
THE PAST, PRESENT AND FUTURE OF PERIODONTOLOGY

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Asian Pacific Society of Periodontology

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Nara, Japan

**Edited by
P Mark Bartold
T Nagata**

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Acknowledgements

The 10th International meeting of the Asian Pacific Society of Periodontology was held in Nara, Japan on 3 & 4 September 2013. This was a very special meeting for the APSP as it celebrated 20 years since its foundation in 1993. Accordingly, the theme for this meeting was “The Past, Present and Future of Periodontology”. Over 360 delegates from 17 countries attended this APSP meeting. The meeting was opened with an address by Professor Toshihiko Nagata, the President of the 10th Asian Pacific Society of Periodontology Meeting. Additional greetings were presented by Mr Yoshihiro Kaneda, Sunstar Group; Mr Takashi Yamamoto, Lion Corporation and Dr Arunee Laiterapong, Johnson & Johnson Consumer Limited.

The two-day program was very full, with 20 presentations from speakers from 17 different countries. In addition, 78 posters were scheduled for presentation.

Over the two days, 3 keynote speakers, 3 special invited speakers and 12 country representative speakers from the Asian Pacific region presented lectures on a wide range of topics including:

- History of the Asian Pacific Society of Periodontology
- Periodontal treatment strategies
- Periodontal teaching and training
- Periodontal/implant interrelationships
- Tissue regeneration around teeth and implants
- Periodontal bone biology

The poster sessions, sponsored by Sunstar, were very successful and in keeping with tradition from previous meetings, four prizes were awarded for the posters judged to be the best on the day.

This volume serves as a record of all of the presentations made at this meeting. I am sure you will agree with me that each of the chapters is very interesting and represents many contemporary concepts and excellent overviews of the past, present and future of periodontics.

The APSP wishes to acknowledge our sponsors who are listed on the following page. Without this support the 10th APSP meeting and the publication of the proceedings would not have been possible. I would like to acknowledge the contribution of my Co-Editor, Professor Toshihiko Nagata (APSP President, 2013-2015) to the publication of the proceedings. As in previous years, I also thank the presenters for providing their manuscripts for publication. Finally this publication would not have eventuated had it not been for the excellent and efficient production editing of Ms Catherine Offler.

P. Mark Bartold
March 2014



Invited lecturers, left to right:

Shinya Murakami, Jin-Cai Zhang, Young Ku, Yeek Herr, Warwick Duncan, Tara Taiyeb Ali, Nannette Vergel de Dios, Yuniarti Soeroso, Huan-Xin Meng, Toshihiko Nagata, Isao Ishikawa, Li-Jian Jin, Stanley Lai, Yulianti Kemal, Benjamin Tan, Jian-Xia Hou, Kong Mun Chung, Narangsak Laosrisin, Yuichi Izumi, Yosvimol Kuphasuk, Mark Bartold, and AR Pradeep.

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Chapter 1

History of the Asian Pacific Society of Periodontology 1993 – 2013

I Ishikawa

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At the 10th meeting of the Asian Pacific Society of Periodontology (APSP) founding member and current Honorary Advisor of the APSP, Professor Isao Ishikawa, presented a lecture detailing the ten year history of this important periodontology organization. Below is a transcript of Professor Ishikawa's presentation, recorded in these proceedings as the first official record of the history of the APSP to date.

The APSP had its initial roots formed in 1992 when Professor Hiroshi Okada asked Professor Isao Ishikawa to convene an Asian Pacific Session held during the 9th International Conference on Periodontal Research (ICPR) held in Osaka, Japan (Figure 1). This special session was held because it was the first time an ICPR had been held in Asia.

As shown in Figure 2, many well-known periodontists from Asian Pacific countries participated in this session. This meeting was judged to be an outstanding success and as a result the Japanese Society of Periodontology requested Professor Ishikawa organize a second Asian Pacific Periodontal Symposium. This meeting was held in Tokyo, Japan in 1993.

At the 1993 meeting the initial idea of forming an Asian Pacific Society of Periodontology was presented by Professor

Ishikawa and Professor Bartold, and the participants at this meeting unanimously agreed to the formation of this society. The inaugural members responsible for the formation of the APSP are shown in Figure 3. At this meeting it was decided that Professor Bartold would be the inaugural President and Professor Ishikawa was elected the inaugural Secretary General.

With representatives from 14 countries in the Asian Pacific region present at the Inaugural Meeting, the objective of the Society was determined as: "to serve as a non-profit medium for the exchange, advancement and dissemination of scientific knowledge related to periodontal research and education in the Asian Pacific Regions". To date, nine symposia have been held under the title "Asian Pacific Periodontal Symposium". These have been very successful meetings, with participants being derived solely from the Asian Pacific region. One of the principal objectives of this Society is to foster collegiality, friendships and perhaps collaborations. The group is very diverse, with many differences in culture, socioeconomics and religion. These have particular ramifications for the manifestation of the periodontal diseases, their treatment and long term management. Such a mix is unique and provides an opportunity for us all to learn from each other's diverse experiences.

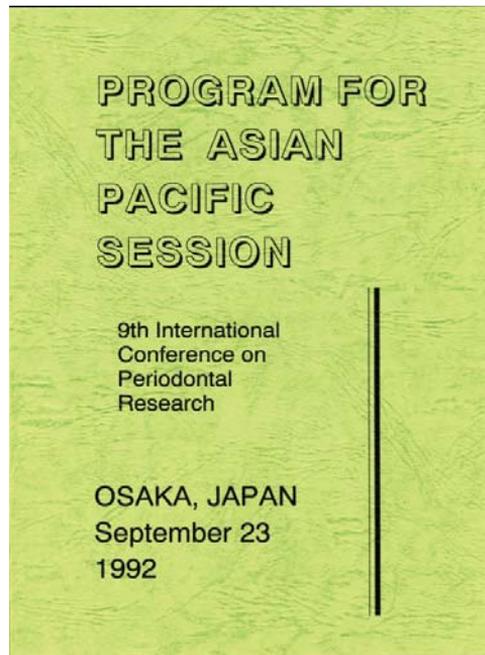


Figure 1. Program of the first Asian Pacific Session of the International Conference on Periodontal Research (Osaka, Japan 1992).



Figure 2. Participants of the first Asian Pacific Session of the International Conference on Periodontal Research (Osaka, Japan 1992).



Figure 3. Participants of the second Asian Pacific Session (Tokyo, Japan 1993).



Figure 4. Participants at the inaugural Asian Pacific Society of Periodontology meeting (Gold Coast, Australia 1995).

China	1,272.2	Korea	46.0
India	955.2	Malaysia	21.6
Indonesia	199.2	Taipei	21.6
Pakistan	129.8	Australia	18.5
Japan	126.1	Saudi Arabia	17.8
Bangladesh	109.2	Hong Kong	6.5
Vietnam	76.5	Jordan	4.1
Philippines	70.2	New Zealand	3.7
Thailand	60.6	Singapore	3.7

Table 1. Population of countries with APSP members as of 1995.

Year	Location of Meeting	President	Secretary General
1995	Gold Coast, Australia	Mark Bartold	Isao Ishikawa
1997	Seoul, Korea	S-H Son	Isao Ishikawa
1999	Bangkok, Thailand	Tipaporn Vongsurasit	Isao Ishikawa
2001	Kuala Lumpur, Malaysia	Tara Taiyeb Ali	Isao Ishikawa
2003	Cebu, The Philippines	Nanette Vergel De Dios	Isao Ishikawa
2005	Chennai, India	D Arunachalam	Isao Ishikawa
2007	Beijing, China	Jincai Zhang	Isao Ishikawa
2009	Singapore	Kong Mun Chung	Shinya Murakami
2011	Hong Kong	Li-Jian Jin	Shinya Murakami
2013	Nara, Japan	Toshihiko Nagata	Shinya Murakami

Table 2. Meetings and Office Bearers of the Asian Pacific Society of Periodontology.

The first meeting of the APSP was held on 25-26 July 1995 at the Gold Coast, Australia (Figure 4). It was organized by Professor Bartold. During the inaugural speech for this meeting Professor Bartold commented “This is the first time that we have met under the auspices of our newly formed Asian Pacific

Society of Periodontology. Therefore, this is indeed an historic occasion.”

Following this successful first meeting, the APSP was born and began its journey of maturation and expansion. The significance of this Society should not be underestimated. As shown in Table 1, the number of countries



Figure 5. Covers of all APSP Proceedings books published to date.

represented by membership of the APSP, even at this early stage, represented a significant proportion of the global population.

Subsequent meetings were then held in Korea (in conjunction with IAP and FDI), Thailand (with Thai Society of Periodontology), Malaysia (with Malaysian Society of Periodontology), the Philippines, India, China, Singapore, Hong Kong and Japan. Over the years attendance at the APSP meetings has increased steadily. Over 360 delegates from 21 countries attended the Hong Kong meeting which had developed into a busy two day program with 20 presentations from 17 different countries. A summary of the meetings held to date, Past Presidents and Secretary General is shown in Table 2.

In addition to organizing conferences in centres all over the Asian Pacific Region, other activities included participation in the Euro-Perio meetings commencing in 2000. This has been a significant development for the APSP as it has given specific recognition of this organization within the European setting.

Another important activity of the APSP

has been the preparation and publication of the meeting proceedings. These have been prepared by Professor Bartold for every meeting held to date. Illustrations of the front cover of each proceedings is shown in Figure 5. These serve not only as a permanent record of the presentations at each of the APSP meetings, but also provided a valuable opportunity for many presenters to have their presentations published in a book which has been distributed to dental libraries around the world.

In conclusion, the establishment of the Asian Pacific Society of Periodontology has been very important, because it provides a forum to discuss periodontology as it affects the Asian Pacific region. It is clear that we have a solid research, clinical and educational base from which to operate.

Chapter 2

The Perio-Systemic Link: Where Are We Now?

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Introduction

Over the past 30 years, the terms “Perio-Systemic Links” and “Periodontal Medicine” have become familiar on a worldwide level, not only to the dental practitioner and the dental researcher, but also to the general public. Following the seminal observations 30 years ago on the associations between tooth loss and cardiovascular disease events, and from earlier reports for other perio-systemic associations, we have witnessed a broad dissemination of the findings from epidemiological studies, from research on the underlying biological mechanisms of periodontal and systemic diseases and conditions, and from reports from pilot and large-scale intervention studies on the potential benefits of periodontal treatment (Linden *et al* 2013). The broad general media attention given to these studies has increased public awareness of the potential benefits of periodontal therapy for not only maintaining the dentition, but also for the potential widespread benefits on overall health. In any region of the world, the dental practitioner in general, and the periodontal specialist in particular, receives questions from their patients such as: “Will my periodontal disease increase my risk for getting a heart attack?”; “If I am pregnant, what would be the benefits of treating my periodontal disease?”, “Will periodontal treatment improve my diabetic

condition?”, “Does periodontal disease increase my risk for Alzheimer’s disease?”. In order to be able to give advice to our patients based on the current level of evidence, a brief critical overview of the current state of knowledge and evidence is presented in this paper, with the hope that the dental practitioner can use some of this information in both giving advice to patients and in their treatment decisions.

