which is similar to the environment existing around a natural tooth. Thus the clinical application of dental implant combined with a cell sheet engineering may be possible as an alternative implant therapy.

Future directions

As mentioned above, a fully functional implant with periodontal tissues that can replace conventional dental implants has yet to be developed. Cementum formation on an implant is necessary to achieve an implant with a functional periodontium. However, it is a very challenging task to induce cementogenesis on titanium surfaces artificially, because the differentiation mechanism of cementoblasts, cementum forming cells on titanium are unknown. Using cell sheet engineering, we have developed a novel tissue engineering method for periodontal formation around implants. Although many unanswered questions remain in the methodology, this new concept can initiate a fully or partially functional implant therapy. This methodology may play an innovative role in the future for periodontal, prosthetic and orthodontic fields in dentistry.

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We thank Professor Teruo Okano and Professor Takanori Iwata for their kind support, and also Dental Corporation TOKUSHIN-KAI group for partial financial support.

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Chapter 2

Immediate Implant Placement and Provisionalization in Periodontally Compromised Site (in the anterior zone) – A 12 Years Follow-up Case Report

J Chua
Private Practice, Kuala Lumpur

Introduction

Over the past two decades, single tooth immediate implant placement and provisionalization (IIPP) has gained acceptance as a safe and preservative procedure for replacing failing anterior teeth. The survival rates for single tooth immediate implant and immediate provisionalization have been reported to range from 89% to 100%. (Whorle 1998, Kan *et al* 2003, Groisman *et al* 2003, Chen *et al* 2004). To date many case series have been published investigating immediate implant placement (Chaushu *et al* 2001, Proussaefs *et al* 2002, Kan *et al* 2003, Kan *et al* 2011, De Rouck 2008, Buser 2009, Chen 2009). However, there are only a few published randomized controlled studies (Schropp *et al* 2003, Lindeboom *et al* 2006, Crespi *et al* 2008, De Rouck *et al* 2009, Yoshino *et al* 2014). Reviews only began to appear in 2014 (Slagter *et al* 2014, Lin *et al* 2014, Rojo *et al* 2016, Kinaia *et al* 2017). It is now recognized that IIPP has a number of benefits including improved patient comfort, quality of life; enhanced preservation of hard and soft tissue thus allowing better gingival contours and good architecture.

Most of the reports to date have emphasized the need for stringent case selection prior to immediate implant placement and provisionalization - especially in the anterior zone. In earlier days immediate implants and provisionalization were considered extremely high risk procedures and thus only performed in fully intact socket walls with no infection. Immediate implant placement and provisionalization is now classified as complex dental implant procedure under the (SAC) classification. (Levine 2017)

Immediate implants in compromised sites

Recent studies are beginning to push the boundaries of what is determined as ideal sites for this procedure, where immediate implants are placed in infected sites or compromised sites (Table 1). The 5 criteria for an ideal site for IIIP that have been identified by Mortan *et al* (2014) as: a) Thick soft tissue biotype b) availability of bone apical and palatal c) intact socket walls d) no acute infection e) facial bone wall at least 1 mm thick.

The above situations do not frequently occur as most of the time when a tooth is deemed hopeless it is due to various conditions such as endodontic, periodontic lesions, combined lesions and internal or external resorption; which often causes destruction of hard or soft tissue around the tooth. These conditions are often the ones that will benefit from single procedures rather than multiple surgeries as preservation of the remaining hard and soft tissue is critical. When flaps are raised during advance ridge preservation procedures, interpapillary soft tissue is often compromised.

Publications reporting outcomes of immediate implants placed into infected sites are scarce (Table 1). Early evidence suggests that implants can be placed into sites with peri-apical and periodontal infections with comparable outcomes. It is also noted that complications may occur more often thus authors caution that this procedure is highly technique sensitive and needs meticulous case selection. More scientific evidence is needed before this can be classified as a safe procedure.

It is also interesting to note that recent publications have demonstrated the possibility of buccal plate reconstruction of up to 3 mm in 10 patients when Immediate Implant Placement (IIP) were done in extraction sockets with labial plate dehiscence defects. (Sarnachiaro *et al 2016*). Thus, IIP in compromised sites may have more benefits than risks as previously thought.

This case report pushes the boundaries of an ideal host and demonstrates IIP on a patient with a failing tooth due to perio-endo lesion.

Reviews	Waasdorp *et al* (2010) Palmer (2012) Chrcanovic *et al* (2015) Lee *et al* (2015) De Oliveria-Neto *et al* (2019)
Retrospective	Meltzer (2012) Fugazzotto (2012a, b) Blus *et al* (2015) Tripodakis *et al* (2016)
Prospective	Lindeboom *et al* (2006) Siegenthaler *et al* (2007) Crespi *et al* (2010) Truninger *et al* (2011) Jung *et al* (2013) Malo *et al* (2015) Al Nashar & Yakoob (2015)
Case Series	Chu et al. (2007) Noelken *et al* (2011) Shibly *et al* (2012) Marconcini (2013) Sarnachiaro *et al* (2016)
Randomized controlled trial	Shibly (2010) Slagter (2016)

Table 1. Publications reporting outcomes of immediate implants placed into infected sites

Case Description

A 31-year-old male patient was referred for periodontal consultation complaining chronic discomfort and

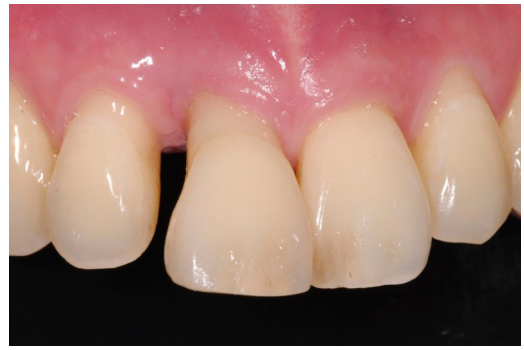

Figure 1. Pre-op of failing #11.

swelling of his maxillary right central incisor (#11). Clinical periodontal evaluation revealed overall good oral hygiene. The failing tooth (#11) was extruded with gingival recession of 5 mm. Buccal swelling of 5 X 5 mm was also noted on this tooth (Figure 1). Bone-sounding measurements of 9 mm, 13 mm and 13 mm were noted on the distal, buccal and mesial aspect of the failing tooth #11 respectively. In addition, bone sounding measurements of the adjacent teeth revealed 5 mm at the mesial of #12 and 4 mm at the mesial of #21. The facial free gingival margin of tooth (#11) was more apically positioned to the contralateral tooth (#21). Radiographic evaluation revealed peri-apical lesion, advance bone loss with vertical defects on the mesial and distal (Figure 2). The tooth was diagnosed as hopeless due to a perio-endo lesion. The patient was given the options of treatment available upon extraction and patient decided on replacement with a dental implant. A CT scan revealed adequate bony architecture apical to the incisor for immediate implant placement. (Figure 3). After discussing the risks and benefits, the patient desired to have immediate implant placement and provisionalization following the extraction of tooth #11.

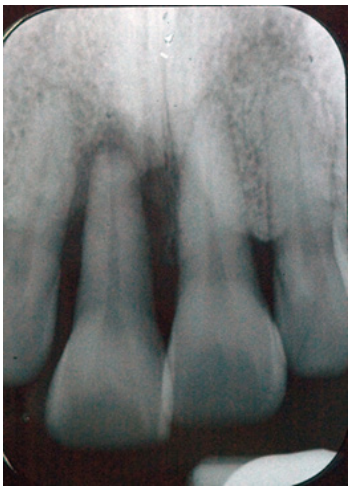

Figure 2. Peri-apical radiograph of #11

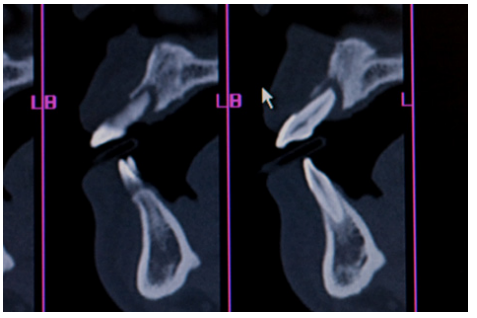

Figure 3. CT Scan

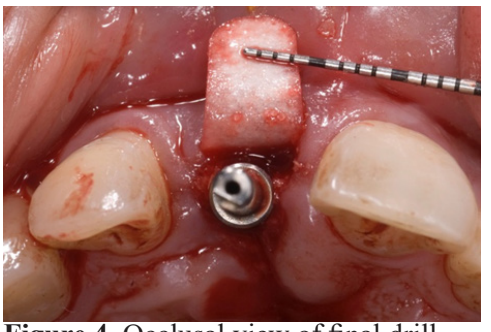

Figure 4. Occlusal view of final drill with membrane and xenograft inserted

Pre-Surgical Procedures
Fabrication of immediate temporary denture

A preliminary impression was made using vinyl poly-siloxane (Monophase, GC.) and diagnostic casts were fabricated. The models were duplicated and an immediate temporary partial denture was fabricated in case an immediate implant and immediate provisionalization could not be performed.

Surgical Procedures
Immediate Implant Placement

Intrasulcular incision was performed around #11. The failing tooth was removed atraumatically while

preserving the gingival architecture. After the socket was excavated using a curette (Younger-Good 7/8 curette, Hu-Friedy, Chicago, Ill.) to remove granulation tissue, it was treated with tetracycline and irrigated with saline. The bone sounding measurements performed without the presence of the tooth was 6 mm, 8 mm and 9 mm at the mesial-buccal, mid-buccal and distal-buccal aspect of the tooth socket respectively. The osteotomy was prepared sequentially to completion by engaging the palatal and apical bone of the tooth-socket. The gap presented between the buccal bony plate and the final drill was filled with xenograft bone material (Bio-oss, Geitslich) and a resorable membrane (Biomend, Zimmer) was used to support the buccal plate (Figure 4). An implant (Nobel Replace Select Tapered Groovy, Nobel Biocare) was then placed with 35 Ncm (Figure 5) and primary implant stability was achieved by engaging the palatal wall and the bone 3 mm to 5 mm beyond the apex of the extraction socket. The implant-prosthetic platform was placed 3 mm from the pre-determined gingival margin.

Preparation of immediate provisional

An immediate temporary abutment (Nobel Biocare) was placed onto the implant. The extracted tooth was sectioned from the root hollowed out to be used as the provisional. It was then retrofitted onto the immediate temporary abutment with light-cure flowable composite. The abutment-provisional complex was then finished and polished to achieve a proper emergence contour and marginal fit. (Figure 6 & 7).

Cementation of provisional crown

The immediate temporary abutment was torque to 15 Ncm and the provisional crown was cemented (Temp-bond, Kerr USA, Romulus, Mich.). Occlusion was checked and adjusted to ascertain that it was free of centric and eccentric contacts. After all excess cement was removed, two

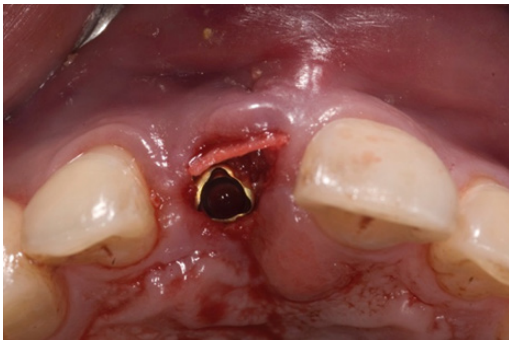

Figure 5. Occlusal view of implant on the palatal with membrane and xenograft on the buccal

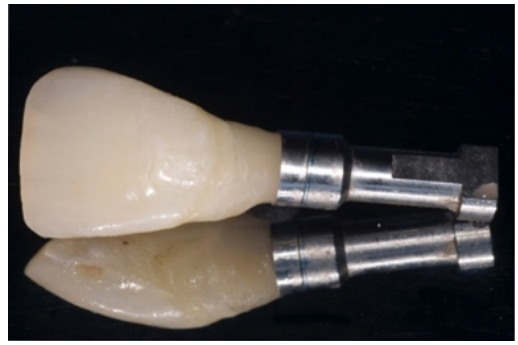

Figure 6. Provisional abutment complex using patient's own tooth

Figure 7. Provisional abutment complex using patient's own tooth

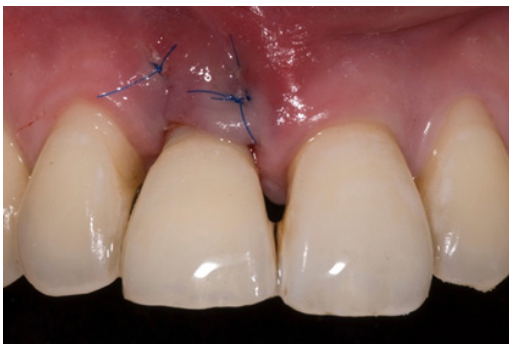

Figure 8. Sutured with temporary crown cemented

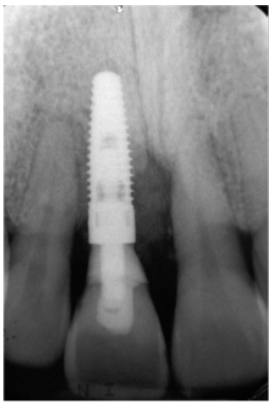

Figure 9. Peri-apical radiograph after cementation

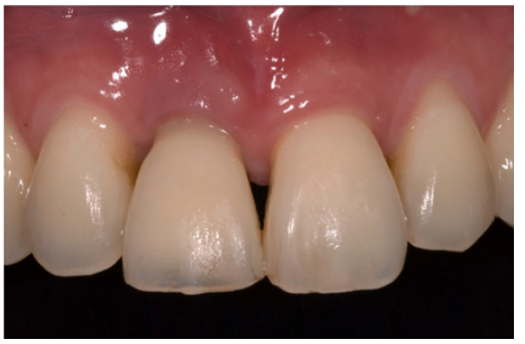

Figure 10. 10 days healing

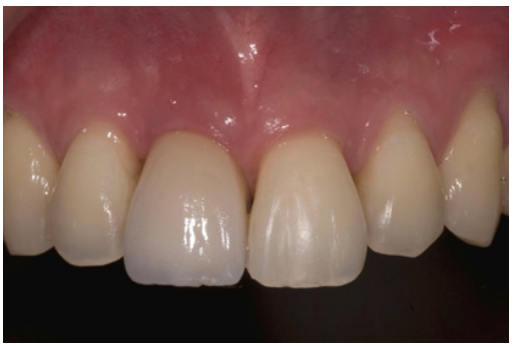

Figure 11. Crown issued

cross-stitches (6-0 polypropylene, HU-Friedy) were placed at the free gingival margin to ensure the bone graft was being held in position during the implant healing phase (Figure 8). Periapical radiograph was taken to ascertain the complete seating and proper fit of the abutment-provisional complex (Figure 9).

Postoperative Instruction

Antibiotics (Augmentin 625mg) and pain analgesic (Arcoxia 90 mg) was prescribed for the patient post-operatively. The patient was instructed not to brush the surgical site, but rinse with 0.12 percent chlorhexidine gluconate (Oradex, Colgate), and be on soft diet for two weeks. The patient was also advised against functioning or activities to the surgical site for at least three months.

Postoperative follow-up

The sutures were removed 10 days following the implant surgery and the healing was uneventful (Figure 10). The final implant impression was made approximately ten months following the implant surgery using vinyl poly-siloxane (Reprosil, Dentsply International Inc.). The zirconium abutment (Procera, Nobel Biocare) was torqued to 35 Ncm (manufacturer's recommendation, Nobel Biocare) and the definitive all-ceramic restoration (Procera, Nobel Biocare) was cemented with rely-X (Unicem, 3M) (Figure 11). The patient was then put on a regular 4 monthly supportive periodontal maintenance therapy and yearly re-evaluation. The yearly monitoring includes photos and peri-apical radiographs (Figure 12). Figure 13 shows that gingival margin at 12 years was very similar at 4 years where recession was noted and has maintained since then. Peri-apical radiographs show stability of the bone level up to 12 years and increased in density of the area which bone was missing previously. (Figure 14)

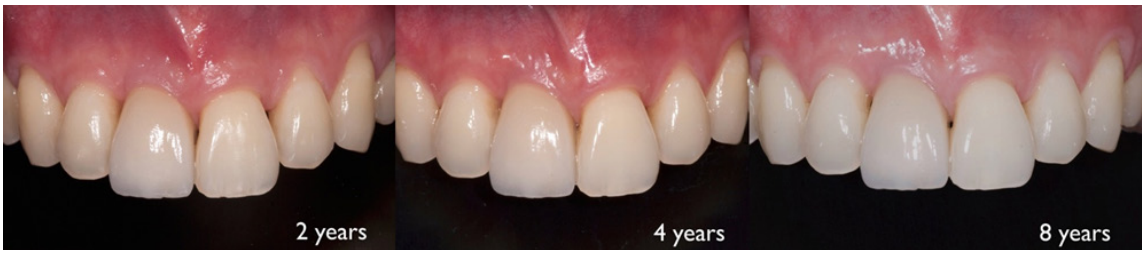

Figure 12. Follow-ups at 2 years, 4 years and 8 years

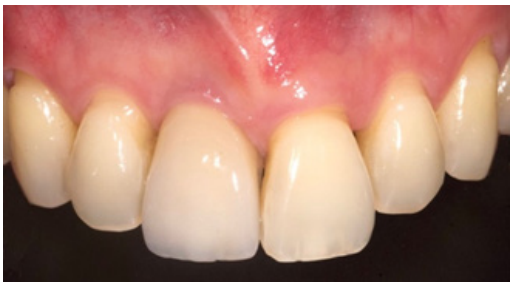

Figure 13. 12 years follow up

Discussion

Periodontally involved cases are usually challenging due to advance bone loss and thus clinical loss of attachment coupled with recession. This often leads to horizontal and vertical defects that often required additional surgeries to correct. Reconstructing and, or preserving the hard and soft tissue in these cases is paramount in order to achieve as reasonable as possible esthetics for these patients. Immediate provisionalization following immediate implants has been shown to have better outcomes in maintaining mid facial esthetics by De Rouck *et al* (2009). Yet, immediate implants have been penalized heavily in literature for increased risk of recession or complications. It is controlling the factors discussed below that allows IIPP to deliver more benefits than harm.

Two recent reviews on IIPP have indicated the criteria for success in this

procedure. Ten keys were identified by Levine *et al* (2017) for successful IIPP, namely 1) esthetic risk assessment 2) CBCT analysis 3) minimally traumatic tooth extraction 4) 3D implant placement in good position 5) use of a narrower implant 6) buccal gap bone graft 7) facial gingival grafting 8) immediate contour management of the emergence profile from the implant 9) custom impression coping technique 10) final restoration with a screw-retained crown. Kan *et al* (2018) also reviewed the literature and showed that 1) immediate loading positively influences the esthetic result 2) flapless procedures reduce surgical discomfort but a skilled clinician is required 3) it is important to fill the buccal gap with slow-resorbable biomaterial 4) in the case of a thin biotype, soft tissue augmentation is suggested. In this case report, all the factors were observed except for soft tissue augmentation.

In a less than ideal clinical situation, two main factors needs to be discussed in IIPP that dictates whether this procedure can be done successfully in a compromised site.

They are :

1. Risk of recession

The risk of recession is one of the complications that can be debilitating for patients especially in the anterior zone. It has been reported by a systematic review by Chen *et al* (2009) to be up to 30% of cases. There were 5 keys that were related to this risk reported by Ross *et al* (2014), namely A) Location of implant platform in the buccopalatal dimension B) Maintenance of buccal bone over implant and horizontal dimension C) Pre-existing gingival biotype D) Use of flapless or minimally invasive surgical flap E) Use of provisional to develop and, or maintain the emergence profile. All of these factors are critical to prevent recession, it is difficult to say if one is more important than the other. Nonetheless, managing these 5 factors will allow better control and prevent recession. Despite missing buccal bone in this case report, the horizontal dimension was regenerated back due to presence of interproximal bone, thus preventing severe recession.

2. Hard and soft tissue remodelling

Increased resorption after tooth extraction is well known especially for thinner buccal bone plates in the anterior region. (Araujo & Lindhe 2005, Botticelli *et al* 2005, Novaes *et al* 2011). It has been suggested that immediate implants may

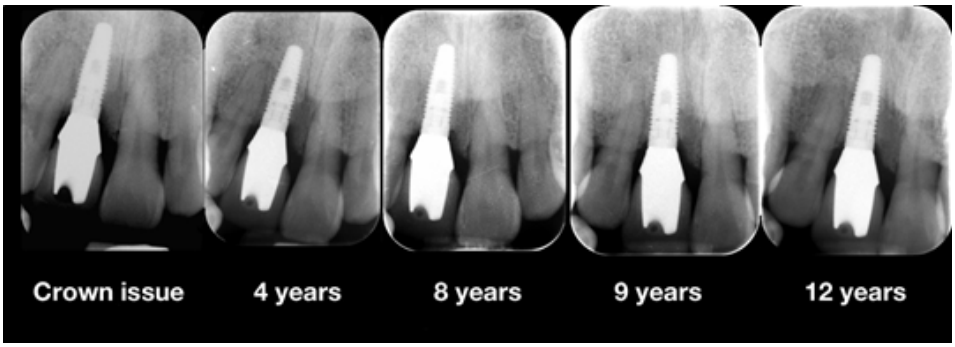

Figure 14. Peri-apical radiographs from crown issue, 4 years review, 8 years review and 12 years review.

reduce alveolar ridge resorption following tooth removal (Denissen 1993, Paolantonio 2001). This buccal bone thickness is a fundamental factor in buccal bone plate resorption even with flapless implantation. Thus, for a successful IIPP, an ideal host with an intact buccal bone is advised.

3. Buccal bone regeneration

Noelken *et al* in 2011 published a case series of 16 patients where IIPP was performed after long-axis root fracture and complete loss of the buccal bone. There were no implant losses and oral hygiene was suggested to be predictive for the esthetic results. Postoperative CBCT confirmed restoration of facial lamella in the majority of the patients. Shilby *et al* (2012) demonstrated regeneration of buccal bone is possible even on patients with a history of periodontal disease. This is similar to the case report where buccal bony plate is restored and confirmed 8 years after IIPP was performed.

In 2016, Slagter *et al* published a randomized clinical controlled trial on forty patients with labial bony defects of $\geq$ 5mm. 20 patients received immediate implants and 20 patients had delayed implant. The change in marginal bone level was not significant between the groups and that immediate implant placement was not inferior clinically. This case report clearly shows that even if buccal bone was missing; it could be reconstructed simultaneously to maintain and regenerate the buccal bone as shown in a CBCT scan taken 8 years post insertion of implant (Figure 15).

4. Gingival biotype and connective tissue graft

Rojo *et al* (2016) published a systematic review on soft tissue augmentation techniques in IIPP. They noted that all of the studies reported a positive behaviour of soft tissue and peri-implant bone. The use of autologous connective tissue could prevent and minimize gingival recession. Maia *et al* (2015) investigated the influence of gingival thickness on buccal plate remodelling in 8 dogs and concluded that the gingival thickness or the addition of a biomaterial in the gap did not influence the results. Thus, soft tissue augmentation may not always be necessary for thick tissue biotype. And in this case, connective tissue augmentation was not performed as buccal plate was missing and connective tissue in direct contact with xenograft may impair it from remodelling to bone. The use of barrier membrane was to prevent repopulation of the lesion with soft tissue precluding bone formation.

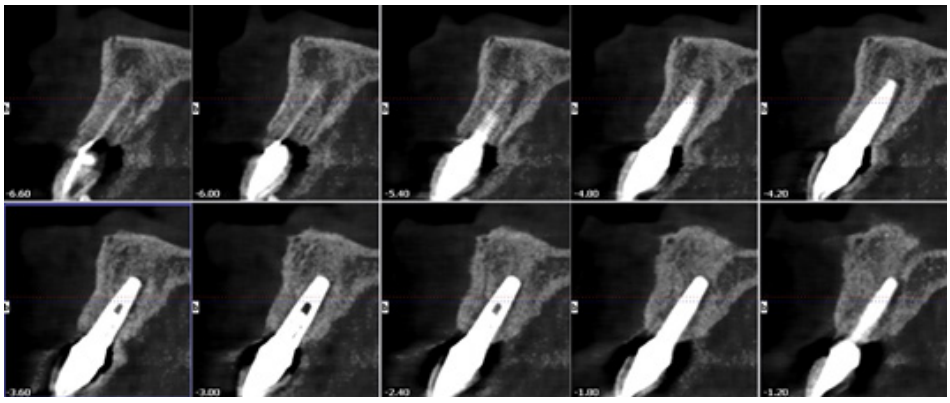

Figure 15. Regeneration of buccal bone shown 8 years after implant placement

A recent meta-analysis and systematic review (Cosyn *et al* 2019) concluded that immediate implant placement demonstrated higher risk of early implant loss than delayed implant placement. The lower survival rate of immediate implant was 94.9% vs 98.9% of delayed implant. This in comparison with Lee *et al* (2015) who reported that immediate implant survival rates were as high as 98.4% in compromised sites. It has been suggested that the protocol for implant placement in sites with peri-apical lesions must include prophylactic antibiotics, thorough socket curettage and irrigation, attainment of primary stability and use of graft or barrier for peri-apical fenestration. Thus, all these factors were considered in this case report to ensure a higher rate of success.

Conclusion

This case report demonstrates that immediate implant placement and provisionalization may be performed successfully on periodontally involved tooth and can maintain and improve the remaining hard and soft tissue architecture.

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Chapter 3

Back to Histology for Predictable Outcomes in Partial Thickness Flap Procedures

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Introduction

Thin gingival phenotype is a risk factor for recession around teeth (Agudio *et al* 2016, Chambrone *et al* 2016). Similarly, thin soft tissue phenotype (Kan *et al* 2011, Nisapakultorn *et al* 2010) and the lack of keratinized mucosa lead to increased risk for recession around dental implants (Lin *et al* 2013, Roccuzzo *et al* 2016) The free gingival graft and the connective tissue graft (CTG) combined with a coronally advanced flap are commonly used to increase the thickness and quality of the soft tissue around teeth and dental implants.

The oral mucosa

The masticatory mucosa in the mouth consists of mucoperiosteum, where epithelium and the underlying lamina propria attach directly to the underlying bone without a submucosa layer. In addition, the attached gingiva contains alveologingival fibres and dentogingival fibres that help attach the gingiva to the underlying cementum surface and alveolar bone. The alveolar mucosa, a lining mucosa, is mobile and distensible because it has a loose lamina propria where the collagen fibres are arranged in a network to allow free movement as well as a high content of elastin (Berkovitz *et al* 1992; Bourke *et al* 2000). The alveolar mucosa commonly has a submucosa, a layer of loose connective tissue that separates the lamina propria from the underlying bone (Berkovitz *et al* 1992).

Partial thickness flap

A partial thickness flap is made up of a portion of the mucosa not including the periosteum. A partial thickness flap is achieved not simply by making an incision that divides up the thickness of mucosa flap excluding the periosteum. The partial thickness incision can be made deep and close to the periosteum (figure 1). The partial thickness incision can also be made more superficial, at one extreme like a de-epithelialization procedure, and also at different depths through the lamina propria, submucosa or muscle layers (figure 2). Making the partial thickness incision at the correct layer is crucial to the predictability and achievement of surgical objectives. This requires the knowledge of histology of the mucosa and the conscientious application of this knowledge in the surgical design of the flap.

Figure 1. Deep incision close to the periosteum. This incision is made with the blade parallel to the contour of the underlying bone.

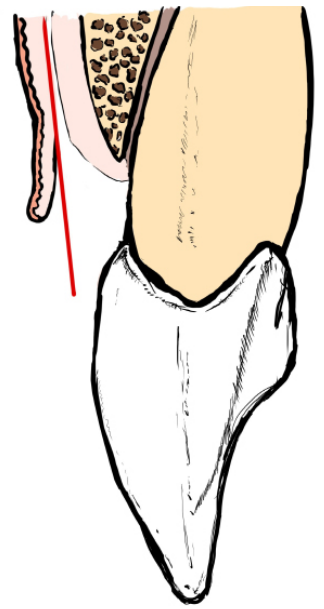

Figure 2. Superficial incision. This incision is made with the blade parallel to the contour of the underlying bone.

Free gingival grafts

The keratinization of the surface epithelium is determined by genetic factors inherent in the connective tissue (Karring *et al* 1971). A graft harvested from the palate should determine and form a keratinized mucosa after healing. The mobility of this healed graft however is affected by the presence or not, of submucosa and loose lamina propria at the recipient site. In order to achieve a stable band of keratinized mucosa after a free gingival graft procedure, and create a mucoperiosteum, the partial thickness incision on the recipient bed in a free gingival graft procedure should be a deep one, at the level between the submucosa and the underlying periosteum. Not doing so will leave the palatal graft positioned on a bed of loose connective tissue, which risks remaining mobile even if the epithelium is keratinized (figures 3 to 6). Because the partial thickness incision is made at the level close to the periosteum, an extreme thin tissue phenotype is not a contra-indication for free gingival grafts (figures 7 to 9).

Coronally advanced flap with CTG

The histology of the gingiva and the lining mucosa is different, therefore the considerations for the depth of the partial thickness incisions are different. The partial thickness incisions made at the anatomical papilla where the surgical papilla will lie on at flap closure is a de-epithelialization incision, while the incision at the surgical papilla should be one that leaves enough connective tissue for the survival of this portion of the flap. The partial thickness incision over an area where a CTG may be

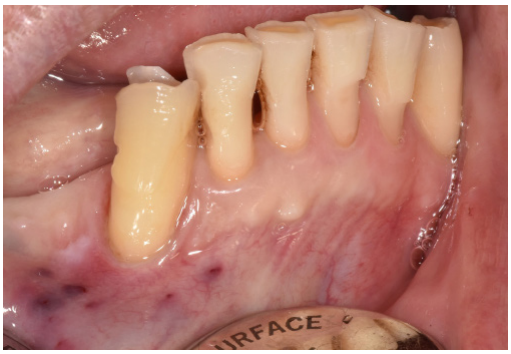

Figure 3. Pre-op appearance of tooth 43.

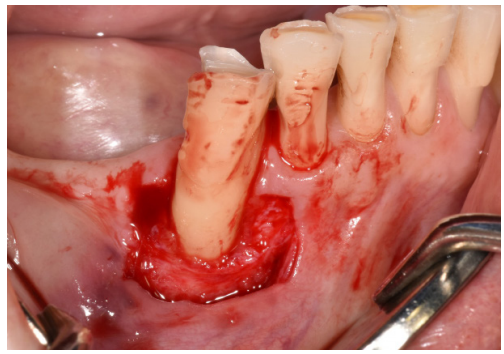

Figure 4. A deep partial thickness incision is made beyond the lamina propria and submucosa layer, in order that the graft can lie on stable immobile periosteum bed.

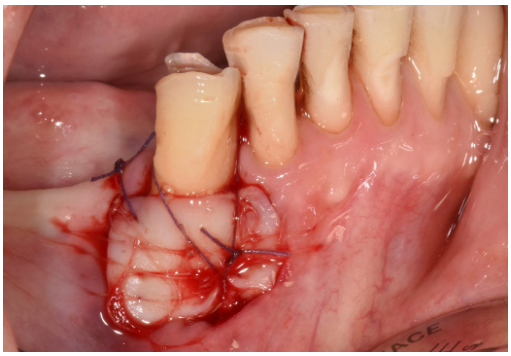

Figure 5. The palatal graft is securely sutured down to the underlying periosteum.

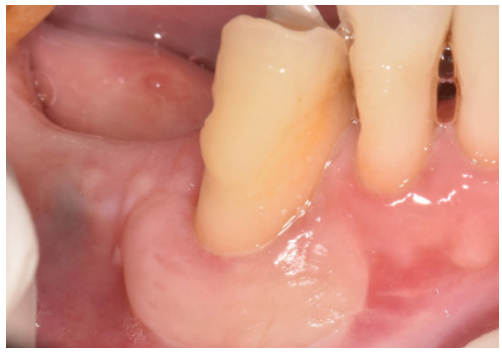

Figure 6. Healing at 3 months. A stable band of thick keratinized mucosa is noted.

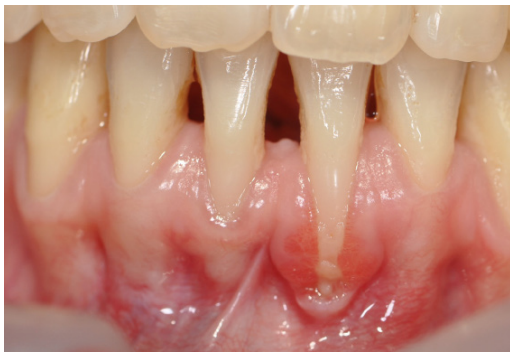

Figure 7. Extreme thin tissue phenotype is not a contra-indication for free gingival graft.

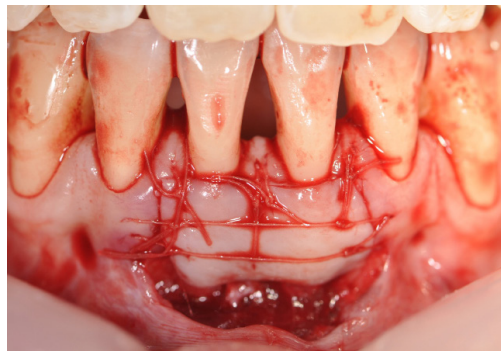

Figure 8. Free gingival graft was done.

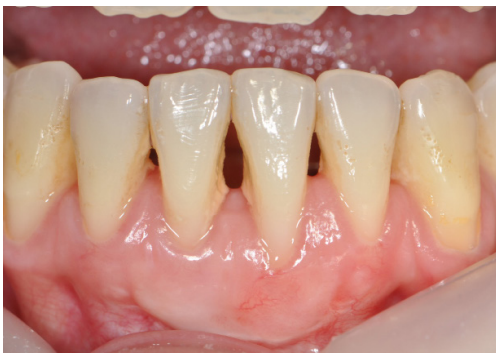

Figure 9. Healing at 12 months.

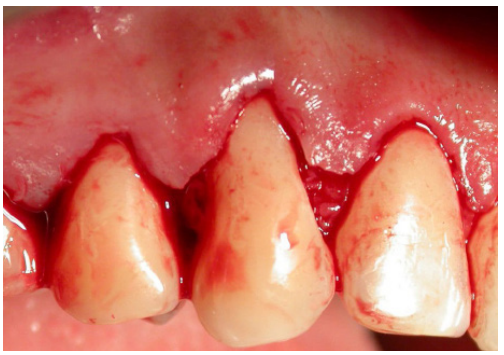

Figure 10. The partial thickness incisions made at the anatomical papilla where the surgical papilla will lie on at flap closure is a de-epithelialization incision, while the incision at the surgical papilla should be one that leaves enough connective tissue for the survival of this portion of the flap.

sutured on needs to be thick enough for this purpose (Zuchelli and De Sanctis 2000, Zucchelli *et al* 2009) (figure 10).

Zuchelli and De Sanctis (2000) described a sharp dissection into the vestibular lining mucosa to release muscle tension (figure 11) and Greenwell *et al* (2004) described a superficial incision to separate the epithelium and connective tissue from the deeper muscle and periosteum (figure 12) to allow extreme flap release and prevent the muscles from retracting the flap during healing.

Harvesting the palatal connective tissue

The superficial part of the palate consists of the lamina propria which contains more fibrous connective tissue and less fatty and glandular tissues. Bertl *et al* (2015) found that it is not the location of the palate where the graft is harvested from, but the method by which the harvesting was done, that determines whether the graft contains more fibrous connective tissue or fatty/glandular tissues. The de-epithelialized palatal graft contains more fibrous connective tissue than sub-epithelial connective tissue graft. The de-epithelialized palatal graft is therefore the harvesting method of choice if the quality of the fibrous connective tissue is parameter of measure.

There is concern that a de-epithelialized graft harvesting method leads to increased post-operative pain for patients. In a randomised controlled clinical trial, Zucchelli *et al* (2010) compared post-op pain reported by patients after a de-epithelialized graft and a sub-epithelial connective tissue harvested via a trap door access method and reported no statistical difference in post-op painkiller consumption, bleeding or reported discomfort. Painkiller consumption increased in cases of primary flap dehiscence/necrosis (28% of the sub-epithelial connective tissue patients).

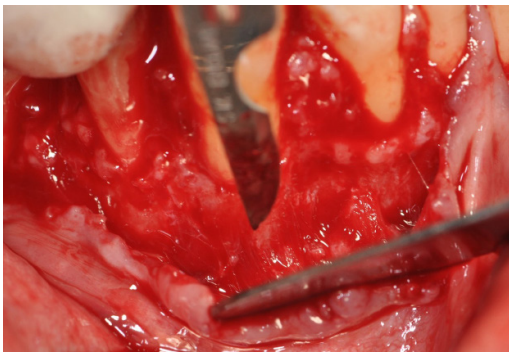

Figure 11. A deep incision is made with blade parallel to bone to separate the muscles from the periosteum.

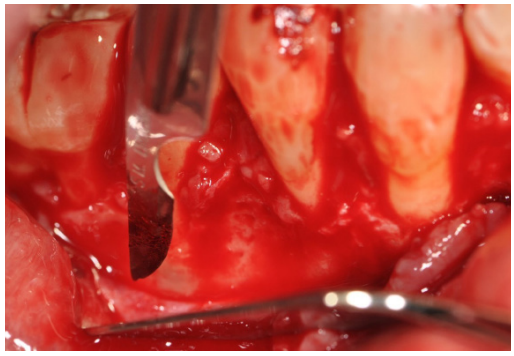

Figure 11. The blade is now angled parallel to the mucosa surface and a superficial incision is made to separate the lamina propria and epithelium from the muscle layer.

This could be because post-operative pain after palatal graft harvesting is related to the thickness and not the size of the graft (Burkhardt *et al* 2015). The de-epithelialized grafts Zucchelli *et al* (2010) harvested in their protocol were thin grafts of average 1.32mm in thickness. Zucchelli *et al* (2015) also found that while no statistical difference was noted in root coverage outcomes, a statistically greater increased in buccal thickness was observed in the de-epithelialized graft group. Ouhayoun *et al* (1988) harvested palatal grafts and then further separated them into two thinner grafts, a superficial epithelial–CTG and a deeper CTG. These were transplanted into contralateral recipient mucosal beds in the lower canine/premolar area that lacked attached gingiva. Analysis of punch biopsies three months postoperatively showed that sites receiving the epithelial–CTG displayed histologic and biochemical characteristics of keratinized mucosa. However, sites receiving the deep CTG predominantly showed features of non-keratinized mucosa.

Conclusion

The partial thickness flap may be split at different depths for different purposes. The careful attention to detail, based on understanding the histology and biology of oral tissues improves the outcomes of periodontal mucogingival surgery. Failure to do so will inevitably lead to sub-optimal outcomes.

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Chapter 4

An Update of Periodontal-Systemic Associations

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Introduction

"Periodontal Medicine" is a term that was first introduced in 1996 as a broad term to define a new field of periodontology that studied how periodontal health/disease interacted with systemic health/disease (Offenbacher 1996). Today the number of conditions claimed to be associated with periodontal disease expanding with little regard for the real significance or otherwise of such associations. It is now appropriate to reflect on this and evaluate the validity of these associations.

The central concept of periodontal medicine is how periodontal disease contributes to the systemic load of inflammation. In this regard our concepts for periodontal disease have been divided between conventional concepts and newer inverted paradigms. For example, our conventional understanding of periodontitis accepts that this condition is a chronic inflammatory reaction to subgingival plaque in systemically susceptible individuals that results in periodontal destruction

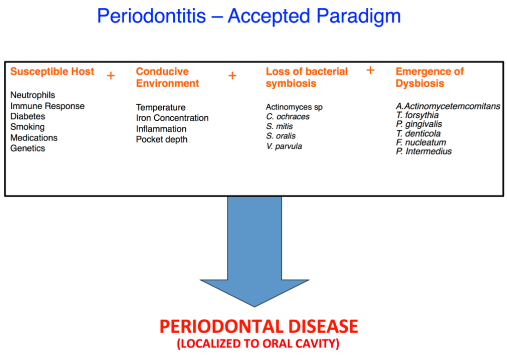

Figure 1. Conventional paradigm of periodontal disease whereby periodontitis arises in systemically susceptible individuals due to a chronic inflammatory reaction to subgingival plaque
(Reproduced from: Bartold PM, Mariotti A. The future of periodontal-systemic associations: Raising the standards. Curr Oral Health Rep 2017;4:258-262).

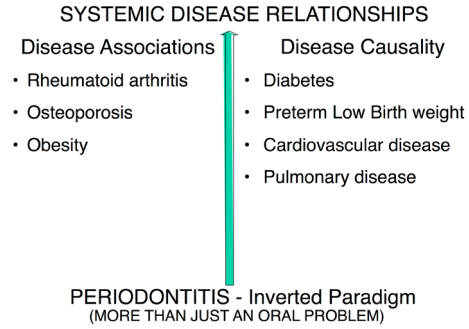

Figure 2. Inverted paradigm of periodontal disease whereby systemic conditions can influence periodontal status and conversely periodontal status can influence systemic conditions.
(*Reproduced from: Bartold PM, Mariotti A. The future of periodontal-systemic associations: Raising the standards. Curr Oral Health Rep 2017;4:258-262*).

(Figure 1). However, another paradigm emerged (Page 1988) whereby the plaque/host/disease model is inverted and periodontitis is noted to impact on systemic conditions (Figure 2). Thus, periodontal medicine embraces a "bidirectional" association between systemic conditions and periodontitis, with periodontitis not only affecting systemic health but systemic health affecting periodontitis.

How are Oral Health and Systemic Health Related and Reported?

How periodontal infection and inflammation affects general health and well-being is poorly understood due to the complexity of the relationships and myriad confounding factors. While emerging evidence supports some relationships between periodontitis and chronic systemic conditions, the validity of many can be questioned. Notwithstanding this there is a very real risk that many of these associations, irrespective of their real significance, become established dogma within the literature. Therefore it is becoming imperative that we understand and appreciate with respect to relationships between periodontal disease and systemic disease in various communities.

In the periodontal/systemic interactions field observational studies have been the most popular means of identifying potential relationships between periodontal diseases and systemic diseases. Understanding the strengths and weaknesses of such studies is critical if we are to truly establish the reality of systemic associations.

A limiting feature of observational studies is that they involve interpretations of subjective observations of individuals within populations over which the investigator has no control over important confounding variables. Thus, the identification of subjects is not controlled by the researcher but is directly dictated by set of specific criteria such as age, gender and disease experience. Such studies allow investigators to assess conditions and individuals within a real-life context to formulate hypotheses. Through observational studies, it is possible to study how subjects behave individually or together and observe outcome measures dependent on detailed criteria that are more robust than simple self-reporting of outcome measures. The principal measures of outcomes on observational studies are usually reported as relative risks, rate ratios, hazard ratios and odds ratios.

Importantly, however, is the important issue that while observational studies can determine associations and assist in formulating hypotheses, they are of no use in determining cause and effect relationships. Hypothesis validation generated from observational studies requires that well planned studies such as randomized controlled clinical trials are undertaken. Because observational studies cannot be considered definitive experimental studies, they suffer from many methodological problems. For example, individual preferences, practice patterns and/or policy decisions can influence observational studies *Mamdani et al* 2005. In addition, because there is no randomization of subjects within observational studies into experimental and control groups, there is a very high risk of selection bias, information bias, investigator bias, confounding variables, effect modification and chance all challenging how the results can be interpreted.

Although observational studies have limitations, as discussed above,

they can shed important light on disease associations (e.g., cholesterol levels and cardiovascular disease) and these can subsequently be confirmed by well planned investigations. However, relying solely on data obtained from observational studies to justify treatment practices and policies is naive. More importantly, observational studies have a very high risk and bias to result in unsubstantiated research claims (Ioannidis 2005). For example, observational studies may support the use of procedures that in reality have no better outcome than less interventional medical management of the problem (Boden *et al* 2007). Indeed there are many examples of medical practices, instituted because of observational studies, being reversed (Prasad *et al* 2013). The reasons for inaccuracies in interpretation of data obtained from observational studies arise because: 1) population sample sizes are too small; 2) noted effects are too small; 3) variability in study design, case definitions, study outcomes and analytical processes; 5) investigator bias or conflict of interest; and/or 6) investigators pursuing a statistically significant result in a very active research field (Ioannidis 2005).

The expanding list of periodontal-systemic associations is of concern

The fundamental basis of periodontal medicine is the tenet that health periodontal tissues are necessary for good general health. This is a very reasonable basis for providing high quality clinical care for our patients. Indeed, this philosophy should remain of considerable importance and help fashion ongoing clinical and scientific endeavours based on logical biologic plausibility. Accordingly, utmost care needs to be exercised to not misinterpret, or over represent, any findings from single observational studies.
Procedures should be in place to ensure that studies in this field have a solid biologic rationale and are well focussed to answer specific questions of clinical significance. However, in the past (and even in the present) this has not always been apparent. As a profession we must be concerned about the abundance of published investigations that have been poorly conceptualized and have disregarded any logical plausibility. Indeed, such concerns were articulated over a decade ago but unfortunately little action has been taken (Hujoel *et al* 2006).

To date, over 100 different periodontal-systemic associations have been reported in the literature all of which rely on observational studies. Of these, only periodontal disease and adverse pregnancy outcomes has been studied using validated, objective clinical endpoints as part of their experimental design. These studies have not confirmed a significant relationship.

Are Periodontal-Systemic Associations Causal, Syndromic or Syndemic?

Many inferences are made in the literature of a causal relationship between periodontitis and various systemic conditions. However, in order for a condition to be causal it must be present before the onset of another condition. However, for periodontal disease and systemic conditions, this does not seem to occur because not everyone who has periodontal disease concurrently suffers from the systemic conditions under investigation In reality it is more probable that there are groups of individuals who have periodontal disease and are at increased risk for developing

other inflammation-associated conditions. In this context, chronic periodontitis could be an indicator for coronary artery disease. Here periodontitis is a condition that is associated with increased risk but is not always an essential component in causation. Thus it may be that disease associations could equally as important as causality because by identifying people at risk of having multiple systemic conditions could assist in their holistic management.

The concept of syndromic relationships is important in light of the manifestation of multiple diseases that may have similar features. By definition a syndrome is a group of symptoms that collectively indicate or characterize a disease, psychological disorder, or other abnormal condition. In this context various periodontal and systemic conditions could cluster and manifest with the traits of a syndrome (Offenbacher *et al* 1999).

A similar concept has also been proposed in which periodontal diseases and systemic diseases may exist as part of a syndemic relationship (Hein and Small 2006). A syndemic relationship refers to a set of two or more linked health problems that interact synergistically to cause an excess burden of disease in the population. Thus seems possible some periodontal disease and systemic disease associations could be syndemic in nature.

The proceedings of a joint workshop of the European Federation of Periodontology and the American Academy of Periodontology investigating all aspects of the relationship between periodontal diseases and systemic diseases were published in 2012 (European Federation of Periodontology & American Academy of Periodontology 2012) The main systemic conditions considered were: cardiovascular disease, diabetes mellitus, adverse pregnancy outcomes, respiratory disease, chronic kidney disease, rheumatoid arthritis, cognitive impairment, obesity, metabolic syndrome, cancer and other associations were considered. It was summarized that while there was good evidence supporting relationships between periodontitis and some conditions further studies are needed to verify these early findings.

Clinicians and researchers need to understand that associations between periodontal and systemic diseases needs to be considered with regards to: a) the risk markers that may bind them together and b) experimental (not just observational) data that define the relationship between them. To assist in this process a “Disease Association Checklist” (Table 1) has been developed (Bartold and Mariotti 2017). This allows for a simple algorithm to be used to make rational assessments of any proposed disease associations. By assembling known information into this checklist evidence will present as to the validity of periodontal and systemic interrelationships. As illustrated in Table 2, when available information is applied to this checklist there is good evidence available to support realistic relationships between periodontitis and diabetes, cardiovascular disease and rheumatoid arthritis, equivocal evidence of an association between periodontal disease and obesity or adverse pregnancy outcomes and negligible evidence for osteoporosis.

Disease Association Checklist
Biologic Plausibility
Strength of Association
Effect of Periodontal Treatment on Systemic Condition
Effect of Treatment of Systemic Condition on Periodontitis

Table 1.

	Diabetes	Obesity	Adverse Pregnancy Outcomes	Cardiovascular Disease	Osteoporosis	Rheumatoid Arthritis
Biological Plausibility	Yes	Yes	Questionable	Yes	Yes	Yes
Strength of Association	Yes		Poor	Yes	No	Yes
Effect of Periodontal Treatment on Disease Condition	Yes	Nil	None	Equivocal / None	No	Emerging

Table 2. Evidence for various periodontal and systemic interrelationships

Conclusions

The emergence of periodontal medicine as a sub-discipline within the field of periodontology has been significant. In the past periodontal treatment focused on the bacterial cause of the disease and was solely based on anti-infective measures to control the disease. However, with current understanding that both periodontal infection and inflammation may have significant consequences for general health and well being, our attention has begun to focus on a more holistic approach and consider how to control any unwanted effects of periodontal disease on overall health. It is not surprising that these developments have caught the public's attention. However, as a profession we must not make any claims that treatment or periodontal disease will affect certain systemic conditions until we have more evidence to support this. It is proposed that by using simple algorithms such as presented in the Disease Association Checklist we can begin to determine rationally assess the various associations as they come to light. It is imperative that only biologically plausible and genuine associations are considered and further investigated. To date, very few conditions show a robust association with periodontal disease. Thus, any proposed periodontal-systemic association must be critically assessed and if the conditions proposed in the Disease Association Checklist cannot

be met then they should not be considered further as credible, plausible and realistic relationships.

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Exploring Innovative Frontiers in Periodontal Health and Implantology
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Chapter 5

Ferritin Plays Key Roles for Homeostasis of Periodontal Ligament Tissue

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Introduction

The periodontal ligament (PDL) is a specialized connective tissue interposed between the roots of teeth and the inner wall of tooth-supporting bone (alveolar bone). The PDL links the teeth to the alveolar bone proper, thereby providing support, protection and sensory input for the masticatory system (Beertsen *et al* 1997). It has also been demonstrated that PDL tissues contain multipotent mesenchymal stem cells that can differentiate into mineralized tissue-forming cells, such as osteoblasts and cementoblasts (Bartold *et al* 2006; Seo *et al* 2004). Thus, the PDL is thought to play crucial roles for not only homeostasis of periodontal tissues but also bone remodeling, wound healing and tissue regeneration (Beertsen *et al* 1997).

Recent reports have demonstrated that ferritin, a key molecule for controlling the iron concentration, regulates osteoblast differentiation (Zarjou *et al* 2009; Zarjou *et al* 2010b). Cytosolic ferritin is a ubiquitous and highly conserved iron storage molecule that is composed of ferritin light polypeptide (FTL) and ferritin heavy polypeptide (FTH) (Torti 2002). Twenty-four ferritin subunits assemble to form the apoferritin (iron-free ferritin) shell. The FTL and FTH subunits are encoded by separate genes and their functions are nonexchangeable (Caskey *et al* 1983; Worwood *et al* 1985). Within cells, ferritin has anti-inflammatory properties *(Fan et al 2014; Zheng et al* 2016) and its expression is regulated by pro-inflammatory cytokines (Pham *et al* 2004).

We investigated the expression of ferritin in the PDL to clarify the molecular characteristics of PDL cells. Interestingly, we found that FTL and FTH were highly expressed in PDL tissues *in vivo* and elucidated the function of ferritin for the cytodifferentiation and inflammation of PDL cells *in vitro.*

Ferritin and its membrane receptors

Ferritin was first discovered in 1894 and purified in 1937 (Crichton 1973). It is involved in iron metabolism and can capture up to 4500 iron atoms (Harrison and Arosio 1996). FTH is abundant in the human heart and has enzymatic activity, which is essential for oxidizing ferrous iron into

ferric iron. FTL is rich in human liver and has carboxy groups, which are associated with iron nucleation, mineralization and long-term iron storage (Rucker *et al* 1996). Both FTL and FTH are critical for maintaining iron homeostasis. The ratio of FTH to FTL varies depending on tissue type and can be modified by pathological conditions, such as inflammation (Torti 2002). Genes encoding FTH and FTL subunits are located on chromosomes 11q and 19q respectively (Worwood *et al* 1985). Ferritins are mainly present in the cytosol, as well as in the nucleus and the mitochondria (Harrison and Arosio 1996). In addition to intracellular form, ferritins are also found extracellularly in serum, synovial and cerebrospinal fluids (Meyron-Holtz *et al* 2011).

T cell immunoglobulin and mucin domain 2 (TIM-2) was the first identified cell surface receptor for ferritin in mice. It is a transmembrane protein expressed by renal tubule cells, splenic B cells, T cells and liver cells. However, TIM-2 does not have a human ortholog. Li *et al* identified Transferrin receptor (TfR1) is a membrane receptor for FTH and accounts for mostly FTH binding to cell surface, and induces FTH entry in lysosomes and endosomes (Li *et al* 2009) Scavenger receptor class A member 5 (Scara5) has been identified as another cell surface receptor of ferritin, which mainly binds FTL (Li *et al* 2009).

Regulation and functions of ferritin

Inflammatory cytokines (e.g. TNFα and IL-1β) are capable of regulating the expression of ferritin on two levels: transcriptional level (mainly FTH) and translational level (both FTH and FTL) via NF-κB signal pathway (Kwak *et al* 1995; Smirnov *et al* 1999; Wei *et al* 1990). Oxidative stress induces the expression of FTH and FTL mRNA in vitro, and increases ferritin expression on a translation level (Tsuji *et al* 2000). Ferritin has a beneficial role when cells are exposed to oxidative stress through minimizing ROS, which can damage cells. Hypoxia increases the expression and accumulation of ferritin in cells obtained from brain and lung tumors (Qi and Dawson 1994).

Intracellular functions of ferritin are iron storage in a non-toxin, bioavailable form and anti-oxidation. Intracellular iron through the Fenton reaction generate reactive oxygen species (ROS), which cause DNA breaks, lipid peroxidation and other forms of damage (Harrison and Arosio 1996). The over-expression of FTH in HeLa cells has been noted to have a protective role against oxidative damage and cytotoxicity (Cozzi *et al* 2003; Pham *et al* 2004). Furthermore, FTH upregulation by NF-κB inhibits TNFα-induced apoptosis by suppressing ROS (Pham *et al* 2004). Deletions of FTH in mice are embryonically lethal (Ferreira *et al* 2000). FTH inhibits immune responses by inducing IL10 production partly.

Extracellular ferritin is a pro-inflammatory signaling molecule that can increase the expression of TNFα and IL-1β in hepatic stellate cells through PI3K, MAPK, and NF-kB signal pathway (Liu *et al* 1991). Moreover, extracellular ferritin promotes the proliferation of epithelial breast cancer cells, MCF7 cells and T47D cells independent of iron. Extracellular ferritin has immunosuppressive effects on myeloid cells and lymphocytes via regulating iron (Fargion *et al* 1991; Matzner *et al* 1979). When lymphocytes are treated with extracellular ferritin, lymphocyte cell activation is inhibited (Fargion *et al* 1991). Extracellular ferritin can also increase

angiogenesis. In a mouse tumor model, the addition of ferritin increases intratumor blood vessel density significantly (Coffman *et al* 2009).

Ferritin and cancer risk

Recently, some studies have shown that ferritin is over-expressed in multiple tumor tissues, such as pancreatic cancer (Marcus and Zinberg 1974), hepatocellular carcinoma (Kew *et al* 1978), breast cancer (Guner *et al* 1992), Hodgkin's lymphoma (Eshhar *et al* 1974). The ratio of FTH/FTL of ferritin varies in different pathological conditions and is increased in cancer patients. studies have demonstrated that the expression of FTH are highly increased in tumorigenic cell lines and tumorous tissues from patients.

Serum ferritin levels are elevated in patients with multiple malignancies, including neuroblastoma (Hann *et al* 1980), melanoma (Gray *et al* 2003), renal cell carcinoma, squamous cell carcinoma of the head and neck (Kukulj *et al* 2010), non-small-cell lung cancer (Kukulj *et al* 2010). This increase is a potential tumor biomarker and associated with shorter survival and more progressive disease. Serum ferritin in cancer patients consists mainly of FTH (Lukina *et al* 1993).

Ferritin in chronic kidney disease

In general, low serum ferritin indicates iron deficiency and high serum ferritin indicates iron overload. However, high serum ferritin is also found in chronic kidney disease, infection, and liver diseases. Inflammation is the probable cause of elevated ferritin in hemodialysis patients (Kirchbaum 2001). If the level of serum ferritin in hemodialysis patients is over 800 ng/ml, CRP level is higher and malnutrition-inflammation score is worse (Kalantar-Zadeh *et al* 2004).

The expression of ferritin in the periodontal tissues

FTL and FTH are highly expressed in human and mouse PDL tissues. We analyzed FTL and FTH expression in various human tissues. Real-time PCR analyses revealed markedly higher expression levels of FTL and FTH in the PDL than in the other human tissues examined (Hou *et al* 2012). These findings prompted us to investigate the specific expression of FTL and FTH in periodontal tissues.

First, we assessed the expression of ferritin in primary cultured cells isolated from human periodontal tissues using the polymerase chain reaction and immunofluorescent staining. Second, we investigated the expression and distribution of ferritin in the periodontal tissues of *Macaca fascicularis*, human gingival tissues using immunohistochemistry. Both protein and mRNA for ferritin were constitutively expressed in human primary cultured cells, including those from the dental apical papilla, periodontal ligament, dental pulp, and gingival epithelium, as well as gingival fibroblasts. In *M. fascicularis* tissues, the immunohistochemical staining was particularly strong in blood vessels and mineralizing areas of the dental pulp and periodontal ligament. Ferritin heavy chain exhibited specific immunopositivity in the stratum basale of the epithelium in human gingival tissue. Ferritin is constitutively present and widely distributed in the periodontal tissues of primates. Ferritin may play roles in epithelial proliferation, vascular angiogenesis, and mineralization in these tissues.

Dental pulp, gingiva, and PDL are all vascularized connective tissues. After injury and inflammation, their healing potential is very good. In part, this healing potential is associated with local cells which secrete factors that promote cell differentiation and neovascularization. Previous research has shown that ferritin has regulatory effects on angiogenesis (Coffman *et al* 2009). Immunohistochemical staining of healthy gingival samples showed that FTL was distributed in the stratum spinosum and vessels, and FTH was distributed in the basal cell layer and vessels.

According to the immunohistochemical results, PDL and pulp cells expressed high levels of ferritin. These cells have mineralization activity like osteoblasts (Mori *et al* 2011). Recently, it has been reported that ferritin, especially FTH, negatively modulates the differentiation of osteosarcoma cells and human smooth muscle cells into osteoblasts via ferroxidase activity (Becs *et al* 2016; Zarjou *et al* 2010). We suggest that an autocrine or paracrine ferritin loop might be formed in the local environment to promote mineralization homeostasis in healthy tissues and enhance osteogenesis in disease sites. Further studies are needed to uncover the roles of ferritin in cytodifferentiation and mineralization.

Role of ferritin in the cytodifferentiation of periodontal ligament cells

FTL and FTH expression is induced during PDL cell cytodifferentiation *in vitro*. To clarify a more complete expression pattern of FTL and FTH, we analyzed the FTL and FTH mRNA and protein expression levels during cytodifferentiation of the mouse PDL cell line MPDL22. Expression of FTL and FTH mRNAs and proteins was induced during the course of MPDL22 cytodifferentiation and peaked at the early stage of the cytodifferentiation (Hou *et al* 2012).

Apoferritin enhances MPDL22 cytodifferentiation and mineralization in a time- and dose-dependent manner (Hou *et al* 2012). Moreover, apoferritin significantly increased the ALPase activity in a dose-dependent manner. In addition, real-time PCR analyses demonstrated that apoferritin significantly upregulated the gene expression for Runx2 and type I collagen during MPDL22 cytodifferentiation. We confirmed that the intracellular Pi concentrations of MPDL cells were not affected by apoferritin during culture for 48 hours.

RNA interference of FTH downregulates MPDL cytodifferentiation and mineralization. To clarify the role of FTH in PDL cells during the course of their cytodifferentiation and mineralization, we established MPDL22 transfectants with FTH knockdown. Downregulation of the cytodifferentiation and mineralization-related genes Runx2, type I collagen and osteocalcin were observed in the group of FTH shRNA transfectants. The FTH shRNA also inhibited mineralized nodule formation. We further found that ceruloplasmin, which possesses ferroxidase activity, enhanced the mineralized nodule formation of PDL cells. These findings clearly suggest that ferritin, probably through the ferroxidase activity of FTH, regulates the cytodifferentiation and mineralization of PDL cells (Hou *et al* 2012).

Ferritin is up-regulated in periodontal tissues of periodontitis

Expression of ferritin (FTH and FTL) are increased in inflammatory tissues of periodontitis. Immunohistochemical staining of inflammatory gingival samples showed that FTL and FTH were up-regulated in connective tissue in areas of inflammatory cell infiltration (Huang *et al* 2019).

To confirm that ferritin is up-regulated in inflamed tissues, we used an animal model of experimental periodontitis. Micro-computed tomography (CT) and histological examination confirmed the establishment of ligature-induced periodontitis in C57BL/6 mice. Micro CT analysis showed resorption of alveolar bone and H&E staining showed massive inflammatory cell infiltration in the tooth-supporting tissues. Furthermore, immunohistochemical staining showed that FTH and FTL were expressed widely in the unligated periodontal tissues. Gingival epithelium, periodontal ligament tissue, and gingival connective tissue are all positively stained. Meanwhile, the locations of positive FTH and FTL staining in the ligated tissues were exactly the same as in the unligated tissues. However, the intensity of positive staining became significantly stronger along with the extent of inflammatory infiltration (Huang *et al* 2019). Ferritin, a ubiquitous iron-binding protein, sequesters iron in a non-toxic but bioavailable form and thus limits free radical generation triggered by inflammation (Muhoberac *et al* 2011). Increased ferritin expression has been reported in atherosclerosis, cancer, and neurodegenerative diseases (Torti and Torti 2002). The increased ferritin may have cytoprotective function and anti-inflammatory effect in some cells (Fan *et al* 2014; Hatcher *et al* 2015). However, further studies are needed in human periodontal ligament cells.

Ferritin regulation occurs at multiple levels (Tsuji *et al* 2000). Lipopolysaccharide and the pro-inflammatory cytokines TNFα, IL-1α, IL-1β, IL-6 modulate ferritin expression (Huang *et al* 2018; Wang and Wei 2012). Previous studies have shown that the levels of *P. gingivalis*, IL-6, and TNF-α are higher in the pathogenic sites affected by periodontitis (Li *et al* 2015; Zhan *et al* 2017). In this study, we found exogenous factors, such as *P. gingivalis*-*LPS*, are not the only factors of promoting ferritin expression, endogenous factors, such as IL-6 and TNF-α, also aggravate ferritin expression in human periodontal ligament cells (Huang *et al* 2019). These results suggested that the up-regulation of the ferritin gene and protein expression induced by pro-inflammatory cytokine in human periodontal ligament cells, may be an early response of cells when they are stimulated by bacterium.

Circulating ferritin level is elevated in systemic inflammation (Branco and Garcia 2017). Furthermore, serum, salivary, and GCF ferritin are elevated in patients with chronic periodontitis. However, the precise mechanisms of ferritin release and the functions of secreted ferritin in the serum and tissue are unclear. T lymphocytes contribute to the synthesis and secretion of ferritin and infiltrate in inflammatory periodontal tissues (Dörner *et al* 1980). In the study, we found ferritin expression up-regulated in the area of T-lymphocytes infiltration (Huang *et al* 2019). This may be a source of extracellular ferritin. Periodontal ligament cells and gingival epithelial cells maintain a lot of ferritin.

We deduce that these cells may release ferritin when they are stimulated or damaged by bacteria. We have found that *P. gingivalis*-LPS is a factor of promoting ferritin secretion and we have also demonstrated that human periodontal ligament cells secrete ferritin (Huang *et al* 2019).

Ferritin promotes inflammatory cytokine expression in human periodontal ligament cells through ERK/P38 MAPK pathways

Previously, studies have reported that ferritin exists extracellularly, contains small amounts of iron and acts as a cytokine to regulate pro-inflammatory function in hepatic stellate cell via NF-κB-regulated signaling (Arosio *et al* 1986; Ruddell *et al* 2009). We have demonstrated that ferritin induces IL-6 and IL-8 production significantly in human periodontal ligament cells (Huang *et al* 2019). IL-6 and IL-8 play important roles in the initiation and progression of periodontitis. This suggests that ferritin may act as a second messenger system to pro-inflammation. This may amplify immuno-inflammatory responses in periodontal ligament during periodontitis.

The intracellular signaling pathways involved in ferritin-mediated IL-6 and IL-8 expression have been further investigated. We found that ferritin activated ERK and P38 MAPK pathways (Huang *et al* 2019). The inhibitors of ERK and P38 also alleviated the pro-inflammatory effects of ferritin in the present study. Therefore, these results support the hypothesis that ferritin induced IL-6 and IL-8 production by mainly activating ERK and P38 MAPK pathways in human periodontal ligament cells.

Conclusion

The expression of ferritin is significantly positive in periodontal tissues of periodontitis. *P. gingivalis*-LPS, IL-6, and TNF-α, which are increased in patients of periodontitis, induce ferritin expression and secretion. Extracellular ferritin promotes pro-inflammatory cytokines production in human periodontal ligament cells, which may enhance the immune inflammatory responses in periodontitis. Taken together, ferritin plays key roles for homeostasis of periodontal ligament tissue and may be involved in the development of periodontitis.

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Chapter 6

No Better Time to Unlearn and Relearn

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Introduction

In line with a clearer understanding of the complex nature of periodontal and peri-implant diseases, a newer and systematic classification for these diseases is necessary. The classification should enable clinicians to properly diagnose and manage patients. At the same time, the classification is also important for scientists to investigate the aetiology, pathogenesis, natural history and treatment of these diseases and conditions.

The new classification for Periodontal and Peri-implant Diseases and Conditions was first introduced to the dental fraternities during the Euro Perio 9 meeting which was held in Amsterdam in 2018. The launch event was attended by many periodontists from around the world. The introduction of the new classification has changed many paradigms in the field of Periodontology and has been well received with much enthusiasm. Nevertheless, this classification poses challenges in terms of knowledge sharing and diagnostic skills training among periodontists as well as general dentists. Since its introduction, the implementation of this classification has been discussed in many countries worldwide. Accordingly, in Malaysia, the Malaysian Society of Periodontology (MSP) undertook a proactive approach by developing a comprehensive plan to implement the classification in Malaysia. The plan included (i) to introduce and standardise knowledge of the new classification to all periodontists in Malaysia, (ii) to propose a standardised curriculum for undergraduate and postgraduate teaching among universities in Malaysia, (iii) to assess the knowledge and practice in Periodontology clinical fraternities, and (iv) to create awareness on new classification to all dentists in Malaysia.

As part of the plan, the Periodontology team at the University of Malaya (UM), also known as "Perio Malaya", was given the task to introduce and standardise knowledge on the new classification to all periodontists in Malaysia. This paper summarises the Periodontology Classification Masterclass organised by the Perio Malaya team in an effort to impart and standardise knowledge from the 2017 Classification of Periodontal and Peri-implant Diseases and Conditions among Malaysian periodontists. The Masterclass was a collaborative effort with University Kebangsaan Malaysia (UKM) and the Malaysian Society of Periodontology (MSP); and the programme was supported by Ministry of Health (MoH)

Malaysia. The Masterclass was held in Petaling Jaya on July 9th 2019 and the plan had already began in January 2019.

Preparation

The Perio Malaya team acted as a local organising committee and was formed to identify the best way to convey the knowledge related to the new classification. The scope of this Masterclass was to disseminate knowledge and align the classification scheme to Malaysian periodontists. The committee reviewed the findings from consensus papers and recommendations from the four workgroups of the World Workshop (Caton *et al* 2017).

Given that the classification had been presented globally 12 months earlier (at Euro Perio in June 2018), most clinicians would have attended repeated lectures, webinars and roadshows available. However, a gap in knowledge remained as many practitioners were still unsure on how to translate the knowledge into practical diagnostic skills. Thus, Perio Malaya decided to include a series of case-studies into the workshop in the programme. The plan was for attendees to be guided step-by-step on how to reach a diagnosis within the context of the new classification system. This hands-on experience was the plus point for the programme. Case studies were carefully selected and vetted for the workshop. In addition, 18 periodontics postgraduate students from UM and UKM were trained and calibrated to assist the participants throughout the workshop.

An expert forum was included in the Masterclass in order to provide a multi-disciplinary as well as multi-sector involvement in identifying the challenges ahead. Service and academic sectors have different concerns when it comes to implementation of the classification. For example, the academic sector is concerned about the direction of research and publication, should there be any delay in the implementation of this new classification. All findings and recommendations of the workshop content were agreed to by consensus.

Format of Workshop

In order to maximise engagement of participants during the workshop, the participants were provided (*via* google drive) with pertinent consensus reports and review papers a week prior to the Masterclass. The participants were seated in groups of 10 (including a postgraduate facilitator), with intention to increase their interaction during workshop. Each group was provided with 2 sets of clinical case materials.

Renowned Emeritus Professor Mark Bartold gave the plenary lecture, talked through the history and philosophy of periodontal classifications and highlighted the changes of the 1999 classification. In addition, a condensed scheme for each of four workgroup sections was also presented.

The session continued with a case-studies workshop, which followed similar four workgroup sections of the World Workshop. The workgroups were chaired by Drs Mohd Zamri Hussin and Norul Husna Hassan from UM; and Drs Shahida Mohd Said and Masfueh Razali from UKM. Each speaker was responsible for a workgroup section and following which, there were cases presented for hands-on experience. It was encouraging when the speakers prepared check list forms that simplified the thinking process throughout the step-

by-step guide to reach diagnosis.

The forum staged Emeritus Prof Dr Mark Bartold and 5 prominent periodontists and a public health specialists from Malaysia. Their diverse backgrounds and experiences enhanced the session, enlightened the forum session, and enabled thought provoking and illuminating deliberation on potential challenges and opportunities in implementing the new classification in Malaysia. Among the issues discussed were the time taken to collect periodontal measurements and/or parameters to reach the diagnosis. It was pointed out that the diagnosis process was simplified during the workshop through the readily available information. In day-to-day actual clinical settings, especially in the high-volume government service sector, there is the potential for delays to the diagnostic processes. Likewise, those in the private sector also may not be able to commit to such extensive periodontal measurement as time is precious. This brought about discussion as to whether the 2017 classification would be suitable to be introduced to the general dental practitioners. This forum opened up opportunities for all to voice out their concerns and considerations on the practicality to implement the new classification.

The Way Forward

Throughout the programme planning, the challenges faced were mainly related to meeting various deadlines for the event. We had many time constraints in terms of preparing the cases and materials for the workshop as well as conducting the training sessions for the facilitators. Nevertheless, we had at least 4 group meetings to prepare for the event.

Organising the programme also was a challenge. The Perio Malaya team succeeded with the help of the Dental Research Management Unit (DRMU) of UM and full support from the faculty and university.

The Masterclass received an overwhelming response with more than 140 participants nationwide attending. The attendees were mainly periodontists from the MOH, universities and armed forces, restorative specialists, public health specialists and dentists with special interest; as well as periodontics and public health postgraduate students. The majority of the participants rated the programme well and would like to recommend it to others. It was concluded that it was timely to implement the 2017 classification in tandem with the worldwide acceptance. Nevertheless, further discussions and decisions are required to look into how this new scheme of classification can be adopted and adapted in accordance with the Malaysian scenario.

In the future the Perio Malaya group will host a focussed group discussion (FGD) session in early October 2019. The objective will be to look into issues and challenges faced by the participants (who have been trained with the new classification), in using the classification at their work places. The participants' input would shed some light on the actual scenario at the dental clinical setting, be it in government or private universities, government or private service sectors as well as armed forces. The outcomes of this FGD will guide MSP in assessing the readiness to implement the 2017 Classification in Malaysia.

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Chapter 7

Relationship Between Periodontitis and Alzheimer's Disease

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Introduction

Alzheimer's disease is the most common cause of dementia. It is estimated that 152 million people worldwide will be living with dementia in 2030 (World Alzheimer Report, 2018). Alzheimer's disease has become a major health problem often associated with elderly people. To date, preventive approaches for this neuroinflammatory-related disease has been extensively studied due to the increasing number of elderly people worldwide that is projected to be 1.4 billion in 2030 (World Population Prospects, 2017).

There is growing evidence supporting a relationship between periodontitis and Alzheimer's disease. Numerous studies have shown that poor oral hygiene is connected to the presence and increased severity of Alzheimer's disease (Syrjälä et al 2012; Aragon et al 2018). Researchers have proposed that periodontitis could lead to the progression of Alzheimer's disease by further increasing levels of pro-inflammatory cytokines (Cestari et al 2016). Moreover, certain periodontopathogens have also been found to have the ability to be present the brain (Dominy et al 2019). Although inflammation is present in the pathogenesis of both diseases, the over-lapping risk factors between periodontitis and Alzheimer's disease are poorly understood. The objective of this systematic review is to elucidate the relationship between periodontitis and Alzheimer's disease.

Methods and Results

The Preferred Reporting Items for Systematical Reviews and Meta-Analysis (PRISMA) guidelines was used to identify

epidemiological and experimental studies investigating the relationship between periodontitis and Alzheimer's disease (Figure 1). Three databases (PubMed, Web of Science, and Biomed Central) were searched from 2009 to June 2019. The search strategy involved the following keywords: "periodontitis" AND "periodontal disease" AND "Alzheimer's disease" AND "dementia". Eighteen studies were included for systematic review. There were 14 epidemiological studies (Table 1) and four experimental studies (Table 2).

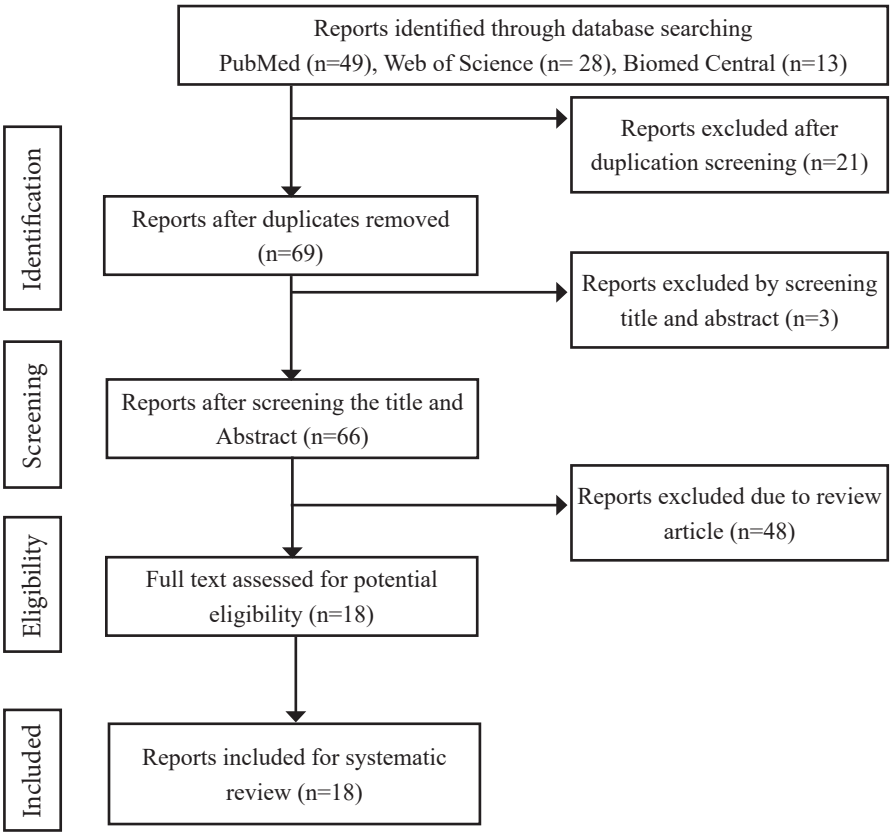

Figure 1. PRISMA flow diagram of the search process.

Authors (Publication Year)	Study Design	Sample Size	Age	Assessment of Periodontitis	Assessment of Alzheimer's disease (AD)	Main Findings
Holmer J, *et al* (2018)	Case-control	154 patients diagnosed with AD, MCI, or SCD, and 76 controls	50-80 years	- Oral hygiene - Probing pocket depth - Bleeding on probing - Suppuration - Tooth mobility - Furcation involvement - Panoramic radiograph	- Medical history and examination - Mini Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA) - Clock drawing test (CDT) - Blood tests - Brain imaging (MRI/CT) - Electroencephalography (EEG) and lumbar puncture with cerebrospinal fluid (CSF) analysis - Neuropsychological assessment	Subjects with AD, MCI, and SCD had more marginal alveolar bone loss and poorer overall oral health status than controls.

Authors (Publication Year)	Study Design	Sample Size	Age	Assessment of Periodontitis	Assessment of Alzheimer's disease (AD)	Main Findings
Aragon F, *et al* (2018)	Case-control	70 Alzheimer's patients and 36 controls	The average age of the Alzheimer's patients 77.4 ± 10.6 years and the controls 62.6 ± 7.1 years.	- Community Periodontal Index - Oral hygiene	- Severe MMSE - Mini-Cog test - CDT - The Functional Assessment Staging of Alzheimer's Disease-FAST - The Clinical Dementia Rating - The Global Deterioration Scale	AD patients exhibited fewer periodontally healthy sextants and worse oral hygiene.
Laugisch O, *et al* (2018)	Case-control	40 patients (20 with AD and 20 with DEM-noAD)	30-70 years	- Number of teeth - Probing depth - Clinical attachment loss - Percentage of bleeding on probing - Full mouth plaque score	- AD was diagnosed according the 2011 guideline of the National Institute of Aging-Alzheimer's 140 Association workgroups - MMSE - MRI	Antibodies against periodontal pathogens may be produced intrathecally but does not support a specific association of periodontal infection with an onset of AD.
Chen CK, *et al* (2017)	Retrospective matched-cohort study	9291 patients with chronic periodontitis (CP) and 18,672 patients without CP	≥50 years	ICD-9-CM diagnostic criteria	ICD-9-CM diagnostic criteria	Patients with 10 years of CP exposure exhibited a higher risk of developing AD than unexposed groups.
Sochoka M, *et al* (2017)	Cross-sectional	128 participants	55-90 years	- Number of teeth - Probing depth - Clinical attachment level - Bleeding on probing - Approximal plaque index - The clinical diagnosis of moderate and severe periodontitis (Page & Eke classification)	- Psychiatric and neurological examinations - MMSE - DSM-V and NINCDA-ADRDA criteria for AD	The level of IL-1, IL-6 , and TNF-α, also anti-inflammatory IL-10 increases with worsened periodontal status and cognitive decline.
Nezu A, *et al* (2017)	Cross-sectional	36 subjects (18 periodontitis and 18 peridontally healthy)	Mean age group periodontitis 62.9±11.0 years and healthy 27.5±4.3 years.	- Bleeding on probing - Probing pocket depth - Clinical attachment level	AD-related genes	Amyloid beta precursor protein and potent amyloid degradation enzyme were up-regulated in periodontitis-affected gingival tissue compared to control.
Cestari JAF, *et al* (2016)	Case-control	25 patients with AD, 19 patients with MCI, and 21 healthy subjects	56-92 years	- O'Leary plaque index - Gingival bleeding index - Probing pocket depth - Clinical attachment level - Cement-enamel junction distance - Absences of teeth	- The criteria of the National Institute for Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ARDRA). - MMSE	The elevated concentration of IL-6 in patients with AD was associated with high serum TNF-α level of subjects with periodontal disease.
Ide M, *et al* (2016)	Cohort (six month)	60 non smoking participants with mild to moderate AD	Mean age at baseline 77.7±8.6 years	- Plaque scores - Pocket depth - Bleeding on probing	- NINCDS-ADRDA criteria for AD with a modified Hachinski Ischaemic Scale - Alzheimer' s Disease Assessment Scale (ADAS-cog) - MMSE	Periodontitis was associated with an increase in cognitive decline (six month follow up period).
Noble JM, *et al* (2014)	Case-cohort	219 subjects (110 incident AD cases and 109 controls)	>65 years	Serum IgG antibody levels to periodontal microbiota	Neuropsychological assessments	An increased risk of AD events was discovered among subjects with high serum *A. naeslundii* IgG antibody.
Martande SS, *et al* (2014)	Cross-sectional	58 individuals with AD and 60 nondemented or cognitively normal individuals	50-80 years	- Gingival index - Plaque index - Probing depth - Clinical attachment level - Percentage of bleeding sites	MMSE	The periodontal status of individuals with AD deteriorates with disease progression.
De Souza Rolim, *et al* (2013)	Case-control	29 patients and 30 control subject	59-91 years	- O'Leary plaque index - Gingival bleeding index - Probing pocket depth - Clinical attachment level	NINCDS-ADRDA criteria for AD classified as mild by the MMSE (score from 18 to 26)	Infections of peridontal were more frequent in patients with mild AD compared to controls.

Authors (Publication Year)	Study Design	Sample Size	Age	Assessment of Periodontitis	Assessment of Alzheimer's disease (AD)	Main Findings
Stein PS, *et al* (2012)	Case-control study nested within a cohort study	158 participants (81 participants developed MCI or AD and 77 controls)	Mean age at baseline AD 74.1±7.5 yeras, MCI 72.1±6.1 years, control 70.0±6.5 years	Chronic periodontitis (American Academy of Periodontology classification)	MMSE score and apolipoprotein epsilon 4 (APOE ε 4) status	Antibody levels to *F. nucleatum* and *P. intermedi*a were significantly increased in the AD patients compared to controls.

Table 1. Summary of epidemiological studies on the relationship between periodontitis and Alzheimer's disease.