It should however be kept in mind that this ever expanding field of periodontal medicine, or perio-systemic links is so broad and deep, that a single paper cannot cover all the possible developments in this field. In fact doing full justice to this field requires the type of extensive monographs published from the proceedings from the various international workshops and conferences devoted this subject. Therefore the author requests the readers indulgence in highlighting some of the principles and insights that he feels would be most valuable for application to their own respective clinical practices.

Some basic principles regarding the perio-systemic link

For the purposes of this paper, we can focus our attention on the perio-systemic links with those periodontal diseases that involve the accumulation of a microbial plaque biofilm, the inflammatory, immune,

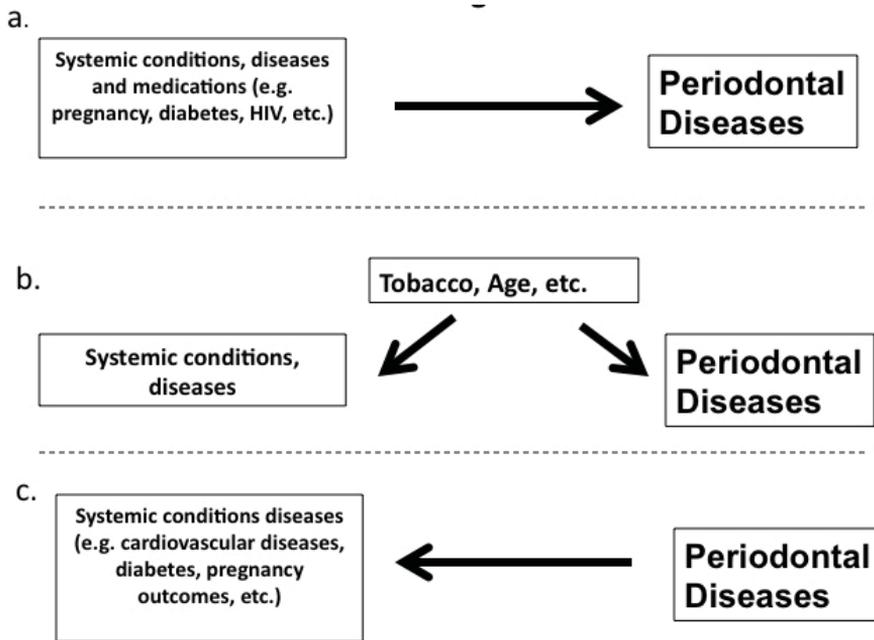


Figure 1. A schematic view of the three types of perio-systemic links that should be considered in a discussion of the types of casual associations or direct relationships between periodontal diseases and systemic diseases and conditions. (A) Systemic conditions, diseases and medications that may initiate or exacerbate periodontal diseases. (B) Systemic conditions, medications, habits, etc. that affect the periodontium and other organ systems in the body in a similar manner. (C) The potential effects of periodontal diseases on systemic conditions.

and other host responses to this biofilm, and the ensuing clinical signs of inflammation and loss of periodontal support. This paper will focus on the links between plaque induced gingival diseases and chronic periodontitis with systemic diseases and conditions. When considering these links between periodontal diseases and the body, there are actually three possible links or directions that can be considered. These are shown in Figure 1.

Systemic conditions, diseases and medications that may initiate or exacerbate periodontal diseases

It is widely recognized that the mouth in general, and the periodontal tissues in particular, are one of the first areas of the body where clinical signs of systemic conditions

may be expressed. We can regard this first possible link as a one-way link from the body to the periodontium. These conditions and diseases include nutritional deficiencies, (of which severe ascorbic acid deficiency, or scurvy is a prime example), hormonal changes in pregnancy, poor glycemic control in various forms of diabetes, medications that promote gingival enlargement, and HIV infection and immunodeficiency, to cite just a few (Ryder *et al* 2012). A common expression in English is that the mouth is like a “Canary in a Coal Mine”, which implies that clinical changes in the periodontium and other areas of the mouth may be the first indication of an underlying systemic problem.

Systemic conditions, medications and habits that affect the periodontium and other organ systems in the body in a similar manner

Perhaps the best example of this link is the use of tobacco in general, and smoking in particular. Both chronic exposure to substances in tobacco in the bloodstream at low doses, and acute exposure to tobacco substances during the act of smoking may lead to destructive inflammatory effects of the periodontium and lung via similar mechanisms (Ryder 2007). Other such overarching conditions may include age, stress, etc.

The potential effects of periodontal diseases on systemic conditions

We can regard this third possible connection as a one-way link from the periodontium to the body. This possible link includes the widely discussed and reported potential effect of periodontal diseases on the incidence, severity, and progression of cardiovascular diseases, pulmonary diseases, diabetes, pregnancy outcomes, and more recently on possible influences of periodontal diseases on kidney function, cancer, cognitive function, rheumatoid arthritis and so on (Grubbs *et al* 2011, Linden *et al* 2013).

The relationship between periodontal diseases and systemic conditions

In this paper we will focus our attention on this third relationship, with the aim of highlighting those possible links where there is supporting evidence based on several areas of investigation or criteria. In addition, as more studies and evidence come to light regarding this third type of link, it is apparent that some of these proposed perio-systemic links also incorporate elements of the first and

second types of links.

Over the past 30 years, investigators in this field have come to a general consensus on several basic criteria which demonstrate a direct relationship between periodontal diseases and systemic conditions and diseases.

Epidemiological studies

These studies do not by themselves demonstrate a direct cause and effect link between periodontal disease and a systemic condition per se, but rather indicate the need for further investigation to demonstrate such a link. Such higher quality epidemiological studies adjust for confounding or mediating factors that may influence the incidence, progression, and severity of both the periodontal disease and the systemic condition to be studied. Such confounding or mediating factors may include smoking, diabetes, age, gender, socioeconomic status or genetics. However since all such confounding or mediating factors may be difficult to identify, these types of studies demonstrate what is termed a “casual association” as opposed to a “direct relationship”. Nevertheless, the observations from these epidemiological studies serve as a basis for the following second and third criteria.

Biological plausibility

These include laboratory and clinical studies which demonstrate a possible biological mechanism linking periodontal disease to a systemic disease/condition. These studies would normally focus on the microbiological, inflammatory, immunological, and genetic influences of periodontal diseases on other systems of the body.

Effects of periodontal treatment on the systemic condition/disease

Of the three criteria cited here, this is perhaps the strongest of the current criteria for the demonstration of a “direct relationship” rather than a “casual association” between periodontal diseases and systemic conditions/diseases.

Areas of investigation

These three criteria will be used as a template in the following discussions of three areas of investigation regarding potential perio-systemic links. These three areas of discussion are:

1. Evidence for a “casual association” but where a “direct relationship” has yet to be determined based on the evidence, e.g. cardiovascular diseases and pregnancy outcomes.
2. There is sufficient evidence from the three criteria listed above to determine a “direct causal relationship”, e.g. bacterial pneumonia and control of diabetes.
3. Intriguing new directions into possible

links between periodontal diseases and systemic conditions, e.g. HIV disease progression and cognitive function/dementia.

Prior to a discussion of individual perio-systemic links, there are two other concepts regarding the perio-systemic link that should be discussed. The first is a definition of how we measure the effects of treatment through either a “true endpoint” or a “surrogate endpoint”. From the perspective of a dental practitioner, a “true endpoint” for a treated patient could be whether the patient retained or lost a particular tooth or teeth as a result of the treatment. A “surrogate endpoint” would be a clinical measure that could predict some future loss of the tooth, such as an increase in pocket depth or further bone loss, with some, but not necessarily a high degree of accuracy. For the effects of periodontal treatment on systemic conditions, it is also important to distinguish what a true endpoint would be, such as a heart attack, stroke or premature birth, as opposed to a surrogate endpoint such as reduction of a systemic inflammatory marker, change in lipid profile, etc. The implications of these two types of endpoints

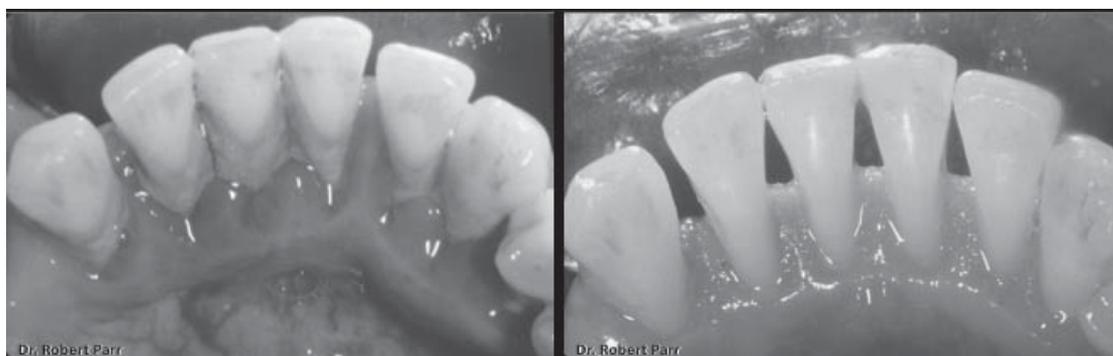


Figure 2. A clinical example of the problems faced when establishing a standard definition of periodontal disease. In the left panel the patient presents with significant deposits of plaque and calculus, marked clinical inflammation, deeper pocket depths, attachment loss and bone loss. After treatment, the same patient would still present with attachment loss and bone loss, but with minimal pocket depths, plaque accumulation and clinical inflammation. Depending on whether the presence and degree of periodontal disease was determined by bacterial load, levels of inflammation, attachment loss, bone loss and duration of the condition, the definition of periodontal disease for this patient would be highly variable.

will be considered when discussing individual systemic conditions/diseases in the following sections.

The second concept is how periodontal disease is measured or assessed in these studies (Eke *et al* 2012). In particular what is the best way to measure periodontal disease? For example: Is self-reporting of periodontal disease a reliable measure?; Are levels of plaque and clinical inflammation more or less important measures of periodontal disease when considering perio-systemic links, as compared to attachment loss and pocket depths? (Figure 2); Are measures of the severity and duration of periodontal disease in a patient more important than measures of the severity of periodontal disease at one time-point?

“The jury is still deliberating” (cardiovascular diseases and pregnancy outcomes)

Long before some of the first intriguing studies were published 30 years ago on the possible links between tooth loss/periodontitis and cardiovascular disease, it was well known to the dental profession that oral bacteria could colonize damaged areas of the heart through dissemination through the bloodstream, leading to endocarditis and sometimes death. The concept of bacterial dissemination from the plaque biofilm, as well as the dissemination and stimulation of inflammatory mediators, forms the basis of the “biological plausibility” for a link between periodontal diseases and cardiovascular diseases and stroke. In particular the role of certain bacterial species and inflammatory mediators (e.g. C-reactive protein, interleukin-6) in initiating the formation of atherosclerotic plaques and in the rupture of the atherosclerotic plaques leading to a clot or thrombus have been demonstrated (Reyes *et al* 2013, Schenkein and Loos 2013). Such a rapid formation of a thrombus that

would almost completely block a blood vessel supply to the heart would lead to a myocardial infarction/heart attack, or if to the brain, lead to a stroke.

While the biological plausibility can be demonstrated, the epidemiological data in general demonstrates more of a casual association between periodontitis and cardiovascular diseases in general, with a somewhat stronger association with stroke in particular (Dietrich *et al* 2013). This may be due in part to the wide variation in how periodontal disease is reported (e.g. self reported, based on attachment loss and pocket depths, based on plaque accumulation and clinical inflammation, or a combination of pocket depths and inflammation). Perhaps the best lines of evidence to support a direct relationship are intervention studies on the benefits of periodontal treatment in reducing the risk for cardiovascular events. Indeed there are several widely quoted studies demonstrating the beneficial effects of a more intensive periodontal treatment approach in reducing inflammatory risk markers for cardiovascular diseases (e.g. C-reactive protein, IL-6, fibrinogen), as well reducing the “stiffness” of peripheral blood vessel walls (D’Aiuto *et al* 2013). This latter assay may be a more accurate surrogate endpoint for the function of the endothelial cells that line the blood vessel wall, which in turn would be influenced by the inflammatory and or atherosclerotic changes to the vessel wall. However, as discussed in the previous section on basic principles, these findings measure “surrogate endpoints”, they do not represent the “true endpoints” of a cardiovascular event such as a heart attack or stroke. Thus at the present time, the central question of whether periodontal treatment can directly prevent these cardiovascular events remains unresolved. Or, to quote the most recent position by the American Heart Association as developed by a team of cardiovascular

and periodontal clinicians and researchers “Observational studies to date support an association between periodontal diseases and atherosclerotic vascular diseases independent of known confounders. They do not, however, support a causative relationship. Although periodontal interventions result in a reduction in systemic inflammation and endothelial dysfunction in short-term studies, there is no evidence that they prevent atherosclerotic vascular diseases or modify its outcomes” (Lockhart *et al* 2012).

So the question remains, can such a long-term study be designed and conducted to determine if periodontal treatment could prevent an atherosclerotic vascular disease event? Detecting such an event would require a relatively long observational study period of years to decades with a very large study population. Such a study could be conducted with the resources of several countries working in collaboration. However, for cardiovascular diseases, perhaps the best approach would be to find a viable surrogate endpoint or endpoints that could predict with a high degree of accuracy the future incidence of a cardiovascular event, which would be the true endpoint. At the present time, such a short-term surrogate endpoint or combination of endpoints using serum markers, imaging techniques or genetic approaches, is yet to be determined. However, it is hoped that such a set of surrogate markers will be developed and confirmed in the near future for investigation into a possible direct perio-cardiovascular disease relationship.

In the case of adverse pregnancy outcomes, as with cardiovascular diseases, both biological plausibility studies on the influence of periodontopathic bacteria and periodontal inflammation, and epidemiological association studies, support a potential perio-systemic link (Ide and Papapanou 2013, Madianos *et al* 2013). However unlike cardiovascular diseases, the true endpoint of pregnancy

complications such as low birth-weight, pre-term birth, and pre-eclampsia can be assessed in a viable and limited time frame. While some pilot studies demonstrated the benefits of periodontal treatment during pregnancy in reducing adverse pregnancy outcomes, more recent large-scale studies have not demonstrated a significant beneficial effect of treatment (Michalowicz *et al* 2013). However in these larger scale studies, if one considers a significant reduction in gingival inflammation to be indicated by a reduction bleeding on sites to minimal levels of 10% or less, then the actual effectiveness of the periodontal treatment in the majority of these studies could be considered inadequate. Specifically in one of the most widely quoted studies, the reported average reduction of bleeding on probing from 69 to 45% would still indicate a moderate to high level of inflammation, which could potentially influence pregnancy outcomes (Armitage 2008). This lack of resolution of periodontal inflammation by treatment during pregnancy is understandable when considering the tendency for increased inflammation as a natural course of the hormonal changes during pregnancy. Thus several investigators have suggested that such therapy would need to be initiated before or during the earliest stages of pregnancy (Michalowicz *et al* 2013). In addition a much greater magnitude of improvement in clinical inflammation would need to be achieved (Armitage 2008). Until such studies are performed, the links between periodontal diseases and adverse pregnancy outcomes, as determined by effects of periodontal treatment, have yet to be determined.

A clearer picture (bacterial pneumonia and diabetes)

Perhaps the simplest and most clearly demonstrated perio-systemic link is between periodontal disease and bacterial pneumonia.

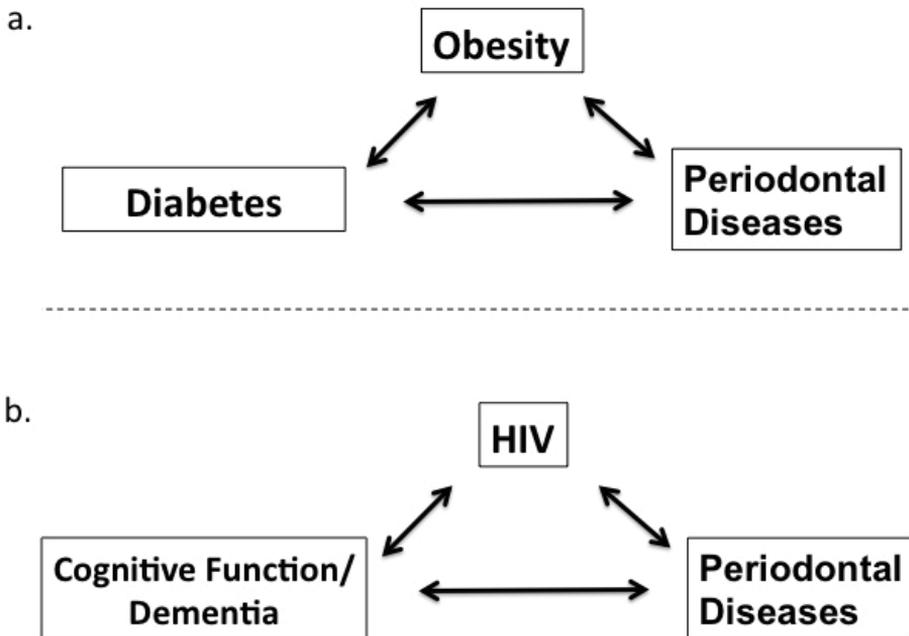


Figure 3. The emerging understanding that the interactions between periodontal diseases and systemic conditions and diseases may not be just a one-way or two-way linear relationship, but may also take on a second and third dimension, as investigations have shown three and often more than three multiple interactions. (A) Recent investigations have pointed to a three-way interaction between periodontal diseases, diabetes and obesity. (B) Similar interactions have been implicated between periodontal diseases, HIV and cognitive impairment/dementia.

Here, as with infective endocarditis, the biological plausibility can be easily demonstrated in elderly and/or hospitalized patients. Specifically, there is a potential for the bacteria that colonize the plaque biofilm and other areas of the oral cavity to directly colonize the lung either through aspiration, inoculation through a respirator tube (when it occurs), and/or dissemination and colonization through the bloodstream (Paju and Scannapieco 2007). In addition, several studies have demonstrated the benefits of some form of local oral microbial treatment on the reduction of the incidence of the true endpoint bacterial pneumonia in hospital settings (Bergmans *et al* 2001).

For the various forms of diabetes, as in the case of bacterial pneumonia, both epidemiologic studies demonstrating the association between periodontal diseases

and diabetes and biological plausibility have been conducted (Borgnakke *et al* 2013, Taylor *et al* 2013). However it should be noted here that in contrast to the discussions of cardiovascular disease and pregnancy outcomes which primarily fell under the one-way influence of periodontal diseases on these conditions (namely, the third type of link), there is strong evidence that the relationship between periodontal diseases and diabetes is a two way relationship with each condition negatively influencing the other. More recently, investigators have added a third leg of obesity of this relationship, with these conditions influencing each other (Figure 3a). One true endpoint for glycemic control in a diabetic patient would be a reduction of serum glycated hemoglobin or HbA1c. The majority of such studies have demonstrated a modest but often clinically significant

reduction in HbA1c in most of these smaller scale intervention studies, comparable to what would be achieved by oral administration of several different oral pharmacological approaches (Engebretson and Kocher 2013, Nathan 2007). Thus, based on the criteria of epidemiological association, biological plausibility, and the benefits of periodontal treatment, there appears to be a direct relationship between periodontal diseases and glycemic control in diabetes. These findings imply that the dental practitioner may play an important role in the management of diabetes.

Some future directions (HIV and dementia)

Up to this point the focus of this paper has been on the four most investigated possible perio-systemic links (cardiovascular diseases, pregnancy outcomes, pulmonary diseases and diabetes). Other possible perio-systemic links are also under current investigation, including those between kidney diseases, rheumatoid arthritis, cancer, HIV, cognitive function/dementia and other conditions. In this section, the possible links and interrelationships between periodontal diseases, HIV and dementia are discussed. As with the previous discussion regarding diabetes and obesity, some recent observations in this field have implicated similar interactive triangular relationships between these three conditions (Figure 3b).

The possible links between periodontal diseases and dementia have gained attention through several epidemiological association studies (Yu and Kuo 2008). In particular, a study of nuns by Stein *et al* (2007), demonstrated a correlation between tooth loss and dementia. However whether tooth loss was due to periodontal disease, or whether the dementia led to worsening oral hygiene and thus to tooth loss, was not determined. Nevertheless, there are some observations

in the area of biological plausibility that support further investigation into this area. In particular, treponeme species from periodontopathic bacterial plaque have been localized in the brain tissue of Alzheimer's disease patients when compared to controls (Riviere *et al* 2002). In addition, the increase in inflammatory mediators in the bloodstream may impair or damage the blood-brain barrier, thereby leading to bacterial infiltration into the brain and a local inflammatory reaction in the brain, which in turn could trigger and/or exacerbate neurodegenerative changes.

New insights in the interactions between HIV infection and periodontal disease and to cognitive function have also been investigated. While it is well known that the HIV virus and resulting immunological and inflammatory changes can adversely affect the periodontium, new evidence is emerging that periodontopathic bacteria as well as an increase in inflammatory mediators can reactivate dormant HIV infection (Gonzalez *et al* 2009, Ryder *et al* 2012). Furthermore, while anti-infective approaches to HIV virus and other opportunistic infections from HIV infection have been shown to be significantly beneficial to the long term survival of HIV patients, several of these Highly Active Retroviral Therapies (HAART) may have adverse side effects that may affect a periodontitis-brain link. These include reports that HAART can result in a shift in the oral microbial flora, with an increase in periodontopathic bacterial species, and reports that the use of protease inhibitors may damage the oral epithelial barrier, leading to translocation of oral bacteria into the bloodstream and across the blood-brain barrier (Danaher *et al* 2010, Navazesh *et al* 2005). As both HIV infection and periodontal disease may adversely affect cognitive function, new investigations into this possible three way relationship are currently under investigation.

Conclusions

From this brief summary of the current state of our understanding of the perio-systemic link, it is hoped that the reader can appreciate both the richness and complexity of this field. From the variety of links discussed, perhaps a few suggestions on where one would hope the field can progress in the near and distant future can be made:

1. For studies that require large numbers of subjects to follow over longer periods, establishment of such larger scale studies on an international scale should be pursued.
2. An international agreement for a standard definition of periodontal disease is urgently needed. This international standard must take into account the bacterial load (plaque level), clinical inflammation, clinical parameters of loss of attachment (pocket depths, attachment loss, bone loss) and length of exposure (history of disease).
3. Further investigation is needed into the development and assessment of individual and combinations of surrogate endpoints that accurately predict true clinical endpoints, particularly in the area of cardiovascular disease.