Authors (Publication Year)	Study Type	Experimental Design	Analysis	Main Findings
Dominy, *et al* (2019)	Human study	Tissue microarrays (TMAs) brain tissue cores of AD patients and control were stained by gingipain-specific antibodies (RgpB and Kgp).	IHC, IF, WB, qPCR	The RgpB and Kgp loads (both tau and ubiquitin loads) in AD brains were significantly higher than control. The RgpB was colocalized in AD hippocampus and associated with neurons, astrocytes, but not microglia. Protein levels of Kgp in AD cerebral cortex were nearly absent. *P. gingivalis* was detected in all saliva samples in 7 out of 10 cerebrospinal fluid (CSF) of AD patients.
	In vitro study	SH-SY5Y cells (human neuroblastoma) were induced with various concentration of *P. gingivalis* or *P. gingivalis* gingipain-deficient mutants.	WB	Tau-5 antibody was down-regulated in a dose-dependent manner by the stimulation of *P. gingivalis*, but not in *P. gingivalis* gingipain-deficient mutants; this indicates that gingipains have a significant role in the loss of the Tau-5 epitope.
	In vivo study	8-week-old BALB/c mice were induced with *P. gingivalis* every other day for 42 days. Further, Kgp inhibitors (COR119, COR271, and COR286) were orally given twice a day accordingly for the next 5 weeks.	IF, WB, qPCR, histological analysis	*P. gingivalis* DNA was found in all brains of infected mice on days 35-70. The Kgp inhibitor COR271 minimized the number of bacteria in the brain and recovered the $Gad67^{+}$ GABAergic interneurons loss compared to *P. gingivalis* infected group.
Ding Y, *et al* (2018)	*In vivo* study	Young and middle-age C57BL/6 J mice were orally infected with *P. gingivalis*, once every 48 h, for 6 weeks.	MWM behavioural test, IHC, WB, qPCR	The protein levels of TNF-α, IL-6, and IL-1β in the brain tissues of the middle-aged infected mice were increased. This shows that *P. gingivalis* infection may lead to an age-dependent brain inflammation.
Zhang J, *et al* (2018)	*In vivo* study	8-week-old C57BL/6 J mice were injected intraperitoneally with *P. gingivalis* LPS, either with or without TLR4 inhibitor (TAK-242) for 7 days.	Behavioural tests (OFT, MWM, and PAT), IHC, WB, qPCR	Administration of *P. gingivalis*-LPS resulted in learning and memory impairment in mice. The expression of TNF-α, IL-6, IL-1β, and IL-8 in the cerebral cortex were up-regulated in *P. gingivalis* infected mice compared to control. This spatial learning and memory impairment induced by *P. gingivalis* LPS was regulated by the activation of the TLR4/NF-κB signaling pathway.
Ilievski V, *et al* (2018)	*In vivo* study	8-week-old C57BL/6 J mice were orally applied with *P. gingivalis*/ gingipain for 22 weeks.	IF, confocal microscopy, qPCR	*P. gingivalis*/gingipain was detected intracellularly in astrocytes, microglia, and neurons in the hippocampi of the mice, suggesting the translocation of applied *P. gingivalis* from oral cavity to the brain. The expression of TNF-α, IL-6, IL-1β, and IL-8 in the hippocampi were up-regulated compared to control.

Table 2. Experimental studies on the relationship between periodontitis and Alzheimer's disease.

Discussion

In general, our results showed a positive correlation between periodontitis and Alzheimer's disease. Most of the epidemiological studies in this review were case-controls and found that subject with Alzheimer's disease had poorer oral hygiene and periodontal condition compared to controls (Holmer *et al* 2018; Aragon *et al* 2018; De Souza Rolim *et al* 2015). Oral health may be significantly compromised in patients with Alzheimer's disease because of the deterioration of memory, motor skills, and personal care.

The exact mechanism of how periodontitis involved in the pathogenesis of Alzheimer's diseases remains unclear. It is proposed that periodontitis can lead to progression of Alzheimer's disease by two probable mechanisms: (1) Periodontitis preceding systemic inflammation/ infection, and (2) invasion of the brain by microorganisms present in the dental plaque biofilm (Gurav, 2014 ; Abbayya et al 2015). Kamer et al (2009) showed that subjects with Alzheimer's disease had a higher number of positive IgG antibody tests against some of periodontal bacteria compared to cognitively normal subjects. Other studies have reported an association between IL-6 and TNF- α in patients with Alzheimer's disease/mild cognitive impairment and periodontitis (Cestari et al 2016).

A recent study has demonstrated strong evidence connecting Porphyromonas gingivalis and Alzheimer's pathogenesis (Dominy et al 2019). This study showed the existence of *P. gingivalis* DNA and gingipain in Alzheimer's disease brains. Further, oral administration of small-molecule inhibitors targeting gingipains reduced *P. gingivalis* load in the mouse brain and decreased neuroinflammation. These findings are potential breakthrough in periodontal medicine research.

Conclusion

There is a link between periodontitis and Alzheimer's disease. Early detection and management of periodontitis may serve as one of the preventive strategies of Alzheimer's disease. Further investigations are required to emphasize a causal association between these two diseases.

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Chapter 8

Threading Informed Consent in Personalized Periodontal Care: The Shift from a Reasonable Doctor to a Reasonable Patient

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Introduction

Paternalism in dental professional culture, recognized through the phrase "doctor knows best", is seeing a decline. In the past, professionals believed that they knew what was best for their patients, leaving said patients out in dental treatment decisions. It was justified as an integral part of effective health care, since patients might refuse treatment against their best interest (Trathen 2015). In a U.S. case, the landmark opinion of Judge Benjamin Cordozo legally defined simple consent—every human being of adult years and sound mind has a right to determine what shall be done with his own body; and a surgeon who performs an operation without his patient's consent commits an assault, for which he is liable for damages (Schloendorff v. Society of New York Hospital 1914).

It is reported that a considerable number of lawsuits originate from misunderstandings and not from treatment errors (Krause 2001). A more recent report showed similar findings that a majority of medical litigation in oral surgery practice involved a lack of communication and rapport between the surgeon and the patient (Marei 2013).

The doctrine of informed consent in Philippine Jurisprudence traces its roots to English common law and was further expanded by common law decisions from other jurisdictions. In the 2011 Philippine case of *Li v. Soliman*, quoting the United States case of *Schloendorff v. Society of New York Hospital*, it was held that "informed consent evolved into a general principle of law that a physician has a duty to disclose what a reasonable prudent physician in the medical community in the exercise of reasonable care would disclose to his patient as to whatever grave risk of injury might be incurred from a proposed course of treatment, so that a patient exercising ordinary care for his own welfare, and faced with a choice of undergoing the proposed treatment, or alternative treatment, or none at all, may intelligently exercise his judgment by reasonably balancing the probable risk against the probable benefits." Autonomy, literally 'self-rule', is the ability of a person to make decisions about their own lives independently and free from coercion (Trathen 2015). The Court in *Li v. Soliman* recognized the inherent autonomy of the patients by requiring doctors, before

administering treatment to their patients, to adequately disclose the material risks and side effects of the proposed treatment. This is known as the Doctrine of Informed Consent. The Court then later applied the Doctrine of Informed Consent in the case of *Rosit v. Davao Doctors Hospital* (2015) to hold the physician liable for withholding material information which would have been vital in the decision of his patient to go through with the operation.

There are four essential elements a plaintiff must prove in a malpractice action based upon the doctrine of informed consent: "(1) the physician had a duty to disclose material risks; (2) he failed to disclose or inadequately disclosed those risks; (3) as a direct and proximate result of the failure to disclose, the patient consented to treatment she otherwise would not have consented to; and (4) plaintiff was injured by the proposed treatment." The gravamen in an informed consent case requires the plaintiff to "point to significant undisclosed information relating to the treatment which would have altered her decision to undergo it (Li v. Soliman 2011).

In 2015, through the case of *Montgomery v. Lanarkshire Health Board*, the United Kingdom Supreme Court shifted the test from a reasonable prudent physician to a reasonable patient when it held that "the court is satisfied that a 'reasonable person in the patient's position would be likely to attach significance to the risk and this would be determined by the Court and not exclusively by experts in the field".

Statement of the Problem

A crux of controversy is by which standard the courts determine what constitutes adequate disclosure of the treatment, its associated risks, and its side effects.

The opinion in the case of *Li v. Soliman*, penned by Justice Martin S. Villarama, saw a divided Court ruling 8 to 5 in favor of the physician, holding that the parents of the deceased were not able to prove through expert testimony that there was failure in the duty of adequate disclosure, despite admission by the physician herself that she failed to disclose to the parents many of the other associated risks and side effects of chemotherapy (including the material used) —infection, sepsis, and death (Li v. Soliman 2011). The dissenting opinion, penned by Justice Antonio Carpio, stated that the standard of what should be disclosed depends on what a reasonable person, in the same or similar situation as the patient, would deem material in deciding whether to proceed with the proposed treatment, as opposed to a testimony by an expert witness (Li v. Soliman 2011). This is echoed in the aforementioned case of *Montgomery v Lanarkshire Health Board* which shifted the standard of reasonable test from focusing on 'reasonable doctor' to 'reasonable patient' (Lee 2017). In the United States, it is reported that the states are split almost evenly on the question of whether the "reasonable professional" or "reasonable patient" standard should govern disclosures required to obtain informed consent (Sfikas 2003). In Wyoming, for example, case law has established that physicians and dentists must disclose risks that a reasonable medical practitioner in similar circumstances would reveal. In contrast, in Georgia, which in 2000 was the last state to adopt an informed consent requirement, physicians and dentists must disclose the risks that a reasonable person in the patient's position would have considered significant in deciding whether or not to

undergo proposed treatment (Sfikas 2003).

Therefore, as proposed by Justice Antonio Carpio in his *Li v. Soliman* dissent and recent jurisprudence in the United Kingdom and United States, to determine whether there has been adequate disclosure may depend on the standard applied, either a "reasonably competent doctor" through an expert testimony or a "reasonable person". The standard applied may be relevant in determining whether the doctor would be held liable for his failure regarding the duty to disclose.

The Majority Opinion in the case of Dr. Rubi Li v. Spouses Reynaldo and Lina Soliman, as parents/heirs of deceased Angelica Soliman

The eight justices that granted Dr. Li's petition were the ponente, Associate Justice Villarama, joined by Chief Justice Renato C. Corona, Associate Justices Jose Portugal Perez, Arturo D. Brion, Antonio Eduaro B. Nachura, Teresita J. Leonardo-De Castro, Lucas P. Bersamin, Jose Catral Mendoza, and Roberto A. Abad.

The facts of the case

Angelica Soliman, 11 years old, was diagnosed with Osteosarcoma, a highly malignant cancer of the bone. Angelica's right leg was amputated by Dr. Jaime Tamayo in order to remove the tumor. As adjuvant treatment to eliminate any remaining cancer cells, and hence minimize the chances of recurrence and prevent the disease from spreading to other parts of the patient's body (metastasis), chemotherapy was suggested by Dr. Tamayo. Dr. Tamayo referred Angelica to another doctor at SLMC, herein petitioner Dr. Rubi Li, a medical oncologist. Angelica then underwent chemotherapy. Unfortunately, she died thereafter (Li v Soliman 2011).

The parents filed a damage suit alleging negligence on the part of Dr. Li and other physicians. They also averred that Dr. Li assured Angelica will recover in view of 95% chance of healing with chemotherapy, with the only side effects being slight vomiting, hair loss, and weakness. The Regional Trial Court of Legazpi City dismissed the complaint. On appeal, the Court of Appeals concurred with the finding that no negligence was committed by Dr. Li; however, the appellate court found that Dr. Li failed to fully explain all the known side effects of chemotherapy. The appellate court stressed that since the respondents have been told of only three side effects of chemotherapy, they readily consented thereto. Had Dr. Li made known to the parent those other side effects which gravely affected their child (such as carpopedal spasm, sepsis, decrease in the blood platelet count, bleeding, infections and eventual death) the parents could have decided differently or adopted a different course of action which could have delayed or prevented the early death of their child.

The issue before the Court

Whether the Dr. Li can be held liable for her failure to fully disclose serious side effects to the parents of the child patient who died while undergoing chemotherapy, despite the absence of finding that petitioner was negligent in administering the said treatment.

The ruling of the Court

The Court held that the petition is meritorious.

The type of lawsuit which has been called medical malpractice or, more appropriately, medical negligence, is that type of claim which a victim has available to him or her to redress a wrong committed by a medical professional which has caused bodily harm. In order to successfully pursue such a claim, a patient must prove that a health care provider, in most cases a physician, either failed to do something which a reasonably prudent health care provider would have done, or that he or she did something that a reasonably prudent provider would not have done; and that the failure or action caused injury to the patient (Li v. Soliman citing Garcia-Rueda v. Pascasio).

Both the trial and appellate courts concurred in finding that the alleged negligence of petitioner in the administration of chemotherapy drugs to respondents' child was not proven considering that Drs. Vergara and Balmaceda, not being oncologists or cancer specialists, were not qualified to give expert opinion as to whether petitioner's lack of skill, knowledge and professional competence in failing to observe the standard of care in her line of practice was the proximate cause of the patient's death. Furthermore, respondents' case was not at all helped by the nonproduction of medical records by the hospital (only the biopsy result and medical bills were submitted to the court). Nevertheless, the CA found petitioner liable for her failure to inform the respondents on all possible side effects of chemotherapy before securing their consent to the said treatment.

Informed consent evolved into a general principle of law that a physician has a duty to disclose what a reasonably prudent physician in the medical community in the exercise of reasonable care would disclose to his patient as to whatever grave risks of injury might be incurred from a proposed course of treatment, so that a patient, exercising ordinary care for his own welfare, and faced with a choice of undergoing the proposed treatment, or alternative treatment, or none at all, may intelligently exercise his judgment by reasonably balancing the probable risks against the probable benefits.

Proficiency in diagnosis and therapy is not the full measure of a physician's responsibility; the physician is not expected to give the patient a short medical education, the disclosure rule only requires of him a reasonable explanation, which means generally informing the patient in nontechnical terms as to what is at stake, the therapy alternatives open to him, the goals expectably to be achieved, and the risks that may ensure from particular treatment or no treatment.

Examining the evidence on record, The Court held that there was adequate disclosure of material risks inherent in the chemotherapy procedure performed with the consent of Angelica's parents.

1. There is reasonable expectation on the part of the doctor that the parent/ patients understood very well that the severity of these side effects will not be the same for all patients undergoing the procedure.
2. It is difficult to give credence to the claim that Dr. Li assured of a 95% chance of recovery.
3. The duty to disclose material risk cannot be reduced to one simplistic formula applicable in all instances.
4. The expert testimony must show the customary standard of care of physicians in the same practice as that of the defendant doctor.

In a medical malpractice action based on lack of informed consent, "the plaintiff must prove both the duty and the breach of that duty through expert testimony. Such expert testimony must show the customary standard of care of physicians in the same practice as that of the defendant doctor."

In this case, the testimony of Dr. Balmaceda who is not an oncologist but a Medical Specialist of the DOH's Operational and Management Services charged with receiving complaints against hospitals, does not qualify as expert testimony to establish the standard of care in obtaining consent for chemotherapy treatment. In the absence of expert testimony in this regard, the Court feels hesitant in defining the scope of mandatory disclosure in cases of malpractice based on lack of Informed consent, much less set astandard of disclosure that, even in foreign jurisdictions, has been noted to be an evolving one.

The Dissenting Opinion in the Case of Dr. Rubi Li vs Spouses Reynaldo and Lina Soliman, as parents/heirs of deceased Angelica Soliman

As opposed to the main opinion, the closely voted dissent, was penned by Associate Justice Antonio T. Carpio, joined by Associate Justices Conchita Carpio-Morales, Presbitero J. Velasco, Jr., Diosdado Peralta, and Maria Lourdes P. A. Sereno. Justice Mariano Del Castillo took no part.

The doctrine of informed consent requires doctors, before administering treatment to their patients, to disclose adequately the material risks and side effects of the proposed treatment. The duty to obtain the patient's informed consent is distinct from the doctor's duty to skillfully diagnose and treat the patient.

Justice Carpio proposed two standards to determine the adequacy of disclosure by the doctors of the associated risks and side effects of the proposed treatment: the physician standard and the patient standard of materiality. Under the physician standard, a doctor is obligated to disclose that information which a reasonable doctor in the same field of expertise would have disclosed to his or her patient (determined through an expert witness testimony). Under the patient standard of materiality, a doctor is obligated to disclose that information which a reasonable patient would deem material in deciding whether to proceed with a proposed treatment.

In Li vs Soliman, Dr. Li, considered the expert, admitted that she assured the parents of an 80% chance that cancer would be controlled and that she failed to disclose 15 out of the 26 possible risks she identified. Infection, sepsis and death are material risk to a reasonable person and to the parents. No expert witness is necessary to establish the materiality. Dr. Li should have adequately disclosed to the parents that there is a chance that their daughter could die of infection as a result of chemotherapy. Such disclosure could have led to seeking an alternative treatment.

In order to determine what the associated risks and side effects of a proposed treatment are, testimony by an expert witness is necessary because these are beyond the common knowledge of ordinary people.

In Canterbury v. Spence, the Court held that, "There are obviously important roles for medical testimony in [nondisclosure] cases, and some roles which only medical evidence can fill. Experts are ordinarily indispensable to identify and elucidate for the factfinder the risks of therapy." The Court also held that, "medical facts are for medical experts."

The oncologist failed to obtain the informed consent of the parents before administering chemotherapy to their 11-year-old daughter.

Dr. Li impliedly admits that she failed to disclose to Reynaldo and Lina many of the other associated risks and side effects of chemotherapy, including the most material (infection, sepsis and death). She failed to disclose as risks and side effects (1) rashes; (2) difficulty in breathing; (3) fever; (4) excretion of blood in the mouth; (5) excretion of blood in the anus; (6) development of ulcers in the mouth; (7) sloughing off of skin; (8) systemic lupus erythematosus; (9) carpopedal spasm; (10) loose bowel movement; (11) infection; (12) gum bleeding; (13) hypovolemic shock; (14) sepsis; and (15) death in 13 days.

Infection, sepsis and death are material risks and side effects of chemotherapy.

To any reasonable person, the risk of death is one of the most important, if not the most important, consideration in deciding whether to undergo a proposed treatment. Thus, Dr. Li should have disclosed to Reynaldo and Lina that there was a chance that their 11 year old daughter could die as a result of chemotherapy as, in fact, she did after only 13 days of treatment.

The Decline of Paternalism and The Doctrine of Informed Conflict Applied in the Philippines: The Case of Nilo Rosit vs Davao Doctors Hospital and Dr. Rolando G. Gestuvo

In Rosit vs Davao Doctors Hospital, the Court held that Dr. Rolando Gestuvo is guilty of withholding material information which would have been vital in the decision of his patient in going through with the operation with the materials at hand. Thus, Dr. Gestuvo was also guilty of negligence on this ground.

In contrast to Li vs. Soliman, the case of Rosit vs. Davao Doctors Hospital did not present an expert testimony to establish the negligence of the doctor but applied the doctrine of *res ipsa loquitor* to establish such negligence. Further, the doctor admits that he did not inform his patient of the required disclosure. When the court asked Dr. Gestuvo whether he informed the patient that smaller screws were available in Manila, albeit at a higher price, he replied "*The reason I did not inform him anymore Judge because what I thought he be already hard up with the down payment. And if I will further introduce him this screw, the more he will not be able to afford the operation."* The larger screws modified with a saw was used to fixate a fractured mandible. It had struck a molar and caused the patient pain and could hardly open his mouth.

The motive of the doctor for failing to inform and ask his patients is characteristic of paternalism. In the past, medical professionals believed that they knew what is best for their patients, a trait often strengthened within the medical professional culture. 'Doctors knows best' was a view which in many ways was socially

endorsed (Parsons 'sick role' exempted an ill person from their usual social duties) but at the same time demanded cooperation with medical professionals (Trathen 2015).

If a patient might refuse what was clearly in their best interest, then better to avoid their involvement. To do otherwise would compromise the beneficent duty of the doctor, and so paternalism was not merely seen as justified, but an integral part of effective medical care (Trathen 2015).

The four essential elements a plaintiff must prove in a malpractice action based upon the doctrine of informed consent stated in Li vs. Soliman were present:

1. Dr. Gestuvo clearly had the duty of disclosing to Rosit the risks of using the larger screws for the operation. This was his obligation as the physician undertaking the operation.
2. Dr. Gestuvo failed to disclose these risks to Rosit, deciding by himself that Rosit could not afford to get the more expensive titanium screws.
3. Had Rosit been informed that there was a risk that the larger screws are not appropriate for the operation and that an additional operation replacing the screws might be required to replace the same, as what happened in this case, Rosit would not have agreed to the operation. It bears pointing out that Rosit was, in fact, able to afford the use of the smaller titanium screws that were later used by Dr. Pangan to replace the screws that were used by Dr. Gestuvo.
4. As a result of using the larger screws, Rosit experienced pain and could not heal properly because one of the screws hit his molar. This was evident from the fact that just three (3) days after Dr. Pangan repeated the operation conducted by Dr. Gestuvo, Rosit was painfree and could already speak. This is compared to the one (1) month that Rosit suffered pain and could not use his mouth after the operation conducted by Dr. Gestuvo until the operation of Dr. Pangan.

Written or Oral Consent in Dentistry in the Philippines

Apart from dentists participating in research projects where signed informed consent forms are expressly required by the Philippine Regulatory Commission Board Res. No. 14. 2008, Code of Ethics for Dentists, Dental Hygienists and Dental Technologists, there is no other requirement that informed consent must be obtained in writing. Most jurisdictions do not require a written consent. A notable exception would be the California Code of Regulations which requires that all dentists must obtain written informed consent before performing I.V. sedation (Curly 2016). Curley reminds that risk management principles recommend the use of written documentation for most informed consents. He states that written documentation is a deterrent to claims of lack of informed consent. Studies have shown that patients do not recall pretreatment discussions, and they can insist with credibility that they were not warned.

In the US, the National Institutes of Health recommended in 1979 that potential surgical risk, including any permanent condition with an incidence higher than 0.5% or any transitory condition with an incidence of 5% or more, should be part of the information provided to patients.

Delegation of Service

The law is silent on whether providing information to obtain informed consent must be done by the dentist him/herself or whether delegation may be allowed. The Code of Ethics for Dentists, Dental Hygienists, and Dental Technologists only expressly prohibits delegation of procedure, services, or operations in the mouth which require his/her personal competence as a professional. Although the dentist will ultimately be liable as the dentist shall at all times be responsible for the actions of his/her employ at the areas of his/her jurisdiction (or areas of assigned practice) during assigned clinic/duty hours (Code of Ethics, §3). In addition, the dentist is required to supervise his/her associates and his/her auxiliaries in the performance of their duties and shall at all times assure delivery of quality standard of care (Code of Ethics, §4).

Section 3. Delegation of service- Dentist shall conduct himself/herself a professional deportment at all times; therefore, he shall not delegate procedure, services, or operations in the mouth which require his/her personal competence as a professional. He/she shall only delegate services to duly licensed dentist, licensed dental hygienist, or licensed dental technologist in his/her employ to perform dental services duly expressed by law. He/she shall at all times be responsible for the actions of his/her employ at the areas of his/her jurisdiction (or areas of assigned practice) during assigned clinic/duty hours. (emphasis supplied)

Section 4. Supervision of work- The dentist shall supervise his/her associates and his/her auxiliaries in the performance of their duties and shall at all times assure delivery of quality standard of care. (emphasis supplied)

Conclusion

As admitted by the main opinion of the Court in Li vs Soliman, the Doctrine of Informed Consent has been evolving towards holistic inclusion in medical decision-making, as evident in the dissent by Justice Carpio. Instead of the doctor solely controlling the steering wheel, the perspective of the medical profession, and consequently also the dental profession is moving towards properly guiding the patient towards a safe harbor.

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Chapter 9

Global Periodontal Health Strategies: An Update and Perspective

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Introduction

Periodontal disease is recognized as one of the global epidemics and a major oral health burden with devastating socio-economic impact and consequences as well as enormous healthcare costs (Pihlstrom *et al* 2005; Petersen & Ogawa 2012; Chapple 2014; Kassebaum *et al* 2014; Listl *et al* 2015; Jin *et al* 2016). The 1st global burden of disease (GBD) study (1990-2010) revealed that severe periodontitis is the 6th most significant disease burden of mankind among all 291 diseases/conditions and injuries investigated, and 11.2% of the world population are affected (Marcenes *et a*l 2013; Kassebaum *et al* 2014; Herrera *et al* 2018). A follow-up GBD study (1990-2016) showed that severe periodontal disease is ranked as the 11th most prevalent disease among all 328 diseases and injuries in 195 countries (GBD 2016 Disease & Injury Incidence and Prevalence Collaborators 2017; World Health Organization 2018). Severe periodontitis accounts for multiple tooth loss and edentulism in adult populations, and profoundly affects oral health and function, thereby significantly disturbing patients' esthetics, limiting nutrient intake, reducing quality of life and contributing to low self-esteem (Pihlstrom *et al* 2005; Jin *et al* 2011; Tonetti *et al* 2017; Herrera et al 2018). Moreover, periodontal disease is a serious inflammatory condition and its impact is beyond the mouth. It is evident that periodontitis is intimately linked with various life-threatening non-communicable diseases (NCDs) such as diabetes mellitus (DM), cardiovascular disease (CVD), certain types of cancer and respiratory illnesses (Tonetti & Kornman 2013; Jin *et al*, 2016; Monsarrat *et al.* 2016; Herrera *et al* 2018). Currently, a major challenge facing oral healthcare professionals is how to increase the public's awareness of oral/periodontal health and healthcare, through proactive prevention and inter-professional collaborations. In the past decade, significant advances have been made in periodontal science and periodontal medicine as well as progressive developments in global oral/periodontal and general health policies and strategies, which are of profound importance for tackling periodontal disease and further promoting global oral/periodontal health and healthcare.

This paper gives an update on the scientific rationale, framework and updates for proactive prevention of periodontal diseases and effective periodontal

healthcare, and then highlights the recent developments and implementation in global periodontal health strategies and actions for oral/periodontal health and general health.

Microbe-host symbiosis and oral/ periodontal health

It is noted that the human body consists of trillions of cells, surprisingly mostly commensal microbes that outnumber the host cells about ten to one; and majority of these commensals reside in gut, respiratory and urinary tracts, skin and mouth (Wilson *et al* 1997; Cohen & Slavkin 2000; Paster *et al* 2001; Hair & Sharpe 2014). In general, a normal individual is recognized as a complex community of 'superorganism' with 'holobiont-hologenome', containing the host cells, all symbiotic microbes (e.g., bacteria, fungi, viruses and protozoa), host genome and total microbiome (Margulis 1991; Zilber-Rosenberg & Rosenberg 2008). It is of fundamental importance to note that host–microbe symbiosis critically contributes to the overall development of anatomical structure and physiological functions as well as the establishment of immunological system via cooperation and competition approaches (Rosenberg & Zilber-Rosenberg 2016). Conceivably, host–microbe symbiosis and homeostasis are central to oral/periodontal health. In this regard, human periodontal tissue is well armed by multifunctional innate defense molecular systems (Jin 2011). An update on periodontal pathogenesis has reflected this important notion by adding the framework of periodontal health (Meyle & Chapple 2015). Moreover, periodontal/ gingival health has been incorporated for the first time in the new classification of periodontal diseases and conditions (Caton *et al* 2018).

It is evident that disturbance of host–microbe symbiosis leads to host–microbe dysbiosis triggering the onset and progression of a number of inflammatory diseases such as periodontal disease. It has been emphasized that understanding how host cell interactions are subverted by microbial pathogens is essential to understand common infections and resultant inflammatory diseases as well as provide a framework for healthcare (Bhavsar *et al* 2007). There are varying multi-scale mechanisms and pathways accounting for these key events.

P. gingivalis as a 'keystone' periodontopathogen – a challenge note

P. gingivalis, one of the 'red complex' periodontopathogens, is claimed to be the 'keystone' periodontopathogen (Hajishengallis *et al* 2012), owing to its unique pathogenicity and virulence (e.g., featured survival strategy, host invasion and evasion, and activation for dysbiosis) (Honda 2011; Bostanci & Belibasakis 2012; Darveau *et al* 2012; Lamont & Hajishengallis 2015). Furthermore, this noxious pathogen at a low abundance may play a crucial role in the shift of host–microbe symbiosis to dysbiosis, thereby resulting in dysregulated immuno-inflammatory responses and periodontal destruction (Hajishengallis *et al* 2011; Honda 2011). Hence, it has been proposed that a promising strategy is to tackle such keystone pathogens, that have markedly negative impacts on commensal microbial community, and to effectively treat polymicrobe-induced inflammatory diseases such as periodontitis (Honda 2011). In recent years, several studies on targeting *P. gingivalis* have been undertaken. A recombinant immunogen against *P. gingivalis* gingipains has been

shown to be a potentially useful adjunct to manage *P. gingivalis*-mediated disease such as periodontitis (Reynolds *et al* 2015). Small molecules like Alop1 and dynasore may protect human oral keratinocytes from the invasion of *P. gingivalis* and its outer membrane vesicles (Ho *et al* 2016). Very recently, work from our laboratory has provided the first evidence that bismuth drugs enable to effectively conquer *P. gingivalis* in its planktonic and biofilm forms. Bismuth drugs also affect invasion of this bacterium into host cells (e.g., human gingival epithelium progenitors, human oral keratinocytes and coronary artery endothelial cells), by influencing the energy metabolism, inactivating multi-virulence components (e.g., fimbriae, hemagglutinin HagA and gingipains) and disturbing iron acquisition (Cheng *et al* 2019). Profoundly, these bismuth drugs can favorably modulate the immuno-inflammatory responses in human gingival epithelium progenitors perturbed by *P. gingivalis* (Cheng *et al* 2019).

Disturbance of microbe-host symbiosis by ecological/ environmental and host conditions

Over the past decade, it has been demonstrated that genetic and ecological/ environmental changes seriously disturb the microbe-host symbiotic interactions including both types of mutualism and commensalism, and subsequently lead to the initiation and progression of inflammatory diseases (Dethlefsen *et al* 2007). As such, this notion is crucial to develop new strategies for healthcare. The emerging ecological plaque hypothesis addresses that environmental and host factors (e.g., life-style variables and inflammatory status) dictate microbial composition, disrupt host-microbe symbiosis and thereby enormously increase the risk for oral diseases such as periodontitis (Marsh & Devine 2011; Marsh & Zaura 2017). For example, *P. gingivalis* produces two isoforms of lipopolysaccharides (LPS) with opposing immunoregulatory effects (Dixon & Darveau 2005). Interestingly, *in vitro* experiments have demonstrated that hemin level and culture temperature significantly alter the activities of *P. gingivalis*. This may be via downregulating the expression of the isoform of *P. gingivalis* LPS 1690 under low hemin/temperature conditions, while also upregulating the expression of pathogenic isoform of LPS 1435/1449 under high hemin/temperature conditions that represent the main elements of uncontrolled inflammatory status (Al-Qutub *et al* 2006; Curtis *et al* 2011). Our work has shown that these two isoforms of *P. gingivalis* LPS differently modulate the expression profiles of various innate defense molecules (e.g., hBD-2, IL-6, IL-8, MMP-3 and LPS bonding protein (Lu *et al* 2009; Herath *et al* 2011; Ding *et al* 2013a,b; Herath *et al* 2013a,b; Ding & Jin 2014; Herath *et al* 2016; Ding *et al* 2017). These findings collectively suggest that periodontal inflammation may stimulate the growth of 'the bad phenotype of *P. gingivalis*', and that inflammation-induced environmental niches may enhance the functional activities of the pathological phenotype of *P. gingivalis* cells hereby contributing to periodontal pathogenesis. Of note, a recent study has assessed and compared the metagenomic profiles of microbiomes at periodontally healthy subjects and patients with generalized chronic periodontitis through whole genome shotgun sequencing (Dabdoub *et al* 2016). Surprisingly, the microbial metagenomes of clinically 'healthy' sites in periodontitis patients are functionally

affiliated with the periodontitis sites, rather than the healthy sites from the periodontally healthy counterparts (Dabdoub *et al* 2016). Taken together, these findings indicate that genetic, environmental and host factors predominantly determine the microbial profiles and functionality, and therefore play critical roles in health maintenance and disease onset/development. Emerging evidence highlights the importance of effectively controlling inflammation and risk factors like tobacco usage as well as promoting healthy life-style, other than tackling plaque biofilms alone (Marsh & Devine 2011; Bartold & van Dyke 2013, 2017). Recently, we have explored novel approaches to controlling inflammation and related infections more precisely and effectively, such as nano-based drug delivery systems (Seneviratne *et al* 2014; Leung *et al* 2016; Li *et al* 2016a,b; Li *et al* 2017).

Periodontal disease and systemic comorbidities – emerging concepts and implications

Periodontal health is intimately interconnected with general health and wellbeing. It has been documented that a total of 57 diseases and conditions in mankind have been associated with periodontal diseases according to the World Health Organization (WHO) database (Monsarrat *et al* 2016). The GBD study (1990-2010) shows that the global burden of periodontal disease increased by 57.3% in line with the DM (69%), neoplasms (27%) and CVD (23%) (Murray *et al* 2012; Jin *et al* 2016). Clinically, the beneficial effects of periodontal care on the management of common NCDs like DM and CVD have been well documented (Tonetti *et al* 2007; Teeuw *et al* 2010; Lalla & Papapanou 2011) Tonetti et al 2007) as well as improvements of the profiles of endothelial progenitor cells as shown in our previous studies (Li *et al* 2011a,b; Wang *et al* 2017). In addition, periodontal treatments can contribute to reducing medical costs and hospitalization rate in patients with type 2 DM, cerebral vascular disease and coronary artery disease who concurrently suffer from periodontitis (Jeffcoat *et al* 2014).

So far, the identified underlying pathways linking oral/periodontal conditions with systemic conditions include noxious infections, systemic inflammation and microbe-host dysbiosis as well as common genetic/acquired risk factors like tobacco use and obesity (Thoden van Velzen *et al* 1984; Sheiham & Watt 2000; Parahitiyawa *et al* 2009; Bochenek *et al* 2013; Genco *et al* 2014; Nakajima *et al* 2014; FDI World Dental Federation 2019a). It has been speculated that periodontal health may be a sign of full-body 'strength' for certain individuals. Our previous 16-year longitudinal study demonstrated that young adults with periodontitis experience significantly increased risk of premature death before age 60 due to life-threatening diseases (e.g., circulatory system diseases, neoplasms and digestive system diseases) (Söder *et al* 2007). Moreover, we recently carried out an 18-year retrospective cohort study, and our findings showed that an individual's periodontal condition reflects overall host susceptibility to the onset and progression of a number of common systemic comorbidities, including the four major killers of life (DM, CVD, cancer and chronic obstructive pulmonary disease) as well as cognitive impairment, hypertension and dyslipidemia (Zhao *et al* 2019).

With further studies, the sub-group of susceptible individuals in a given population may be potentially identifiable by assessing their periodontal

status (Zhao *et al* 2019). This work could inspire dental and medical professionals to deliver personalized healthcare proactively and effectively, via inter-professional teamwork for better oral/periodontal health and general health. Notably, our novel finding is in line with the emerging notion of interactome networks of human disease modules, highlighting that a series of overlapping and inter-connected diseases/ disorders may occur through co-expression patterns of multiple systemic comorbidities with possibly similar signs, symptoms and underlying pathological pathways (Menche *et al* 2015).

Global strategies, policies and actions

The four common NCDs, namely DM, CVD, cancer and chronic respiratory diseases, are the predominant causes for two thirds of mortality worldwide and contribute to a global socio-economic crisis (Beaglehole *et al* 2011a.b; Ezzati & Riboli 2012). Oral disease is considered as one of the main global disease burdens (Petersen et al 2005). Remarkably, the 2011 United Nations High-level Meeting on Prevention & Control of NCDs addressed that these life-threatening NCDs and oral diseases can be better managed through the Common Risk Factor Approach (CRFA) (Sheiham & Watt 2000; United Nations 2011) for proactive health promotion and disease prevention. As tobacco smoking is one of the leading risk factors for periodontitis, full implementation of CRFA is of profound importance to enhance the public and professional awareness of the importance of global oral health and general health.

To further advocate and advance on health equity as well as achieve the sustainable social development goals, WHO initiated and developed the 'Health in All Policies' (HiAP) Framework in 2014, calling for global actions on the critical elements identified (WHO 2014). This important global approach requires all public policies in social sectors to address the health implications of various important decisions and enhance the cooperation and synergic health impacts for global health and health equity. In responding to this important strategic framework, dental professionals should promote oral health in all health-related policies (OHiAHP) in national health agendas (FDI 2019b). As such, periodontal healthcare professionals also advocate for periodontal health in all oral health-related policies for improving awareness of periodontal health and promoting oral/periodontal health literacy (Jin 2015: Kumarswamy *et al* 2015). It is also noted that the United Nations approved the 2030 Sustainable Development Goals (SDG) in 2015 (United Nations 2015a), and one of the targets set aimed to achieve the Universal Health Coverage (UHC) – "all individuals and communities receive the health services they need without suffering financial hardship" (WHO 2019). Hopefully, oral/periodontal healthcare could be considerably covered in the UHC framework of actions and goals in the near future for achieving the SDG 3 – "Ensure healthy lives and promote well-being for all at all ages". (United Nations 2015b).

Meanwhile, it is very inspiring to see the great initiative and progress made in promoting global periodontal health across the global oral/periodontal professional communities over the past three years. It is worth noting that the 'Perio-Green Paper' reached for the first time a global consensus on setting essential strategies and approaches for proactive prevention (10 recommendations), early diagnosis

(2 approaches) and effective periodontal treatments (14 recommendations), in line with the global healthcare policies and strategies of United Nations and WHO (Tonetti *et al* 2017). Some of the notions and important points have been reflected in the new classification of periodontal diseases and conditions (Caton *et al* 2018; Tonettis *et al* 2018). Indeed, the European Federation of Periodontology (EFP) has published a series of consensus reports and useful guidelines for promoting periodontal health and primary/secondary prevention of periodontal diseases (EFP 2014a-e; Sanz *et al* 2015; Tonetti *et al* 2015). Currently, there are over 1.1 million oral healthcare professionals in the world, and how to actively engage them as well as other main stakeholders (e.g., oral health policy-makers, other healthcare professionals, school systems, social workers, payers, dental industries, patient groups and the public at large) for global periodontal health is of great significance and urgently needed. It is noteworthy that FDI World Dental Federation officially kicked off its 'Global Periodontal Health Project' (GPHP) through the engagement of the FDI GPHP Task Team at the World Oral Health Forum during the 2017 FDI World Dental Congress in Madrid, for reducing the global burden of periodontal diseases (FDI GPHP Task Team and panelists 2017). In this important forum, three key questions were discussed and elaborated interactively including 'Why are periodontal diseases important?', 'Are periodontal diseases preventable and treatable?' and 'Current problems and recommended actions', led by the speakers and expert panel members. Subsequently, a series of actions have been undertaken and GPHP assets have been developed, published in FDI website, widely disseminated and used by many members of national dental associations and other stakeholders, including the white paper (Herrera *et al* 2018), promotion toolkits (FDI GPHP Task Team 2018a), chair-side guide (FDI GPHP Task Team 2018b), proceedings and symposium (FDI GPHP Task Team 2018c, 2019a), and the first global survey report of GPHP (FDI GPHP Task Team 2017). Furthermore, FDI's first policy statement on 'Global Periodontal Health' was also highly approved by the General Assembly during the 2018 FDI World Dental Congress in Buenos Aires (FDI 2019a). Notably, 61 national dental associations from 59 countries actively participated in the 2017 global survey (FDI GPHP Task Team 2017), and subsequently 69 national dental associations from 67 countries completed the follow-up survey in 2019 (FDI GPHP Task Team 2019b). In this survey, the same set of questionnaires was used allowing for assessing the impacts of this global periodontal health initiative on national oral health policies as well as measuring the outcomes and implementation of the actions recommended on a national level. These two surveys cover four aspects including i) national health agendas and policies; ii) health and healthcare information systems; iii) periodontal health education and workforce; and iv) for periodontal health preventive and promotional activities undertaken by FDI members of national dental associations (FDI GPHP Task Team 2017, 2019b). The findings are very positive and encouraging. Two thirds of the national dental associations have actively committed to address the challenges of NCDs, and are concurrently engaged in the promotion of periodontal health that has increased from 50% in 2017 to 61% in 2019, and prevention of periodontal diseases rising from 50% to 59% during the same period. Performing periodontal screening during dental checkups has increased by 15%

(50% to 65%). It is also noted that 69% of national dental associations will continue to organize campaigns for periodontal health in the public community. This FDI initiative for global action has increasingly contributed to promoting periodontal health and healthcare for oral health and general wellbeing.

Periodontal health is an important component of everyone's general health and wellness, and it is crucial for promoting good quality of life and healthy aging. It is believed that oral/periodontal health is beneficial to all as a unique component of total health for a lifetime. The important goal of global periodontal health can be achieved through a strong teamwork worldwide in the future.

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Chapter 10

New Era of Periodontal Regeneration

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Introduction

Periodontal disease is a chronic inflammatory disease caused by bacterial biofilms (dental plaque). Progression of this disease leads to the destruction of periodontal tissue. The principle of periodontal treatment is to mechanically remove the biofilm and calculus along with necrotic cementum on the root surface and alleviate inflammation in the periodontal tissue. Although conventional periodontal therapies such as scaling and root planing control periodontal disease, these therapies alone are not enough to induce regeneration of the destroyed periodontal tissue. Thus, several treatment procedures have been clinically applied to regenerate periodontal tissue damaged by periodontal disease. Bone grafting, guided tissue regeneration (GTR), and local administration of an enamel matrix derivative (EMD) have been applied clinically and their efficacy and safety have been confirmed. However, more effective and highly predictable regenerative therapies are still desired.

Cytokine Therapy

Several novel therapies have been developed to effectively induce regeneration of periodontal tissue by local administration of recombinant human cytokines to periodontal tissue defects. This therapy is called "cytokine therapy". In the US, the efficacy to induce periodontal tissue regeneration by platelet-derived growth factor-BB and β-tricalcium phosphate has been assessed clinically, and in 2005, this medical device was approved by the Food and Drug Administration and commercialized (GEM-21s®). More recently, our research group has developed a novel therapy for periodontal tissue regeneration by topical administration of recombinant human fibroblast growth factor-2 (hrFGF-2) to periodontal tissue defects to induce periodontal regeneration at the site.

Cytokine therapy using FGF-2

A nationwide phase IIb clinical trial was undertaken at 25 dental facilities in Japan to verify the safety and efficacy of the FGF-2 treatment by comparison with a placebo. The study showed that FGF-2 induced a statistically significant increase in alveolar bone neogenesis at locally applied sites, and that the optimal concentration of FGF-2 for periodontal regeneration was 0.3% (Kitamura *et al*. 2011) (Figure 1).

Thereafter, we carried out a nationwide non-inferiority trial (phase III), comparing Enamel Matrix Derivative (EMD) with

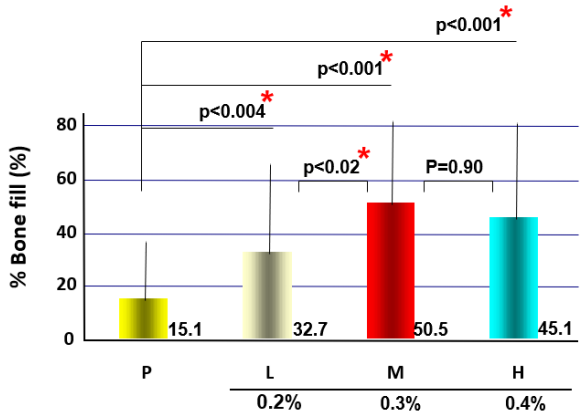

Figure 1. Percentages of new alveolar bone formation induced by FGF-2 medicine (Regroth®): The outcome of Phase IIB clinical trial. By the standardized dental X ray examination, the percentages of new alveolar bone formation at the test sites was analyzed. This result demonstrates that the optimal concentration of FGF-2 for periodontal regeneration is 0.3%. (Modified from *Kitamura et al*. 2011)

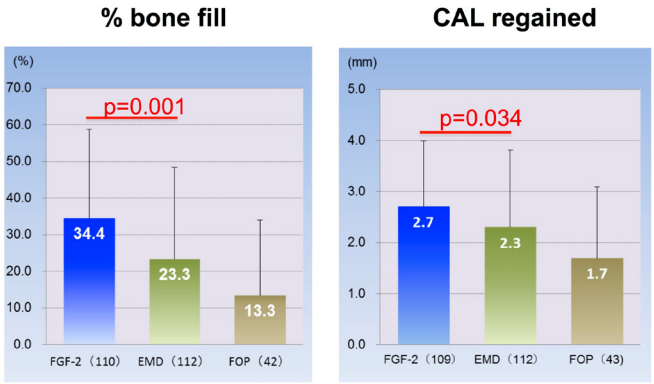

Figure 2. % bone fill and CAL regained 9 months after treatment with FGF-2 medicine (Regroth®) or EMD:
The outcome of Phase III clinical trial.
Regarding % bone fill and CAL regained, the superiority of FGF-2 medicine (Regroth) to EMD was demonstrated. (Modified from Kitamura et al. 2016)

0.3% FGF-2 at 15 dental facilities in Japan to further investigate the clinical efficacy and relevance of the 0.3% FGF-2 treatment. Based on the results of a series of clinical trials, the FGF-2 medicine (Regroth®) was officially approved by the Ministry of Health, Labour and Welfare of Japan. This clinical trial clearly revealed not only the non-inferiority, but also the superiority of FGF-2 as an EMD in terms of clinical efficacy for the induction of periodontal tissue regeneration at 9 months after administration (Kitamura *et al*., 2016) (Figure 2). During these clinical

trial periods, no serious side effects were observed.

Future regenerative therapy

Guided tissue regeneration, EMD, and cytokine therapy for periodontal regeneration promote periodontal regeneration by stimulating proliferation, migration, and/or differentiation of endogenous stem cells within the periodontal tissues. However, the number and functions of stem cells in our body are known to decrease with age. Similarly, the endogenous stem cell population in periodontal tissues is believed to have decreased cellular abilities such as proliferation and cyto-differentiation into hard tissue forming cells. Moreover, tissue destruction caused by severe periodontitis results in a shortage of the number of endogenous stem cells, leading to insufficiency of inducing periodontal tissue regeneration. Thus, there may be a need for "cell therapy" whereby stem cells collected from other tissues are transplanted into the defective periodontal tissue.

Cell therapy

"Cell therapy" aims to induce periodontal tissue regeneration by transplanting stem cells, which play a central role in tissue regeneration, into periodontal tissue defects. The selection of stem cells to be transplanted is a crucial issue for cell therapy. Potential candidates include embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and somatic stem cells. Although ES and iPS cells may possess treatment potentials for regeneration medicine, they still have many challenges that need to be resolved, such as ethical and safety issues including neoplastic transformation. For these reasons, somatic stem cells, especially mesenchymal stem cells (MSCs) collected from the patients themselves are currently under intense investigation for clinical use in a number of clinical studies. Detailed mechanisms of tissue regeneration by transplantation of MSCs are being elucidated gradually (Figure 3). It is well understood that transplanted MSCs directly differentiate into various cell types constituting the target tissues by

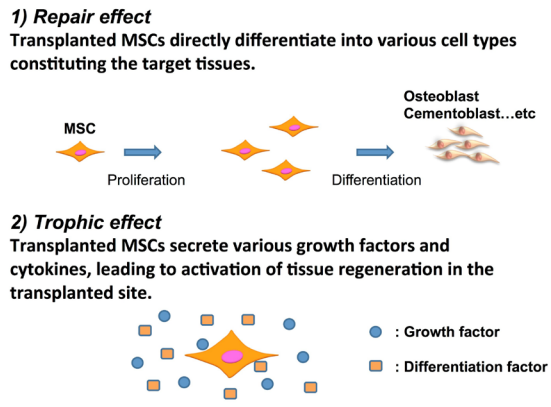

Figure 3. Mechanisms of tissue regeneration by transplantation of mesenchymal stem cells (MSC). Two mechanisms, repair effect and trophic effect, by which auto-transplantation of ADMPC stimulates periodontal regeneration are expected.

which the "repair effect" plays a key role in inducing periodontal tissue regeneration in the transplanted site. Additionally, recent studies have indicated the importance of "trophic effects" by which transplanted MSCs secrete various growth factors and cytokines, leading to activation of tissue regeneration in the transplanted site.

Periodontal regeneration therapies using bone marrow-derived MSCs, periodontal ligament (PDL) stem cells, and alveolar bone periosteal cells have been studied and their efficacy and safety has been demonstrated (Yamada *et al*., 2006; Iwata *et al*., 2018; Okuda *et al*., 2013). MSCs within the PDL and alveolar bone periosteal cells may be promising candidates as stem cell sources, but are limited in terms of the harvestable amount of tissue, whereas bone marrow-derived MSCs involve an invasive harvesting procedure and are known to exhibit low proliferation.

Adipose tissue-derived multilineage progenitor cells (ADMPCs) are highly purified adipose tissue-derived mesenchymal stem cells (ADSCs)

To overcome these issues, the use of adipose tissue-derived stem cells (ADSCs) has been investigated. ADSCs can be harvested less invasively and relatively easily compared with stem cells derived from other tissues. Additionally, ADSCs possess a high renewal capacity and proliferative ability, and produce an abundance of cytokines that stimulate regenerative processes. Clinical studies have demonstrated that autologous transplantation of ADSCs induces a wide variety of tissue regeneration effects including improvement of urethral sphincter functions, bone regeneration in skull defects and maxillary and mandibular defects, and closure of the intestinal cutaneous fistula and tracheal fistula.

To establish a novel cell therapy for periodontal regeneration, we focused on adipose tissue-derived multi-lineage progenitor cells (ADMPCs), which can be isolated from subcutaneous adipose tissue as described by Okura *et al*., as a promising a stem cell source. ADMPCs are highly purified MSCs isolated from ADSCs by additional purification with EDTA treatment and can efficiently differentiate into a variety of cell types such as hepatocytes, islet cells, and cardiomyocytes *in vitro* (Okura *et al*., 2011). Furthermore, *in vivo* experiments using beagles have demonstrated that transplantation of ADMPC together with fibrin gel (Bolheal®) into periodontal tissue defects induces significant periodontal regeneration accompanied by neogenesis of the cementum and alveolar bone (Ozasa *et al*, 2014).

Repair effect of ADMPCs in periodontal regeneration

To examine the differentiation ability of human ADMPCs into periodontal tissue-forming cells, the ADMPCs were cultured in mineralization medium (M-Med). Real-time PCR revealed expression of mineralization-related genes *RUNX2* and the PDL-specific gene *PLAP-1*mRNA (Figure 4). Furthermore, the ADMPCs cultured in M-Med had increased mRNA expression and enzyme activity of alkaline phosphatase and formed calcified nodules detected by alizarin red staining (Figure 5). These results suggest that human ADMPCs have a differentiation ability for PDL cells and hard tissue-forming cells such as osteoblasts and cementoblasts.

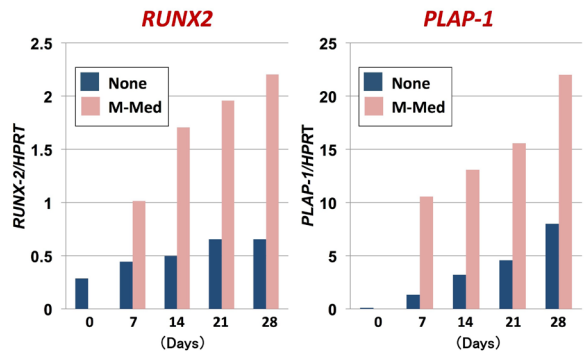

Figure 4. *Mineralization-related gene expression in human ADMPC.* Human ADMPCs were cultured in control or mineralization medium (M-Med) for 7, 14, 21, 28 days. *RUNX2* and *PLAP-1* mRNA expressions in ADMPC were examined by Real-time PCR. Values are indicated as ratios relative to *HPRT* (hypoxanthine phosphoribosyltransferase).

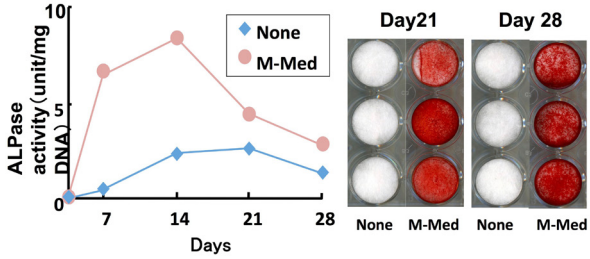

Figure 5. *Alkaline phosphatase (ALPase) activity and calcified nodule formation of human ADMPC.* Human ADMPCs were cultured in control or mineralization medium (M-Med) for 7, 14, 21, 28 days. ALPase activity and calcified nodule formation were assayed by Bessey-Lowry method and alizarin red staining, respectively.

Trophic effects of ADMPCs on cytodifferentiation of PDL cells into hard tissue-forming cells

To examine the effects of ADMPC-derived cytokines on the proliferation and cytodifferentiation of human PDL (HPDL) cells into hard tissue-forming cells, the culture supernatant of ADMPCs (ADMPC-CM) was harvested and 50% ADMPC-CM was added to M-Med to stimulate HPDL cells. A proliferation assay demonstrated that ADMPC-CM had no effect on the proliferation of HPDL cells. However, real-time PCR analysis showed significantly higher expression of *ALP*, *RUNX2*, and *COL1a* mRNAs in the ADMPC-CM-supplemented group after 6 days of culture compared with the control group (Figure 6). Furthermore, significantly higher ALP activity and significantly enhanced calcified nodule formation were observed in the ADMPC-CM-supplemented group compared with the control group. These results suggest that ADMPC-derived cytokines stimulate the differentiation of HPDL cells into hard tissue-forming cells (Sawada et al., 2015).

The cytokines contained in ADMPC-CM were investigated by the Human Growth Factor Array®. The analysis revealed the presence of several growth factors, such as insulin-like growth factor binding protein 6 (IGFBP6), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF), in ADMPC-CM. Enzyme-linked immunosorbent assays showed the production of high levels of IGFBP6, HGF, and VEGF. Among them, IGFBP6 was the most abundant in ADMPC-CM. Interestingly, the positive effects of ADMPC-CM on the cytodifferentiation of HPDL cells were suppressed significantly by transfecting ADMPCs with IGFBP6 siRNA. This suggested that IGFBP6 plays important roles in ADMPC-induced periodontal regeneration (Sawada *et al*., 2015).

Efficacy and safety of autotransplantation of ADMPCs for periodontal regeneration

A clinical study was carried out to examine the efficacy and safety of autotransplantation of ADMPCs for periodontal regeneration. In this clinical study, 12 severe periodontitis patients were registered. The ADMPCs were isolated from abdominal adipose tissue of the patients and autotransplanted into intraosseous alveolar bone defects together with fibrin gel (Bolheal®). This clinical study showed no serious adverse events throughout the study period and a considerable amount of alveolar bone formation was confirmed

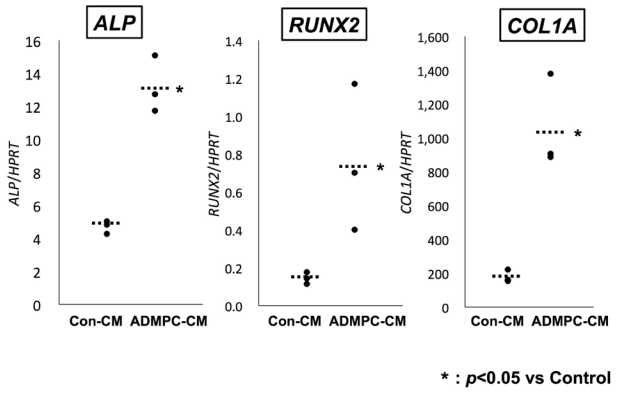

Figure 6. Effects of ADMPC-derived humoral factors on HPDL differentiation into hard tissue-forming cells. Human ADMPC were cultured for 6 days in M-Med with (ADMPC-CM) or without (Con-CM) 50% ADMPC-CM. The expressions of ALP, RUNX2, and COL1a mRNA were examined by Real-time PCR. Values are indicated as ratios relative to HPRT (modified from Sawada et al. 2015)

at the transplanted sites by standardized dental X-ray analysis.

Discussion

The ultimate goal of periodontal treatment is to restore not only the anatomical structure, but also the functions of periodontal tissue damaged by periodontitis. Conventional periodontal treatments, such as scaling and root planing, can stop progression of the disease, but do not stimulate meaningful periodontal regeneration. Introducing the concept of tissue engineering, which effectively combines the use of stem cells, scaffolds, and signaling molecules, is essential to develop ideal regenerative therapies to treat severe periodontal tissue defects. The results of our studies suggest that FGF-2 can be used as a "signaling molecule", and that ADMPCs play important roles in inducing periodontal tissue regeneration as "stem cells". Interestingly, ADMPC-derived cytokines promote periodontal tissue regeneration as "signaling molecules" in autocrine and paracrine manners. When we can develop a novel scaffold customized for periodontal regeneration and successfully merge the abovementioned three elements of tissue engineering in a successful manner, we can maximize the outcome of the therapy and induce ideal periodontal regeneration in any type of severe bony defect such as 1- or 4-wall bone defects and horizontal bone defects. Further investigation in this field is still strongly desired.

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Chapter 11

Novel Grafting Materials for Bone Regeneration

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Oral applications of different grafting technologies and materials include periodontal regeneration (Richardson *et al*. 1999; Velasquez-Plata *et al.* 2002; AlGhamadi *et al.* 2010) , peri-implantitis (Schwarz *et al*. 2006), sinus elevation to enable implant placement (Jensen *et al.* 1996; Yildirim *et al.* 2001), and bone regeneration following tooth loss due to trauma or alveolar ridge preservation. Grafting materials may be placed into a tooth socket prior to implant placement (Vittorini Orgeas *et al.* 2013; Avila-Ortiz *et al.* 2014; Atieh *et al.* 2015) or accompany immediate implant placement if there is a disparity between implant and tooth socket diameters (Araújo *et al.* 2011), or be used to cover exposed implant surfaces when there are deficiencies of alveolar bone (Hämmerle *et al.* 2004). Even when a autogenous block bone graft is used to augment a healed, deficient alveolar ridge, this is often accompanied by the use of bone replacement grafting materials and/or resorbable membranes (Cosyn *et al.* 2013).

The development of guided tissue regeneration using membrane technologies to regenerate firstly periodontal tissues (Pontoriero *et al*. 1988) and then bone (Dahlin *et al*. 1988) led to the concept of guided bone regeneration, either prior to or contemporaneous with implant placement (Nyman *et al*. 1990). This evolved over forty years from non-resorbable to resorbable membranes (Hurzeler *et al*. 1996) with bone replacement grafting materials (Zitzmann *et al*. 1997) and bioactive substances (Sharma and Pradeep, 2011; Lekovic *et al*. 2003; Miron *et al*. 2016); this history has recently been comprehensively reviewed (Scantlebury and Ambruster, 2012). Research continues with a view towards the development of so-called “third generation” biofunctionalized membrane and grafting technologies (Sam

and Pillai, 2014), with an eye towards fourth-generation technologies where appropriate carriers may be engineered for the transplantation of mature periodontal ligament cells (Ishikawa *et al*. 2009) or appropriate multipotent "stem" cells (Bartold *et al*. 2006). In a review of this therapeutic approach, Monsarrat *et al*. (2014) commented that "the challenge remains to identify the best combination of cells, biomaterials, and biomolecules for various clinical situations, using animal models that best represent the etiopathophysiology" of the human clinical situation.

Clinical translational research requires a multidisciplinary team approach and involves multiple steps from initial product concept to human phase 1 clinical trials (Figure 1). A key part of this is the use of animal models to validate in vitro findings; such preclinical work provides the supporting base for the so-called "evidence pyramid' (Varoni *et al*. 2015). In New Zealand we have a long history using sheep as an animal model, for examination of regenerative periodontal therapy, initial implant healing, stem cell therapy, sinus grafting, peri-implantitis and alveolar ridge preservation; this work has been presented at previous meetings of the Asian and Pacific Society of Periodontology (Duncan, 2014, 2016; Duncan *et al*. 2018). Our lab uses four main sites in sheep, each reflecting different conditions in humans: the healed edentulous mandibular ridge, the maxillary sinus, the femoral epicondyle, and mandibular tooth sockets (Figure 2). We have used these sites for testing commercially-available products as well as for our own experimental work developing new materials. Our experimental designs attempt to address various considerations that dictate the possibility of translating our work, from laboratory to chair-side use by dental practitioners (Figure 3).

Building on the work of Lindhe and colleagues in the dog tooth socket model (Cardaropoli *et al*. 2003; Araújo and Lindhe, 2005) we established a similar model using sheep mandibular premolar sites. We then used this large animal model for testing a novel regenerative biomaterial that consisted of tricalcium phosphate (TCP) nanoparticles, flame-spray synthesised from calcium hydroxide and electrospun with poly(lactide-co-glycolide) (PLGA) at a ratio of 2:5 (40% TCP) into 10 micron fibres. This formed a flexible, highly-porous cottonwool-like material. Previous testing by the laboratory that developed the material, showed encouraging results in a small animal (rabbit) cranial defect model (Schneider *et al*. 2008). In our lab, comparison with sheep tooth sockets grafted using a bovine bone xenograft and cross-linked collagen membrane, demonstrated comparable results after 18 weeks healing, but without the presence of residual un-resorbed grafting material in the tooth sockets (Liu *et al*. 2016). Furthermore, these results confirmed the utility of this animal model. Simultaneous grafting into a peri-implant critical-size defect in the femoral epicondyle of the same animals also provided valuable information. However, one drawback of this material was the extremely slow process required for production, making it unlikely that this could be scaled-up for commercial production.

Subsequent experiments focused on testing of another novel material in 11 sheep, again with simultaneous use of two sites (mandibular premolar sockets and maxillary sinus).

1.	Initial product development
	a. Xenografts, de novo alloplasts
	b. Processes eg: 3-D printing, sol-gel, heat-treatment
2.	Biomechanical characterization
	a. Strength, elastic modulus, robustness during handling
	b. Surface roughness, surface charge and hydrophilicity
3.	In vitro cell culture & microbiology
	a. Cell attachment, proliferation, deposition
	b. Stem cell chemotaxis, transformation
	c. Angiogenesis
	d. Antimicrobial activity
4.	Testing in appropriate, FDA-approved animal models
	a. Small animal critical size defect (CSD)
	b. Large animal clinical simulation
5.	Human phase 1 clinical trials

Figure 1. Steps in Clinical translational research

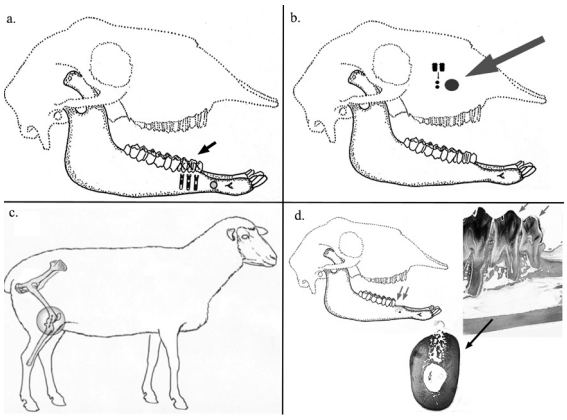

Figure 2. Anatomical sites in the Otago sheep model. (a) Healed mandibular premolar sites with dental implants (b) maxillary sinus with graft and implants (c) femoral epicondyle (d) mandibular premolar extraction sockets.

1.	Safety and efficacy
2.	Scalable processes
3.	Cost benefit to patient
4.	Add value to existing treatments
5.	Protection of intellectual property
6.	Route to market
7.	International connections, marketing
8.	Clinician approval and acceptance

Figure 3. Over-arching considerations during clinical translational research

The tested material consisted of a commercially-available cone-shaped device made of type I equine collagen, reinforced with biphasic calcium phosphate granules (60% hydroxyapaptite (HA) and 40% TCP) ((Parasorb ConeOss®, Resorba Medical GmbH). Controls included un-grafted tooth sockets and bovine xenograft with collagen membrane (BioOss® and BioGide®, Geistlich Pharma New Zealand Ltd.). After 16 weeks, histomorphometric analysis showed equivalent results comparing

the test materials against BioOss® for formation of new bone, both in tooth sockets (Lander, 2016) and in the maxillary sinus (Sheftel *et al*. 2019), however unlike BioOss®, the test material did not prevent alveolar ridge atrophy. In this experiment, dental implants were not placed into the grafted sinus sites, however the results we achieved with both the test material and the bovine xenograft were comparable to our previous work (Phillip *et al*. 2014), where implants were placed into sheep maxillary sinus with simultaneous grafting using a synthetic alloplastic material consisting biphasic calcium phosphate particles, 60% HA and 40% beta-TCP (Straumann® Bone Ceramic, Institut StraumannAG, Basel, Switzerland).

Our current work has evolved from two streams of investigation. Building on previous work with dental restorative materials that incorporated nano-silver (Garden *et al*. 2013), we developed a nano-silver antibacterial gel that we tested for safety and efficacy in a split-mouth periodontitis/peri-implantitis model in sheep (Duncan *et al*., 2018.) Simultaneously we had been working on a novel bovine xenograft material called MoaBone® (Molteno® Ophthalmic Ltd., Dunedin, New Zealand), a by-product of the milling of hydroxyapatite spheres used for implantation into eye-sockets following orbital enucleation (Jordan *et al*. 2000; Suter *et al*. 2002). Implantation of these highly-processed bovine hydroxyapatite granules into our sheep sinus model resulted in comparable histomorphometric outcomes to the BioOss® controls, however the test material showed greater osteoclastic resorption; it was apparent that this material needed further optimisation in order to make its handling characteristics and resorption times acceptable for clinical use (Smith *et al*. 2018).

We have now combined our patented nano-silver technology (Cotton *et al*. 2015, 2017) with the optimised MoaBone® in both particulate and block forms, with a view towards developing a xenograft that is resistant towards infection, without the need for antibiotics. Our initial in vitro work has shown that silver nano-particles (AgNP) are bacteriostatic in low concentrations against S. mitis, S mutans and E coli, and less cytotoxic towards cultured human gingival fibroblasts than chlorhexidine or silver diamine fluoride. The bacteriostatic nature and low cytotoxicity of AgNP shows promise for its potential use as an antimicrobial agent in bone grafting applications. We have published results showing the safety and efficacy of our silver nanoparticle technology (Gee *et al*. 2018) and are now working on the optimisation and biofunctionalization of MoeBone® xenograft blocks, the in vitro assessment of AgNP toxicity towards osteoblasts and osteoclasts, and the development of a hydrogel incorporating AgNP loaded hydroxyapatite particles, with the intention of testing these in vivo in a small animal model (rabbit cranial CSD) in the near future.

Whilst we feel that our AgNP-xenograft has potential for clinical use, we recognise that the long term goal will be the development of cellular-based solutions for bone and tissue regeneration. In our lab, we have worked with adipose derived stem cells (Godoy Zanicotti *et al*. 2017; Zanicotti *et al*. 2018) and periosteal-derived stem cells (Naung *et al*. 2019), but at present we consider dental-pulp derived cells to have the greatest potential (Coates *et al*. 2019). Currently we are working to extend our findings using 3-D printable

hydrogels, with a view towards bioprinting appropriate scaffolds containing dental pulp cells for oral regeneration.

Conclusions

Guided tissue and guided bone regeneration are successful clinical treatment options, widely used to regenerate bone and periodontal ligament around diseased teeth and implants, to preserve bone in tooth sockets and to regenerate lost bone prior to implant placement. However, currently-available therapies for GTR and GBR largely involve passive scaffolds and/or tissue excluding membranes. The next generation of membranes and grafts will involve bioactive materials, which need further development. A focus on cell-promotion and anti-infection should be priorities for these next-generation products. The "next-after-next" therapeutic treatments are likely to involve personalised 3-D printed constructs carrying the patient's own cells. Each step in the development of the technologies requires multi-skilled teams working with appropriate preclinical in vitro and in vivo models in order to demonstrate safety, efficacy, scalability and cost-benefit, prior to commencing human clinical trials.

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Chapter 12

Molecular Markers in Periodontal Disease and Health Involving Type II Diabetes Mellitus.

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Introduction

Chronic Periodontitis (CP) is a polymicrobial dysbiotic disease resulting in imbalance of the host defense mechanism, leading to immune-inflammatory responses that can cause destruction of the periodontal connective tissues and bone. Although CP is of multifactorial origin, chronic bacterial exposure is a pre-requisite for gingival inflammation, periodontal pockets, attachment loss and alveolar bone loss (Socransky and Haffajee 1992). Host and bacterial interactions during periodontal inflammation result in an acute inflammatory response which progresses into a chronic stage and is dominated by B lymphocyte and macrophages followed by an intense T lymphocyte stage. This sequence of events is mediated by cytokines (Gemmel *et al* 2001). Many cell types other than immune cells such as epithelial cells, keratinocytes, gingival and periodontal ligament fibroblasts are shown to produce cytokine upon stimulation (Baek *et al* 2013).

Diabetes Mellitus (DM) is a complex metabolic disorder characterized by abnormal sugar, fat and protein metabolism with the precipitation of hyperglycemia due to disruption in insulin production or defects in its action, which may lead to various systemic complications (Winer and Sowers 2004). Periodontal disease is now considered the sixth complication of DM (Loe 1993). Diabetic subjects with periodontal infection have a much higher risk of worsening glycemic control over time compared to diabetic subjects without periodontitis thus suggesting a bidirectional relationship between these diseases (Taylor 2001).

Researchers have made efforts to unravel molecular markers for periodontitis, resulting in the identification of several genes, transcripts, proteins, and metabolites related to periodontitis. In this pursuit, gingival crevicular fluid (GCF) has been the main bio fluid used to screen molecular profiles of periodontal disease, as it can reflect both the local oral microenvironment and the systemic environment related to health status (Trindade *et alet al* 2014).

Fibroblast growth factor-21 (FGF-21) is a member of the FGF family. The mature predominant FGF-21 polypeptide

is 181 amino acids (Nishimura 2000). It is a potent metabolic regulator, leading to its characterization as a new adipokine. It is predominantly expressed in the liver, pancreas, muscles, testis and to a lesser extent in adipose tissues (Dostalova *et al* 2009). FGF-21 is induced under several stressful conditions which may facilitate adaptation and protection. The differential production of *FGF-21* in various pathological conditions is regarded as a potential biomarker for various metabolic diseases (So and Leung 2016). Macrophage migration inhibitory factor (MIF) is a multipotent lymphokine with a molecular weight of 12.5 kDa, implicated in the pathogenesis of numerous inflammatory and autoimmune disorders (Cvetkovic and Stosic-Grujicic 2006). It is produced by a variety of cell types such as monocytes/ macrophages, B and T cells including endocrine, endothelial and epithelial cells (Calandra and Roger 2003). MIF is rapidly released in response to stimuli such as microbial products, proliferative signals and hypoxia. It may be a major cytokine in the pathogenesis of different diseases that include systemic infections, sepsis, autoimmune disease, cancer and metabolic disorders. Evidence suggests that circulating MIF can be elevated in serum of type 2 Diabetes (Yabunaka *et al* 2000). Human IL-34 is a 27.5 KDa secreted dimeric glycoprotein consisting of 242 amino acids which is expressed in human tissues including brain, heart, liver, kidney , spleen, mammary glands and prostate (Lin *et al* 2008). It is identified as the second functional ligand for colony stimulating factor receptor (CSF-1R) in human monocyte proliferation screening assay. IL-34 plays an important role in RANKL induced osteoclastogenesis as it can substitute for M-CSF and support osteoclast differentiation in the same way that M-CSF does (Chen *et al* 2010). IL-34 has been shown to be a potential inflammatory biomarker for the prediction of the risk of vascular diabetic complications (Zorena 2016; Guruprasad and Pradeep 2018). It contributes to inflammation and osteoclastogenesis in bone degenerative disease such as periodontitis (Bostrom and Lundberg 2013).

To date, no studies have been reported on FGF-21, MIF and IL-34 levels in GCF and serum in diabetic subjects with CP. It is a first of its kind clinical biochemical study designed to estimate and correlate the levels of FGF 21, MIF and IL 34 in GCF and serum in healthy and CP subjects with and without type II DM.

Materials and methods

This study was conducted from August to November 2017. The study was conducted in full accordance to the Declaration of Helsinki 1975, revised in 2013. The research protocol was approved by the Institutional Ethical Committee and Review Board of the Government Dental College and Research Institute, Bangalore, India. Following the ethical clearance was granted; 40 subjects (20 male and 20 females) were recruited from the outpatient section of Department of periodontology, Government Dental College and research Institute, Bangalore, India. A written informed consent was obtained from those who agreed to participate voluntarily in the study.

Inclusion criteria consisted of subjects in the 25-60 year age group, with at least 20 natural teeth, diagnosed with chronic periodontitis and had not received periodontal therapy within the preceding six months.

Exclusion criteria consisted of subjects suffering from aggressive periodontitis, rheumatoid arthritits, ulcerative colitis and Crohn's disease, chronic kidney disease, heart disease and hypertension or any other systemic disease that can alter the course of periodontal disease. Pregnant and lactating females or those subjects who had not received any medication that can affect periodontal status. Subjects with a history of smoking and alcoholics were excluded.

A full mouth periodontal probing and charting was undertaken for each subject and intra oral peri-apical radiographs were taken for each subject using the long cone technique. BMI charting was carried out according to the WHO Guidelines (World Health Organization 2000) and diabetic status evaluated based on glycated hemoglobin levels (HbA1c) criteria of the American Diabetes Association 2012.

Subject Grouping

Subjects were selected randomly and categorized into 4 groups based on gingival index (GI) (Loe & Silness), probing pocket depth (PPD), Clinical attachment level (CAL), radiographic evidence of bone loss and HbA1c levels. The group I (Healthy) consisted of 20 samples (10 GCF and 10 serums) from 10 subjects with clinically healthy periodontium. Following assessing the gingival status using GI, the score obtained was 0, PPD<3 mm and CAL=0 with no crestal bone loss as assessed from the radiograph. The group II (CP without type 2 DM) consisted of 10 subjects who had shown clinical signs of inflammation with GI score >1, PPD>5mm, CAL>3mm with radiographic evidence of bone loss. The HbA1c values reported were <6.5%. The group III (CP with well controlled type 2 DM) consisted of 10 subjects with clinical signs of gingival inflammation. The GI score recorded was >1, PPD>5mm, CAL>3mm with radiographic evidence of bone loss. The HbA1c values were <7%. The group IV (well controlled type 2 DM without CP) consisted of 10 subjects with clinically healthy periodontium with no evidence of disease. Following assessment GI score=0, PPD<3mm, CAL=0 with no crestal bone loss determined from radiograph. The HbA1c values were <7%.

Site selection

The clinical examinations, intra oral radiographic examinations, group allotment, sampling and site selection were carried out by one examiner. Following the detailed examinations and allocation of the subjects to respective groups; on the subsequent day samples were collected. This was done to prevent the contamination of GCF with blood associated with probing of inflamed sites. The clinical assessment was performed with a University of North Carolina (UNC-15) periodontal probe (Hu- Friedy, Chicago, IL, USA). The collections of GCF from test sites were based on the signs of inflammation, highest CAL and radiographic evidence of bone loss. In healthy subjects to standardize site selection and to obtain adequate fluid volume, samples were obtained from mesio buccal region of maxillary right first molar.

GCF collection

The subjects were allowed to sit comfortably in an upright position on the dental chair, after which the air drying and isolation with cotton rolls of the selected

test sites were performed. Supragingival plaque was removed gently using a Universal Columbia curette #4R/4L to avoid contamination and blocking of micro capillary pipette. GCF was collected by placing white color coded 1-5µl calibrated volumetric micro capillary pipettes obtained from Sigma- Aldrich Chemical Company, USA. A standardized volume of 3µl was collected in 15- 20 minutes from each test site by placing the tip of the pipette extracrevicularly. After collection of GCF, the samples were assigned to a particular group based on GI score, PPD, CAL and radiographic evidence of bone loss. Any samples contaminated with blood and saliva were discarded. The GCF was pooled from test and healthy sites and were transferred to a sterile eppendrof vial containing 200µl of phosphate buffer saline (PBS) covered with a tin foil and stored at -70 °C until analyzed. Periodontal treatment (scaling and root planning) was performed for gingivitis and periodontitis subjects at the same appointment following the GCF collection.

Serum collection

2ml of blood was withdrawn from the skin over the antecubital fossa by venipuncture using 20 gauge needles with 2ml of syringe and immediately transferred to the laboratory. Blood samples were allowed to clot at room temperature and after one hour it was centrifuged at 1000 rpm for 15 minutes to separate serum component. Serum was extracted from blood and stored at -70 °C till the assay procedure.

FGF-21, MIF and IL-34 Analysis

The samples were assayed for FGF-21, MIF and IL-34 using their respective enzyme linked immunosorbant essay (ELISA) kits according to the manufacturer's instructions (Ray Biotech, Inc, USA) for FGF-21 and MIF and (Duo Set, R & D System's, USA) for IL 34. These assays employ a pre-coated antibody specific for each FGF-21, MIF and IL-34 on 96 well plates. Standards and samples were pipetted into the wells and any FGF-21, MIF and IL-34 present was bound by immobilized antibody and captured by biotinylated antihuman FGF-21/ MIF/ IL-34 polyclonal antibody (as per the kit). Addition of HRP conjugated streptavidin was done. A substrate solution was added after washing. The colors developed in proportion to the bound biomarkers quantified and were monitored using a micro plate reader until a maximum optical density was reached. 50 µl of stop solution was added and the optical density was read at 450 nm. The concentrations of each biomarker (FGF-21, MIF and IL-34) were estimated based on the optical density values of the standards provided with the respective kits.

Statistical analysis

The data were analyzed using a statistical software program (SPSS Inc. Version 10.5, Chicago, IL, USA). For comparison of FGF 21, MIF and IL-34 in GCF and serum between the groups, Analysis of Variance (ANOVA) was performed. At the initial stage of the study; power of the study was calculated. Based on the power of the study and confidence interval of 95% ($p<0.01$), sample size was determined. The correlation between GCF and serum for each molecule and other clinical parameters was statistically significant or not was determined by Pearson's correlation coefficient. P value<0.01 was considered statistically significant. The mean intra examiner

standard deviation of differences in PPD and CAL measurements were obtained using single passes of measurements with a UNC 15 Probe 9 (Hu- Friedy, Chicago, IL, USA).

Results

The descriptive statistics along with the mean GCF and serum concentration for all groups are tabulated in Tables 1 for FGF-21, MIF and IL-34. The mean FGF-21, MIF and IL-34 concentrations in both GCF and serum were highest for group III followed by group IV, group II and least concentration was observed in group I.
The equality of means between the groups for each molecule was evaluated by ANOVA test. A significant difference between in the GCF and serum levels of FGF-21, MIF and IL-34 was found between the groups as depicted in table 2.

The correlation of GCF and serum fluid levels of FGF-21, MIF and IL-34 to HbA1c was statistically significant ($p<0.01$) in all the four groups. The Pearson's correlation co efficient test found significant correlation between GCF and serum levels for all the molecules as tabulated in tables (3A-C).

Discussion

The present cross-sectional study assessed GCF and serum levels of FGF-21, MIF and IL-34 in periodontally healthy, CP and CP patients with and without type-2 DM. The groups showed a variability of their levels of these cytokines which could be attributed to differences in the inflammatory burden and the stage of disease process at the time of collection of GCF and serum samples. The mean GCF and serum concentrations of the molecular markers were highest in group with CP and well controlled DM followed by well controlled DM group alone. The concentrations were lowest for the healthy subjects with no CP and DM. This further demonstrates that periodontal disease can be an originator of systemic burden and these molecules can contribute as potential markers of periodontal inflammation.

Study group	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
Age (in years)	39.5±6.63	40.7±4.96	40.8±3.22	42.4±4.32
GI		1.6±0.51	1.6±0.51	
PPD	2.4±0.51	6±0.66	6.3±1.15	2.8±0.63
CAL		4.3±0.82	4.2±1.03	
BMI (kg/m^2)	21.94±1.55	21.88±1.66	23.11±1.08	23.31±0.74
HbA1c	4.54±0.21	4.53±0.27	6.71±0.15	6.66±0.21
***FGF-21* (in pg ml) in GCF**	44.3 ±11.47	144 ± 37.91	537.7 ±69.8	387 ± 78.29
***FGF-21* (in pg/ml) in Serum**	101 ±17.26	276 ±64.24	587 ±64.36	445 ± 54.22
MIF (in pg/ml) in GCF	1091 ±189.33	2583.3 ±164.99	5595 ± 176.99	4231 ±189.33
MIF (in pg/ml) in Serum	1158.5 ±200.85	2651 ±165.33	5662 ±167.20	4299 ±168.73
IL 34 (in pg/ml) in GCF	232 ± 43.65	675 ± 72.45	1043 ± 101.63	425 ± 56.14
IL 34 (in pg/ml) in serum	45 ± 16.34	412 ± 165.54	543 ± 121.65	176 ± 112.32

Table 1. Descriptive statistics of study population of *FGF-21* (mean ± SD).

	FGF 21		MIF		IL-34	
	GCF	serum	GCF	serum	GCF	serum
Groups I-IV	F value= 161.66 p-value <0.001*	F value= 153.92 p-value <0.001*	F value= 1177.74 p-value <0.001*	F value= 1236.39 p-value <0.001*	F value= 785.86 p-value <0.001*	F value= 175.43 p-value <0.001*

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

	Correlation co-efficient	**P value**
Group I	0.975	0.001*
Group II	0.961	0.001*
Group III	0.970	0.001*
Group IV	0.960	0.001*

Table 3A. Pearson correlation co-efficient between GCF and serum concentration of FGF21. *Significant at p value <0.001

	Correlation co-efficient	**wP value**
Group I	0.986	0.001*
Group II	0.918	0.001*
Group III	0.974	0.001*
Group IV	0.992	0.001*

Table 3B. Pearson correlation co-efficient between GCF and serum concentration of MIF. *Significant at p value <0.001

	Correlation co-efficient	**P value**
Group I	-0.131	0.253
Group II	0.231	0.001*
Group III	0.453	0.542
Group IV	0.289	0.001*

Table 3C. Pearson correlation co-efficient between GCF and serum concentration of IL 34. *Significant at p value <0.001

The systemic inflammatory burden caused a local spillover of FGF-21 in GCF and acted as a homeostatic mechanism of the tissues to exert the protective role of FGF-21 in inflammatory state which was in accordance with previous studies where serum FGF-21 levels were increased in diseases such as obesity and type 2 DM (Marz *et al* 2009). The increased concentrations of MIF reported in this study are similar to other published reports (Mitchell *et al* 1999; Leech *et al* 1999)

where MIF stimulated the expression of large panel of proinflammatory molecules, nitric oxide, COX 2, products of arachdionic acid pathway and several matrix metalloproteinases. This implicates role for MIF in the pathogenesis of acute and chronic inflammatory and autoimmune diseases. IL-34 expression in human adipose tissues and the circulating concentration are significantly elevated in obese patients, and is associated with insulin resistance as evaluated by Chang *et al* (2014). IL-34 m-RNA expression was observed in periapical lesions and was significantly higher than that of the normal periodontal ligament tissue. This implies that IL-34 could be closely related to inflammation seen in chronic apical periodontitis. Thus, the increase in GCF and serum concentrations of IL-34 in CP and type-2 DM in the present study might be attributed to the pro-inflammatory properties of the protein.