It is hoped the dental practitioner, and in particular the periodontal practitioner, will be able to use some of the observations in this paper during their consultations with their patients to answer questions on the potential effects of periodontal disease and benefits of treatment to their overall health, and help develop a plan of treatment for their patients.

In conclusion, the author would like to present one appropriate anecdote regarding this field of study. Amongst investigators in this field, some stronger proponents of perio-systemic links have used a slogan in their presentations (which is partially tongue-in-cheek): “Floss or Die”. Another investigator

with a more sceptical view would reply: “Floss and Die Anyway”. At the present time, both messages have validity. The perio-systemic link field is still in its infancy. Future developments should continue to be of great interest and impact to both the dental practitioner and to the general public.

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Chapter 3

MCP-4 and Progranulin: Novel Biomarkers Linking Periodontitis and Obesity

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Introduction

Chronic periodontitis (CP) is a multifactorial disease which involves the destruction of the supporting structures of the teeth, including the periodontal ligament, bone and gingival tissues (Kinane and Attstrom 2005). The presence of microorganisms is a crucial factor in inflammatory periodontal disease, but the progression of the disease is related to host-based risk factors. Other risk factors, including plaque or oral hygiene modifications, systemic health including diabetes and human immunodeficiency virus, socio-economics, stress, obesity, smoking, genetics, age and gender are all relevant and may interact to render subjects at increased risk (Kinane and Bartold 2007).

Obesity is becoming the most prevalent health problem, not only in developed countries, but also in many developing countries. Obesity is well-known to be a significant risk factor for various adult diseases, such as type 2 diabetes, hyperlipidemia, hypertension, cholelithiasis, arteriosclerosis, and cardiovascular and cerebrovascular diseases (Kopelman 2000). Recently, obesity has emerged as one of the risk factors for periodontal disease and conversely, the remote

effects of periodontal disease on various systemic diseases has been proposed (Dahiya *et al* 2012). It has been suggested that obesity is second only to smoking as the strongest risk factor for inflammatory periodontal disease (Nishida *et al* 2005).

Obesity might represent a systemic condition capable of influencing the onset and progression of periodontal disease. The first obesity-periodontitis link was noted using a ligature-induced periodontitis model in rats (Perlstein and Bissada 1977). An analysis of the Third National Health and Nutrition Examination Survey (NHANES III) showed that waist to hip ratio, body mass index (BMI), fat-free mass and log sum of subcutaneous fat had significant correlations with periodontal disease, suggesting that abnormal fat metabolism may be an important factor in the pathogenesis of periodontal disease (Wood *et al* 2003). The possible causal relationship between obesity and periodontitis and the molecular mechanisms by which obesity may affect the periodontium are not well understood. However, adipose tissue derived cytokines and hormones involved in inflammatory processes point towards similar pathways involved in the pathophysiology of obesity and periodontitis (Pischon *et al* 2007).

Monocyte chemoattractant protein-4 (MCP-4) is a CC chemokine which is a potent chemoattractant for monocytes and eosinophils, and stimulates histamine release from basophils (Garcia Zepeda *et al* 1996). MCP-4 has been implicated in conditions such as rheumatoid arthritis, symptomatic carotid atherosclerosis, asthma and renal inflammation (Breland *et al* 2010, Chakravorty *et al* 2001, Iwamoto *et al* 2006, Lamkhioued *et al* 2000). Recently a study showed that MCP-4 is a critical molecule linking obesity and chronic inflammation, and serum levels of MCP-4 correlated with BMI and other obesity related parameters (Hashimoto *et al* 2006). Progranulin (PGRN), also known as granulin/epithelin precursor, proepithelin, PC cells-derived growth factor and acrogranin, is a glycosylated protein released by a variety of cells (Bateman and Bennet 1998). PGRN has been also implicated in inflammation and wound healing (He *et al* 2003, Zanocco-Marani *et al* 1999, Zhu *et al* 2002). During the wound repair response, PGRN is upregulated and stimulates neutrophil and macrophage infiltration and neovascularization of wound tissue (Zhu *et al* 2002). Circulating PGRN is found to significantly correlate with BMI, macrophage infiltration in omental adipose tissue, high sensitivity C-reactive protein (hs-CRP) serum concentrations, HbA1C values and total cholesterol (Youn *et al* 2009).

Until recently, no studies have reported levels of MCP-4 and PGRN in gingival crevicular fluid (GCF) and serum in subjects with CP among obese and non-obese individuals. Therefore this clinico-biochemical study was undertaken to evaluate the GCF and serum levels of MCP-4 and PGRN in obese and non-obese subjects with clinically healthy periodontium and CP.

Material and methods

The study group consisted of 40 age and

gender balanced subjects (25 to 45 years; 20 males and 20 females) attending the outpatient section at the Department of Periodontics, Government Dental College and Research Institute, Bangalore. Written informed consent was obtained from those who agreed to participate voluntarily. Ethical Clearance was obtained from the Institutional Ethical Committee and Review Board.

Inclusion criteria

Selection of subjects was as follows: aged 25 to 45 years; presence of at least 20 natural teeth; diagnosis of CP based on clinical parameters of probing depth (PD), clinical attachment level (CAL), gingival index (GI); subjects having BMI, in the range of 18.5 to 22.9 kg/m² in non-obese subjects and in obese subjects >25 kg/m², and waist circumference (WC) >90 cm in men and >80 cm in women (Glavind and Loe 1967, Loe 1967, WHO 2000). Radiographic bone loss was recorded dichotomously (presence or absence) to differentiate subjects with CP from other groups.

Exclusion criteria

Subjects with aggressive periodontitis, hypertension, smoking habit, type 2 diabetes, gross oral pathology, heart disease, rheumatoid arthritis, osteoarthritis, dyslipidemia, tumors, or any other systemic disease which can alter the course of periodontal disease, or those who had any course of medication affecting periodontal status or had received periodontal therapy in the preceding 6 months were excluded from the study. Subjects with other acute or chronic medical conditions or infectious diseases like pneumonia and other febrile illnesses or inflammatory states that could have an impact on the levels of these inflammatory mediators were also excluded.

Grouping criteria

The subjects were categorized into 4 groups based on GI, PD, CAL, BMI and WC. Group I (healthy non-obese) consisted of 10 subjects with clinically healthy periodontium with no evidence of disease. The score obtained after assessing the gingival status using GI was zero, PD <3 mm and CAL = 0 with no crestal bone loss as determined from radiograph and BMI value more than 18.5 kg/m² and less than 22.9 kg/m² and WC <90 cm in men and <80 cm in women. Group II (healthy obese) consisted of 10 subjects with clinically healthy periodontium with no evidence of disease. The score obtained after assessing the gingival status using GI was zero, PD <3 mm and CAL = 0 with no crestal bone loss as determined from radiograph and BMI value more than 25 kg/m² and WC >90 cm in men and >80 cm in women. Group III (non-obese with CP) consisted of 10 subjects, who showed clinical signs of gingival inflammation, PD >5mm and attachment loss, i.e. CAL >3 mm with radiographic evidence of bone loss. The GI score 1 was obtained for these subjects and BMI value more than 18.5 kg/m² and less than 25 kg/m² and WC <90 cm in men and <80 cm in women. Group IV (obese with CP) consisted of 10 subjects, who showed clinical signs of gingival inflammation, PD >5 mm and attachment loss, i.e. CAL >3 mm with radiographic evidence of bone loss. GI score >1 was obtained for these subjects and BMI value more than 25 kg/m² and WC >90 cm in men and >80 cm in women.

Clinical evaluation of subjects

Group allocations and sample site selections were performed. A calibrated examiner performed a clinical evaluation measuring the clinical parameters including PD, CAL, GI using a University of North Carolina-15 periodontal probe (Hu-Friedy,

Chicago, IL, USA). The same examiner also performed the radiographic evaluation and collected the GCF samples.

Site selection and GCF collection

Two test sites for GCF sample collection were selected based on the highest scored sites in the oral cavity. In group III and group IV subjects, the two sites showing the greatest CAL and signs of inflammation, along with radiographic confirmation of bone loss, were selected for sampling. One of the two sites selected in each subject was used for MCP-4, with the other for PGRN analysis. In the healthy group, to standardize site selection and obtain adequate fluid volume, sampling was predetermined to be from the mesio-buccal region of the maxillary right first molar. In the absence of a tooth in this site, the left first molar was sampled. First, the selected site was cleaned, isolated and air dried using sterile cotton rolls and supragingival plaque was removed gently using a Universal gracey curette #4R/4L (Hu-Friedy, Chicago, IL, USA) to avoid contamination of the paper strips. The paper strips (Periopaper, OraFlow Inc., Amityville, NY, USA) were placed gently at the entrance of the gingival sulcus/crevice until light resistance was felt, with care being taken to avoid mechanical injury, and left in place for 60 seconds (Loe and Holm-Pederson 1965). The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, OraFlow Inc., Amityville, NY, USA). Samples that were suspected to be contaminated with blood and saliva were excluded from the study. After collection of the gingival fluid, the two periopaper strips for each site that absorbed GCF in each subject were pooled and immediately transferred in microcentrifuge tubes (pre-marked with the biomarker names) containing 400 µl of phosphate buffer saline and stored frozen at -70°C for subsequent analysis. Periodontal

treatment (scaling and root planing) was performed for CP subjects at the same appointment after GCF collection by the clinical operator.

Blood collection

2 ml of blood was collected from the antecubital fossa by venipuncture using a 20 gauge needle with 2 ml syringe and immediately transferred to the laboratory. The blood sample was allowed to clot at room temperature and after 1 hour serum was separated from blood by centrifuging at 3000 g for 5 minutes. The serum was immediately transferred to a plastic vial and stored at -70°C until the time of assay.

MCP-4 and PGRN analysis

The GCF and serum samples were then assayed for MCP-4 and PGRN using enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions (MCP-4 was procured from Raybiotech, Inc, USA and PGRN from Adipogen International Inc, Incheon, South Korea) by a technician

who was blinded to the groups allotted and who was not involved in sample collection. The GCF sample tubes were first homogenized for 30 seconds and centrifuged for 5 minutes at 1500 g to elute. The elute was then used as the sample for ELISA estimation from GCF samples. The concentrations of MCP-4 and PGRN in the tested samples for both GCF and serum were estimated using the reference calibrated standard curve, plotted using the optical density values of the standards (provided with the kit). The MCP-4 concentrations were determined in pg/ μ l for GCF and pg/ml for serum while PGRN concentrations in ng/ml for both GCF and serum.