Chair side kits can be refined for easier and faster diagnosis using these molecular markers. Multicenter interventional studies should be carried out to find the role of these markers in periodontitis and diabetes in various ethnic groups of population. Further research has to be carried out to explore the clinical implications of these molecular markers on improvement in disease complications.

Conclusion

Within the limitations of the present study, it can be postulated that increased concentration of FGF-21, MIF and IL-34 can be detected in GCF and serum in CP with or without type 2 DM. These molecular markers can be considered as potential inflammatory marker of CP and DM. The levels of concentrations of these markers could be valuable in detecting high-risk individuals with periodontitis and systemic diseases, such as diabetes. Further multicenter, longitudinal, prospective studies must be carried out to confirm these findings and for better understanding of the possible roles of these molecular markers in the pathogenesis of periodontal diseases and diabetes.

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Exploring Innovative Frontiers in Periodontal Health and Implantology
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Chapter 13

Efficacy of Locally Delivered Minocycline in Advanced Periodontitis. A Clinico-Microbiological Study- Preliminary Findings.

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Rationale and background

Periodontitis is a complex, multifactorial disease characterized by the loss of connective tissue attachment with destruction of periodontium (Vandekerckhove *et al* 1998). Microbes involved in periodontal disease are mainly Gram-negative anaerobes with many anaerobic spirochetes in a biofilm habitat. However, recent investigations have implicated new pathogens such as *Filifactor alocis*, *Synergistetes*, *TM7*s being associated with advanced periodontitis (Al hebshi *et al* 2015).

The primary goal in periodontal therapy includes removal of the etiological factors by mechanical periodontal therapy (Hanes and Purvis 2003). The efficacy of mechanical plaque removal is limited and transient in subgingival area. Mechanical therapy alone fails to eliminate the anaerobic infection at the base of the pocket, within the gingival tissue or in furcation defect (van Steenberghe *et al* 1993). These areas act as a bacterial reservoir from which recolonization of the tooth surface can occur after instrumentation.

The use of antimicrobial agents as an adjunct to mechanical therapy has proven to be effective in the treatment of chronic periodontitis. (Ellen and McCulloch 1996) Moreover, systemically administered antibacterial agents achieve relatively low concentrations in the pocket even at high dosage. Also, the unwanted effects such as development of resistant strains and superimposed infections preclude the use of these agents as the sole treatment modality **(**Haffajee *et al* 2003).

Local delivery of antimicrobial agents on the other hand reduces the above problems besides providing higher concentrations in the availability of the drug at the specific infected sites with the advantage of sustained release (van Steenberghe *et al* 1999). Periocline is a long acting, sustained release local drug delivery system consisting of 2% minocycline hydrochloride in an ointment containing microcapsule type particles. Periocline contains 20mg of minocycline in 0.5 gm of gel in a disposable polypropylene applicator (2% minocycline HCl).

Research has yielded promising results with the local application of minocycline in the treatment of periodontal

disease, compared with other non-surgical therapies (Nagakawa *et al* 1991, Dean *et al* 2003, Williams *et al* 2001, Hanes and Purvis 2003, Lu and Chei 2005). However, there is scarcity of reports on the use of local delivery agents with respect to new range of putative pathogens in advanced periodontitis, wherein the tissue invasive anaerobic organisms are present and possibly compromised host response, hence resulting in an exaggerated breakdown of periodontal tissues at the affected sites. Also, limited evidence is available on efficacy of the local anti-infective approach in the Malaysian population where prevalence of periodontitis is high (Jaafar *et al* 2014).

The effect of Minocycline on new putative pathogens, such as *Filifactor alocis* and oral phylotypes of phyla *Synergistetes* and *TM7* (referred to hereafter as oral Synergistetes and oral TM7s), has not been investigated yet. Hence, the aim of the present study is to evaluate the efficacy of a local delivery agent containing minocycline (Periocline, Sunstar, Japan) as an adjunct to SRP in the treatment of deep periodontal pockets around teeth in advanced periodontitis and the antimicrobial effect on the red complex and the new putative pathogens.

Methodology

A randomized, double-blinded, placebo controlled, parallel arm study was designed and carried out. The study proposal was approved by the Research Management committee and Institutional ethical clearance was obtained from RMC, MAHSA University (***RMC/AL01/2018***). After explaining the nature of the study, the type of intervention and the follow-up protocol, a voluntary signed informed consent was obtained from the participants. Fifty patients with Advanced Periodontitis (Stage III & Stage IV 2018 classification) were enrolled in the study. The inclusion criteria comprised of systemically healthy patients with age range between 20–60 years, diagnosed with untreated advanced periodontitis with pocket depths of ≥6 mm around at least 4 teeth, in two or more quadrants with radiographic evidence of bone loss. At least 20 teeth had to be present for the subject to be included in the study. The patients who took antibiotics or anti-inflammatory drugs in the past 3 months, with history of periodontal therapy within past 2 years, allergy to tetracycline, pregnant or nursing females, those using regular mouth rinse or any other oral pathology or on medication that has effect on gingiva were excluded from the present study.

Plaque sample site selection criteria

In each patient at least 4 teeth (non-adjacent) with PPD of 6-9 mm and bleeding on probing at the initial visit were selected for collection of plaque sample and Periocline application. The teeth were selected from at least 2 different quadrants and if possible, all from different quadrants. In the selected patients, individual teeth with either a prosthesis, grade II mobility or more, or with hopeless prognosis as evident by degree of bone loss were not included for plaque sample collection.

Clinical procedure

Full mouth scaling and prophylaxis was done after initial examination. In 2-3 weeks after initial examination and supragingival scaling, baseline plaque samples and clinical parameters including plaque score, bleeding score, probing

pocket depth, clinical attachment loss were recorded. UNC Probe was used to record all periodontal parameters.

***Randomization*:** Subjects were randomly divided into control and test groups based on a predetermined computer-generated randomization code.

Test group: Treated with SRP followed by placement of minocycline ointment.

Control group: Treated with SRP followed by placebo application. Ultrasonic scaler and Gracey's curettes were used to perform the SRP.

***Probing depth*:** Probing depths were measured using UNC -15 probe at six sites per tooth calibrated in millimeters in six sites at baseline,6 weeks and 12 weeks.

***Clinical attachment loss*:** Using UNC -15 probe, clinical attachment loss was measured from CEJ to base of the periodontal pocket at all the six sites per tooth.

***Bleeding on probing*:** Full mouth bleeding on probing was recorded at six sites per tooth using Muhlemann's criteria (Muhlemann and Son 1971).

***Plaque Index*:** Plaque index was recorded using Sillness and Löe (1964) plaque index recorded on mesial, buccal, distal and palatal surfaces of the teeth.

Periodontal Inflamed Surface Area: PISA quantifies the inflammatory burden posed by periodontitis to cause systemic inflammatory response. PISA was calculated for the corresponding tooth by filling in clinical attachment level, recession and bleeding on probing on six sites per tooth in the freely downloadable spread sheet available from www.parsprototo.info. (Nesse et *al* 2008).

Adverse events: Incidence of Adverse events were recorded after application of the medicament in both the groups during the recall visits. All the observed and reported adverse experiences were recorded. The reported adverse experience and its relationship with the application of the medicament was evaluated by the investigators.

Blinding: Randomization was done by statistician. Instrumentation and periodontal assessments were done by two well trained calibrated periodontists, who were blinded of the code. Randomization code was given to the dental nurse who coordinated with patients. All the medicament/placebo applications were done by another trained investigator.

Application of minocycline ointment or placebo was done at baseline visit and repeated at 2 weeks and 4 weeks (van Steenberghe *et al* 1993). During these visits, supragingival scaling, OHI reinforcement was done as needed for subjects in both the groups. For the application of the medication or placebo, the selected sites were dried, isolated, and the subjects were instructed not to drink or rinse for at least 30 minutes after application. No dressing was placed. Subjects were informed to refrain oral hygiene procedures for next 24 hours.

Recording of clinical parameters and collection of subgingival plaque samples were repeated at 6 weeks and 12 weeks in both the groups. (Figure 1) The susceptibility of the pathogens to Periocline was analyzed using RT -PCR.

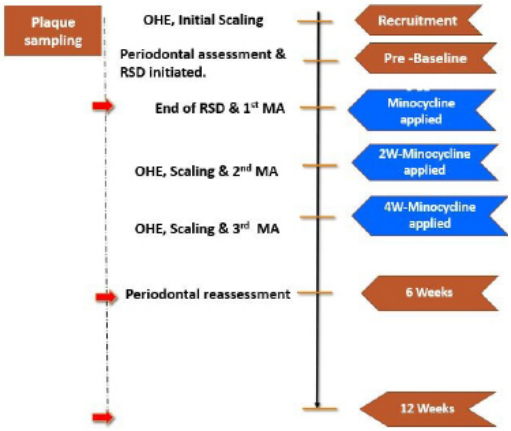

Figure 1. Recording of clinical parameters and collection of subgingival plaque samples at baseline 6 weeks and 12 weeks.

Sample collection and DNA extraction

Real-time PCR (qPCR) is the most sensitive method used to detect and quantify nucleic acid, especially in low-abundance templates. PCR can be divided into three steps. First, double-stranded DNA (dsDNA) is separated at temperatures >90 °C. Second, oligonucleotide primers are annealed at 50 °C to 60 °C. Finally, optimal primer extension occurs at 70 °C to 78 °C. Subgingival plaque samples were collected using paper point and submerged in 1 mL sterile normal saline. Bacterial DNA was extracted from samples using NucleoSpin® Tissue DNA extraction kit (Macherey-Nagel, Germany) according to manufacturer protocol. Extracted DNA was quantitated and quantitated using Nanodrop Spectrophotometer (Thermo Fisher, USA). qPCR was used to detect and quantify all the pathogens in the samples using PrimeTime qPCR Probe Assay (Integrated DNA Technologies, USA) on Applied Biosystem 7500 fast real-time PCR system as described previously (Al-hebshi et al 2015). The target and primer sequences used in this study were listed in Table 1. The delta-delta Ct method, also known as the 2–ΔΔCt method was used to analyse relative quantification of bacterial abundance in the samples.

Data and statistical analysis

Means and standard deviations were calculated for all the clinical parameters in both the treatment groups for different time intervals. SPSS 25 statistical package was used for data analysis. For detection of intergroup statistical significance independent t-test was used, whereas for detecting the significance within each group between the time intervals, repeated measures ANOVA was applied for time effect. Results were considered significant at $p \leq 0.05$.

Results

Before performing the study, the sample size was determined using G * Power software with significance level of 0.05 and power of 80%. In a previous study (Paquette, *et al.* 2003) the true difference in the experimental and control means was 0.5. In line with this, 25 experimental subjects and 25 control subjects have to be recruited to be able to reject the null hypothesis. In addition, the inter examiner agreement between the two clinical investigators was calibrated and was estimated to have a good agreement.

The CONSORT flowchart outlines the recruitment, randomization, intervention and analysis in Table 2. Twenty-two (22/24) subjects completed the study (12 test and 10 control) by July 2019. There was one drop out in control group and in test group one subject was excluded due to antibiotic use for other problems.

Target	Primer sequences 5'-3'	Product size (bp)
Total bacteria	F-primer: AAACTCAAAGGAATTGACGGGG R-primer: TTGCGCTCGTTGCGGGACT	205
P. gingivalis	F-primer: ACGAATCAAAGGTGGCTAAGTT R-primer: TTAGTCGCATTTTCGGCTGAT	85
T. forsythia	F-primer: GATAGGCTTAACACATGCAAGTC R-primer: GTTGCGGGCAGGTTACATAC	99
T. denticola	F-primer: GGGCGGCTTGAAATAATRATG R-primer: CTCCCTTACCGTTCGACTTG	92
P. micra	F-primer: TGAGCAACCTACCTTACACAG R-primer: GCCCTTCTTACACCGATAAATC	112
Oral Synergistetes [a]	F-primer: GGAGTACGGTCGCAAGATTG R-primer: GTAAGGTTCTTCGGTTTGCATC	98
F. alocis	F-primer: ACCCTCAAGTTGCCAAAATTATTAT R-primer: TACTCCCTTTCTTCTGGTTAAATCT	101
Oral TM7s [b]	F-primer: GCTCGTGTCGTGAGATGTTT R-primer: ATCCCCTCCTTCCTCCCCG	107

Table 1. List of target bacteria and primer sequences

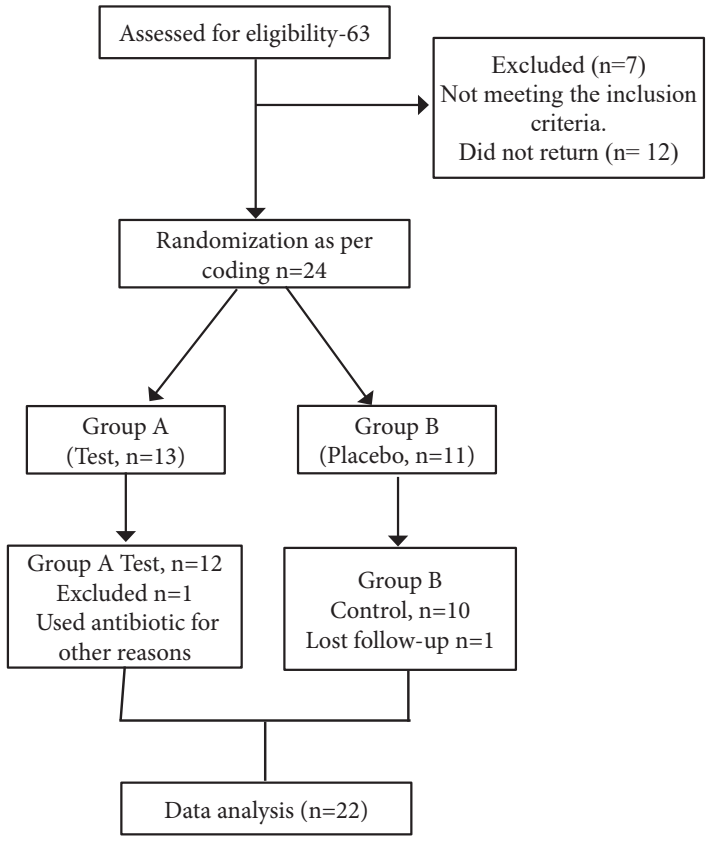

Table 2. CONSORT flow chart

Variable	Test (n=12) n (%)	Control (n=10) n (%)	Whole group (n=22) n (%)	*p*-value
Age	42.00 ± 10.26	47.00 ± 10.96	44.27 ± 10.64	0.283≠
Gender:				0.639≠
Male	6 (50.0)	4 (40.0)	10 (45.5)	
Female	6 (50.0)	6 (60.0)	12 (54.5)	
Race:				0.900≠
Malay	5 (41.7)	3 (30.0)	8 (36.4)	
Chinese	5 41.7)	6 (60.0)	11 (50.0)	
Indian	1 (8.3)	1 (10.0)	2 (9.1)	
Others	1 (8.3)	0	1 (4.5)	

Table 3. Sociodemographic characteristics of the subjects (n=22). Chi-Square test was applied; ≠ Statistically non- significant at P ≤0.05

The sociodemographic characteristics of the subjects at baseline and their distribution among the test and control groups is presented in table 3.

Mean age of the subjects in test and control groups was 42 ±10.26 and 47±10.96 years respectively and was not significantly different (p=0.283). Both the groups matched closely for gender and race distribution. Distribution of diabetic subjects and smokers was also not significantly different between the groups. (p>0.05).

The mean number of sites treated in test group was 8.5 ±1.41 and 8 ±2.18 in control group (Non-Significant; p=0.435). No adverse events were reported by the patients in test or control group.

Inter group Comparison-Overall mean

Plaque index showed slight elevation at 6 weeks and reduced by 12 weeks in both the groups, while the test group had greater reduction of PI by 12 weeks. However, these observations were not significant. For bleeding score, both the groups showed reduction in bleeding at 6 weeks and 12 weeks. Although the reduction is greater in test group, the intergroup difference was not significant. (Table 4)

Periodontal parameters

PPD reduced from 3.67 ± 0.58 at baseline to 2.98 ± 0.55 by week 6 and further to 2.76 ± 0.46 mm by week 12 in test group. In control group, PPD reduced from 3.65 ± 1.01 at baseline to 3.00 ± 0.63mm by 6^{th} week and 2.99 ± 0.50 mm by 12^{th} week. The mean difference observed is better in test group (0.9 vs 0.6) but was not statistically significant (Table 5) Similarly, CAL in test group was 4.51 ± 0.95 at baseline and improved to 3.79 ± 0.93 by week 6 and 3.64 ± 0.86 by week 12. On the other hand, in control group, CAL was initially 4.44 ± 1.61mm at baseline which reduced to 3.81 ± 1.46 mm by 6^{th} week and 3.73 ± 1.32 mm by 12^{th} week. Again, test group showed marginally better improvement which, however, is not significant. (Table 5)

PI		Baseline	6 weeks	12 weeks
Test group (n=12)	Mean ±**SD**	0.76 ± 0.36	0.88 ± 0.43	0.63 ± 0.33
	Mean difference		-0.12	0.13
Control group (n=10)	Mean ±**SD**	0.88 ± 0.55	0.95 ± 0.53	0.84 ± 0.41
	Mean difference		-0.07	0.04
p-value *(Intergroup difference)*		0.563	0.727	0.103
BoP		Baseline	6 weeks	12 weeks
Test group (n=12)	Mean ±**SD**	**1.51 ± 0.57**	**1.02 ± 0.41**	**0.92 ± 0.30**
	Mean difference		0.49	0.59
Control group (n=10)	Mean ±**SD**	**1.47 ± 0.64**	1.16 ± 0.37	1.12 ± 0.40
	Mean difference		0.31	0.35
p-value *(Intergroup difference)*		0.852	0.424	0.178

Table 4. Comparison of plaque and bleeding scores between the two groups at different time intervals.

Intragroup comparison- overall mean

Within the groups, the mean difference in plaque and bleeding scores at week 6 were not significant in either of the groups. Both PPD and CAL showed a highly significant improvement from baseline to 6 weeks and 12 weeks in test group ($P \leq 0.001$**), whereas in control group the observed parameters were not significant ($P > 0.05$, Table 5)

Following the periodontal parameters, mean PISA value at baseline was 1887.95 ± 590.42 in test group which reduced to 1273.40 ± 660.04 by 12 weeks. In control group, PISA at baseline was 1738.19 ± 988.35 with a reduction to 1309.95 ± 427.53 by 12 weeks. No significant difference between the test and control groups was observed, but within the test group the reduction of PISA was significant over 6 and 12 weeks. Control group failed to show any significant difference in PISA values over the time intervals. (Table 6)

PPD		Baseline	6 weeks	12 weeks	P value *(Intragroup difference from BL to12 weeks)*
Test group (n=12)	Mean ±**SD**	3.67 ± 0.58	2.98 ± 0.55	2.76 ± 0.46	
	Mean difference (C.I)		0.69 (0.410, 0.967)	0.91 (0.586, 1.231)	**<0.001****
Control group (n=10)	Mean ±**SD**	3.65 ± 1.01	3.00 ± 0.63	2.99 ± 0.50	
	Mean difference (C.I)		0.64 (-0.365, 1.649)	0.65 (-0.313, 1.615)	**0.237**
p-value *(Intergroup difference)*		**0.954**	**0.999**	**0.261**	
CAL		Baseline	6 weeks	12 weeks	
Test group (n=12)	Mean ±**SD**	4.51 ± 0.95	3.79 ± 0.93	3.64 ± 0.86	
	Mean difference (C.I)		0.72 (0.410, 1.028)	0.86 (0.542, 1.186)	**<0.001****

Table 5. Comparison of periodontal parameters (Overall means)

PISA	TEST MD (95% CI)	*p*-value	CONTROL MD (95% CI)	*p*-value
BL vs. 6 wks	525.33 (132.829, 917.831)	**0.009****	334.46 (-584.133, 1253.058)	**0.679**
BL vs. 12 wks	614.56 (157.306, 1071.811)	**0.009****	428.24 (-404.456, 1260.944)	**0.422**
6 vs. 12 wks	89.23 (-146.900, 325.358)	0.673	93.78 (-132.198, 319.761)	0.588

Table 6. Periodontal Inflamed Surface Area (PISA)

Observations at tested sites

Mean values of PPD of all the sites may not reflect the true disease burden. Hence, the means of PPD and CAL for tested sites (≥6mm sites at baseline) were estimated, wherein treatment intervention was carried out. The mean PPD of the tested sites (≥ 6mm) in test group was 7.23 ± 0.45 mm and 6.81 ± 0.78 mm in the control group which reduced to 5.75± 1.43 mm and 5.40 ± 1.30mm by 12 weeks respectively. The mean PPD reduction in test and control groups (1.6mm vs 1.4mm) was not significantly different (P≥0.05). Similar observation was noted with CAL. Within each group, test group showed a highly significant reduction of PPD and CAL at 6th week and 12th week (P ≤0.001)

whereas within the control group both PPD and CAL difference at 6 weeks was non-significant and by 12 weeks the difference was marginally significant. (P= 0.004 for PPD; P=0.005 for CAL) as shown in the table 6. (Table 7)

Site based response analysis

Table 8 demonstrates the site-based responses observed with the adjunctive application of minocycline versus SRP and placebo over 12 weeks. When the baseline PPD of 6-8mm were considered, 21.4% vs 11.2% of sites treated with adjunctive minocycline or SRP alone respectively, demonstrated probing depth reduction of at least 3mm over 3 months following treatment (P=**0.026*)** whereas in the sites with > 8mm baseline PPD, 29.4% of sites showed 3mm PPD reduction as compared to 8.5% sites with SRP alone (P=0.041*). Other observations were not significant between the two groups.

PPD		Baseline	6 weeks	12 weeks	P value *(Intragroup difference from BL to12 weeks)*
Test group	Mean ±**SD**	**7.23 ± 0.45**	**4.62 ± 1.21**	**4.73 ± 0.87**	
	Mean difference (C.I)		2.61 (1.684, 4.360)	2.15 (0.153, 4.145)	**<0.001****
Control group	Mean ±**SD**	**6.81 ± 0.78**	**4.66 ± 2.20**	**4.90 ± 1.01**	
	Mean difference (C.I)		2.5 (2.013, 2.970)	1.91 (0.754, 3.528)	**0.004**
p-value *(Intergroup difference)*		**0.416**	**0.822**	**0.688**	

Table 7. Intragroup mean difference in PPD in tested sites (≥6mm)

Baseline to 12 weeks		1mm	2mm	3mm	≥4mm
6 to 8mm	Test	19.8%	20.7%	21.4%	23.7%
	Control	17.15%	20.0%	11.2%	31.3%
	p-value	0.874	0.900	**0.026***	0.327
>8mm	Test	17.1%	19.6%	29.4%	27.5%
	Control	26.1%	7.3%	8.5%	49.0%
	p-value	0.428	0.101	**0.041***	0.141

Table 8. Site Level PPD reduction between the groups based on baseline probing depths

Preliminary Microbiological Observations:

Overall, test groups and control groups showed reduced abundance of target bacteria after SRP treatment. Minocycline showed reduction in novel pathogens except Oral TM7s and a definitive result couldn't be deduced with the limited data. (Figure 2a, 2b).

Variation in response for each bacteria target have been observed across the selected patients in both test and control groups (Figure 3).

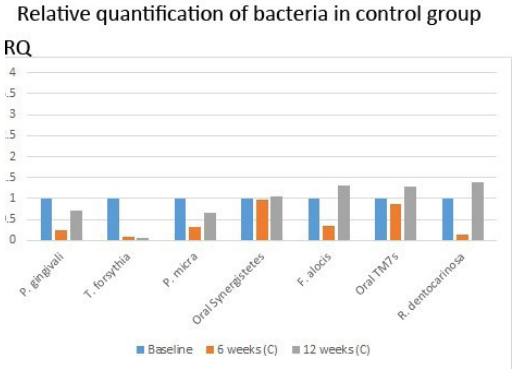

Figure 2a. Effect of Minocycline of target bacteria after SRP treatment. A. Control group.

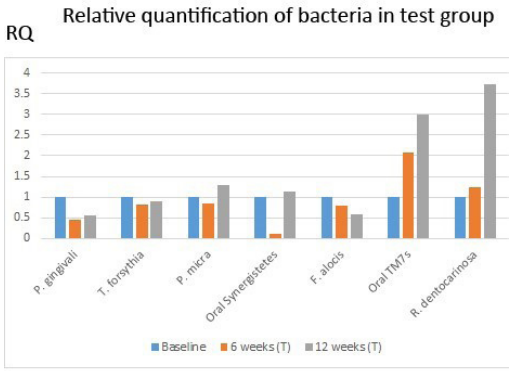

Figure 2b. Effect of Minocycline of target bacteria after SRP treatment. B. Test group.

Discussion

Intra pocket delivery of antibiotics has been shown to achieve a greater concentration of the drug locally, proving bactericidal for most periodontal pathogens, while exhibiting negligible impact systemically (Bonito *et al* 2005). Minocycline is a semisynthetic derivative of tetracycline which has a wider spectrum of action and is one of the most active antibiotics for microorganisms associated with periodontitis (Abbas *et al* 2016). Advantages of minocycline include its marked substantivity, slow release, superior lipophilicity and direct inhibition of collagenase activity (O'Connor *et al* 1990, Javed and Kohli 2010).. In a study the antimicrobial activity of minocycline was found to be greater than doxycycline and tetracycline *in vivo* for dermatological treatment *(*Leyden *et al* 1996).

Adjunctive application of minocycline gel in untreated periodontitis patients had shown improvement in periodontal and microbiological parameters (van Steenberghe *et al* 1993, Graca et.al 1997, Williams *et al* 2001, Hanes and Purvis 2003, Lu and Chei 2005). Contrarily, Timmerman *et.al* (1996) stated no additional benefit over SRP with adjunctive application of minocycline gel in pockets ≤5mm, but possible benefit was observed in pockets ≥7mm. In a recent systematic review with panel of experts on nonsurgical periodontal therapy with and without adjuncts, didn't include minocycline (microspheres) to have an added benefit over SRP (Smiley *et al* 2015). Later studies have also observed no additional improvement with the adjunctive application of minocycline microspheres. (Killeen *et al* 2016). Also, the disease severity and patient response to therapy could be affected by the culture,

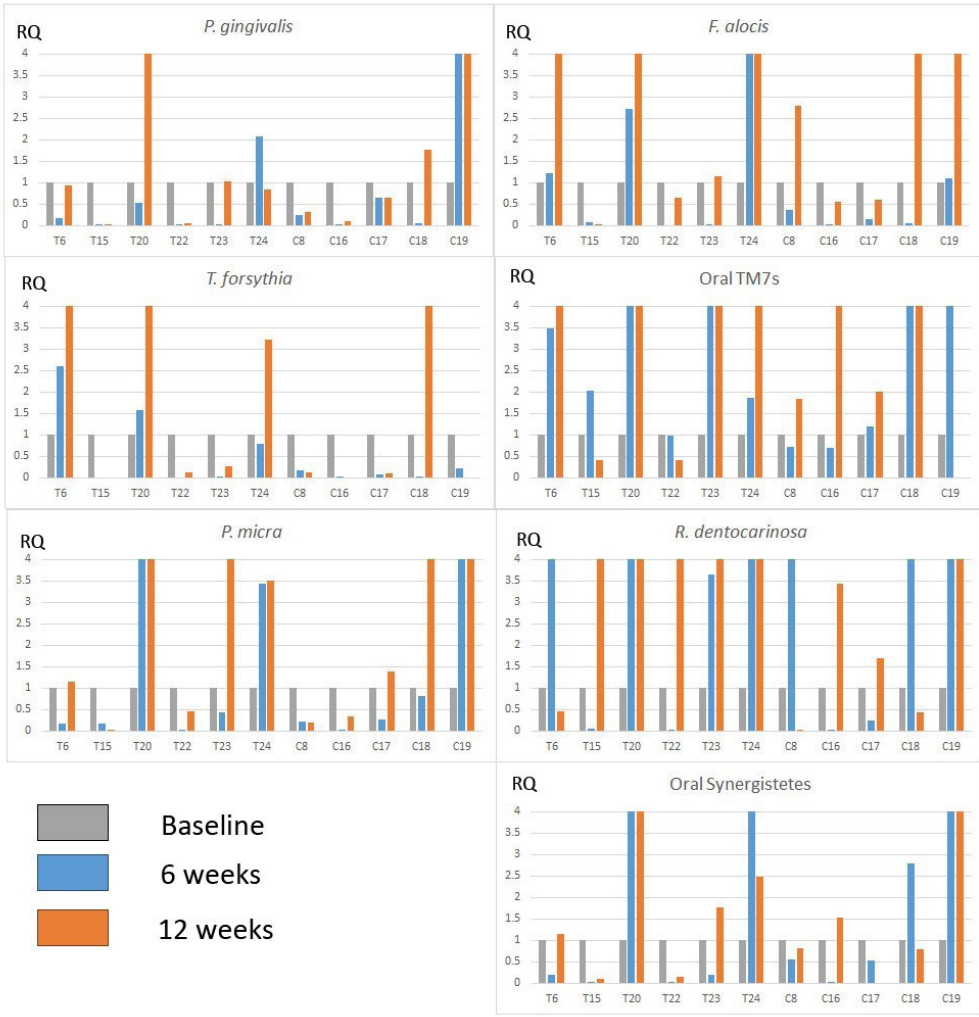

Figure 3. Variation in response for each bacteria target in test and control groups

habits, socioeconomic and genetic status of the population studied.

In the present study, clinical and antimicrobial efficacy of adjunctive local application of minocycline gel in untreated advanced periodontitis was assessed in a double-blind randomized placebo controlled clinical trial in the Malaysian population. Periocline™ is a bio-absorbable long acting, sustained release local drug delivery system consisting of 2% minocycline hydrochloride in a gel containing microcapsule type particles.

Studies employing repeated applications of 2% minocycline gel once a week for 4 weeks, 2 applications every weekly or biweekly or 3 applications at biweekly intervals were tried earlier. However, Ishikawa *et al* have shown all these modes of application were equally effective (Ishikawa *et al* 1988). Hence, in this study biweekly application for 3 times was chosen to favor the patient

compliance. This is in accordance with the manufacturers' instructions (three to four applications, once in every 15 days). Baseline is considered 2 weeks after initial screening and oral prophylaxis wherein periodontal assessment was done and subgingival samples were collected, so that the assessment of probing pockets depths would be accurate and minimize the influence of pseudo pockets reflecting true disease assessment.

Plaque and bleeding scores improved in both groups by the end of 12 weeks but did not show any significant difference between the test and control groups although the improvement in BoP is greater in test sites. Similar results in plaque score were reported by van Steenberghe *et al* 1993 and Jones *et al* 1994. This could be explained based on the study protocol that was followed with OHI reinforcement and oral prophylaxis (supragingival) that was performed every recall visit. For bleeding scores previous studies have shown a significant difference in bleeding index between test and control groups. (Atilla *et al* 1996 and McColl 2006 *et al*). However, it can be noted that there were methodology variations such as, single blinding and non-placebo control group and a 12-month duration that could have influenced these results.

Results of this study showed significant improvement in sites treated with minocycline gel by the greater probing depth reduction and greater clinical attachment gain. When an overall mean PPD was considered, the mean pocket depth reduction achieved in test group was 0.9mm versus 0.6mm in control group and is not statistically significant. Similar non-significant results in PPD have been reported earlier. (Zingale *et al* 2012, Soeroso *et al* 2017). This observation agrees with previous reports by van Steenberghe *et al* (1993) in which PPD reduction of 0.8mm and 0.5mm respectively was observed. CAL gain observed was 0.8 mm in test group and 0.7 mm in control group. Similar observations for CAL were also observed in previous studies even when the number of applications of the minocycline was more. (van Steenberghe *et al* 1993). Several other studies have reported a significant improvement of adjunctive minocycline application (either microspheres or gel formulations) compared to SRP alone. (Williams *et al* 2001, Dean *et al* 2003, Paquette 2004). Possible reasons that could explain lack of significant difference in test group compared to placebo group are: (i) Repeated SRP and OH reinforcement appeared to enhance PD reduction in both groups. (Killen *et al* 2016). (ii) A reduced pocket probe penetration because of the decreased gingival inflammation is the most prominent cause of the apparent gain of attachment. The present protocol design involving a mechanical subgingival cleaning at the start of the observation period reduces the chances for a significant difference during several weeks. iii. Using a placebo in comparative studies of subgingival minocycline administration might perturb the ecosystem of the subgingival plaque in the periodontal pocket and induce transient therapeutic actions in the control group. (Lu and Chei 2005).

PISA scores derived from the periodontal parameters, did not show any significant difference between the test and control groups. However, it is notable that only in test group there is significant difference over time interval of 6 and 12 weeks unlike in placebo group (intragroup results). In agreement with this, analysis of results in tested sites revealed that application of minocycline can result in

an early reduction of PPD (6 weeks) and inflammation which sustained till 12-week follow-up. Whereas in the placebo group, reduction by 6 weeks is not notable although similar treatment protocol and OHI regimen was followed. These results were maintained up to 12 weeks in the test group.

In moderate to severe periodontal disease, a mean reduction of 2 mm in probing pocket depth can be expected following scaling and root planning (Hill *et al* 1981 and Lindhe *et al* 1978). Site based analysis was considered clinically more relevant that verify the primary observations (Paquette *et al* 2004). In this study, site based response observed showed that a significant number of sites in test group showed a reduction of 3 mm over control group. 3 mm difference is clinically significant that can modify the clinical treatment decisions. The above observations need to be further verified as these are preliminary results of 22 subjects. The results may not reflect the true difference in both the groups at this stage with the limited sample. For microbiological parameters, no adjunctive effect of minocycline application observed over test group in inhibition of pathogens. Regardless of intervention given, recolonization was seen by 3 months. Oral TM7s appear to be less susceptible to periodontal therapy rendered. due to the inadequate available samples, the following observations cannot be used to make any conclusions. Study is further ongoing, and the readers can refer to the subsequent results for further details.

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Chapter 14

Supportive Periodontal Therapy – Long-term Benefit

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Introduction

Periodontal disease is a multifactorial disease of the oral cavity with microorganisms playing a key role in its initiation and pathogenesis (Page and Kornman 1997). The host immune system triggers an inflammatory response to microbial insult that both defends and destroys the periodontium (Page and Schroeder 1976). Gingivitis, the mildest form of periodontal disease, is highly prevalent and readily reversible by simple, effective oral hygiene. Inflammation that extends deep into the tissues and causes loss of supporting connective tissue and alveolar bone is known as periodontitis which results in the formation of soft tissue pockets between the gingiva and tooth root. Severe periodontitis can result in loosening of teeth, occasional pain and discomfort, impaired mastication, and eventual tooth loss (Philstrom *et al* 2005). The aim of periodontal therapy is the long-term retention of natural teeth in a healthy, functional, aesthetically acceptable, and painless state (Hirschfeld and Wasserman 1978).

Supportive periodontal therapy (SPT) is defined as the procedure performed at selected intervals to assist periodontal patient in maintaining oral health. As part of periodontal therapy, an interval is established for periodic ongoing care. Maintenance procedures are under supervision of dentist and include an update of medical and dental histories, radiographic review, extraoral and intraoral soft tissue examination, dental examination, periodontal evaluation, removal of bacterial flora from crevicular and pocket areas, scaling and root planing where indicated, polishing of teeth and a review of patient's plaque control efficacy (Periodontology AAP 2001). In supportive periodontal therapy, the most important part requires that the patient should understand the purpose of a maintenance program and the dentist must emphasize that preservation of teeth in question are dependent on this.

The 3rd World Workshop of American Academy of Periodontology (1989) renamed this treatment phase "supportive periodontal therapy" (SPT). This term expresses the essential need for therapeutic measures to support a patient's efforts to control periodontal infections and to avoid reinfection. Preservation of periodontal health of treated patient requires a positive program to assist in the elimination of periodontal disease. After Phase I therapy is completed, patients are placed on a schedule of periodic recall visits for

maintenance care to prevent recurrence of disease (Newman *et al* 2012). SPT has been given many names, including recall maintenance and preventive maintenance, but the name was changed at the 1989 World Workshop in Clinical Periodontics to reflect the fact that the long-term treatment provided for patients during supportive periodontal treatment is of critical importance to survival of dentition. In 2003, a position paper ws published titled "Periodontal Maintenance Therapy" (Cohen 2003). In most cases, this form of therapy is used following the completion of active periodontal therapy, but it can be used in other phases of treatment. Supportive periodontal treatment evolved from traditional dental prophylaxis and now emphasizes treatment of areas of previous attachment loss and areas where clinical signs of inflammation are found (Wilson 1996).

In 1991, the American Academy of Periodontology published Guidelines for periodontal therapy. This publication states that, upon completion of active periodontal treatment, an appropriate program of supportive periodontal treatment, specific to individual circumstances, must be recommended to the patient. Furthermore, the patient must be informed that SPT is essential to future and long-term control of disease (Kerry 1995).

SPT is an integral part of periodontal therapy in which periodontal diseases and conditions are monitored and etiological factors reduced or eliminated. It is initiated after completion of active periodontal therapy and continued at periodic intervals for life of dentition or its implant replacement. A patient may move from active treatment into supportive periodontal maintenance treatment and back into active treatment if the dentist detects an exacerbation of the disease. In 1916 Widman stated, "If one succeeds in having the patient carry out effective mouth hygiene after the operation, there is no return of pyorrhea" (Kerry 1995). In SPT, periodontal diseases and conditions are monitored, etiological factors reduce or eliminated and continued at periodic intervals for the life of the dentition or its implant replacement (Kerry 1995).

SPT has three main therapeutic objectives. The first is to prevent progression and recurrence of periodontal disease in patients who have previously been treated for gingivitis and periodontitis and is determined by absence of clinical signs of inflammation and stabilization of attachment levels. It is important to recognize sites of recurrent periodontitis at an early stage before irreversible changes occur. A second therapeutic objective is to reduce incidence of tooth loss by monitoring the patient's dentition, including any prosthetic replacements of natural teeth. The third therapeutic objective of SPT is to increase the probability of recognizing and treating other diseases or conditions found within the oral cavity (Wilson 1996).

However, disease does reoccur in a small group of individuals who often are identified as high risk or extreme downhill patients. In the absence of SPT there is an increased risk in these patients for tooth loss. It has been demonstrated that in patients with advanced periodontally compromised teeth, microbial monitoring and use of systemic antibiotics as an adjunct to nonsurgical SPT can effectively reduce need for tooth extractions. This demonstrates that carefully designed SPT is of the utmost importance for successful periodontal therapy (Chace 1951, Becker *et al* 1984, Loesche *et al* 2002).

Frequency of SPT

Studies have indicated patients who return for regular periodic visits of scaling, root planning, oral hygiene reinforcement and disease reassessment demonstrate better periodontal health and a better prognosis in the long term than patients who do not return for these appointments (Wilson 1991). The interval between recalls may range from a few weeks to 6 months or more, but is generally between 2-4 months (Shick 1981, Wilson *et al* 1984), with the shorter intervals reserved for patients with persistently high plaque scores or patients susceptible to recurrences of gingivitis or periodontitis. The interval between maintenance visits is determined by many factors. These include nature and extent of periodontal problem; type of therapy performed and category of maintenance program; level of healing; effectiveness, frequency, and possibility of tooth abrasion or tissue irritation. Other factors are rate of calculus formation; patient's systemic status; any bleeding or exudation on probing; and pathogenic subgingival plaque (Scallhorn and Snider 1981).

The effectiveness and importance of regular prophylactic visits has been well documented (Suomi *et al* 1971, Knowles *et al* 1979, Axelsson and Lindhe (1981a, Axelsson & Lindhe 1981b, Ramflord *et al* 1982). If recall visits are spaced too far apart (Nyman *et al* 1975, Rosling *et al*. 1976), the periodontal status of treated patients may be compromised, whereas with good oral hygiene reinforcement and maintenance visits properly spaced, even cases of advanced disease can be maintained (Nyman *et al* 1975, Lindhe & Nyman 1984).

Based on epidemiologic studies, a maintenance program should provide adequate therapy for previously existing periodontal conditions (Schick 1981). Initially, the patient should be provided with a thorough prophylaxis and complete reinforcement instructions in oral hygiene procedures every 3 months. The 3-month interval should be increased, maintained, or decreased depending on an evaluation of stability of supporting structures. Close monitoring will indicate appropriate time interval for each individual patient, and if necessary retreatment determined for those areas that may be deteriorating.

Maintenance of treated periodontal patient should be carefully considered and a definite routine established (Chace 1951) The essential factors include patient education, oral hygiene reinforcement; full-mouth radiographs every 2 years, and 2 to 3 months recall intervals. All patients treated for periodontal disease require professional maintenance and the degree of professional care depends on severity of original involvement, the skill and motivation of patient in oral hygiene procedures, and above all, susceptibility of the patient to periodontal disease. A typical preventive treatment consists of checking plaque control, examining occlusion, careful subgingival curettage, and charting of mouth.

It is suggested that patients treated for periodontal disease may be susceptible to recurrent periodontitis. Some patients tend to have recurrences despite regular care, necessitating retreatment. Reasons for regression must be thoroughly evaluated and may include oral hygiene regimen, surgical technique, occlusal factors, and systemic factors. Patients should be prepared psychologically and informed of the possibility of additional future treatment (Chace 1977). De Vore

et al (1986) had assessed bone levels around individual tooth groups in 23 patients treated for periodontal disease and followed with infrequent maintenance (< 1 visit per year) with clinical measurement and radiographs. Results showed increased bone loss and tooth loss when compared to initial presentation. Molar teeth were found to be at more risk than incisors and canines and a lack of periodontal maintenance care and inadequate plaque control resulted in progressive bone loss following treatment.

Brand *et al* (2013) conducted a study in fifty-six previously treated periodontal patients who were in maintenance. Bleeding on probing scores (BOP), plaque index (PI), pocket depths (PD), motivation, autonomous regulation controlled regulation and oral health knowledge were assessed at baseline, 6 weeks and 12 weeks. Statistically significant decreases were found over time for BOP, PI and PD 4–6mm for both groups. Differences in clinical parameters between groups were not evident at either 6 or 12 weeks. Thus the results show that a one-time motivational interviewing session is insufficient for improving oral hygiene in long-standing maintenance patients.

In studies conducted by Axelsson and Lindhe (1981a, 1981b), all patients received detailed oral hygiene instructions, a scaling and prophylaxis, removal of ill-fitting margins of restorations, and surgery as needed. One group of patients was then returned to the referring dentist while the other group entered a carefully designed clinic maintenance care program. Results demonstrated that patients placed on a carefully designed recall program were able to maintain excellent oral hygiene standards and stable attachment levels over a 6-year period after treatment for periodontitis. The non-recall group lost on average 1.8 mm of attachment over the 6-year period. Patients who were not maintained in a supervised program were more prone to develop recurrent disease. In a group of 442 patients treated in private periodontal practice with maintenance period ranging from 5 to 17 years with an average of 10.1 years, tooth loss was evaluated. When patients were seen on average every 4.6 months in a recall visit, results indicated that periodontal disease could be effectively treated and that tooth loss due to periodontal disease can be prevented (Oliver *et al* 1969).

In a long term maintenance programme over an average of 22 years, only the percentage of tooth loss was 7.1% (1,110 out of 15,666 teeth), and 31.4% for teeth with furcation involvement were lost (Hirschfeld and Wasserman 1978). Lindhe and Nyman (1984) reported on the long-term maintenance of 61 patients treated for advanced periodontal disease. Patients with 50% or more of their periodontal support lost were given detailed oral hygiene instructions, scaling and root planing, and surgical elimination of periodontal pockets and then placed on a 3 to 6 month recall and followed for 14 years. During this time 92 to 99% of all sites maintained PD < 4 mm, while less than 1% of the sites developed probing depths > 6 mm. The mean attachment level was reduced from 6.1 mm to 5.4 mm and maintained at this level. During the 14 years of maintenance, 30 of the 1,330 (2.3%) teeth were lost during the course of the study, 26 for periodontal reasons. Results demonstrated that treatment of advanced forms of periodontal disease resulted in clinically healthy conditions and that patients could maintain this state over a period of 14 years. A small number of sites lost a substantial amount of attachment at different times of the maintenance period but mean plaque and gingival indices did not prove helpful in monitoring the isolated

sites. Axelsson *et al* (2004) found that the main reason for tooth loss was root fracture during 30 years maintenance delivered in a private dental office; only few teeth were lost because of progressive periodontitis or caries and concluded that incidence of caries and periodontal disease as well as tooth mortality is relatively low.

Several well-controlled longitudinal studies have documented that irrespective of the periodontal treatment performed, pocket probing depth reductions and probable attachment levels can be maintained over several years provided that a high standard of oral hygiene and repeated scaling and root planning are applied in a regular maintenance care program (Knowles *et al*. 1979, Rosling *et al*. 1976, Philstrom *et al*. 1983, Westfelt *et al*. 1983, Lindhe & Nyman 1984). Generally, recall intervals of 3 months have been chosen to guarantee optimal periodontal maintenance (Knowles *et al*. 1979, Philstrom *et al*. 1983) while other groups have preferred to apply some flexibility to the frequency of recall visits (Westfelt *et al*. 1983, Lindhe & Nyman 1984). Similarly, the recall intervals in other study have been chosen between 2 and 6 months depending on the incidence of bleeding on probing and the status of the periodontal tissues as judged by a dentist. Although the accurate interval for optimal maintenance rate interval for optimal maintenance visits is not known and most likely varies with the individual, there is evidence from a microbiological point of view that recall visits should be scheduled every 3rd or 4th month in order to alter the sub gingival micro flora in sites at risk at optimal time intervals. Lang *et al* (1986) reported that the patients who had been treated for advanced periodontitis successfully, the recall interval rarely exceeded 4 months. The frequency of SPT should be dictated by disease stability and not by calendar. On average, patients with gingivitis are seen over the time frame of a year, those with chronic periodontitis four times per year, and those with aggressive forms more often (Wilson 1996).

Compliance in SPT

Compliance can be defined as extent to which a person's behavior coincides with medical or health advice". As chronic diseases became treatable, compliance becomes more important. A review of the medical literature shows that, in general, compliance decreases as treatment time or complexity of required behavioral changes increases. When short-term (up to 3 months) compliance involving taking of prescribed medications is studied, compliance tends to be high (in many instances greater than 75%). But with passage of time, compliance falls, the percentage often dropping below 35%. Noncompliance is seen in patients with problems ranging from trivial to life threatening. The less threatening the patient perceives the problem to be, the lower the compliance.

It has been shown that when patients stop cleaning their teeth, bacterial plaque collects and clinical signs of gingivitis appear; these are reversed when cleaning is restarted (Loe, Theilade and Jensen 1965). It is known that those patients who clean or have their teeth cleaned have decreased caries and periodontitis when compared with controls (Wilson 1984).

The importance of SPT in minimizing greater tooth loss and controlling disease progression and relapse has ben demonstrated (Renvert and Persson 2004). It has been suggested that chronic periodontitis progresses in patients

who drop out during non-surgical therapy and in those who discontinue or not comply with SPT (Kocher *et al*. 2000, Konig *et al*. 2001, Fardal *et al*. 2004, Miyamoto *et al*. 2006, Costa *et al*. 2011, Ng *et al*. 2011). Different social, behavioral, cultural and economic factors as well as personality traits have been identified as determinants of compliance pattern during periodontal maintenance (Demetriou *et al*. 1995, Costa *et al*. 2011).

The first study reporting the degree of compliance with supportive periodontal treatment schedules was first published in 1984 and reviewed all the patients whose progress could be followed after treatment for periodontitis in a private periodontal office (Wilson *et al* 1984). 961 patients from a private periodontal practice were monitored for compliance with suggested maintenance schedules over a period of 8 years. Patient compliance was encouraged by informing them of importance of maintenance, notifying either by telephone or mail to schedule an appointment. Of these patients, only 16.44% complied with recommended maintenance schedules, erratic compliance was found in 49.43% of the patients, and 34.13% never reported for any maintenance therapy. It was suggested that as a result a patient's past history of compliance may modify the therapeutic approach employed. It has been noted that the more often a patient presented for maintenance, the less likely he was to lose teeth (Wilson *et al* 1984, Wilson *et al* 1987).

In a study conducted by Hu *et al* (2017), two groups (compliant and noncompliant) differed significantly in frequency distributions for sex, educational level, and histories of substance use, periodontitis, and root planing or flap surgery. These results suggest that in patients who received a permanent prosthesis on implant placement, root planing or flap surgery was the crucial factor in determining compliance with supportive periodontal treatment.

Causes of failure of compliance

Fear is a major reason for noncompliance in dentistry. Several approaches have been suggested to diminish some of these concerns. Relaxation and symbolic modeling, group education or videotapes can be used for fear reduction, and changing behavior of patients toward dentists. The latter suggests that a system using positive reinforcement of good behavior in children helps to improve compliance and alleviate fear. Improving the patient compliance must be individualized to each patient and therapist. The factors essential to improve compliance are to simplify procedures, remind patients of appointments, keep records of compliance, inform, provide positive reinforcement, identify potential non-compliers and ensure the dentist's involvement (Wilson 1996).

Classification of post treatment patients (Newman *et al* 2012)

The first year after periodontal therapy is very important in terms of motivating patient in a recall pattern and reinforcing oral hygiene techniques (Table 1). It may take several months to evaluate accurately results of some periodontal surgical procedures and some areas may have to be retreated, as results may not be optimal. Furthermore, during the first-year post treatment a patient may have etiologic factors that could have been overlooked and may be more amenable to treatment at this early stage.

For these reasons, recall intervals for first-year patients should not be longer than 3 months. Patients who are on a periodontal recall schedule are a varied group. When one dental arch is more involved than other, patient's periodontal disease is classified by arch with worse condition.

Merin classification	Characteristics	Recall Interval
First year	First-year patient: routine therapy and uneventful healing First-year patient: difficult case with complicated prosthesis, furcation involvement, poor crown-to-root ratios, or questionable patient cooperation	3 months 1-2 months
Class A	Excellent results well maintained for 1 year or more Patient displays good oral hygiene, minimal calculus, no occlusal problems, no complicated prostheses, no remaining pockets, and no teeth with less than 50% of alveolar bone remaining	6 months to 1 year
Class B	Generally good results maintained reasonably well for 1 year or more, but patient displays some of the following factors: 1. Inconsistent or poor oral hygiene 2. Heavy calculus formation 3. Systemic disease that predisposes to periodontal breakdown 4. Some remaining pockets 5. Occlusal problems 6. Complicated prostheses 7. Ongoing orthodontic therapy 8. Recurrent dental caries 9. Some teeth with less than 50% of alveolar bone support 10. Smoking 11. Positive family history or genetic test 12. More than 20% of pockets bleed on probing	3-4 months (decide on recall interval based on number and severity of negative factors).

Class C	Generally poor results after periodontal therapy and/or several negative factors from the following list: 1. Inconsistent or poor oral hygiene 2. Heavy calculus formation 3. Systemic disease that predisposes to periodontal breakdown 4. Many remaining pockets 5. Occlusal problems 6. Complicated prostheses 7. Recurrent dental caries 8. Periodontal surgery indicated but not performed for medical, psychologic, or financial reasons 9. Many teeth with less than 50% of alveolar bone support 10. Condition too far advanced to be improved by periodontal surgery 11. Smoking 12. Positive family history or genetic test 13. More than 20% of pockets bleed on probing	1-3 months (decide on recall interval based on number and severity of negative factors; consider re-treating some areas or extracting severely involved teeth.

Table 1. Merin Classification

Components and maintenance procedure in SPT

SPT is usually commenced after completion of the active phase of periodontal therapy and continues at varying intervals for life of dentition or its implant replacements. The patient may move back into active care if the disease undergoes a period of exacerbation (Wilson 1996). SPT should include an update of medical and dental histories, radiographic review, extra oral and intraoral soft tissue examination, dental examination, periodontal evaluation, removal of bacterial plaque from the supra gingival and sub gingival regions, scaling and root planing where indicated, polishing of teeth and a review of patient's plaque control efficacy and other appropriate behavior modification. These procedures are performed at selected intervals to assist periodontal patient in maintaining oral health.

Schallhorn and Snider (1981) reviewed the practical management of periodontal maintenance and performed a time course study to determine how the therapist's time is spent during a maintenance visit. The authors found that prevention of periodontal disease occurs at 3 levels: preventing inception of disease; preventing progression of existing disease; and preventing recurrence of disease following treatment. The authors defined 4 types of periodontal maintenance therapy (PMT): preventive PMT; trial PMT (allows time for decisions regarding definitive therapy); compromise PMT (i.e., palliative maintenance); and post-treatment PMT (provided to prevent disease recurrence). It was stated that most periodontal therapy should include a 3-month recall but that intervals may range from 1 to 6 months, with a typical appointment taking 52.61 minutes. Factors influencing maintenance interval include oral hygiene, level of

calculus formation, and various host factors. Important clinical parameters for monitoring periodontal health during supportive periodontal treatment are:

- Loss of attachment of 2 mm or more and the associated deepening of the periodontal pocket or gingival recession
- Bleeding on probing
- Suppuration or exudate; and
- Others, including gingival recession, furcation involvement, caries, open contacts and status of occlusion and arch relationship, including any anomalies (Kerry GJ 1996).

The different components included in SPT are chart review, operatory and instruments infection control, health history update. Dental examination includes tooth loss, dental caries and prosthetic examination, fremitus test, periodontal examination including probing depth, Clinical Attachment Level (CAL) measurement, gingival recession, Plaque index, gingival index and personal hygiene overview (Schallhorn and Snider 1981).

Microbiological monitoring

For patients who have an aggressive form of periodontitis, microbiological monitoring and antibiotic sensitivity testing can be useful. When the possible causal organisms remain after this therapy or if generalized attachment loss or inflammation continues or recurs, periodic microbial monitoring is suggested (Slots 1996). When used in these cases, and combined with traditional approaches to therapy, including sub gingival scaling and root planing, the appropriate antibiotic and antimicrobial therapy often yields results superior to scaling and root planing alone.

Dismissal and reappointment

If there are no adverse changes from baseline records and the visit is completed, the patient may leave. Any posttreatment instructions are discussed and the date of the next maintenance visit is decided. If the patient's condition is stable, with lack of inflammation, minimal calculus deposits, and excellent plaque control, the interval can be extended between maintenance visits until a plateau is reached; if there are adverse findings, the interval should be shortened until the optimal recall time for the patient is determined.

Maintenance procedure

As suggested by Hancock and Newell (2001), it consists of III parts.
Part I- Examination (Approximate time- 14 minutes)
Part II- Treatment (Approximate time- 36 minutes)
Part III- Report, clean up and scheduling (Approximate time- 10 minutes)

The 1989 World Workshop in Clinical Periodontics advised that gingivitis and early (chronic) forms of periodontitis be treated in the general dental practitioner's office, but for more advanced disease, a periodontist may be required. Using the final diagnoses as a guide Wilson 1996 suggested following modifications to the World Workshop:

- The general dentist should have primary responsibility for patients with a form of plaque-associated gingivitis or chronic periodontitis with early attachment loss (no bone loss in the furcations).
- The periodontist should treat and maintain other forms of gingivitis.

- Patients with chronic periodontitis with moderate attachment loss (bone loss in the furcations) usually do well by alternating between general dentist's and periodontist's office.
- Although patients with aggressive forms of periodontitis should see the periodontist for supportive periodontal treatment, it is important for these patients to have periodic restorative check-ups by their general dentist.

Risk assessment of recurrence of disease

Chronic periodontitis represents a multi-factorial opportunistic infection (Socransky and Haffajee 1992), known or putative risk factors should be evaluated concomitantly in order to identify susceptibility of patients for periodontitis recurrence. The creation of multi-factorial risk assessment models including relevant risk factors for future disease progression has been highlighted decades ago (Beck 1994). More recently, a Periodontal Risk Assessment (PRA) tool using six parameters to evaluate risk for recurrence of periodontitis at a patient level was proposed for clinical use (Lang and Tonetti 2003). A number of systemic and local risk factors and indicators have been documented, and several risk assessment systems have been proposed (Lang and Tonetti 2003). The Periodontal Risk Assessment (PRA) model has been increasingly used in dental practice and has been validated in a longitudinal study (Matuliene *et al* 2010). It shows that a high-risk patient is more susceptible to recurrence of periodontitis and periodontally – related tooth loss after active periodontal treatment than those with a moderate or low risk, and the risk for disease recurrence among high risk patients may be partially reduced through strictly following a tailor-made supportive care protocol (Matuliene *et al* 2010).

In a study on 109 patients (42 males; mean age: 42.2 ±10.2 years, range 22–62) enrolled in a SPT program for a mean period of 5.6 years the mean number of teeth lost per patient during SPT varied from 0 to 1.8 ± 2.5 for patients with a risk score of 1 and 5 respectively ($p = 0.041$). Mean radiographic bone loss during SPT was ≤0.5 mm in all risk groups, without significant inter-group differences (Trombelli *et al* 2017). Thus it was concluded that the periodontal risk assessment might help to identify patients at risk for tooth loss during SPT.

Assessment of the risk level for disease progression in each individual patient will enable the practitioner to determine the frequency and extent of professional support necessary to maintain the attachment levels obtained following active therapy. The determination of such risk levels would thus prevent both under-treatment, and excessive overtreatment, during SPT.

Maintenance of implant

Although, techniques and various materials have been developed which are capable of a high degree of attaining clinical success, the ultimate long-term success of implants is dependent upon efforts from both patient and dentist in maintaining good health of peri-implant tissues. Therefore, the goals of implant therapy should be to establish and maintain a healthy peri-implant soft tissue seal and high bony attachment levels. Diagnostic techniques, such as probing depths, radiographic evaluation and microbial

sampling have been used to measure tissue health of dental implants. With exception of parallel connective tissue fiber orientation around implants, biologic width and the lack of periodontal ligament, peri-implant tissues have been shown to be similar to that of the natural dentition.

In addition, microbial colonization of dental implant has been shown to be analogous to teeth. Although a periodontal probe is a widely used instrument to evaluate peri-implant tissue health, the diagnostic value of probing around dental implants remains somewhat controversial. Radiographic procedures, either conventional or digital subtraction, are very effective in assessing crestal peri-implant bone heights. Optimal peri-implant health, like teeth, depends on (1) prevention of plaque formation, (2) inhibition of early plaque attachment, (3) elimination of existing plaque, and (4) interference with bacterial succession from non-pathogenic plaque. In a dentate mouth, it has been shown that microflora surrounding a dental implant is similar to that of adjacent teeth. Therefore, the removal of plaque and calculus throughout the dentition is essential to maintain health of soft tissue surrounding dental implant. Several techniques and systems have been proposed in an attempt to remove deposits from implant surfaces. The use of metallic instruments should be avoided. Stainless steel curettes have potential to cause galvanic action and resultant corrosion. Metal ultrasonic tips may severely disrupt the titanium dioxide layer, resulting in plaque-retentive grooves and surfaces. Generally, rubber-cup polishing with non-abrasive paste appears to be adequate for plaque removal (Oliguin *et al* 2002). The general techniques for calculus and plaque removal from implants are similar to natural teeth with three differences. Implants require: (1) non-sharp instrumentation that will not scratch the implants, especially if used for calculus removal, (2) avoidance of prophylactic agents containing acidic fluoride and (3) the use of non-abrasive prophylaxis pastes (Oliguin *et al* 2002).

Agerbaek *et al* (2006) conducted a study in 56 patients and collected data from 127 implants and all teeth. They concluded that BOP and smoking had no impact on bacteria at implant sites but influenced bacterial load at tooth sites. Tooth sites harbored more bacteria than implant sites with comparable probing pocket depth. A 4mm probing pocket depth cutoff level influenced distribution and amounts of bacterial loads and is important with regards to bacterial load at both tooth and implant sites. In a study conducted by Heitz-Mayfield *et al* (2016) twenty-four partially dentate patients with 36 dental implants diagnosed with peri-implantitis, were treated using an anti-infective surgical protocol followed by regular supportive therapy. Supportive peri-implant therapy included removal of supra- and sub mucosal biofilm at treated implants using titanium or carbon fiber curettes, or ultrasonic devices. In addition, professional prophylaxis (calculus/ biofilm removal) at other implants/teeth and oral hygiene reinforcement was provided. Thus, they concluded that five years following regular supportive therapy, peri-implant conditions established following peri-implantitis surgery were maintained in majority of patients and implants. Some patients had recurrence of peri-implantitis and some lost implants over the 5-year period.

In a systematic review conducted by Ramanauskaite and Tervonen (2016) frequency of recall visits varied between studies from a minimum of one visit every

three months to an individually tailored regimen. In all the studies a lack of SPTs or poor adherence to them resulted in significantly higher frequencies of sites with mucosal bleeding, deepened peri-implant pockets or alveolar bone loss and higher implant loss. Thus they concluded that to prevent peri-implantitis, an individually tailored supportive program based on patient motivation and re-instruction in oral hygiene measures combined with professional implant cleaning seem to be crucial for maintenance of implant and peri-implant mucosa.

Complications of SPT

Desite SPT aiming at preventing further loss of teeth as a consequence of periodontitis or treatment of periodontitis, it can lead to some complications such as caries, sensitivity, abscesses (Renvert and Persson 2004).

Caries

Few studies have specifically addressed root caries as a complication during SPT. However, studies suggest that the prevalence of root caries in periodontally treated patients is very high. One of the consequences of periodontal therapy is removal of root cementum. It has been suggested that intact root cementum prevents dentin caries. Due to potential of exposed root surfaces without root cementum as result of initial cause related therapy (ICRT), and further removal of dentin during SPT, subjects susceptible to caries are at a high risk for root caries. In a study of patients who had received ICRT and were on routine SPT, the findings suggested an association between level of oral hygiene and number of root surface lesions and likewise an association with salivary *Streptococcus mutans* counts (Reiker *et al* 1999). However, no relation was found between previous experience of coronal caries, salivary flow rate, or salivary buffer capacity and root lesions. Studies have also shown a relationship between root caries and subgingival presence of *S. mutans*. Molars treated with root resection also carry a higher risk of root caries, resulting in treatment failure in spite of SPT. Therefore, repeated oral hygiene instructions and adjunctive preventive measures including diet counseling and fluoride rinses, as well as fluoride and chlorhexidine varnishes, should be advocated in high-risk patients on SPT.

Endodontic lesions

Endodontic complications during SPT may result in tooth extraction. Data suggest that approximately 30% of all extractions of teeth over a 4-year period of SPT are the consequence of peri-apical lesions (Tonetti *et al* 2000).

Periodontal abscesses

Periodontal abscesses appear to occur in approximately 35% of subjects on SPT and predominantly in subjects who can be identified as rapid downhill cases (McLeood *et al* 1997). It appears that subjects on SPT who only received nonsurgical therapy during the ICRT may be at a greater risk of periodontal abscesses during the SPT phase (Kaldahl *et al* 1996).

Root surface sensitivity

It is well established that following ICRT, root sensitivity is common, especially if treatment involved surgical procedures. In most cases such sensitivity decreases

over time. Reports on root sensitivity during SPT vary from 15% to 98% and are often associated with root surface exposure and gingival recession (Karodottir *et al* 2002, Taani *et al* 2002). The very high prevalence of root sensitivity reported was based on patients previously treated for periodontitis. Data confirm that meticulous plaque control will diminish root sensitivity. Treatment of root sensitivity is consistent with preventive measures of root caries.

Conclusions

Supportive Periodontal Therapy is integral part of maintenance after the initial treatment of periodontal disease. Patient must be motivated and well educated for better compliance of maintenance therapy. Improvements of periodontal health and decrease in progression of periodontal disease are seen with long-term periodontal maintenance therapy. Different patients are classified according to their disease severity and maintenance requirement for adequate time interval during maintenance period. Every component of SPT is most important for thorough examination of the patient and for the efficient treatment.

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Exploring Innovative Frontiers in Periodontal Health and Implantology
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Chapter 15

Long-term Radiographic Outcomes of Various Conventional Periodontal Treatments Without Using Bone Grafts and/or Barrier Membranes.

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Introduction

Periodontists are often asked by patients whether their periodontal disease is treatable. Patients ask whether it is better to continue with periodontal treatment or to get dental implants after extractions. Periodontists should be able to answer that periodontal diseases are curable and maintainable (Greenstein, 2002). All periodontal treatments including successful supportive periodontal therapy must be attempted to save natural teeth. Patients should be aware that dental implant therapy should not be the best choice but the last resort (Gotfredsen *et al* 2008).

Gingivitis and periodontitis are chronic infectious diseases, and their primary etiologic factor is known to be microorganisms (Nisengard *et al* 2006). The onset of periodontal disease is caused by an alteration of the balance between bacterial challenge and host defenses. If the host resistance is superior or the pathogenic etiologic factor is below the threshold value, periodontal diseases will not occur.

In general, the treatment of disease is to remove the causes and therefore, a common periodontal treatment method is to eliminate the pathologic etiologies. It is ideal to completely remove plaque and calculus, the most important cause of periodontal disease; but this is clinically impossible. Therefore, one of the goals of periodontal treatments is to reduce plaque and calculus as much as possible below a threshold that the patient can tolerate. There is an old adage, “once a periodontal patient, always a periodontal patient” (Socransky *et al* 1982). This means if the cause of the periodontal disease has been reduced to below the threshold level, periodic follow-up is necessary to maintain such improved condition. Only patients’ meticulous self-performed plaque control and regular supportive periodontal therapy will maintain their periodontal health (Lindhe and Nyman 1975).

In this Chapter, clinical cases of various conventional periodontal treatments without use of bone grafts and/or barrier membranes will be presented to emphasize the importance of supportive periodontal treatment.

Non-surgical periodontal therapy

The effects of non-surgical periodontal therapy and surgical periodontal therapy have been studied extensively around the world. In a 2-year clinical study, Hill *et al*. reported scaling and root planing as effective as modified Widman flap and pocket elimination surgery in the multi-rooted teeth in 1981 (Hill *et al* 1981). Non-surgical periodontal therapy has been shown to be effective over a 6-year clinical study comparing scaling and root planing with surgical therapy such as modified Widman flap and reverse beveled flap (Isidor *et al* 1984). In similar clinical trials, Lindhe and Nyman (1975) demonstrated that self-performed plaque control in patients had the most decisive influence on the long-term effects of each periodontal treatment.

Importance of supportive periodontal treatment

Where surgical pocket elimination has been carried out to manage advanced periodontitis, proper maintenance therapy is essential to establish and maintain periodontal health, and if not performed, periodontal treatment will be less successful (Lindhe and Nyman 1975). Without any periodontal treatment, the mean annual loss of attachment is reported to be between 0.04 - 1 mm (Machtei *et al* 1993). However, in patients who are well maintained after periodontal treatment the average annual loss of attachment is reduced to around 0.03 mm (Suomi *et al* 1971). Furthermore, for patients who do not receive any periodontal treatment the rate of tooth loss can be up to 0.1 per year (Lindhe and Nyman 1984), 0.2 per year without supportive periodontal treatment (Becker *et al* 1984) and 0.6 per year for untreated periodontitis patients (Becker *et al* 1979). Compared to compliant patients, non-compliant patients have a 5.6-fold increase in tooth loss (Checchi *et al* 2002). The subgingival microbial flora of a patient who has received periodontal therapy and also has regular professional maintenance and thorough self-performed plaque control is similar to that of an individual with a healthy periodontium (Haffajee *et al* 1998).

Objectives of supportive periodontal treatment

The objectives of supportive periodontal treatment are correction of suboptimal plaque control, maintenance of stable clinical attachment level, and regular clinical re-evaluations with appropriate interceptive treatment. Supportive periodontal therapy should not be started after periodontal surgery, but immediately after the initial scaling and root planing phase of treatment. In general, this consists of radiological examinations, periodontal evaluations, removal of supra- and subgingival plaque, scaling and root planing if indicated, and assessment of patients' plaque control.

Optimal frequency of supportive periodontal therapy

According to research in 1970s, intervals of 3-4 months were recommended for the optimal maintenance of periodontal support (Ramfjord *et al* 1973, Lindhe and Nyman 1975). From microbiological studies, it was reported that the subgingival periodontal microbiota takes about 9-11 weeks to return to the baseline level (Greenstein 1992, Cohen 2003). However, intervals of supportive periodontal therapy

should be individualized for each patient. Patient focused recall intervals is essential to allow periodontal health monitoring.

Improving patient compliance

Most dental patients have dental anxiety, fear dental treatments and may experience an economic burden. Some patients underestimate the importance of supportive periodontal therapy and often do not re-visit their periodontists. In order to improve patients' compliance, it is advisable to remind patients of their next appointments via texts or phone calls, to keep records of patient compliance, to put your recommendations in writing and give a copy to the patients, and to provide continuous positive reinforcement (Echeverria *et al* 2019).

Simple site risk assessment

A simple site risk assessment process consists of monitoring bleeding on probing (BOP), probing pocket depth, and suppuration. The BOP indicates whether or not periodontal inflammation exists at the site where the periodontal probe is placed. The probing pocket depth shows the history of the periodontal disease progressed from the previous time of the examination. Suppuration helps to predict how the disease will progress (Kye *et al* 2012).

Clinical cases

Radiographic bone fill after non-surgical therapy

Case 1. In 1996, a private local dental clinic referred a patient for periodontal treatment of the mandibular first premolar which was an abutment for a removable partial denture. The probing pocket depth was 7 mm at the distal line angle of the buccal and lingual surfaces, and radiographic evidence of vertical bone loss was observed. Deep and meticulous root planing was performed twice, and supportive periodontal therapy was carried out every 3-4 months. Following this conservative periodontal treatment, radiographic bone fill was observed at the distal surface of the tooth and the probing pocket depth was reduced to 2 mm. This improved clinical result has been maintained through to 2005.

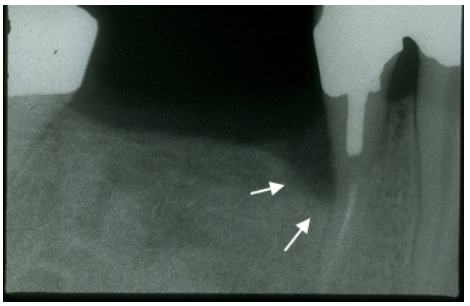

Figure 1. Periapical view. Vertical bone loss in the distal surface on the right mandibular first premolar in 1996 (Arrows indicate the boundary of bone loss).

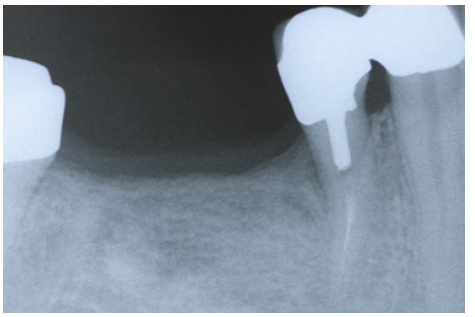

Figure 1. Periapical view. Bone fill in the distal surface of the tooth.

Case 2. A 44-year-old male patient visited the department of periodontics for periodontal and implant treatment. Severe bone loss around the root apex of the right mandibular first premolar was observed on a panoramic radiograph. Probing pocket depth around this tooth was 7-8 mm. Degree of tooth mobility was 2. Patient wanted to save the tooth, and therefore deep and thorough root planing was performed twice, and then supportive periodontal therapy was provided every 2-3 months. At the one year follow-up, radiographic bone fill was observed in the panoramic view. Continuing supportive periodontal therapy maintained a stable bone level.

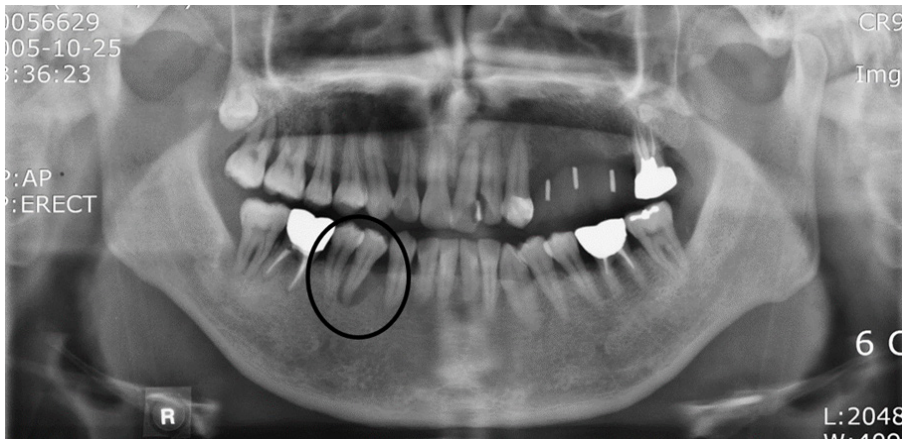

Figure 3. Panoramic view. Vertical bone loss in the mesial surface on the right mandibular first premolar in 1996.

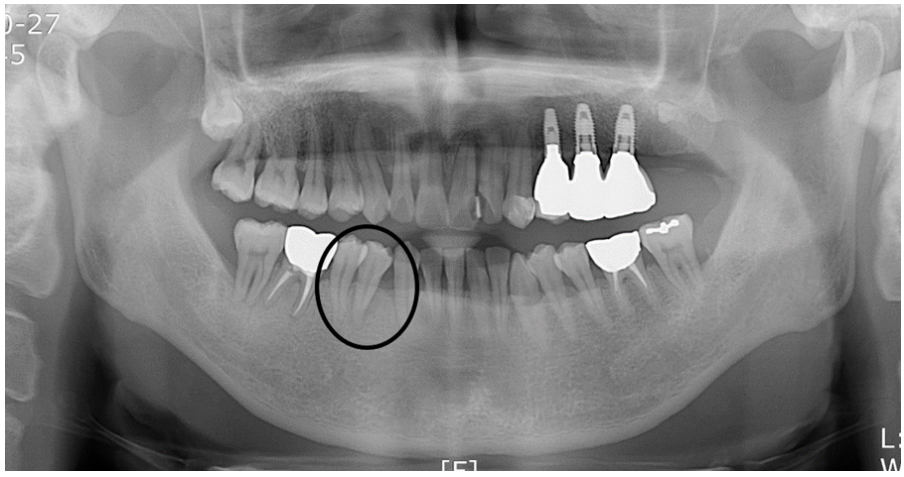

Figure 4. In 2006, radiographic bone fill was observed on the panoramic view.

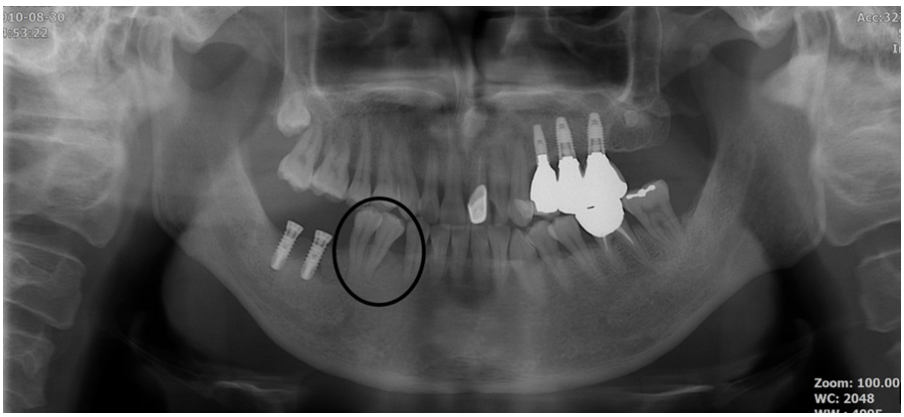

Figure 5. In 2010, bone level was well maintained on the panoramic view.

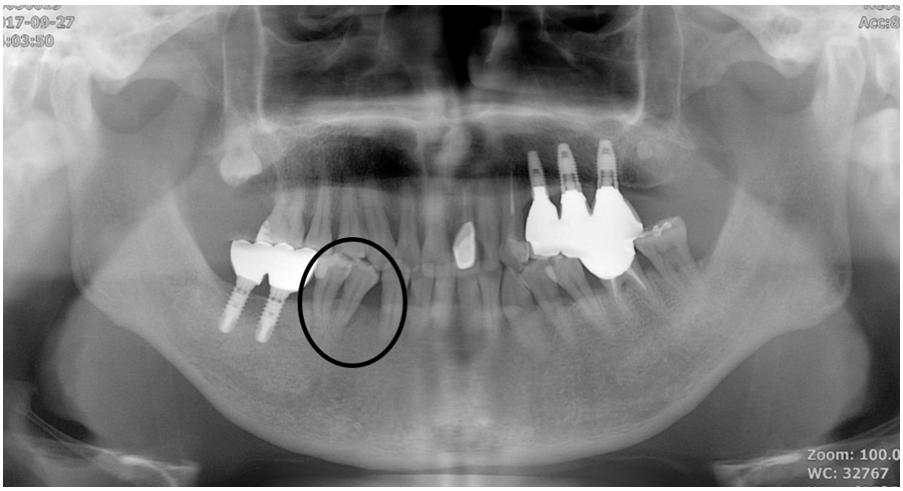

Figure 6. Bone level was still well maintained in 2017.

Significance of supportive periodontal therapy in aggressive periodontitis

Case 3. This 33-year-old female patient was referred from a local clinic to the department of periodontics for further periodontal treatment in 2009 and diagnosed as having generalized aggressive periodontitis. Severe bone destruction throughout the entire dentition was observed in the panoramic view. Deep periodontal pockets (5-10 mm) and severe tooth mobility were evident following a periodontal examination. A number of "hopeless" teeth were extracted and the remaining teeth were utilized for abutments of maxillary and mandibular removable partial dentures. To maintain the remaining teeth, supportive periodontal therapy was regularly and strictly performed every 2-3 months. The teeth have been well maintained for 10 years.

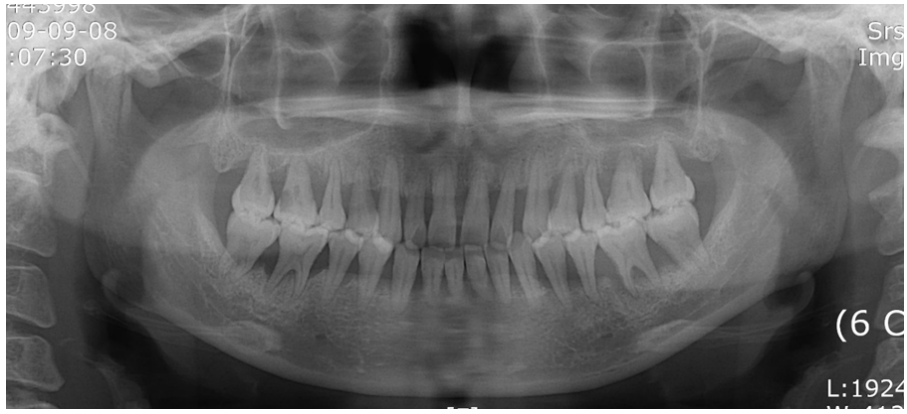

Figure 7. Horizontal and vertical bone loss to the root apex in the entire dentition on the panoramic view in 2009.

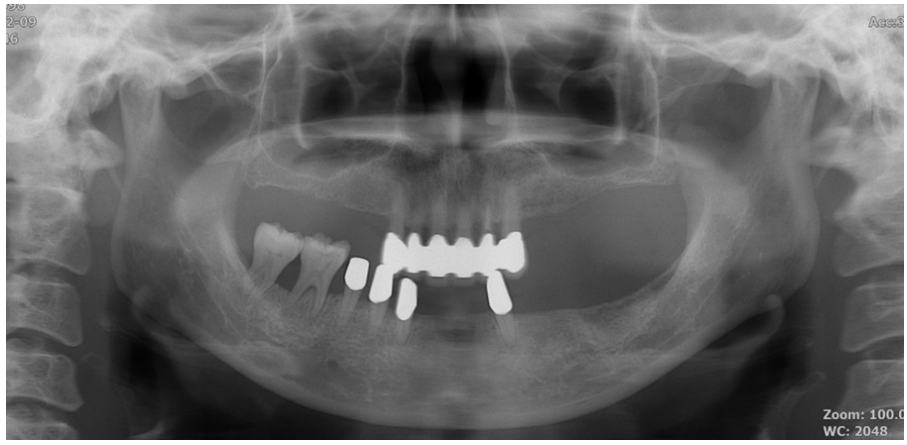

Figure 8. Panoramic view in 2010. Maxillary removable partial denture (RPD) used on the six abutments. Konus RPD used #33, #43, #44, and #45 teeth as abutments.

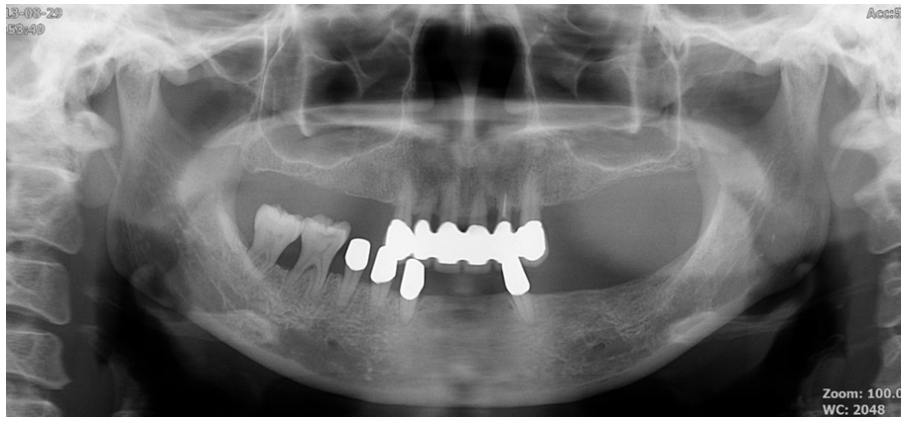

Figure 9. Panoramic view in 2013. Well-maintained residual bone height of remaining teeth

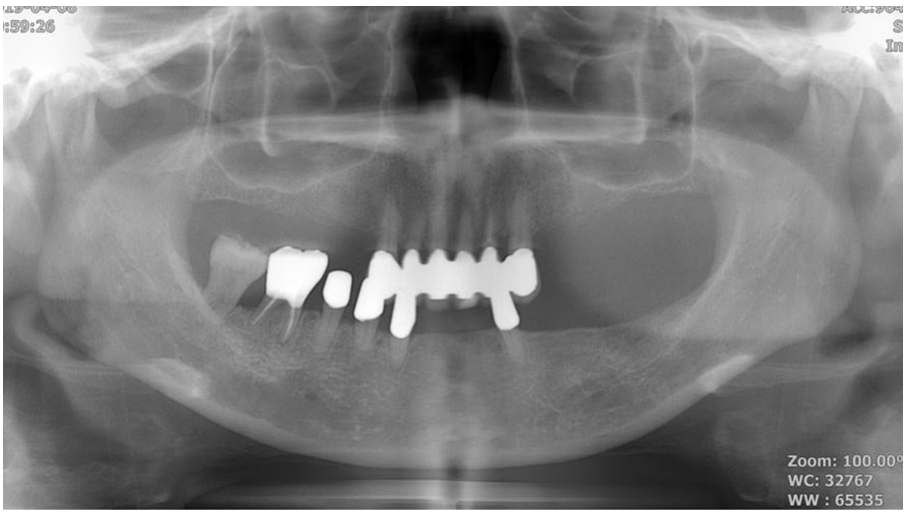

Figure 10. Panoramic view in 2019. Stable residual bone height is observed.