Statistical analysis

The data were analyzed using a statistical software program (SPSS Inc. version 16.0, Chicago, IL, USA). Based on the power of the study and the confidence interval of 95% ($p < 0.05$) four groups with 10 subjects each were created. Using Pearson's correlation coefficient, the relationship between MCP-4 concentration and the clinical parameters were

Study group	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
Age (in years)	31.40 \pm 5.02	33.20 \pm 3.65	32.80 \pm 4.76	31.60 \pm 3.81
Sex (M/F)	5/5	5/5	5/5	5/5
BMI (kg/m ²)	21.71 \pm 0.80	28.65 \pm 1.04	21.23 \pm 0.96	28.46 \pm 1.21
GI	-	-	2.14 \pm 0.21	2.05 \pm 0.48
PD (mm)	2.30 \pm 0.67	2.00 \pm 0.82	7.70 \pm 1.34	7.30 \pm 1.16
CAL (mm)	0.00	0.00	6.40 \pm 1.07	6.40 \pm 1.07
GCF MCP-4 (pg/ μ l)	6.90 \pm 1.52	19.60 \pm 3.92	34.30 \pm 5.06	53.40 \pm 5.04
Serum MCP-4 (pg/ml)	92.20 \pm 5.20	241.70 \pm 9.58	175.10 \pm 15.03	274.30 \pm 14.52

Table 1. Descriptive statistics of study population for MCP-4 (mean + SD).

Study group	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
Age (in years)	32.42 ± 3.20	35.20 ± 3.54	35.22 ± 3.11	36.87 ± 3.32
Sex (M/F)	6/4	5/5	4/6	5/5
BMI (kg/m ²)	20.84 ± 2.55	28.16 ± 2.22	20.63 ± 2.58	31.95 ± 2.71
GI	-	-	2.21 ± 0.54	2.15 ± 0.51
PD (mm)	2.21 ± 0.22	2.12 ± 0.12	8.01 ± 1.29	7.32 ± 1.88
CAL (mm)	2.12 ± 0.45	1.91 ± 0.33	6.22 ± 1.12	5.61 ± 1.56
Serum PGRN (ng/ml)	78.2 ± 2.22	205.9 ± 2.88	182.2 ± 2.37	237.6 ± 2.74
GCF PGRN (ng/ml)	71.8 ± 2.54	197.8 ± 3.22	176.7 ± 2.45	231.5 ± 3.11

Table 2. Descriptive statistics of study population for PGRN (mean + SD).

Study groups	Mean difference	Std error	p-value
Group I & Group II	10.10	0.72	<0.001*
Group I & Group III	25.80	1.31	<0.001*
Group I & Group IV	45.30	1.48	<0.001*
Group II & Group III	15.70	1.30	<0.001*
Group II & Group IV	35.20	1.47	<0.001*
Group III & Group IV	19.50	1.83	<0.001*

Table 3. Pair-Wise comparison using Scheffé's test for MCP-4 GCF concentration (pg/μl). (Mean difference significant at p value < 0.05)

analyzed and p values < 0.05 were considered statistically significant. Analysis of Variance (ANOVA) was carried out to compare GCF and serum levels of MCP-4 and that of PGRN between the groups.

Results

The descriptive statistics along with the mean ± SD of both serum and GCF (of all groups) for MCP-4 and PGRN are tabulated

in Tables 1 and 2. The mean MCP-4 and PGRN concentrations both in serum and GCF were highest for group IV, followed by group III, group II and lowest in group I. Multiple comparisons using Scheffé's test, were carried out to find out which pair or pairs differ significantly at 5% level of significance. When pair wise comparisons were made between the groups, means were statistically significant in both serum and GCF for MCP-4 (Tables 3 and 5) and for PGRN (Tables 4 and 6).

Study groups	Mean difference	Std error	p-value
Group I & Group II	111.60	3.91	<0.001*
Group I & Group III	137.40	6.05	<0.001*
Group I & Group IV	168.50	5.58	<0.001*
Group II & Group III	25.80	6.45	<0.001*
Group II & Group IV	56.90	6.01	<0.001*
Group III & Group IV	31.10	7.58	<0.001*

Table 4. Pair-Wise comparison using Scheffé's test for PGRN GCF concentration (ng/ml). (Mean difference significant at p value<0.05)

Study groups	Mean difference	Std error	p-value
Group I & Group II	148.60	3.02	<0.001*
Group I & Group III	83.30	4.80	<0.001*
Group I & Group IV	182.20	4.51	<0.001*
Group II & Group III	65.30	4.99	<0.001*
Group II & Group IV	33.60	4.71	<0.001*
Group III & Group IV	98.90	6.01	<0.001*

Table 5. Pair-Wise comparison using Scheffé's test for MCP-4 serum concentration (pg/ml). (Mean difference significant at p value<0.05)

Study groups	Mean difference	Std error	p-value
Group I & Group II	148.60	3.02	<0.001*
Group I & Group III	83.30	4.80	<0.001*
Group I & Group IV	182.20	4.51	<0.001*
Group II & Group III	65.30	4.99	<0.001*
Group II & Group IV	33.60	4.71	<0.001*
Group III & Group IV	98.90	6.01	<0.001*

Table 6. Pair-Wise comparison using Scheffé's test for MCP-4 serum concentration (ng/ml). (Mean difference significant at p value<0.05)

		Pearson Correlation Coefficient			
		with BMI	with GI	with PD	with CAL
GCF MCP-4 (pg/μl)	Group I	0.8009	-	0.8966*	-
	Group II	0.9287	-	0.9023*	-
	Group III	0.9293*	0.1431	0.8691*	0.9158*
	Group IV	0.9043*	0.4333	0.8902*	0.6648*
Serum MCP-4 (pg/ml)	Group I	0.8782	-	0.8986*	-
	Group II	0.8636	-	0.8380*	-
	Group III	0.8952*	0.0475	0.9303*	0.9051*
	Group IV	0.9577*	0.3647	0.9377*	0.5680*

Table 7. Pearson Rank Correlation Coefficient Test comparing GCF and serum MCP-4 to BMI, GI, PD and CAL *Significant at p value<0.05

		Pearson Correlation Coefficient			
		with BMI	with GI	with PD	with CAL
GCF PGRN (ng/ml)	Group I	0.4851	-	0.2059	-
	Group II	0.0074*	-	0.7591	-
	Group III	0.1173	0.4144	0.0015*	0.0977
	Group IV	0.0977	0.7796	0.0494*	0.0494*
Serum PGRN (ng/ml)	Group I	0.2686	-	0.2900	-
	Group II	0.0082*	-	0.8384	-
	Group III	0.0702	0.4658	0.0008*	0.0491*
	Group IV	0.0015*	0.9503	0.0089*	0.0331*

Table 8. Pearson Rank Correlation Coefficient Test comparing GCF and serum PGRN to BMI, GI, PD and CAL *Significant at p value<0.05

Pearson correlation coefficient between the clinical parameters and MCP-4 and PGRN levels (serum and GCF) are tabulated in Tables 7 and 8. A positive correlation ($p < 0.05$) could be detected between serum and GCF MCP-4 with their respective clinical parameters (GI, PD and CAL). GCF and serum MCP-4 showed significant positive correlation, with PD in all groups and with BMI in group III and IV. The serum and GCF levels of PGRN were found to be significantly correlated ($p < 0.05$) to BMI in group II and IV; and to PD in group III and IV.

Discussion

The current study evaluated the association between obesity and periodontitis, both of which are chronic inflammatory diseases that can potentially exacerbate the systemic inflammatory response (Pischon *et al* 2007). Pro-inflammatory cytokines and proteins may play a crucial role in the close relationship among periodontitis, obesity, and chronic diseases and this association may be multidirectional (Genco *et al* 2005). A variety of substances, such as TNF- α , IL-6, adiponectin and MCP-1, which play an important role in metabolic complications in obesity have been identified (Kim *et al* 2006). Inflammation is thought to contribute to the development of the sequelae of obesity.

Certain cytokines are thought to reduce adiponectin expression (Brunn *et al* 2003). Adiponectin production is reduced with obesity. Cytokines are central to the initiation and maintenance of immune responses to periodontal bacteria. However, inappropriate cytokine secretion, whether quantitative (i.e. excessive cytokine release) and/or qualitative (e.g. imbalance between the proportions of pro- and anti-inflammatory cytokines), is a manifestation of dysregulated immune responses and leads to destruction of periodontal tissues and the clinical signs of

disease (Preshaw *et al* 2007).

Recent studies have shown that obesity has a significant association with periodontitis in terms of BMI, body fat, and maximum oxygen consumption (Al-Zahrani *et al* 2005, Dahiya *et al* 2012). In fact, adipose tissue secretes several cytokines and hormones that are involved in inflammatory processes, suggesting that similar pathways are involved in the pathophysiology of obesity and periodontitis.

The results of the present study indicated that concentrations of MCP-4 and PGRN in serum and GCF increased progressively from healthy to periodontitis sites, while in periodontitis sites in obese subjects the mean concentration of MCP-4 and PGRN was higher than the concentrations obtained in groups I, II and III, suggesting that these markers reflect chronic inflammation as the periodontal disease advances from health to CP and their levels are much higher in CP subjects with obesity. This also implies that obesity had a potential influence on the secretion of MCP-4 and PGRN in GCF and serum.

The results of this study are in accordance with those of previous studies, where MCP-4 levels were found to be elevated in Japanese overweight subjects (Hashimoto *et al* 2006). Similarly, previous studies have shown circulating levels of MCP-1 and CRP were related to obesity-related parameters such as BMI, waist circumference, HOMA and HDL-cholesterol (Kim *et al* 2006). These findings suggest that circulating MCP-4 may be a potential candidate linking obesity with obesity-related metabolic complications (Christiansen *et al* 2005, Kim *et al* 2006). In another study, circulating PGRN was found to significantly correlate with BMI, macrophage infiltration in omental adipose tissue, hs-CRP serum concentrations, HbA1C values, and total cholesterol (Youn *et al* 2009).

In periodontal disease, macrophages

occur in high numbers in inflamed gingival tissues, and are thought to play a significant role in the killing of pathogens and release of pro-inflammatory mediators and cytokines (Baker 2000). MCP-4 is a CC chemokine and has revealed high homology with MCP-1 (62% identities) and MCP-3 (61%) (Garcia-Zepeda *et al* 1996). CC chemokine receptor expression has been reported in basophils, T-lymphocytes, macrophages, mast cells, neutrophils, and endothelial cells (Baggiolini 2001, Garcia-Zepeda *et al* 1996). It has been found that obesity leads to a local increase in the release of MCP-1, which is one of the chemokines secreted from adipose tissue. MCP-1 has been found to recruit macrophages, resulting in altered metabolic and endocrine activities of fat cells, including insulin resistance (Dahlman *et al* 2005). Therefore, white adipose tissues may probably be one of the major sources of serum MCP-4 in obesity (Hashimoto *et al* 2006). On the other hand, PGRN is thought to play a role in the association between obesity, type 2 diabetes, and inflammatory response and in macrophage accumulation into adipose tissue in subjects with obesity (Youn *et al* 2009). In a recent study we found that PGRN levels were elevated in CP subjects with and without type 2 diabetes (Priyanka *et al* 2013).

Obesity has been suggested to be associated with an increased susceptibility to bacterial infection. In a study the effect of obesity on innate immune responses to *Porphyromonas gingivalis* infection, an infection strongly associated with periodontitis, was investigated. After oral infection with *P. gingivalis*, mice with diet induced obesity had a significantly higher level of alveolar bone loss than the lean controls (Amar *et al* 2007). Thus, high GCF MCP-4 concentration may be due to induction by periodontal pathogen like *P. gingivalis*, causing local production of MCP-4 by various cells at diseased periodontal sites, as bacterial lipopolysaccharide has

been found to be a strong inducer of MCPs (Pype *et al* 1999). Similarly increased GCF levels of PGRN in periodontal disease also indicates that *P. gingivalis* infection may be a cause for increased release of proinflammatory molecules. Moreover it has been suggested that obesity leads to altered cytokine production and certain alterations in structure of the periodontium in rats (Perlstein and Bissada 1977).

The present study showed that the levels of MCP-4 and PGRN were highest in obese subjects having periodontal disease. Thus the levels of these molecules increased from periodontal health to disease and positively correlated with BMI and periodontal parameters. Therefore it can be postulated that MCP-4 and PGRN can form a potential novel molecular link between chronic inflammation, obesity and periodontitis. Further longitudinal, prospective, multi-centred studies with larger sample sizes should be carried out to affirm the findings of the study and the role of MCP-4 and PGRN in linking periodontitis and obesity.

Conclusion

The conclusions drawn from the study were:

1. MCP-4 and PGRN are present in GCF and serum in healthy and CP subjects with and without obesity.
2. There is a substantial increase in the concentration of MCP-4 and PGRN in GCF and serum in CP and obesity.
3. There was a significant positive correlation between clinical parameters such as BMI and PPD with MCP-4 and PGRN concentrations in GCF and serum in all the groups.

Thus it can be concluded that MCP-4 and PGRN can be considered as potential inflammatory biomarkers linking periodontitis and obesity.

Future perspectives

1. MCP-4 and PGRN need to be further assessed for confirmation as markers of inflammation linking obesity and periodontitis.
2. Further longitudinal, multi-centre, prospective research with larger sample sizes should be conducted to confirm their role in obesity and periodontitis.
3. MCP-4 and PGRN levels need to be assessed to determine their possible role in linking periodontitis to other systemic diseases like type 2 diabetes, cardiovascular diseases and other inflammatory disorders.
4. These findings can be helpful in developing chairside kits for the rapid assessment of inflammatory status in periodontitis and other related systemic disorders.
5. Further treatment modalities for periodontitis and systemic diseases focusing on reducing the levels of MCP-4 and PGRN should be evaluated in future.

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Chapter 4

Modern Periodontal Therapy - What Could be Achieved and How Relevant is it to our Patients?

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Introduction

In the past 20 to 30 years the management of periodontal disease in humans has evolved, with many new strategies developed which would hopefully increase the longevity of teeth in the oral cavity. Since the report by Loe *et al* (1965) of plaque biofilm as the causative agent triggering soft tissue disease around the tooth, the focus in the following 10 to 20 years has been the use of periodontal therapy to remove the causative factors, as well as the development of different surgical approaches in managing those conditions which respond unfavorably to a non-surgical approach or when recontouring of the periodontium is indicated. No matter whether treatment is non-surgical or surgical, they both share the same aim, which is to eliminate subgingival biofilms/deposits in order for wound healing to occur in the previously infected periodontium. Numerous clinical studies have obtained promising results, leading to a better chance to maintain teeth rather than the tooth exfoliation or extractions common previously.

It has been stated that the ultimate goal of periodontal therapy is to regenerate the lost periodontium to its original state. Techniques and materials have therefore been developed in order to attempt to achieve this goal (Sculean *et al* 2008). These procedures are invariably surgical in nature, such as

guided tissue regeneration (GTR) with or without bone filling materials, and the use of enamel matrix derivative (EMD). There are many studies providing evidence that these procedures may be effective in increasing clinical attachment level (Casarin *et al* 2010, Mellonig 1999, Parashis and Tsiklakis 2000, Sculean *et al* 2008, Tonetti *et al* 2002). From such perspectives, one may deduce that the regenerative approach should therefore be recommended whenever feasible and should be common practice in modern periodontology.

At the same time however, periodontal regenerative surgery also involves considerable training, time and financial investment. It is not surprising that in the past 10 years there have been many hands-on workshops worldwide that aim to provide continuing education for the dental practitioner to learn these very technique-sensitive procedures. Practitioners need to invest additional money and time to learn these advanced techniques and patients have to pay to undergo one or more surgical procedures and endure possible post-operative complications. In return, patients may be rewarded with decreased probing pocket depth or gained clinical attachment. Since regenerative techniques are becoming common, it could be inevitably foreseen that more and more patients are offered such treatment options. Therefore, an exploration

of how such procedures may impact on our patients is required.

Regeneration: a brief history

Non-surgical periodontal therapy results in healing by repair, whereas periodontal regenerative procedures aims to reconstruct alveolar bone and periodontal ligament with inserting collagen fibers into new cementum (Caton and Zander 1976, Karring and Lindhe 2008). In order for this to occur, epithelial downgrowth, which would in turn result in formation of a long junctional epithelium, needs to be separated from the defect and cells with regenerative potential be allowed to repopulate the wound (Melcher 1976). With previous studies confirming that bone and gingival connective tissue lack cells with such potential, Karring and colleagues confirmed that new attachment was only found on roots with non-impaired periodontal ligament (Karring *et al* 1980, Karring *et al* 1985, Nyman *et al* 1980). This was also supported by the observation of Buser *et al* (1990) that cementum with inserting collagen fibers was formed on implant surfaces placed next to retained root tips, which provided a source of periodontal ligament cells. Guided tissue regeneration, therefore, makes use of the concept in which a barrier membrane prevents epithelial growth into the defect and allows repopulation of defect by periodontal ligament cells (Gottlow *et al* 1984). The use of bone grafting materials may or may not be utilized as supporting fillers, depending on the types of membranes used.

Biologically active materials have been more recently developed for the purpose of periodontal regeneration. One of the few commercially available products is Enamel Matrix Derivative (EMD). It is believed that enamel matrix proteins, mainly amelogenins which are found on the root surface just before cementogenesis occurs,

are responsible for stimulation of processes including differentiation and proliferation of osteoblasts, stimulation of growth factors, and may even have antimicrobial properties (Haase and Bartold 2001, Hammarstrom 1997, Kawase *et al* 2000, Lyngstadaas *et al* 2001, Schwartz *et al* 2000, Van der Pauw *et al* 2000). Through the stimulation of these processes, it is believed that the use of EMD can result in the formation of new cementum and periodontal ligament cells. The attachment gain resulting from the use of this product is thus considered true periodontal regeneration (Hirooka 1998).

Clinical performance and its significance

The amount of regeneration achievable is of great concern in the evaluation of clinical significance, as it should be significant enough to justify the time and expense of the surgery (Jeffcoat 2002). There are many reports of clinical outcomes from the use of regenerative materials (Casarin *et al* 2010, Mellonig 1999, Parashis and Tsiklakis 2000, Sculean *et al* 2008, Tonetti *et al* 2002). For the purpose of this discussion, the results from two systematic reviews will be used.

Needleman *et al* (2006) investigated the benefits of GTR over conventional open flap debridement (OFD). They reported an extra gain of attachment level of 1.22 mm for GTR and 1.25 mm for GTR + bone substitutes over OFD alone, and 1.2 mm of reduction of probing pocket depth. The number needed to treat (NNT) was 8, i.e. at least 8 patients had to receive GTR to have one patient gain at least 2 mm of probing attachment level (PAL). It was concluded that GTR has an advantage for infrabony defects, but noted significant heterogeneity in the results of the studies included in the analysis and therefore cautioned the validity of this estimation (Needleman *et al* 2006).

Esposito *et al* (2009) conducted a similar meta-analysis on the use of EMD, with 13 studies included. They found similar improvement of attachment level (1.1 mm) and probing pocket depth reduction (0.9 mm) compared to GTR techniques. However, significantly more post-operative complications are reported in studies using GTR and it was therefore concluded that the use of EMD may be preferable over GTR. The authors also commented that it is “the patient’s and clinician’s decision whether the clinical gain of periodontal attachment found is of clinical relevance”, having reported that the NNT for EMD result in a gain of at least 2 mm of PAL is nine (Esposito *et al* 2009).

In summary, meta-analyses and most randomized controlled trials support the use of regenerative approaches and these procedures give highly variable but generally favorable results. There are also reports indicating the outcomes can be maintained over many years, but no significant difference in attachment gain after 10 years is noted when OFD and GTR is compared (Cortellini *et al* 1996, Cortellini and Tonetti 2004, Gottlow *et al* 1992, Nickles *et al* 2009, Sculean *et al* 2008).

Assessment of periodontal regeneration

Despite the positive conclusions by the meta-analyses of the periodontal literature, most studies on regenerative procedures have been assessed only indirectly. By definition, periodontal regeneration results in the formation of new cementum with inserting collagen fibres. Therefore, true periodontal regeneration can only be confirmed by histological means. Human studies have provided histological evidence demonstrating the success of GTR (Armitage *et al* 1977, Becker *et al* 1987, Gottlow *et al* 1986, Listgarten *et al* 1976, Nyman *et al* 1982). However, in clinical practice, the outcomes

are mainly assessed by radiographic bone fill, probing attachment levels, probing pocket depths and sometimes by re-entry procedures. These outcomes cannot provide direct evidence of true regeneration versus formation of long junctional epithelium. Junctional epithelium is found between newly formed bone and the root surface, and clinical probing is not accurate in determining the level of connective tissue attachment, as it can be significantly affected by the healthy or inflamed status of periodontium (Armitage *et al* 1977, Caton and Zander 1976, Listgarten *et al* 1976). Nevertheless, the Proceedings of the 1st European Workshop on Periodontology suggested that radiographic bone fill and clinical attachment gain could be accepted as evidence of periodontal regeneration (Lindhe and Echeverria 1994).

Relevance to patients

The outcome of the regenerative procedures in terms of operator-based assessments has been discussed. These include parameters such as clinical attachment level, probing depth and gingival recession. These are surrogate endpoints. True endpoints are parameters that are tangible to patients and in the periodontal context it can be tooth loss or patient quality of life (Hujoel 2004). In order for results of clinical studies to be relevant to patients’ daily life, true endpoints should be chosen as the primary outcome in clinical trials. Unfortunately, this is not the case for the vast majority of studies. This is because surrogate parameters can be measured by the clinician objectively, assuming no bias, whereas patients’ well-being is usually assessed by asking for their opinion, which is considered a subjective assessment.

It can be expected that when explaining the reasons for undertaking a surgical procedure, the goals of controlling disease progression and improving long-term prognosis of the

tooth/teeth involved should be included. But how do patients understand the benefits of additional attachment gain in their context? On the other hand, since periodontal disease may have an impact on the patient's quality of life in terms of functional limitation, discomfort, psychosocial and social disability, any means that may improve limitation or impairment would be very meaningful to the patients (Ng and Leung 2006, O'Dowd *et al* 2010). If the gain in attachment level can be translated to an amount of reversal of the compromised perception, then the surrogate outcomes could be linked to a patient-centered outcome and thus become very relevant to patients.