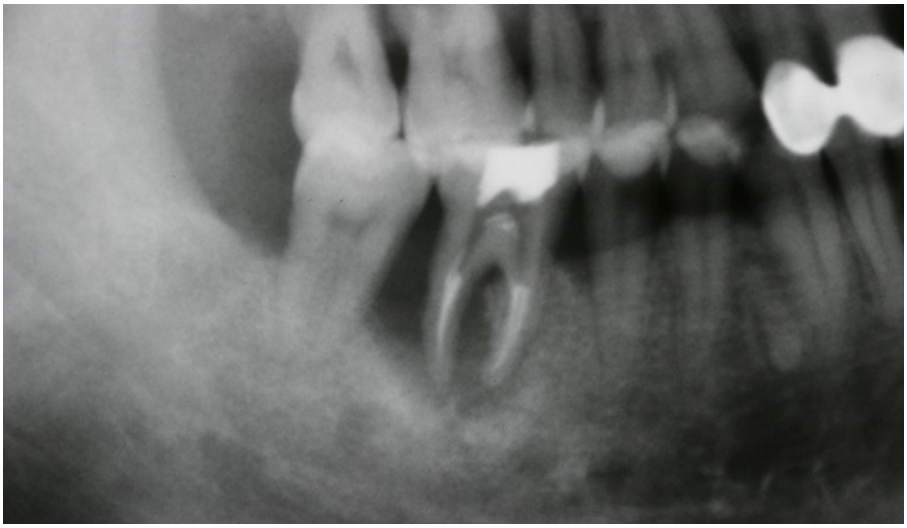

Figure 11. Panoramic view of right mandibular molar area in 1999. Completion of endodontic treatment.

Long-term functioning of a hemisectioned abutment

Case 4, in 1999, the department of conservative dentistry requested to department of periodontology hemisection of the distal root and crown portion of the right mandibular first molar. The hemisectioned tooth was to be used as an abutment for bridgework. Following hemisection, supportive periodontal therapy was provided every 4-6 months for 12 years.

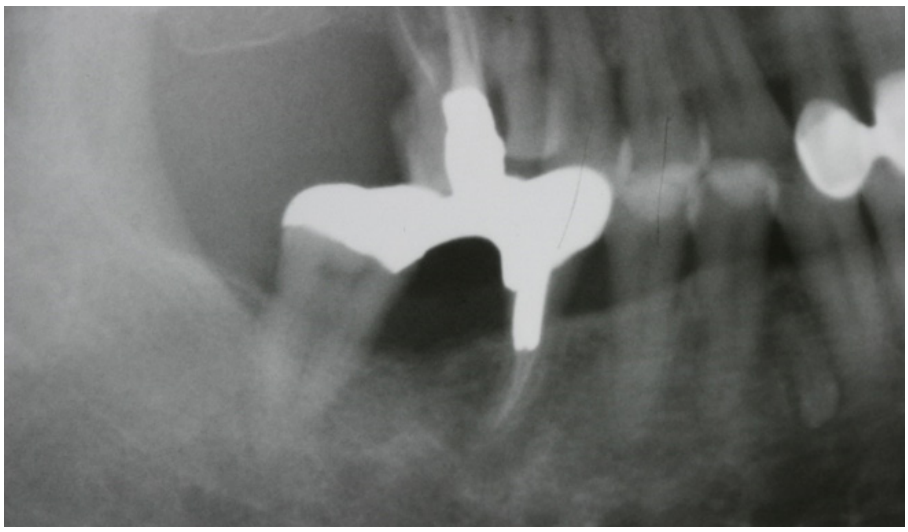

Figure 12. Follow-up panoramic view of right mandibular molar area in 2004. Bridgework on the hemisectioned tooth as abutment.

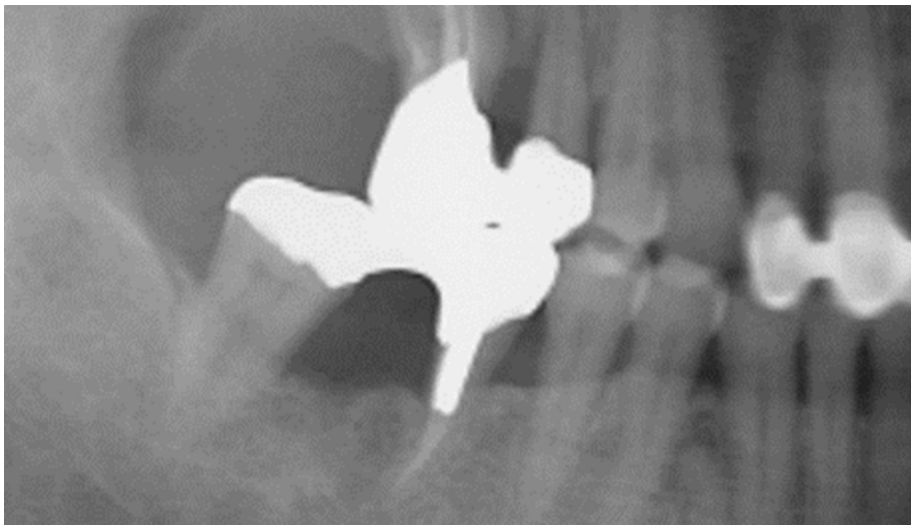

Figure 13. Follow-up panoramic view in 2011. Adequate residual bone height still remains after 12 years of hemisection surgery.

Bone fill after Prichard technique (Infrabony technique)

Case 5. In 1957, the infrabony technique for management of periodontal defects was utilized (Prichard 1957). From the aspect of exclusion of gingival epithelium, the later concepts of guided tissue regeneration seemed to have been developed from the "Prichard technique". The original Prichard surgical procedures were modified in this case. After reflection of partial-thickness flap on the surgical site, granulation tissue was completely removed and the roots thoroughly root planed. The partial thickness flap was sutured to the periosteum apical from the alveolar crest of the surgical site. Surgical pack was not used and bone formation was formed by secondary wound healing.

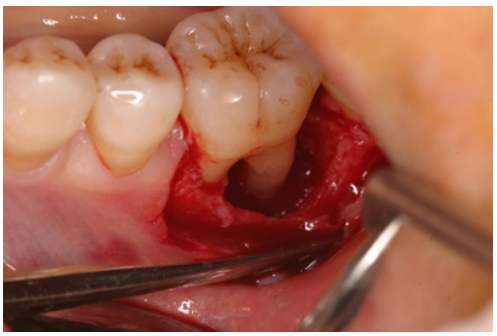

Figure 14. Complete removal of granulation tissue from the infrabony defect and thorough root planing

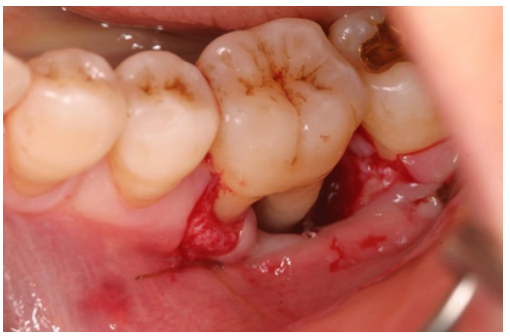

Figure 15. Suturing the flap apical to the alveolar crest of the infrabony defect

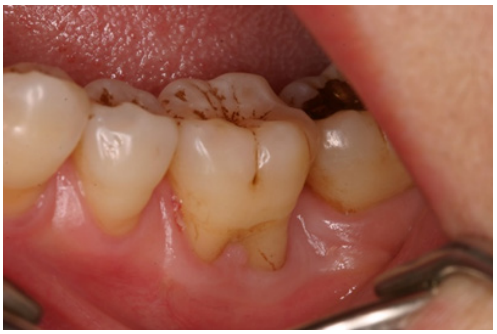

Figure 16. Clinical view after surgery.

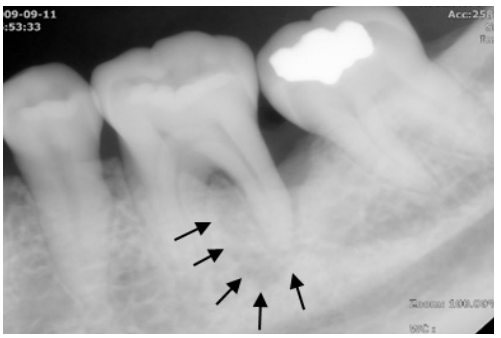

Figure 17. Periapical view in 2009. Severe bone loss around the distal root apex of the mandibular left first molar (Arrows indicate the boundary of bone loss).

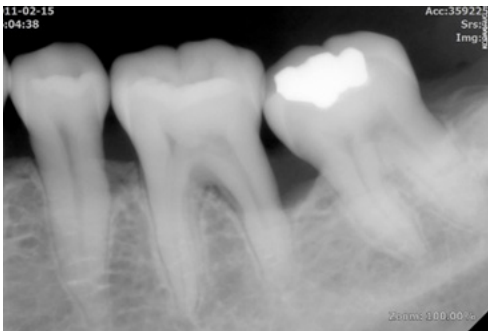

Figure 18. Periapical view in 2011. Radiographic bone fill around the distal root of the mandibular left first molar is observed.

Discussion and Conclusion

Recently implant therapy has become very popular around the world. Many dentists consider tooth extraction and implant placement as their treatment plan of choice. However, all dentists including periodontists, should do their best to save natural teeth. In many cases, only conventional non-surgical periodontal treatment is sufficient to save natural teeth. If not effective, further periodontal surgical treatment should be performed (Lang *et al* 2019).

Non-surgical therapy is still considered to be the 'gold standard' compared to other periodontal treatment methods. Scaling and root planing are fundamental approaches of all periodontal treatments, and should be considered as the first treatment method. Although such treatment methods seem clinically very easy, we should bear in mind that those are the most difficult techniques to perform, especially in the furcation areas, deep pocket sites, and infrabony defect areas. As shown in the radiographic views in clinical cases, non-surgical therapy using repeated root planing was capable of restoring bone levels. The effectiveness of the repeated root planing may be controversial because this procedure is carried out via blind instrumentation. Repeated blind instrumentation has its own limitations because calculus can often be missed on the second attempt for the same reason it was missed the first time. Even in generalized aggressive periodontitis patients, non-surgical therapy must be considered as a first line of management. Repeated root planing is effective in reducing the probing pocket depths of generalized aggressive periodontitis patients (Varela *et al* 2011), and the remaining dentition can be well maintained through meticulous plaque control and regular supportive periodontal therapy.

Root resection can be a treatment option for multi-rooted molars. In a recent systematic review, root resection and hemisection resulted in high survival rates, making it a reliable option for treatment of furcated molars that should be considered before extraction (Mokbel *et al* 2019). In Korea, root resection has been widely used in the past, but its frequency is very low now (Park *et al* 2009). The reason for the lower frequency of this procedure in clinical practice is that the prognosis is poor for resected teeth. Therefore, extraction was preferred to avoid friction with the patient in the local clinics. Hemisection to be done in two-rooted molars may have a better prognosis over the trisection in the three-rooted molars. Hemi-sectioned tooth could be utilized as an abutment for a long time. It is convenient and easy to control the plaque. In addition, it can withstand occlusal forces.

In 1957, the infrabony technique was introduced by Dr. Prichard and has been continuously developed (Prichard 1957, 1977, 1983). However, more recently it has not been a very popular surgical procedure. As shown in the clinical case above, radiographic bone fill was achieved using this procedure. The biggest advantage of this surgical approach is that the procedure does not require the usage of any regenerative materials. But, due to wound healing by secondary intention, patients complain of postoperative pain and healing might be delayed and less predictable than other forms of periodontal surgery.

In order to maintain long-term stable outcomes of non-surgical therapy, hemisection, and infrabony technique, patients and operators must cooperate.

The patients should do their best with self-performed plaque control, and visit the dental clinic for regular follow-ups (Lindhe and Nyman 1975). The operator should check patients' plaque control at each visit, and educate repeatedly how to brush uncleaned areas. In addition, it is necessary to examine whether there are recurrent sites of inflammation through clinical examination at each revisit. If recurrence of infection and inmflammation are noted, interceptive periodontal treatment should be undertaken and efforts made to improve patients' plaque control (Mombelli 2019).

In conclusion, patients' strict plaque control and regular supportive periodontal therapy are the most important methods to maintain the long-term outcomes of all periodontal treatments.

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Exploring Innovative Frontiers in Periodontal Health and Implantology
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Chapter 16

Effect of Honey on Clinical Periodontal Parameters

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Introduction

Chronic periodontitis is an inflammatory condition affecting the supporting tissues of the teeth and is most commonly caused by dental plaque. Inflammatory responses triggered in response to periodontal pathogens are the major events responsible for periodontal destruction (Darveau *et al* 1997). Treatment of chronic periodontitis aims to destroy dental plaque or alter its structure, hence altering the subgingival microflora. Initial therapy for chronic periodontitis starts with proper plaque control by the patient and non-surgical therapies which include scaling and root planing (Chan *et al* 2005). However, scaling and root planing alone may not be sufficient to eliminate all the subgingival microflora especially in areas inaccessible to instrumentation (Herrera *et al* 2002). Thus, Nagarakanti *et al* (2015) in a systematic review concluded that subgingival irrigation may provide additional benefits compared to scaling and root planing alone. Hence, proposed antimicrobial agents such as antiseptics and antibiotics have been proposed to improve scaling and root planing outcomes. Apart from well-investigated and established antiseptics frequently used in gingivitis/ periodontitis prophylaxis and treatment protocols such as chlorhexidine or iodine, special attention has been paid to the use of natural products with health promoting benefits, (Cutting 2006).

Honey has been used historically as a natural medication to treat burns and infected wounds (Molan 2001) and a recent study showed the anti-inflammatory and antibacterial properties of honey as a therapeutic agent. (Tan *et al* 2009).

In Malaysia, Tualang Honey is collected from the tualang tree (Koompassia Excelsia) where the Asian rock bees build their bee hives. This honey is commonly used as food and for traditional medication. Like Manuka honey, which has been well researched, Tualang honey has antimicrobial properties. There are differences between these two types of honey including higher phenolic flavonoids and percentage of hydroxymethyl furfural (HMF). Manuka honey is believed to be more effective against gram negative bacteria which is a predominant bacterium in subgingival dental plaque. (Ahmed *et al* 2013)

A pilot study has been carried out to investigate Manuka honey's antibacterial properties to reduce plaque and bleeding scores. The results suggested that there may be a potential therapeutic role for manuka honey confectionery in the treatment of gingivitis and periodontitis (English *et al*

2004). Samani *et al* (2011) have reported that honey significantly augmented and accelerated the wound healing after periodontal surgical flap with a notable improvement in gingival index.

With the current interest in natural remedies and well-investigated benefits of honey as an adjunct to periodontal treatment until recently, studies on the application of Malaysia Tualang Honey on periodontal treatment are still very limited. Hence, this study was carried out to evaluate the effectiveness of Tualang honey irrigation as an adjunct to scaling and root planing in chronic periodontitis patients.

Material and Method

The study was a randomized controlled clinical trial conducted at Periodontics Specialist Clinic, KP Gunung Rapat. Ethical clearance was obtained prior to the study. A total of 54 patients were recruited for the study. The procedures were explained to the patients and a written informed consent was obtained. Subjects were enrolled if they presented with chronic periodontitis with probing pocket depth (PPD) of 6mm and above on at least two contralateral sites, in good health and non-smokers. Those who were under antibiotic therapy within the past 3 months prior to the treatment, had systemic diseases (diabetes mellitus, rheumatic arthritis, systemic lupus), pregnant and lactating mothers, current smokers and pollen / honey allergy were excluded from this study.

Honey

Honey used in this study was Tualang Bee Honey brand name Borneo Honey which is commercially available in the market for consumer to take orally. It comes in a 350gm bottle and was kept under room temperature. The honey was sent for analysis in MARDI (Malaysian Agricultural Research and Development Institute) to verify the purity and its nutrition content. There was no mercury contamination detected and the acidity of the honey is at 0.0μg/100gm. The pure honey was directly withdrawn from the bottle using 5cc syringe. No dilution was allowed to maintain the purity of the honey. An amount of 1.0cc of the honey was then irrigated to the deepest depth of the pocket using 21G bent needles.

Clinical Procedure

Following initial periodontal assessment, subjects with moderate-severe chronic periodontitis received oral hygiene instruction. Supra and subgingival scaling were carried out until patients full mouth bleeding scores and full mouth plaque scores reached ≤20%. Root surface debridement was then carried out on sites with probing pocket depths of ≥6mm under local anesthesia using Gracey curettes. Upon completion of this non- surgical therapy, the periodontal sites with clinical probing depths of ≥6mm in contralateral sites were randomly irrigated with either 1.0cc of Normal Saline (NS) or Tualang honey (TH). The clinical parameters were re-evaluated after 6 weeks of the last treatment. The participants were advised to refrain from eating, drinking or gargling within one hour after the procedure ensuring the irrigation material were sustained in the mouth. Normal oral hygiene regime was encouraged to all the participants.

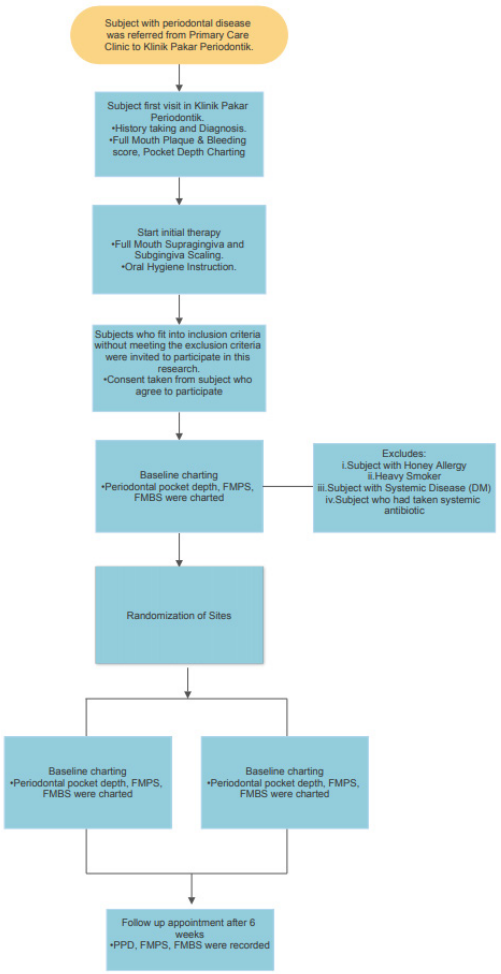

Figure 1. Flowchart of the procedure.

Statistical Analysis

Statistical analysis was performed using SPSS software (IBM SPSS statistic 22.0). For all variables in each group, mean values and standard deviation (SD) were extracted. Paired t-Test and Independent T-test was used to compare the parameters within and intergroup respectively. P values < 0.05 were considered statiscally significant.

Results

Table 1 shows the baseline characteristics of study participants. A total of 54 participants were finally enrolled in both groups consisted of 20 males and 34 females. The mean age of participants in the honey treated group was 44 years old and 49 years old in the control group.

Variables	Test n (%)	Control n (%)
Age in year (Mean, SD)	43.7 (11.9)	49.3 (11.0)
Gender		
Male	20 (37.0)	20 (37.0)
Female	34 (63.0)	34 (63.0)

Table 1. Participant characteristics

Safety Assessment

All subjects had turned up during the evaluation stage. The flow -chart of this trial is shown in Figure 1. Among all the study subjects, none of them reported of pain, allergic to honey or experience any complications after the procedure. No adverse events were noticed.

Periodontal Parameters Evaluation

Among all the study subjects, there was a significant reduction in full mouth bleeding score (FMBS) (p=0.04) from baseline and at six weeks. Percentage of reduction was 1.1%. However, there were no significant differences in full mouth plaque scores (FMPS) were compared between baseline and later time point; instead the plaque score displayed in a pattern of accumulation (p=0.06).

Variables	N	Baseline	Review	p value
Full mouth plaque score (%) (FMPS)	54	17.6 (11.6)	20.4 (12.1)	0.059
Full mouth bleeding score (%) (FMBS)	54	9.4 (5.1)	8.3 (5.5)	0.040*

Table 2. Periodontal parameters

	Baseline	**Review**	**(95% CI)**		**Baseline**	**Review**	**(95% CI)**	
FMPS (%)	17.8 (14.6)	19.8 (13.4)	-2.0 (-7.2, 3.2)	0.436	19.1 (14.2)	20.2 (14.6)	-1.0 (-6.3, 4.3)	0.698
FMBS (%)	10.3 (8.4)	8.0 (7.4)	2.3 (-0.2, 4.8)	0.069	9.5 (6.5)	8.3 (6.8)	1.2 (-1.1, 3.4)	0.300
PPD (mm)	6.7 (0.7)	4.7 (1.1)	2.0 (1.8, 2.3)	<0.001*	6.7 (0.6)	5.3 (0.9)	1.4 (1.1, 1.6)	<0.001*

Table 3. Comparing of treatment site within group

When periodontal parameters within each group were compared, the mean probing pocket depth (PPD) was significantly reduced after treatment with $p<0.001$ in both the experimental and control groups with higher mean difference in the experimental group. As shown in Table 3, in both groups FMBS were noticed to be reduced in time, though with the better results in the honey treated group.

The difference in full mouth plaque scores, full mouth bleeding scores and probing pocket depths between baseline and six weeks were then compared intergroup using independent T-test. Only the mean probing pocket depth was statistically significantly different between groups after six weeks of treatment ($p=0.003$). Other parameters showed no statistically significant differences between groups at baseline and after six weeks (Table 4).

	Baseline		**Mean diff (95% CI)**	**p value**	**Review**		**Mean diff (95% CI)**	**p value**
Parameters	**Test**	**Control**			**Test**	**Control**		
FMPS (%)	17.8 (14.6)	19.1 (14.2)	-1.3 (-7.0, 4.2)	0.632	19.1 (13.6)	20.2 (14.6)	-1.0 (-6.3, 4.3)	0.698
FMBS (%)	10.3 (8.4)	9.5 (6.5)	0.9 (-2.1, 3.8)	0.564	7.9 (7.4)	8.3 (6.8)	1.2 (-1.1, 3.4)	0.300
PPD (mm)	6.7 (0.7)	6.7 (0.6)	0.0 (-0.2, 0.3)	<0.943	4.7 (1.1)	5.3 (0.9)	1.4 (1.1, 1.6)	
*Independent t-test								

Table 4. Intergroup comparison

Discussion

Complementary and alternative medicine represents a group of diverse medicinal and health care systems, practice and products that are not considered to be part of conventional medicine (Little 2004). Apitherapy or therapy with bee products (eg; honey, pollen, propolis etc) is an old tradition that has been revitalized in recent research (Ahuja & Ahuja 2011). Honey has been used to treat a wide variety of ailments due to its antimicrobial, anti-inflammatory and anti-oxidant properties. (Pasupuleti *et al* 2017).

The use of honey in dentistry has been reported, however studies related to periodontal therapy are very limited (Samani *et al* 2011, English *et al* 2004). The traditional therapy for periodontal disease includes scaling and root planing to disrupt the subgingival microflora (Baehni 1997). However, advances in technology have resulted in the introduction of a range of new methods in periodontal therapy. The present study was designed to evaluate the clinical effect of Tualang Honey irrigation after non-surgical periodontal therapy in the treatment of chronic periodontitis. All recruited subjects in this study had the mean age of within the range of 40 – 50 years old; of which, 60% were female. No proven documentation exists demonstrating gender or age dependent effects on wound healing; hence it seems that this uncontrolled bias had minimal effect on our outcomes. The full mouth plaque scores and full mouth bleeding scores were also almost similar at baseline in both groups.

Whole mouth clinical results noted in the present study were similar to other studies using SRP and demonstrating significant improvement of clinical parameters (Badersten *et al* 1981). However, in this study no significant differences for plaque scores of all subjects were observed. Assessing the plaque and bleeding scores of all subjects was to ensure optimal oral hygiene throughout the study. This is consistent with Taib *et al* (2018) and Samani *et al* (2001) who reported an increasing trend of plaque scores in the study subjects. On the other hand, English *et al* (2004) reported high significant reduction in the mean plaque score in their honey group compared to no changes in the control group. These inconsistent findings might be due to the different types of honey used and method of honey application. This could also indicate that the active antibacterial active ingredient in honey might have been diluted to the degree that reduce its effectiveness.

All subjects in the honey and normal saline group in this study showed significant reduction in full mouth bleeding scores, from baseline and at six weeks review with the superiority of honey treated group. Many studies have considered gingival bleeding as a sensitive index for evaluating primary gingival inflammation. Bleeding on probing is considered a good marker for periodontal health (Newman *et al* 2002). A decrease of full mouth bleeding scores may be linked to the fact that the patients successfully improved their brushing habits following the given oral hygiene instructions and good oral hygiene was ensured prior to commencement of scaling and root planing. These findings are comparable with those reported by Samani *et al* (2011), who reported gingival indices had improved with time. However, a study by Nupoor *et al* (2016) showed no statistically significant difference on gingival health. The reduction in the gingival bleeding score in the honey group in this study may be the result of the anti-

inflammatory properties of honey. The potent anti-inflammatory action of honey has been noted in many clinical reports of its use in healing burn and other wounds.

When probing pocket depth was compared, the mean reduction in probing pocket depth was significant. These findings indicate that healing occurred in all subjects as a result of remission of inflammation and can be attributed to the mechanical debridement which removed calculus and altered cementum from the tooth which contributed the most to periodontal disease. There was a superior effect of Tualang Honey over scaling and root planing alone when comparing honey and control groups. This is consistent with studies by Gebara *et al* (2013) and Coutinho (2012,) who reported significant reduction in probing pocket depths and microbial count when comparing test and control groups. Another study conducted by Sanghani *et al* (2016) concluded that subgingival delivery of propolis honey showed promising results when used as an adjunct to scaling and root planing in patients with chronic periodontitis as evidenced by improvements in clinical and microbiological assessments. On the other hand, Haslina *et al* (2018) reported no significant superior effect of Tualang Honey over scaling and root planing alone when compared to honey and control groups. They suspected that sustainability of honey in the periodontal pocket were affected by the wash-out effect of honey by gingival crevicular fluid. Similarly, this present study was designed to evaluate the effect of honey on healing of periodontal pocket by application of honey directly in the periodontal sites immediately after scaling and root planing procedure. Therefore, the effect may be only local.

Since the present study has limitations, further randomized clinical trials using Tualang Honey are recommended to evaluate the possible clinical benefits of Tualang Honey in periodontal therapy

Conclusions

The present data remain inconclusive, but it is suggested that Tualang Honey could be considered for use as an adjunct to scaling and root planing in patients with chronic periodontitis. It may be of value when root planing is less than ideal due to anatomy or other factors. However, the greatest shortcoming is the quick elimination of the applied honey from the periodontal pocket. Further high-quality studies are highly recommended. Acknowledging the limitations of this study, this relatively new approached remedy with ancient background is recommended.

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Chapter 17

Innovation of Thai Rice Frontier for Periodontology in Thailand

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Introduction

Rice *(Oryza sativa L.)* is a kind of grass that has been one of the main foods for half of the world's population, especially in Thailand. Rice is a major source of energy and nutrients because it contains starch, lipids, as well as proteins. Furthermore, antioxidant properties can be found in rice bran - the outer layer of the grain, which means the ground powder of rice and rice bran also contains nutrients and antioxidant properties (Devi *et al* 2007). Our research group has developed and modified adhesive rice films, which can efficiently adhere to the buccal mucosa in the human oral cavity (Okonogi *et al* 2014). A rice gel, which is a trans-mucosal formulation, has also been developed for oral applications (Okonogi *et al* 2015a). Since rice is a biodegradable material and edible, both rice products can be effectively and safely used in pharmaceutical delivery systems. There are several benefits of using rice gel and rice films due to their high adhesiveness to mucosal membranes. While rice gel is convenient and easy to use in patients, rice film can last longer.

Most commercial local drug delivery products contain chemical base agents. This means that uptake of chemical agents into the body via oral cavity can occur while rice gel product can eliminate this problem. However, the amylose content in each kind of rice is different (Frei *et al* 2003, Li *et al* 2008), therefore characteristics of the rice products made from rice varieties also differ. For this reason, a research team from the Faculty of Dentistry and Faculty of Pharmacy, Chiang Mai University was formed to examine the physicochemical characteristics of rice varieties and evaluate the efficacies of rice products in an attempt to develop the best possible rice gel base agent.

Rice gel development

In order to develop rice gel, various rice varieties were selected to determine which ones were suitable candidates. Rice powders are found in both glutinous and non-glutinous forms. For this study two samples of each variety: glutinous rice - Niaw Sanpatong (NSP) and Niaw Koko-6 (NKK), non-glutinous rice - Jasmine (JM) and Saohai (SH), were compared. Each kind of rice powder was chemically modified as

previously described (Okonogi *et al* 2014). The physicochemical characteristics and properties of the rice gels were determined by using X-ray diffractometer, rheometer, and amylose content evaluation. The results showed that the non-glutinous varieties had higher mucoadhesive property with pseudoplastic behavior and high amylose content.

For this reason, non-glutinous rice was chosen to prepare the gel base for local delivery systems in the oral cavity (Okonogi *et al* 2015a). We then added two more kinds of non- glutinous rice to compare. At this point, two white rice grains - Jasmine (JM) and Saohai (SH) - and two reddish purple rice grains - Homnil (HN) and Doisket (DS) - were chemically modified. Each rice gel base was incorporated with an anesthetic agent (2% lidocaine hydrochloride). Four kinds of lidocaine hydrochloride containing rice gels were further studied to check the outer appearance, pH, color, gel strength, foaming property, adhesion, and *in vitro* drug release. The outer appearance of all products showed a homogenized texture. However, we found that they influenced other tested properties differently, and Saohai (SH) - white rice grain and Homnil (HN) - reddish purple rice grain demonstrated the best physical properties. Therefore, anesthetic rice gels were developed from these two kinds of rice and were further evaluated for clinical efficacy in 100 normal volunteers recruited from Chiang Mai University (50 for each product). Onset and duration of the anesthetic action were determined after applying 0.1 ml of each anesthetic gel on the tip of the tongue. Saohai (SH) anesthetic gel exhibited faster onset of numbness and longer duration of action than Homnil (HN) anesthetic gel. Furthermore, Saohai (SH) anesthetic gel exhibited *in vitro* drug release profile within 60 minutes better than Homnil (HN) anesthetic gel. This finding was similar with clinical outcomes in human volunteers. Consequently, we selected Saohai (SH) - white rice grain to be used as a gel base in our development of oral products (Okonogi *et al* 2015b).

However, rice can be rapidly digested by enzymes in saliva. Therefore it is important to consider resistance of the rice gels to oral enzymes. Chemically modified starch from several rice varieties were tested for amylase resistance using a modified method described by Shi and coworkers (2013). Amylase enzyme was added to the rice gel varieties as well as gel from raw rice starch. Separation of rice gels could be clearly seen in raw rice starch whereas chemically modified rice starch showed homogenization during 24 hours of experiment (unpublished data).

Rice gel containing antibiotic for periodontal therapy

Our research group has been investigating the development of a dental gel as a local delivery drug for adjunctive treatment of periodontal therapy. Glycerylmonooleate (GMO) has been used as the main composition of the gel base. Various agents such as glycerylmonostearate (GMS), methylcellulose (MC), surfactants, and triglycerides have been added. Rheologic and swelling properties as well as releasing characteristics have been determined. It was found that surfactants and triglycerides affected rheologic behavior while GMS and MC influenced both rheologic and swelling properties. When tetracycline hydrochloride (TCN-HCl) was incorporated into the developed gel base, in vitro the releasing profile was

evaluated using a horizontal-type diffusion cell apparatus, separated by a dialysis membrane (MW cut-off 10,000-12,000), and the results showed that an aqueous solution of TCN-HCl could be dispersed in the lipid gel base. The character of TCN-HCl gel was smooth, homogeneously dispersed, and pale yellow similar to the color of antibiotics. The polyols, mixture of gel base, especially GMO made TCN-HCl from the surface zone immediately leaked into the surrounding liquid whereas MC promoted the increase of product viscosity which could prolong drug release from the gel. Furthermore, we found that glycerol was the best to help maximize the enhancing power on TCN-HCl released from the base (Okonogi *et al* 2004).

The developed TCN-HCl gel has been studied with respect to its releasing profile and anti-anaerobic bacteria properties in five active periodontal pockets (pocket depth > 5 mm with bleeding on probing) on two patients during periodontal maintenance phase. Gingival crevicular fluid from each pocket was collected at baseline, 1-hour, 2-hour, and 3-hour, as well as at 1-day, 2-day, and 7-day after a single application of 40% TCN-HCl gel in order to determine in vivo drug releasing profile. Rapid spectrophotometric assay (Needleman *et al* 2001) as well as anaerobic bacterial counts were performed. The median concentration of TCN-HCl was at the highest value 1-hour after the application and then it gradually decreased throughout the last day. For the antimicrobial effect, the total anaerobic bacteria decreased significantly after one hour of the application and it continued to decrease until the last day. The reduction of black-pigmented anaerobic bacteria was noted after the second hour and became undetectable from day 2 through to the end of the study (Khongkhunthian *et al* 2003.)

Our research group has also examined the clinical efficacy of using the developed TCN-HCl gel compared to scaling and root planing (SRP) on patients with periodontitis during the maintenance phase of treatment. Twenty-seven persistent pockets (pocket depth > 5 mm with bleeding on probing) from 19 patients were randomly divided into 2 groups: TCN-HCl gel group (12 sites from 9 patients as a test group) and SRP group (15 sites from 10 patients as a control group). TCN-HCl gel was applied into the persistent pockets without mechanical instrumentation. Clinical parameters including probing pocket depth (PPD), loss of clinical attachment level (CAL), bleeding on probing (BOP) and total anaerobic bacteria count were measured at the baseline, 1-week, 4-week, 8-week and 12-week after a single course of each treatment. At baseline, PPD and CAL of the test group were similar to those of the control group. After TCN-HCl gel application, PPD and CAL showed significant improvement. The total anaerobic bacterial count started to decrease from week 1 through the end of treatment significantly. In the control group, PPD significantly decreased only from week 1 until 8 while the total anaerobic bacterial count reduced significantly after week 1 through the end (Sookkhee *et al* 2004). Since the TCN-HCl gel showed superior effectiveness to conventional method in the treatment of deep pockets, it is suggested that TCN-HCl gel can be effectively used in the periodontal treatment of persistent sites during maintenance phase.

However, using local drug delivery in the oral cavity may cause unwanted uptake of chemical agents in the gel base into the tissues. The idea of eliminating the chance of this occurring led to the research and development of local drugs for periodontal therapy by replacing the

gel base from chemical agents with natural polymer from rice.

The safety of using rice gel on oral tissue has been reported by Khongkhunthian *et al (*2017). In order to observe cell vitality, cytotoxicity on oral epithelium has been tested in vitro by using the F-actin and 4',6-diamidino-2-phenylindole staining technique. Furthermore, a lactate dehydrogenase assay was performed to confirm the safety. The results showed no significant differences between cells treated with rice gel and untreated cultures. The inflammatory inducing effect of rice gels was further examined in vivo by monitoring levels of tumor necrosis factor alpha (TNF-α) after rice gel application in the gingival sulcus. The results showed no significant difference of the expression of TNF-α at any given times when compared to the baseline. In conclusion, no evidence of cytotoxicity on oral epithelium when using the developed rice gel was observed (Khongkhunthian *et al,* 2017).

In vivo releasing efficacies of the TCN-HCl rice gel has been studied on 20 patients with untreated periodontitis who exhibited 72 deep periodontal pockets (>5 mm). For each patient, the base rice gel was randomly applied at a control site, whereas 40% (weight/volume) TCN-HCl rice gel (Figure 1) was applied in at least 1 periodontal pocket as test sites. Subgingival plaque samples were collected at baseline, 1-hour, 3-hour; as well as at 1-day and 3-day after the gel application. Total bacteria and *Porphyromonas gingivalis* were analyzed by real-time PCR. Matrix metalloproteinase 2 and 9 (MMP 2 and MMP 9) were analyzed by gelatin zymography. The results showed decreasing amounts of bacteria and enzymes after gel application. It was found that significant reduction of both total bacteria and *P. gingivalis* between the control and test groups occurred at 3-day ($p = 0.025$ and 0.001 respectively). For MMP 2, significant reduction between control and test groups in percentage was shown at 3-day ($p = 0.036$), while MMP 9, the significant difference was seen at 1-day ($p = 0.01$). Therefore it has been concluded that rice gel could be used as a vehicle for drug delivery system, and the drug could be released from the developed rice gel effectively because the amount of bacteria and enzymes reduced respectively (Limtragool *et al* 2016). However, it is necessary for us to continue further clinical research.

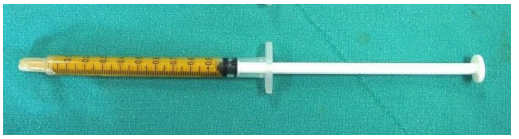

Figure 1. TCN-HCI rice gel

Other applications of rice gel for oral usage

Besides the anesthetic rice gel and TCN-HCl rice gel, our research team has also developed other oral products which contain rice gel base such as antifungal rice gel, fluoride rice gel, and bleaching rice gel. To validate effectiveness of our products, our research team has compared each of them with an equivalent commercially available product.

Rice gel containing 2% miconazole and 2% miconazole Daktarin oral gel® (OLIC (Thailand) Limited, Thailand, under contact with Janssen-Cilag) has been tested in 20 elderly persons who were wearing acrylic dentures (10 for each group). Before and after immersing the

dentures in antifungal gel every night for a week, the amount of cultivable *Candida albicans* was counted. It was found that the amount of *C. albicans* reduced over 90% in both antifungal product groups with no significant difference (unpublished data).

Rice gel containing fluoride has also been tested against a commercial fluoride gel (PreviDent Gel, Colgate®) with the same fluoride concentration (5000 ppm), in a group of 30 volunteers (15 for each group). The remaining fluoride in saliva was measured using a fluoride ion-selective electrode (Orion 9609BNWP Fisher Scientific Co. Itasca, IL, USA) according to manufacturer's instructions before and after a single course of gel application, at 5-min, 10-min and 30-min. No significant differences between the groups at the baseline were noted. However, at 5-min, the result showed significantly higher concentration of fluoride in saliva in the group using our rice gel than the group using commercial product. At 10-min and 30-min, the concentrations of fluoride gradually decreased with no significant difference between the groups (unpublished data.)

Another developed rice gel product is a bleaching nanoemulgel containing 10% carbamide peroxide. To evaluate biochemical effects, Thai rice bleaching nanoemulgel and commercial product - Opalescence® (Ultradent Products Inc., South Jordan, UT, USA) were tested on gingival inflammation of upper front teeth of 100 volunteers (50 for each group.) Before bleaching, no significant difference of IL-1β between the groups was recorded. After bleaching, IL-1β decreased in nanoemulgel group, and a significant difference between the groups was found at 7-day and 14-day. This indicated that the nanoemulgel from Thai rice caused less biochemical inflammation of the gum than the commercial product. Tooth hypersensitivity and tooth color change between the two groups were also compared. Tooth hypersensitivity and self-perception of tooth whitening were determined by using VAS, and tooth color change at different intervals were evaluated by using Vita classical shade guide. Before bleaching, similar distribution of gender, average age and time of bleaching as well as the patients' behaviors were observed. After the first day of bleaching, tooth hypersensitivity increased in both groups, but at the 14th day the intensity of tooth hypersensitivity in the nanoemulgel group was less than the commercial group. The color change evaluated by self-perception and by using Vita classical shade guide showed the same results in both groups at any interval. It can be concluded that the bleaching efficacy of nanoemulgel from Thai rice was similar to the commercial product but with less hypersensitivity (unpublished data).

Conclusions

In conclusion, this report can be used to highlight the potential and effective properties of rice for developing suitable drug delivery systems in oral cavity, especially antibiotics or antiseptic for periodontal therapy. The modified rice products are very interesting for oral applications due to their high adhesive property to the teeth and oral mucosal membrane. Since there are many rice varieties, and different varieties can yield different levels of adhesive strength and other advantage properties, selection of suitable rice variety is essential for practical formulation development and applications.

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Chapter 18

Soft Tissue Management Around Dental Implant: Case reports

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Introduction

Dental implants have been used to replace lost teeth, and have a very high survival rate and long-term stability (Moraschini *et al* 2015; Gomes *et al* 2018). In addition to improving function, esthetics are highly important in dental implant treatment (Stimmelmayr *et al* 2010). Although, the nature of the biological width of natural teeth and dental implants are different (Makigusa 2009), the way soft tissues around dental implants should be managed is similar to tissues around natural teeth. However, there are some important criteria that need to be understood when managing dental implants. It is now becoming more relevant that the placement of dental implants has shifted from a prosthodontic- to periodontal-driven procedure.