Unfortunately, such emphasis is scarce in the periodontal literature. There have been only a limited number of cross-sectional studies or studies with a short observation period reporting the influence of periodontal treatment on oral health-related quality of life (OHQoL), but there is evidence that there are improvements in patient's OHQoL of life after non-surgical periodontal treatment and that patients receiving non-surgical treatment or surgery with application of EMD have a better perception of improvement compared with patients receiving surgical treatment alone (D'Avila *et al* 2005, Needleman *et al* 2004, Ozcelik *et al* 2007, Saito *et al* 2010, Wong *et al* 2012). Nevertheless, the evidence of long term impact is still lacking. If both non-surgical and regenerative treatment has little impact on patient perception during immediately post-operative period, then it is questionable whether there will be more difference as healing continues.

In a study of a group of Chinese periodontal patients, it was found that initial non-surgical periodontal treatment was associated with an improvement in OHQoL (Wong *et al* 2012). Therefore, if regenerative procedures can cause a significant improvement in patient-centered outcomes their use would be well-

justified because they do not solely improve surrogate outcome parameters. On the other hand, if the perceived impact on quality of life is minimal, or is similar to what non-surgical periodontal treatment alone can achieve, we will have to reflect on how well the surgery may help in achieving the goal of controlling disease progression and ultimately preventing tooth loss. It is worth mentioning here that tooth loss is most likely related to the quality of supportive periodontal care instead of the types of periodontal therapy provided. Studies have shown that a well-complied maintenance program performed by a specialist can provide long term periodontal stability, whilst irregular supportive care significantly increases the risk ratio of tooth loss (Eickholz *et al* 2008, Leung *et al* 2006, Rosling *et al* 2001).

Many authors understand the importance of patient-centered outcomes and indeed, in the Cochrane review on GTR for infrabony defects by Needleman *et al* (2006), the primary outcomes included tooth loss, change in attachment levels, and patient well-being or quality of life. However, of all the studies included, none reported tooth loss or patient well-being aspects except post-operative complications, which were concluded to be minimal in general. Only attachment levels and secondary outcomes such as change in probing depth and gingival recessions are reported. The reviewers concluded that GTR gives an average of 1.2 mm more attachment gain over open flap debridement alone, but noted this has to be interpreted with caution because of the huge heterogeneity of the outcome, both between and within studies. It was also noted that some data of importance, such as patient-evaluated outcomes (e.g. aesthetic outcomes), was not available (Needleman *et al* 2006).

In another review, Esposito *et al* (2009) compared the effect of EMD with GTR and other procedures. While the primary outcomes were tooth loss, change in probing attachment

level, aesthetic aspects from patients' point of view as well as complications, again tooth loss was not reported in the included studies, and only two reported aesthetic outcomes, with both finding no difference in reports from patients. Only one study compared the post-operative complications of EMD and GTR and found that there were significantly more complications with GTR than use of EMD (Sanz *et al* 2004). Considering these few studies together, it may be deduced that from the patient's point of view, receiving GTR has more post-operative complications even when under antibiotic prescription, gives no better aesthetic outcome than using EMD, and both procedures have unknown impact on prognosis of the tooth because studies give results of great heterogeneity (Esposito *et al* 2009).

It seems unfortunate that what matters most to the patient is being overlooked when the success of a periodontal procedure is being studied and evaluated. In the future, it is hoped that more studies on patient-centered outcomes will be conducted and provide guidance for operators in the decision making process. Meanwhile, what may be left to discuss could be another more realistic issue: the financial cost.

Economic perspective

Regenerative materials are costly, but are they worth it? Considering the great heterogeneity of clinical outcome, it is very difficult to conclude whether periodontal regenerative procedures are cost-effective. In their systematic review, Needleman *et al* (2006) found no reports at all on cost/benefit with the use of GTR technique in infra-bony defects. This issue was not even mentioned by Esposito *et al* (2009) in their EMD review. The difficulties that financial cost may cause in clinical decision making may be very minimal when carried out in academic environment,

where costs could be waived or greatly reduced for participating patients for research purposes. In private practice however, cost plays a much larger role in treatment planning as a whole.

The costs for regenerative materials vary greatly between different manufacturers. For the following brief discussion, only the most widely used commercially available products are considered. The bovine bone graft Bio-Oss® (Geistlich) costs about HK\$850 (small granules, 0.5 g) while the porcine resorbable collagen membrane Bio-Gide® (Geistlich) costs about HK\$1500 (25 mm x 25 mm). Emdogain® (0.3 ml) with PrefGel (0.5 ml EDTA) costs about \$2000 (prices quoted by Advance Dental Consulting Ltd, November 2010). Therefore, the material fee alone would cost about HK\$2000-2500 for a GTR or Emdogain procedure. Fees for the dentist/periodontist and the surgery also differ greatly, but no survey on the range of charges has been undertaken and therefore estimations cannot be made accurately. However, if the surrogate outcome of attachment gain is used for comparison between OFD plus regenerative procedures and OFD alone, we may consider the extra material cost as an investment in any additional gain of attachment over OFD alone. If there is no significant difference in PAL gain between GTR and EMD technique, then the use of Emdogain will cost slightly lower than GTR in terms of material fees (Esposito *et al* 2009). On the other hand, if GTR with bone substitutes results in an extra attachment gain of 1.25 mm over OFD alone, this extra 1.25 mm of clinical attachment gain from the materials used costs around \$2350 (\$850 for Bio-Oss® and \$1500 for Bio-Gide®), resulting in a cost of around \$1880 per millimeter of attachment gain (Needleman *et al* 2006). How significant this cost is to the patient and the operator will vary widely and how crucial this extra gain of PAL is to a tooth will differ from case to case. Calculations

of this kind should still be considered as part of the risk:benefit and cost:benefit ratio assessments (Jeffcoat 2002).

There is also wide interest in using EMD in combination with different grafting or bioactive materials (Guida *et al* 2007, Jepsen *et al* 2008, Kuru *et al* 2006, Lekovic *et al* 2001, Sculean *et al* 2005). While reports gave variable results and conclusions, a recent network meta-analysis concluded that there was little evidence of additional benefit in using extra regenerative materials in combination with EMD (Tu *et al* 2010). Therefore, from the current evidence available, it seems that if a regenerative approach is to be opted, the use of EMD alone may be the most cost-effective choice.

Meanwhile, in order for this additional gain of attachment to be stable, meticulous plaque control and regular maintenance care is fundamental. It has been estimated that, in order to achieve 1 mm less clinical attachment loss over 30 years, a cost of €1500 (or €50 a year) in a periodontal maintenance by specialist is necessary (Gaunt *et al* 2008). It may then be deduced that the monetary price to gain 1 mm of attachment through regenerative materials is three times more expensive than the prevention of its loss in one year. However, it must be borne in mind that there is great heterogeneity of clinical outcomes and the lack of consistent results make the generalization of cost-effectiveness very difficult.

If the gain in attachment level can decrease the risk of tooth loss, then this is of great clinical significance and becomes directly relevant to the patient. It has been calculated that for a German population, the cost of replacement of a tooth is a minimum of €1650 for a bridge, €2000 for an implant, and €800 for a denture (Pretzl *et al* 2009). Therefore, should a tooth's prognosis be improved and thus retained for longer period of time through either simple non-surgical or periodontal

regenerative procedures, it will be both very cost-effective and cost-beneficial to do so (Braegger 2005). No matter what treatment modalities have been adopted, periodontal stability is only possible through good plaque control and compliance to the maintenance program. It is well documented that regular supportive periodontal care can result in minimal attachment loss and even “hopeless” teeth may survive for many years (Joss *et al* 1994, Hirschfeld and Wasserman 1978, Kaldahl *et al* 1996, Pretzl *et al* 2008, Tonetti *et al* 2000). In the end, periodontal health affects the patient's well-being and quality of life and those are the aspects that patients care about, whereas attachment level may be more interesting to the practitioner rather than the patient. Therefore, when making clinical decisions on the regenerative approach to be used the clinician must ask themselves whether this is likely to be truly beneficial to the patient and if it would outweigh the financial and/or other intangible costs, such as post-operative complications.

Conclusion

With a long history and on-going research development, as well as dedication in critical analyses of literature, evidence-based periodontology is made possible and accessible (Needleman *et al* 2005). This is something we are proud of, but should be used carefully and skillfully. It is important to bear in mind that the evidence in the literature for periodontal regeneration as a modern periodontal therapy assists the clinician in treatment planning decisions, but may not necessarily equate to what is the best for the patient's well-being or is most relevant to our patients. Just as the most important treatment for patients susceptible to caries is primary prevention (dietary counseling, application of fluoride, etc.) rather than filling of the cavities alone, the ultimate goal of periodontal

treatment should be control of the disease for long-term retention of natural teeth in a healthy and functional state, rather than simply regenerating bony defects (Eickholz *et al* 2008, Hirschfeld and Wasserman 1978). It is, of course, ideal if sites with periodontal attachment loss can be restored to their original state; but when current science is yet to show a consistent way to achieve this, the translation of surrogate endpoints to their real impact on patients and consideration of the cost-effectiveness of any regenerative attempts are equally important. We have to acknowledge that at present we do not have sufficient reports in the literature for clinicians to refer to. At the same time, we know that long junctional epithelium is not necessarily inferior to connective tissue attachment, and a reduced but healthy periodontium can also provide satisfactory function in the long run (Nyman and Ericsson 1982). Therefore, any treatment procedures, non-surgical or surgical, using regenerative materials or not, must be carefully selected, taking into account all factors possibly involved, including patient, tooth, defect and operator factors, so as to obtain a more predictable outcome, improving the prognosis of the teeth involved and hence hopefully be ultimately beneficial to the patient's overall quality of life (Cortellini and Tonetti 2000). The paradox is, it is sometimes because of very careful case and defect selection that it becomes difficult to assess whether the change of prognosis of the tooth, if any, is truly reflecting the benefits from the use of regenerative materials, rather than the surgical or non-surgical treatment itself.

In addition, one must not forget that no matter what types of periodontal therapy are currently available, it does not alter the inherent susceptibility of the host to periodontal disease. Success in periodontal therapy lies in long term maintenance by both the patient and the clinician, and it is well known that outcomes are heavily

influenced by patients' oral hygiene practices (Axelsson and Lindhe 1981a, Axelsson and Lindhe 1981b, Corbet and Davies 1993, Lindhe and Nyman 1975, Lindhe and Nyman 1984). Without compliance to a periodontal maintenance program, attachment gained by any means can be easily lost. Interestingly, conveying this message to both clinicians and patients can sometimes be more difficult than achieving periodontal regeneration.

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Chapter 5

Effects of Flavonoids on the Expression of Bone Sialoprotein Gene

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Introduction

Flavonoids are micronutrients that are widely present in foods of plant origin. Various biological properties have been attributed to them, such as anti-cancer, anti-oxidant, anti-inflammation, anti-viral, anti-mutagenic, gastroprotective, protein kinase C inhibition, topoisomerases II inhibition and the prevention of age-related pathologies (Akama *et al* 1996, Manna *et al* 1999, Shimizu and Ogata 2002). Flavonoids are present mainly as glycosides, in which hydrogen is substituted by the sugar moiety (Kim *et al* 2007, Murota *et al* 2000, Murota *et al* 2002). Soybeans are rich in isoflavones, such as genistein and daidzein, and have been reported to reduce the occurrence of osteoporosis (Devareddy *et al* 2006, Morabito *et al* 2002). A synthetic analogue, ipriflavone, is effective in inhibiting bone resorption in post-menopausal osteoporosis, although its action may not occur directly through estrogen receptors (ER) (Petilli *et al* 1995).