"Peri-implant plastic surgery", a new term derived from "Periodontal plastic surgery", is a surgical procedure to correct soft and/or hard tissue defect in morphology, position and/or amount of gingiva around dental implant (Palacci and Nowzari 2008). The procedure aims to harmonize the peri-implant structures with the final prosthetic restoration by performing soft and/or hard tissue engineering. Soft tissue management around implants might be performed prior to implant placement, during the second-stage surgery before the prosthetic phase, or after loading of the definite crowns.

Preliminary considerations

The fundamental consideration for the long-term success of implant restoration starts from appropriate patient selection, correct diagnosis, comprehensive treatment plans discussed together with patient, and adequate surgical and final prosthodontic protocols. Soft tissue management in the anterior area can represent the most critical and difficult part of the entire oral rehabilitation, especially in the high demanding patients (Ioannou *et al* 2015).

Although the absence of keratinized mucosa may not compromise the long-term success of implans, its presence improves esthetics (Geurs *et al* 2010), reduces plaque accumulation by improved cleansibility (Ladwein *et al* 2015) and guarantees a stable mucosal seal (Bruschi *et al* 2012). Several surgical techniques have been introduced to increase the amount of

keratinised mucosa, or to even re-establish keratinized tissues where it was previously absent (Nasr 2006).

Apically positioned partial-thickness flaps can be used to increase the amount of keratinized gingiva when there is presence of the gingival tissue of at least ≥3mm thick, good depth of the vestibule, and thick alveolar bone (Stappert and Romeo 2015; Parthasarathy *et al* 2013); Free gingival grafts associated with a partial-thickness flap is desirable in case of complete absence of keratinized gingiva to protect the underlying connective tissue and bone (Buyukozdemir *et al* 2015).

In some clinical situations, especially in the anterior area where an adequate width of keratinized tissue is present but poor volume in all dimensional aspects, autogenous connective tissue graft is considered to improve the emergence profile of implant-supported fixed restorations (George and Dhir 2015).

Materials and Methods

Case 1: Re-establishment of Keratinised Mucosa using Free Gingival Graft

A 37-year-old female patient had been referred to the periodontal department, Faculty of Odonto-Stomatology, University of Health Sciences, after implant placement with simultaneous bone grafting. Clinical findings demonstrated a shallow vestibule with unattached mucosa approximating the crestal level of gingival ridge (Figure 1). It was proposed to take a free gingival graft from the palate and reposition the mucogingival junction more apically to increase patient comfort and create a good soft tissue seal around the dental implants at the time of implant exposure.

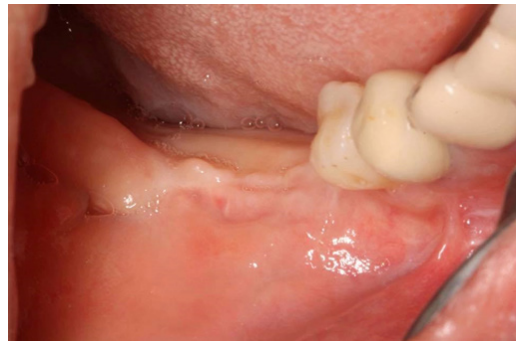

Figure 1. Clinical examination showed shallow vestibule with unattached mucosa.

Surgical Procedures:

A linear incision in the keratinized gingiva close to the crest of the ridge was performed and a split-thickness flap was raised with complete muscle dissection (Figure 2).

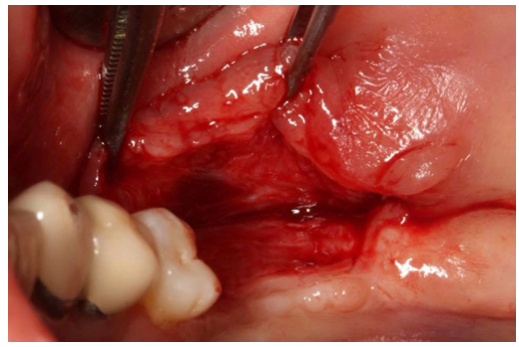

Figure 2. Split-thickness flap was raised with complete muscle dissection.

The flap was firmly secured to the periosteum with single interrupted sutures with an absorbable polyglycolide-co-l-lactide (PGLA) 5-0 suture (Novasorb® suture; Novamedic Co.,Ltd, Thailand) to avoid any apical movement (Figure 3). A free gingival graft was harvested from the hard palate (Figure 4) and reshaped to fit the recipient sites.

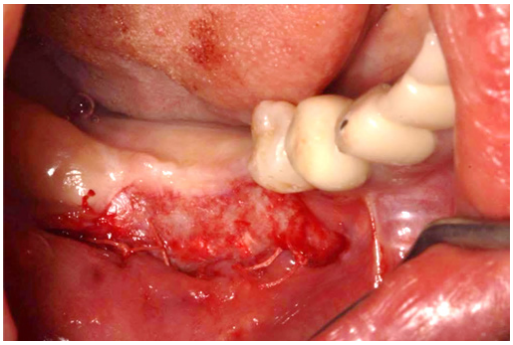

Figure 3. The flap was firmly secured to the periosteum with single interrupted sutures.

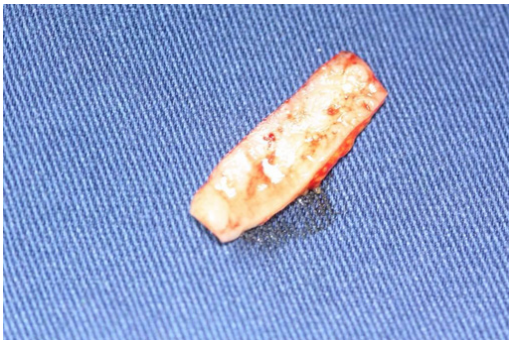

Figure 4. Free gingival graft was harvested from the hard palate.

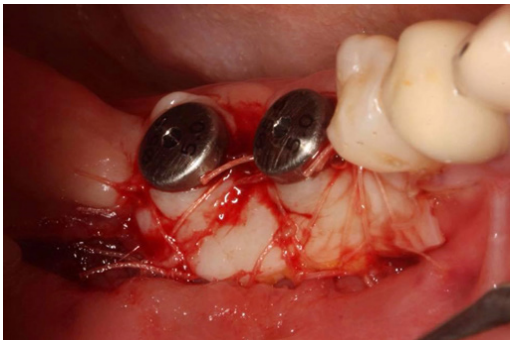

Figure 5. Free gingival graft was harvested from the hard palate.

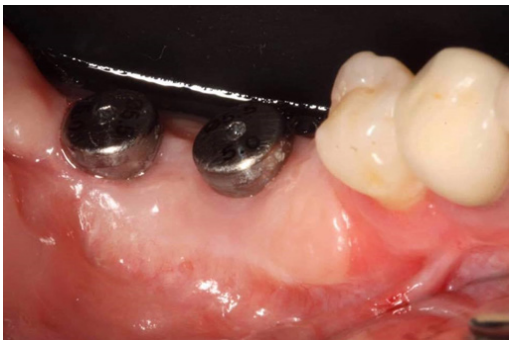

Figure 6. A new depth of the vestibule was established.

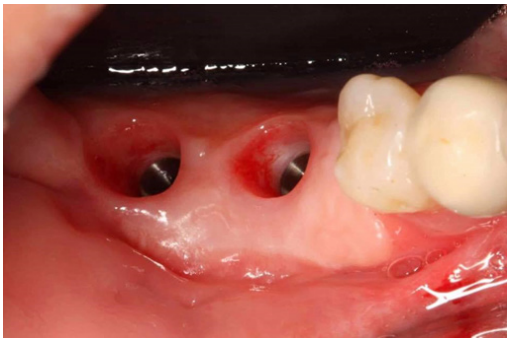

Figure 7. Abutments were removed prior final impression.

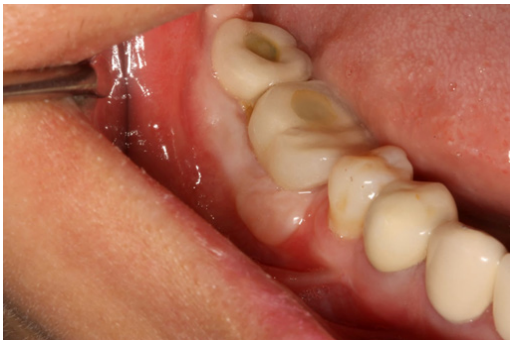

Figure 8. 1-year follow-up after final prosthetic restoration.

The free gingival graft was sutured to the buccal mucosal connective tissue and to the underlying periosteum with absorbable PGLA 5-0 and 6-0 sutures (Novasorb® sutures; Novamedic Co.,Ltd, Thailand) (Figure 5). After 2 months, a new depth of the vestibule was established (Figure 6). The mucogingival junction was repositioned approximately 8-10 mm apical to its pre-surgical level (Figure 1). Soft tissue around the crestal of dental implants looked fit and tight after healing abutments were removed for final impression (Figure 7). The 1-year follow-up showed stable tissue results with no sign of inflammation

after prosthetic restoration (Figure 8).

Case 2: Soft Tissue Augmentation using Connective Tissue Graft

A 34-year-old healthy female patient was referred to the periodontal department, Faculty of Odonto-Stomatology, University of Health Sciences, for management of a soft-tissue complication over an implant site. The dental history revealed that there had been an immediate implant placement at site #21 due to a vertical root fracture. Free gingival grafting had been performed to cover the wound. Unfortunately, post-operative complications occurred and resulted in a soft tissue defect (Figure 9a-d). Clinical examination showed a soft-tissue deficiency at the site #21 (Figure 10). Connective tissue grafting was planned to rescue the situation. At the surgical re-entry, the loss of buccal plate was also detected, therefore both hard and soft tissue augmentation were performed.

Surgical procedures:

The full-thickness flap with a vertical incision was elevated. The surgical entry revealed that buccal plate was missing at the cervical part of the implant (Figure 11). Freeze Dried Bone Allograft (FDBA, Bone RegenOss; Cellumed Co., Ltd., Seoul, South Korea) was placed under an absorbable collagen membrane (Membra-Gen; Membra bio, South Korea (Figure 12). A connective tissue graft was harvested from the hard palate (Figure 13) and then placed over the collagen membrane. The tension-free flap was closed with single interrupted sutures and the CTG was secured underneath using non-absorbable 6-0 suture (Polyamide monofilament, Sofilon; Connek, Bankok, Thailand) (Figure 14). 6-month follow-up showed the graft blended well with the surrounding keratinised gingiva, and the soft-tissue volume was gained (Figure 15-B).

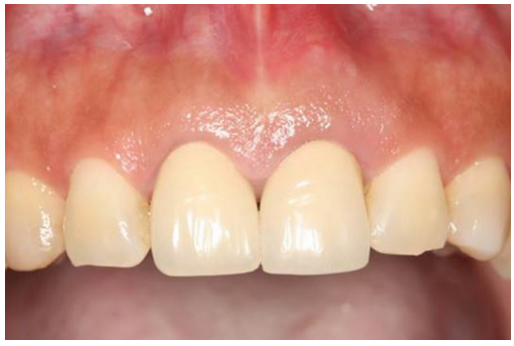

Figure 9a. Initial situation before referral.

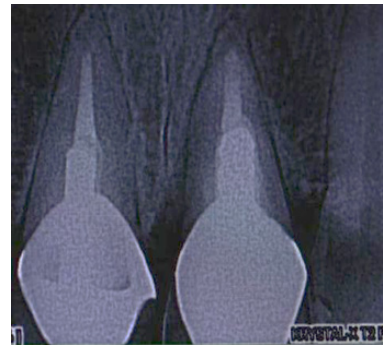

Figure 9b. Initial radiographic examination had revealed vertical root fracture at metal post-core of #21.

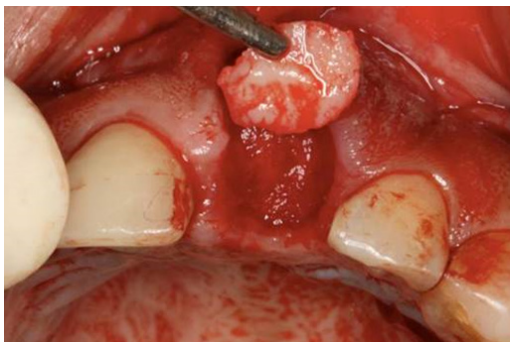

Figure 9c. Free gingival graft harvested from hard palate had been placed on the implant site.

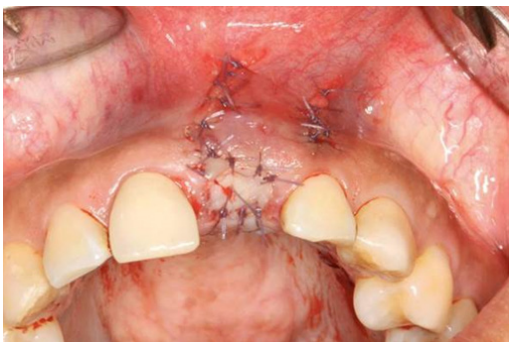

Figure 9d. The free gingival graft had been sutured over the socket without de-epithelizing the socket border first.

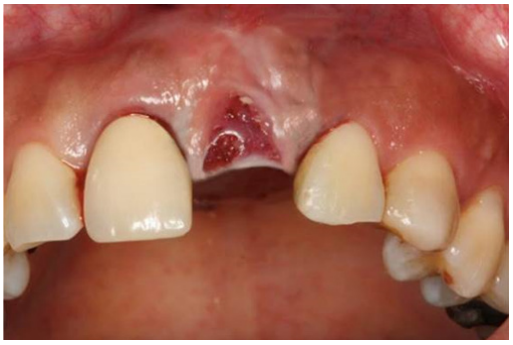

Figure 10. Soft-tissue deficiency at the implant site #21 at the time of referral.

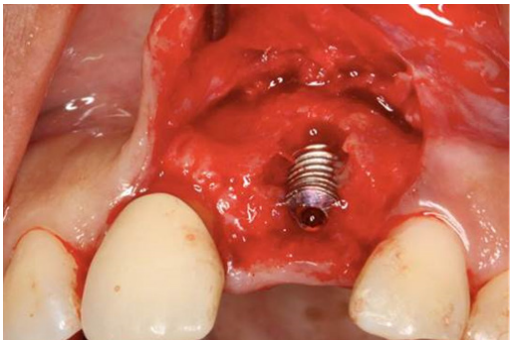

Figure 11. Full-thickness flap was elevated revealing missing buccal plate at the cervical part of the implant.

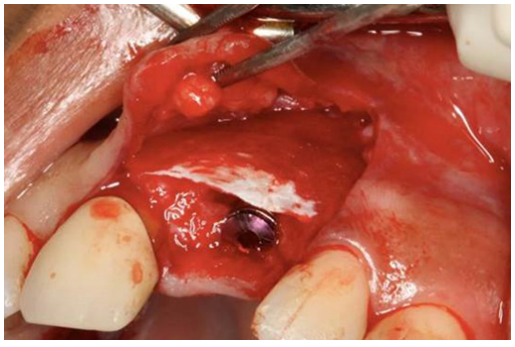

Figure 12. Absorbable collagen membrane in place before adding bone substitutes.

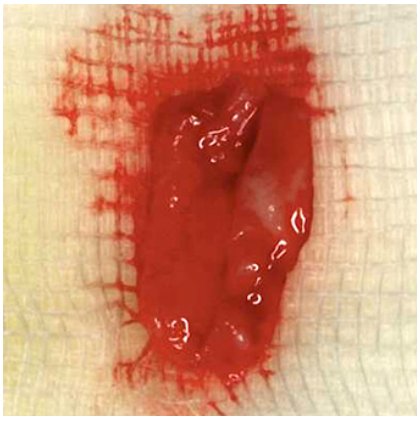

Figure 13. Connective tissue harvested from the hard palate.

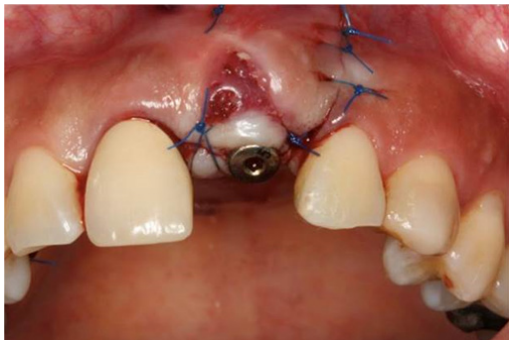

Figure 14. Connective tissue was secured underneath the tension-free flap.

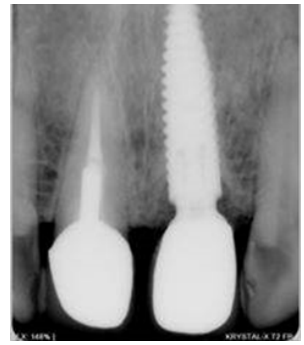

Figure 15a. Radiograph at 6-month follow-up.

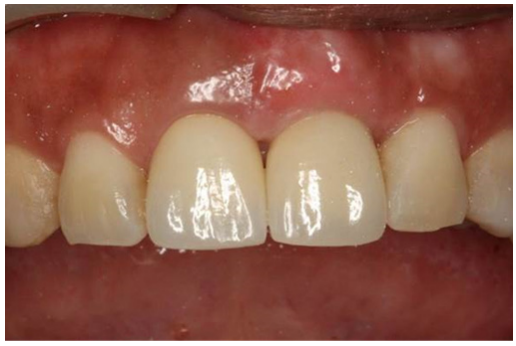

Figure 15b. Clinical situation at 6-month follow-up.

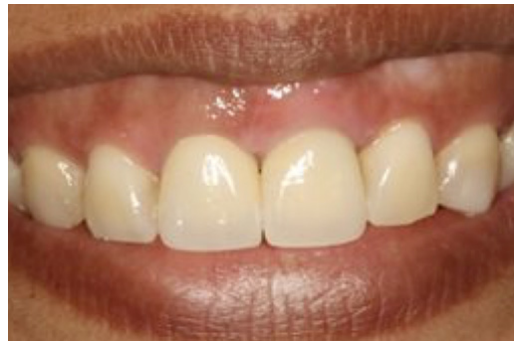

Figure 15c. Clinical situation at 18-month follow-up.

Discussion

Soft tissue management around dental implants is considered to be one of the important key factors for long-term success. The decision whether to increase the width of keratinized gingiva and when to perform such procedures remains controversial. The appropriate timing of soft tissue management around dental implants is critically important and the soft tissue status should be the first factor to be evaluated (Kadkhodazadeh *et al* 2017). The case reports presented in this Chapter emphasize the re-establishment of adequate keratinized tissue around dental implants using free gingival graft in the posterior mandibular region and the enhancement of soft tissue volume around dental implant using connective tissue graft in the anterior maxillary region.

Although the effect of keratinized mucosa width on peri-implant health remains inconclusive, the presence of keratinized mucosa has been reported to be positively associated with ease of self-performed oral hygiene (Schwarz *et al* 2018). With this regards, in case 1 where inadequate gingival width and shallow vestibular depth were identified, vestibuloplasty together with a free gingival graft were performed. From a clinical perspective, keratinized tissue augmentation was preferable as keratinized mucosa may be more resistant to possible soft tissue recession due to inflammatory or non-inflammatory recession, and brushing trauma during the course of

years. A 8-year cross-sectional study revealed that the absence of keratinized mucosa at the implant sites were associated with significantly higher plaque score and marginal bleeding compared to the sites with adequate keratinized mucosa (Ladwein *et al* 2015). In a recent study at 10 years after implant placement, it was reported that the lack of keratinized mucosa was associated with higher plaque score even the patients exercised sufficient oral hygiene and received the supportive periodontal therapy (Roccuzzo *et al* 2016).

The concept of >2 mm thickness of keratinized mucosa is more relevant in the anterior esthetic zone. Clinical studies showed that the amount of soft-tissue volume can greatly influence the esthetic outcome and even partially compensate the hard-tissue defects (Thoma *et al* 2014a; Thoma *et al* 2014b). The transplantation of an autogenous connective tissue graft with/without epithelium has been the gold standard for both esthetic and biological purposes. In the second case report, a connective tissue graft was employed to correct the inadequate healing of a free gingival graft that had been performed at the time of implant placement resulting in a soft tissue defect. Following the soft tissue augmentation with autogenous connective tissue graft, the soft-tissue volume remained stable and the color and texture of the gingiva were greatly improved at 18-month follow-up. Stable results have been reported with a mean gingival recession of 0.2 mm within 1 year after crown insertion using similar surgical procedure as demonstrated in the present case reports (Schneider *et al* 2011). To date, the long-term evidence supporting whether augmented soft tissues can be maintained over time is still limited (Rotundo *et al* 2015). Longer follow-up periods and further clinical studies are mandatory to validate the long-term stability of these procedures.

Conclusions

Esthetic implant placement is strongly influenced by the soft tissue condition around the implant. Biological considerations and the approrpriate choice of surgical techniques are key factors for successful surgery outcomes. Peri-implant plastic surgery using free gingival grafts and connective tissue grafts remain the standard of care to re-establish inadequate keratinised mucosa and to enhance the soft tissue profiles.

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Poster Presentations

Winners of Sunstar Poster Awards

The following is a record of the posters awarded prizes at the 13th Meeting of the Asian Pacific Society of Periodontology

Clinical Research Category: 1st Place - Nuzul Izwan Omar (Malaysia)

The effectiveness of *Ficus deltoidea* in preventing aveolar bone loss osteoporotic rats

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Background and Objectives: *Ficus deltoidea*, a Malaysian traditional leaf known as "mas cotek" has been shown to possess antioxidant properties that may prevent chronic inflammatory bone diseases from developing. The aim of the study was to investigate the inhibitory effect of *Ficus deltoidea* on alveolar bone loss in rats experiencing induced osteoporosis by ovariectomy (OVX).

Materials and Methods: Twenty-six female Wistar (12 weeks old) rats were randomly divided into four groups (n=6): ovariectomy sham-operated (SO), non-treated ovariectomized (OVXN), ovariectomized with estrogen (OVXP) and ovariectomized with *Ficus deltoidea* (OVXF). For the baseline (BL), two non-ovariectomized healthy rats were euthanized at the beginning of the study. Ovariectomy was done under anesthesia and the treatment was started two weeks postoperatively. Both SO and OVXN groups had received deionized water, group OVXP had received Premarin (64.5µg/kg body weight) and group OVXF had received *Ficus deltoidea* (800mg/kg body weight). All treatments were given by oral gavage daily and rats were sacrificed after two months. The interradicular bone of first molar (M1) was observed for the alveolar bone loss height and density morphometric analysis by micro-CT scan and, histological analysis was determined by quantitation of osteoclasts and osteoblasts.

Results: Morphometric analysis demonstrated that alveolar bone density and height were reduced in OVXN group. However, in OVXF group, the trabecular thickness was maintained similar to BL and, trabecular separation and alveolar bone loss height was less compared to OVXN group. In the histological analysis, OVXF group had demonstrated statistically significant a smaller number of osteoclasts and a higher number of osteoblasts compared to OVXN ($p=0.008$ and $p=0.019$; $p<0.05$ respectively).

Conclusion: It has been found that oral administration of *Ficus deltoidea* can prevent alveolar bone loss in osteoporotic induced rats by its potential to preserve trabecular bone density, decrease osteoclasts and increase osteoblasts cell count to reduce bone resorption and stimulate bone formation respectively.

Clinical Research Category: 2nd Place - Lew Pit Hui (Malaysia)

Evaluating presence of antibodies against citrullinated proteins in patients with rheumatoid arthritis and periodontitis

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Background and Objective: Presence of antibodies against citrullinated proteins (anti-CitP) are highly specific for rheumatoid arthritis (RA). Recent studies have revealed the occurrence of citrullination in periodontitis (PD) which can induce anti-CitP production, allowing for a potential link between PD and development of RA. However, conflicting findings have been reported to associate RA and PD in relation to the expression of anti-CitP. This study aimed to compare the presence of serum anti-CitP with clinical periodontal and RA parameters in patients with RA and/or PD.

Materials and Methods: A total of 80 participants were recruited, each of 20 from those diagnosed with both RA and PD (RAPD), only RA, only PD, or no RA or PD (healthy). Full mouth periodontal examination was conducted and 10ml of serum sample was colected from each subject for quantification of anti-CitP using enzyme-linked immunosorbent assay (ELISA). ESR levels and RA disease duration were also recorded.

Results: Highest mean (±SD) levels of serum anti-CitP were found in RAPD group (228.82±219.09 IU/mL) followed by RA group (204.01±202.41 IU/mL), PD group (102.62±75.46 IU/mL) and lowest in healthy group (68.73±52.49 IU/mL). Anti-CitP levels were significantly higher in RAPD and RA groups compared to that of healthy group ($p < 0.05$). Multiple regression analysis to control for age and gender confirmed the significance. Anti-CitP levels were not correlated with any of the clinical periodontal parameters between all 4 groups. For RAPD and RA groups, no significant correlations were observed between serum anti-CitP levels with ESR and RA disease duration.

Conclusion: There is a trend for an increase in anti-CitP levels from healthy to PD to RA and RAPD groups. The underlying biological mechanisms for the relationship between PD and RA needs to be further investigated in future studies.

Clinical Research Category: 3rd Place - Hu Kang-Shuo (Taiwan)

Aesthetic result of immediate implant placement in extraction sockets with intact versus deficient walls

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Background and Objective: To assess whether immediate implant placement in intact sockets yields more favourable aesthetic outcomes and higher survival rates compared with immediate placement in sockets with deficient walls (deficient sockets).
Materials and Methods: This retrospective study included patients with unrestorable teeth who were treated with tooth extraction, defect debridement, and immediate implant placement between March 1996 and September 2017. Fifty patients with unrestorable teeth (incisor, canine, or premolar) were treated with immediate implant placement (50 implants; follow-up, 4–46 months; median 6.5 months); 25 implants were placed in deficient sockets, and 25 implants in intact sockets. The pink aesthetic score (PES) was used for outcome analysis. All cases were evaluated using the PES, and only upper anterior sites (incisor and canine) were re-evaluated using the PES.
Results: The implant survival rate was 100%. The average PES was 8.4 ± 1.29 in the deficient socket group and 8.52 ± 1.05 in the intact socket group (p=0.72). In upper anterior sites, PES was 8.59 ± 1.18 in the deficient socket group and 8.63 ± 1.09 in the intact socket group (p=0.93). These results were not statistically significant different.
Conclusion: Immediate implant treatment in deficient or intact socket sites yielded comparable outcomes. Longer-term follow-up of aesthetic results and clinical outcomes are required for the evaluation of the validity of immediate implant placed into deficient sockets.

Laboratory Research Category: 1st Place - Ishii Shiori (Japan)

Effects of toothpaste containing pyrroridone carboxylic acid on dentin demineralization and collagen degradation

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Background and Objective: In super-aged society such as Japan, the risk for root caries is growing because the number of the remaining teeth is increasing. Thus, the prevention of root caries is important issue. As dentin consists of mineral, organic component (mainly collagen) and water, preservation of both mineral and collagen are necessary to prevent root caries progression. In this study, we focused on Pyrrolidone carboxylic acid (PCA) which is known to have a protective effect on skin and evaluated the inhibitory effects of the toothpaste containing PCA on dentin demineralization and collagen degradation. Furthermore, we investigated the mechanism of inhibitory effect of PCA.
Materials and Methods: 1. Evaluation of the inhibitory effects on demineralization and collagen degradation. Collagen-exposed bovine dentin disks were treated with either of the following toothpastes: [1,450 ppm F (NaF)], [3,000 ppm F] and [1,450 ppm F+PCA], then immersed in artificial saliva containing collagenase. After the disks were demineralized, the amount of released Calcium ions was measured by atomic absorption spectroscopy. Furthermore, the amount of hydroxyproline, a component of collagen, of dentin surfaces was measured by Confocal Raman Microscopy. 2. Assessment of the interaction of PCA with dentin components Adsorption affinity of PCA on collagen and hydroxyapatite were respectively examined by quartz crystal microbalance with dissipation monitoring.
Results: 1. In group [1,450 ppm F+PCA], the amount of released Calcium ions was significantly smaller than that in group [1,450 ppm F]. The amount of hydroxyproline in group [1,450 ppm F+PCA] was significantly larger than those in groups [1,450ppm F] and [3,000 ppm F]. 2. PCA adsorption affinity on collagen was higher than that on hydroxyapatite.
Conclusion: It was indicated that PCA toothpaste had inhibitory effects on dentin demineralization and collagen degradation. The protective effect of PCA on collagen might contribute to the notable efficacy of PCA toothpaste.

Laboratory Research Category: 2nd Place - Yuliana Ayob (Malaysia)

Elucidation of the antibacterial effect of Malaysian coconut oil against Aggregatibacter actinomycetemcomitans

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Background and Objective: Periodontal disease is associated to high levels of periodontopathogenic microorganisms such as *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* and *Prevotella intermedia*. Several agents have been used to inhibit the growth of these bacteria towards achieving healthy periodontium. This study is aimed to determine the antibacterial effect of coconut oil and compare the effectiveness between fermented and cold press oils against Aa (ATCC 43718).

Materials and Methods: Malaysian coconut oil was provided by AZIMPRO, a local company. Antimicrobial Susceptibility Testing (AST) was carried out to test for antibacterial susceptibility to both fermented and cold press coconut oil using the agar well diffusion method. The culture media used was brain heart infusion agar and broth. Broth serial microdilution test was used to evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). A Scanning Electron Microscopy (SEM) was also conducted. The configuration changes on the bacteria were observed by SEM. The negative control used was DMSO and the positive control was chlorhexidine. Fermented and cold press coconut oil were compared for their antibacterial effect.

Results: The highest mean zone of inhibition was chlorhexidine, followed by 50% cold press coconut oil and the lowest was 50% fermented coconut oil (35.0 mm,14.56 mm and 13.56 mm respectively). No significant difference was found between both types of coconut oil (P value >0.05). Both fermented and cold press coconut oil showed an antibacterial effect against the bacteria (MIC 6.25% and 3.125%, MBC 50% and 3.125% respectively). SEM shows alterations of Aa configuration after treated to the coconut oil.

Conclusion: This finding exhibits the growth of Aa was inhibited by both types of coconut oil with similar effectiveness seen in fermented and cold press coconut oil. This result is promising to suggest that coconut oil has an antibacterial effect against Aa.

Laboratory Research Category: 3rd Place - Wahidatunnur Musa (Malaysia)

Hydroxytyrosol from Olive Oil: Antibacterial Effects on Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Fusobacterium nucleatum

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Background and Objective: Hydroxytyrosol is a fraction of phenolic compound, a secondary metabolite in olive oil that has a role as an antimicrobial agent. Studies have shown that it has antibacterial activity towards wide spectrum of bacteria. However, little is known on the antibacterial activity of this component towards periodontal pathogen. This study was conducted to evaluate the potential of hydroxytyrosol in olive oil as an antibacterial agent towards periodontal pathogens, namely Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Fusobacterium nucleatum.

Materials and Methods: Hydroxytyrosol was prepared in various concentrations (0.78mg/ mL to 100mg/ mL) to determine its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the three periodontopathogens. The anti-adhesion activity of the hydroxytyrosol was quantified using crystal violet staining. The effects on the morphology of the periodontopathogens were examined through scanning electron microscopy (SEM).

Results: The MICs of the hydroxytyrosol on A. actinomycetemcomitans, P. gingivalis and F. nucleatum were 6.25 mg/mL, 25.0 mg/mL and 25.0 mg/mL, respectively. The MBCs of the hydroxytyrosol on A. actinomycetemcomitans and F. nucleatum were 12.5 mg/mL and 25.0 mg/ mL while no bactericidal effects observed on P. gingivalis. The adhesion of all the tested bacteria was interrupted by this component at lowest concentration (0.78mg/ mL). Based on SEM findings, there were disruption of bacterial cell surfaces such as blebs formation and cell shrinkage after exposure with the hydroxytyrosol.

Conclusion: Hydroxytyrosol exhibited antibacterial activity against the tested periodontal pathogens, with bactericidal effects on A. actinomycetemcomitans and F. nucleatum, and bacteriostatic effects on P.gingivalis.

Clinical Case Reports Category: 1st Place - Balaji Manohar (India)

Tooth as a graft - application in periodontal regeneration

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Introduction: Tooth and bone share many similarities. Tooth, cartilage, nerve, and maxillofacial bones originate embryologically from the neural crest, sharing identical origin and many proteins are common to bone, dentin and cementum. Generally, the extracted teeth have been discarded as infective medical waste in the world. Researchers focused on bone-inductive, absorbable properties of tooth and introduced medical recycle of human tooth as a novel graft material for bone regeneration.
Case: Patients with vertical intrabony defect as well as tooth indicated for extraction were selected for this case series. Non -smokers with no systemic disease were considered. Extracted tooth was processed using a domestic mixer grinder to prepare the graft material and placed at defect site. Baseline and 26 weeks post-operative evaluation of the primary endpoint, bone fill on Cone Beam Computed Tomography (CBCT) and secondary outcomes including clinical parameters were assessed. During the entire observational period of 26 weeks, soft tissue healing was uneventful. CBCT revealed the homogeneous incorporation of tooth graft at the defect site. The procedure was not associated with any wound infection or adverse events in both the cases.
Discussion: Use of autogenous tooth bone graft material resulted in significant reduction of probing pocket depth, gain in clinical attachment and defect fill. The possible explanation for better results with autogenous tooth bone graft is validated by various in vitro and animal studies which have demonstrated its biocompatibility, osteoinductive, and osteoconductive potential. Furthermore, the use of domestic mixer grinder ensures cost effectiveness of the procedure.
Conclusions: Autogenous grafting is considered as gold standard. Recycling of biomedical waste as graft biomaterial, not only attracts predictable clinical results but adequate patient compliance and value too. This novel procedure may be further investigated with larger sample size in intrabony defect.
Keywords: tooth graft, intrabony defect, CBCT, regeneration

Clinical Case Reports Category: 2nd Place - Nor Shafina Mohamed Nazari (Malaysia)

Volume stable collagen matrix in comparison to autogenous CT graft in the treatment of multiple gingival recession a case report

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Introduction: Volume-stable collagen matrix (VCM) is a recently-introduced soft tissue substitute. Based on pre-clinical studies, VCM is able to support fibroblast ingrowth and angiogenesis for the purpose of soft tissue augmentation. Besides, it also has the ability to maintain volume over time in comparison to other soft tissue substitutes.
Case: This case report describes a female patient who presented with generalised gingival recession Miller Class I to III and abrasion cavities. She was concerned about her appearance. Following oral hygiene education and non-surgical periodontal management, the gingival defects were then planned for root coverage procedure and the abrasion cavities were restored with tooth-coloured materials. The root coverage procedure was performed by utilising coronally advanced flap (CAF) and subepithelial connective tissue graft (CTG) for the maxillary right area, while CAF and VCM (Fibro-Gide®) were used for the maxillary left area. The surgical areas were reviewed and follow up visits were performed at least for six months after the surgery.
Discussion: Root coverage was obtained over the recession defects following both CAF + CTG and CAF + VCM. The results from the two treatment modalities yield comparable outcomes. Other than that, the use of VCM avoids complications and morbidity related to graft harvesting procedure.
Conclusion: VCM is a viable soft tissue substitute for soft tissue augmentation procedure around teeth. Keywords: root coverage, soft tissue substitute, gingival recession

Clinical Case Reports Category: 3rd Place - Wan Mahadzir Mustafa (Malaysia)

Dental implant in the rehabilitation of an adult cleft patient - a case study

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Introduction: Successful dental rehabilitation of the cleft lip and palate patient can be difficult to achieve without careful planning and sustained effort.

Case: A case is reported where tertiary osteoplasty using cancellous autogenous iliac bone graft was performed as dental implant site development prior to restoration. Once the graft has taken this was followed by the insertion of a Frialit 2 dental implant and later the provision of suprastructure. Good long term results at 15 years post insertion was obtained in this case.

Discussion: Dental rehabilitation of the cleft lip and palate patient involves the creation of a continuous dental arch. This can be achieved by fixed prosthesis, removable prosthesis or osteointegrated dental implants, by surgical and/or by orthodontic means.

Sometimes orthodontics cannot create a continuous dental arch with space between teeth in alveolar cleft patients. The reasons for this may include: agenesis of lateral incisor, loss of tooth buds due to primary surgery, early loss of teeth as a result of dental malformation, caries or trauma. The use of dental implants can improve the condition for later prosthetic treatment.

Prior to implant insertion implant site can be developed by ridge splitting, guided bone regeneration (GBR), bone grafting or distraction osteogenesis. Bone grafting is advantageous as it is suited to the knife edge nature of the defect and is relatively inexpensive. Superior bone to metal contact is obtained by implants in grafted bone. Although simultaneous implant is not indicated in the present case the many advantages of bone grafting persuaded us to opt for this approach. Successful long term results vindicated our decision to choose this option.

Conclusion: A dental implant placed in the bone grafted area of a cleft patient is an effective long term solution to replace missing upper lateral tooth in the patient with alveolar cleft.

Systematic/Literature Reviews Category: 1st Place - Tan Oi Leng (Malaysia)

Contemporary local drug delivery and adjunctive agents used in non-surgical periodontal therapy

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Background and Objective: Periodontal infections tend to be site-specific, mostly confined to the periodontal pocket. Therefore, much attention has been garnered towards local drug delivery and adjunctive agents as to prevent potential side effects and increased antibiotics resistance with systemic antimicrobials use. However, the question of the most efficient local drug delivery or adjunctive agent for dental practitioners to utilise and provide the maximum benefits to their patients remains unanswered.

This review aimed to provide insight on the efficacy of current commercially available local drug delivery and adjunctive agents used in non-surgical periodontal therapy in adults treated for periodontitis.

Data/Sources: The PubMed/MEDLINE (via Ovid), EMBASE (via Ovid) and CENTRAL databases were searched to identify any randomised controlled human intervention studies with professionally applied local subgingival drug delivery and adjunctive agents used in the treatment of periodontitis. The search considered works published till April 2019 using specific keywords pertaining to the topic. Bibliographies from previous systematic reviews on the topic were scrutinised. Only articles published in the English language were selected, and the use of experimental or discontinued drugs were excluded.

Study selection: The longest follow-up studies of each local delivery system were selected, and 24 randomised controlled trials that compared non-surgical mechanical therapy with and without an adjunctive agent were identified. The details of each system and their clinical results were summarised in table form.

Conclusion: Overall, various commercially available local subgingival drug delivery and adjunctive agents have been clinically tested in the non-surgical treatment of periodontitis. However, the methodologies and clinical results vary within and between each system. Therefore, it is difficult to conclude and support the superiority of one local adjunctive agent over another. Further well-designed medium to long term studies are needed as their usefulness is still debatable, considering the cost-benefit ratio with modest clinical results.

Systematic/Literature Reviews Category: 2nd Place - Ang Yee (Malaysia)

Tooth mobility simulation in laboratories studies: comprehensive review

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Background and Objective: Tooth mobility simulation can be used to overcome some limitations of laboratory studies. This narrative review aimed to review several materials and methods used for tooth mobility simulation. This work will provide a platform to researchers aiming to develop new models for dental material testing.

Data/Sources: PubMed and Google Scholar were searched for English articles (years 1969 to 2018) related to laboratory studies that simulate tooth mobility.

Study Selection: Only studies published in English were selected. Elastomeric impression materials and tissue conditioners are widely applied to mimic the elasticity of periodontal ligaments in periodontium simulation. Acrylic resin and polystyrene resin are examples of materials utilized to simulate alveolar bone. The centre of rotation (CRO) will exist at the middle third of the tooth root when a tooth deflects within its socket. The CRO is commonly positioned at the apex to increase tooth mobility in numerous studies. Several materials and methods, such as socket enlargement, ball-ended root systems, and screw-retained plastic models, as well as the simulation of alveolar bone loss, were reviewed and discussed in detail.

Conclusion: Natural human teeth can be standardized through simple composite build up. Embedding teeth in a designated socket dimension while replacing spaces with silicone and rubber foam might be the best combined approach for producing a highly accurate model for periodontium simulation.

Keywords: Periodontal ligament simulation, tooth mobility, alveolar bone, tooth mobility simulation methods

Systematic/Literature Reviews Category: 3rd Place - Trisantoso Rezdy Asalui

Effectiveness of platelet rich fibrin in coronally advanced flap: A systematic review

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Background and Objective: Gingival recession is displacement of marginal gingiva tissue apical to cemento-enamel junction. Gingival recession can be caused by incorrect brushing technique, teeth malposition, inflammation of the gingiva, abnormal frenum attachment and iatrogenic dentistry. There are various techniques to treat gingival recession and coronally advanced flap is one of them. Platelet-rich fibrin is the second generation of platelet concentrate and it supports migration of cell, accelerates wound healing, angiogenesis, and tissue regeneration. The aim of this study is to know the effectiveness of platelet-rich fibrin in treating gingival recession using coronally advanced flap.

Data/Sources: Two search engines were used in this research which were PubMed and Wiley Online Library from 2009 until 2019 to identify articles published in dental journal that using specific keywords, they were "coronally advanced flap" AND "gingival recession" OR "root coverage" AND "platelet-rich fibrin". Full-text articles and related articles were manually reviewed.

Study Selection: The initial database search resulted 68 articles. Only full-text reviews were selected in this study. A total of nine studies discussed about coronally advanced flap and platelet-rich fibrin but only four studies that met the inclusion criteria, with 97 patients as subjects. All of the studies showed that treating gingival recession using coronally advanced flap and platelet-rich fibrin was not better than using coronally advanced flap alone.

Conclusion: Treatment of gingival recession using coronally advanced flap and platelet-rich fibrin is not better than using coronally advanced flap alone.