Kaempferol and quercetin belong to the flavonol family (Comalada *et al* 2006). Flavonols, in contrast to soybean isoflavones, are the most abundant phytoestrogens in western diets, being present in fruits,

vegetables, nuts, seeds, tea, red wine and dark chocolate. Quercetin and its glycoside derivative rutin reduced osteoclastic bone resorption by inhibiting the receptor activator of nuclear factor κ B (NF κ B) (RANK) protein and activating caspases (Rassi *et al* 2005). Kaempferol, but not quercetin, inhibited tumor necrosis factor α (TNF α)-induced production of IL-6 and monocyte chemoattractant protein-1 (MCP-1) in MC3T3-E1 osteoblast-like cells (Pang *et al* 2006). Kaempferol and quercetin increases alkaline phosphatase (ALP) activity in MG-63 osteoblast-like cells through extracellular regulated kinase (ERK) and the estrogen receptor (ER) pathway (Prouillet *et al* 2004). These findings indicate that kaempferol is a potent anti-osteoclastic agent due to its action on both osteoclasts and osteoblasts.

Bone sialoprotein (BSP) is a mineralized connective tissue-specific protein that is glycosylated, phosphorylated and sulfated (Ogata 2008). Studies on the developmental expression of BSP have shown that BSP mRNA is expressed at high levels at the onset of bone, dentin and cementum formation (Chen *et al* 1992). BSP is also expressed in breast, lung, thyroid and prostate cancers (Waltregny *et al* 2000). Thus, it has been

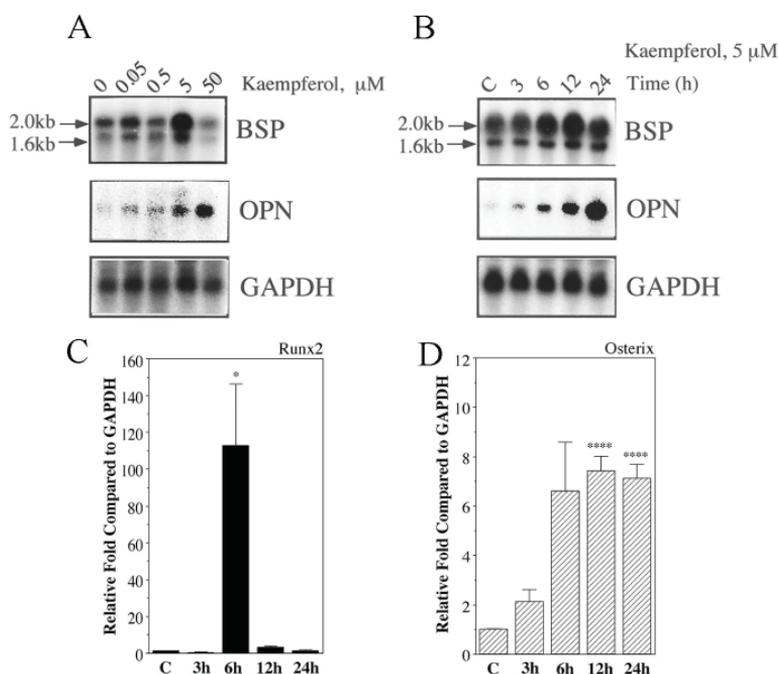


Figure 1. Effects of kaempferol on BSP, OPN, Runx2 and Osterix mRNA levels in UMR106 cells. (A) Dose-response effect of kaempferol on BSP mRNA levels in osteoblast-like UMR106 cells treated for 12 hours. (B) 24 hour time course revealed an increase in BSP mRNA following administration of 5 μ M kaempferol to UMR106 cells. Total RNA was isolated from triplicate cultures harvested after stimulation at 3, 6, 12, 24 hours and used for Northern hybridization using BSP, osteopontin and GAPDH DNA probes. Results of representative hybridization analysis for control and kaempferol-treated cells are shown. (C&D) The expressions of Runx2, Osterix and GAPDH mRNA treated with 5 μ M kaempferol for 12 hours in UMR106 cells were measured by real-time PCR. The relative amounts of mRNA of Runx2 (C) and Osterix (D) to GAPDH were calculated. The experiments were performed in triplicate for each data point. Quantitative analyses of triplicate data sets are shown with standard errors. Significant differences from control: *($P < 0.1$); ****($P < 0.01$).

suggested that BSP may be involved in the osteotropism of metastatic cancer cells through its ability to bind to hydroxyapatite and to mediate cell attachment through cell-surface integrins (Ganss *et al* 1999). To study the transcriptional regulation of BSP, rat, human and mouse BSP gene promoters have been characterized (Benson *et al* 1999, Kim *et al* 1994, Li and Sodek 1993). These promoters have an inverted CCAAT box (-50 to -46), which is required for basal transcription (Kim and Sodek 1999, Shimizu and Ogata 2002). In addition, a cAMP response element (CRE; -75 to -68), a fibroblast growth factor 2 (FGF2)

response element (FRE; -92 to -85), a pituitary specific transcription factor-1 (Pit-1; -111 to -105), and a glucocorticoid response element (-920 to -906), overlapping an AP-1 site (-921 to -915) have also been characterized (Ogata *et al* 1995, Ogata *et al* 2000, Samoto *et al* 2003, Shimizu-Sasaki *et al* 2001, Yamauchi *et al* 1996).

To determine the mechanism of BSP gene regulation by flavonoids, we analyzed the effects of kaempferol on the expression of BSP gene in osteoblast-like cells. These studies have revealed that kaempferol induced the BSP gene transcription that was mediated

through inverted CCAAT, CRE and FRE elements in the rat BSP gene promoter.

Stimulation of BSP mRNA expression in UMR106 cells

To study the regulation of BSP expression by kaempferol, we performed Northern blot analyses of total RNA extracted from UMR 106 osteoblast-like cells. First, a dose-response effect of kaempferol on the BSP mRNA levels was established by treating the UMR 106 cells with different concentrations of kaempferol for 12 hours. Kaempferol increased BSP mRNA levels at 0.05 to 5 μ M and had a maximal effect at 5 μ M (Figure 1a). This optimal level of kaempferol was used to determine the time course of BSP mRNA expression. Kaempferol up-regulated BSP mRNA accumulation at 6 and 12 hours, whereas no effect on GAPDH mRNA was observed (Figure 1b). Kaempferol induced the expression of osteopontin (OPN) mRNA levels in either a dose- and time-

dependent manner (Figure 1a&b). The results of real-time PCR showed that kaempferol (5 μ M) significantly increased Runx2 mRNA expression at 6 hours, and Osterix mRNA levels at 3 hours and reached maximal at 12 and 24 hours respectively in UMR106 cells (Figure 1c&d).

Transient transfection assays of rat BSP promoter constructs

To further determine the effects of kaempferol on the activation of BSP transcription, various-sized rat BSP promoters ligated to a luciferase reporter gene (pLUC1 - pLUC6, pLUC1; -18 to +60, pLUC2; -43 to +60, pLUC3; -116 to +60, pLUC4; -425 to +60, pLUC5; -801 to +60 and pLUC6; -938 to +60) were transiently transfected into UMR106 cells. The results of luciferase assays showed an increase in transcription after 12 hours treatment with 5 μ M kaempferol using pLUC3 constructs, which encompass nucleotides from -116 to +60, as well as longer constructs

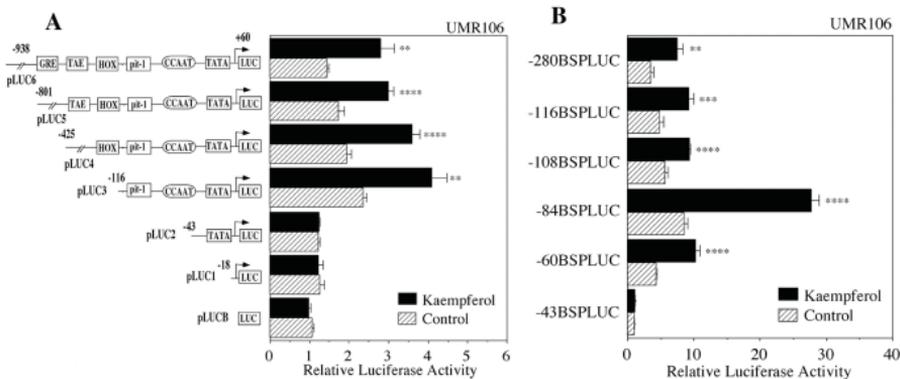


Figure 2. Kaempferol up-regulates BSP promoter activity in UMR106 cells. (A) Transient transfections of UMR106 cells, in the presence or absence of kaempferol (5 μ M) for 12 h, were used to determine transcriptional activity of chimeric constructs that included various regions of the BSP promoter ligated to a luciferase reporter gene. (B) Fine 5' deletion mapping of the nts -280 to -43 elements in the BSP promoter. A series of rat BSP promoter 5' deletion constructs was analyzed for relative promoter activity after transfection into UMR106 cells and examined for induction in the presence of kaempferol (5 μ M). The results of transcriptional activity obtained from three separate transfections have been combined and the values expressed with standard errors. Significant differences from control: **($P < 0.05$); ***($P < 0.02$); ****($P < 0.01$).

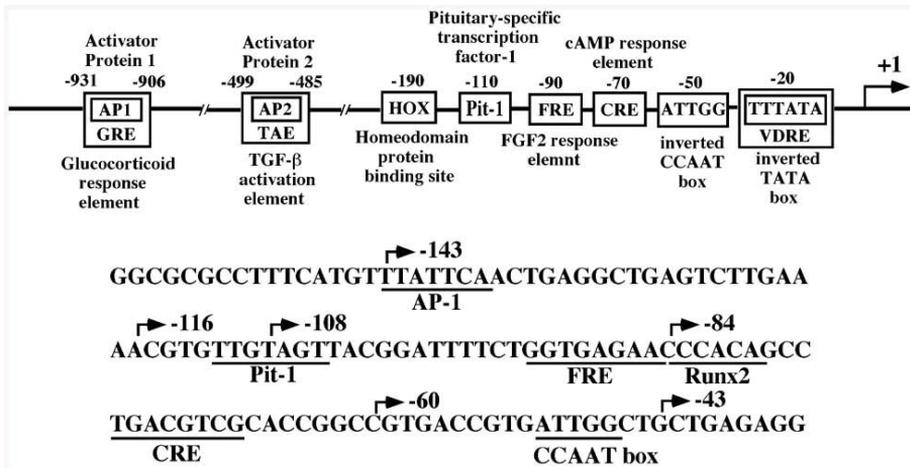


Figure 3. Regulatory elements in the proximal rat BSP promoter. (Upper panel) The position of inverted TATA and CCAAT boxes, CRE, FRE, Pit-1, HOX, TAE and GRE overlapping with AP-1. The numbering of nucleotides is relative to the transcription start site (+1). (Lower panel) The nucleotide sequence of the rat BSP gene proximal promoter is shown from -159 to -35. Inverted CCAAT box, CRE, Runx2, FRE, Pit-1 and AP-1 are present.

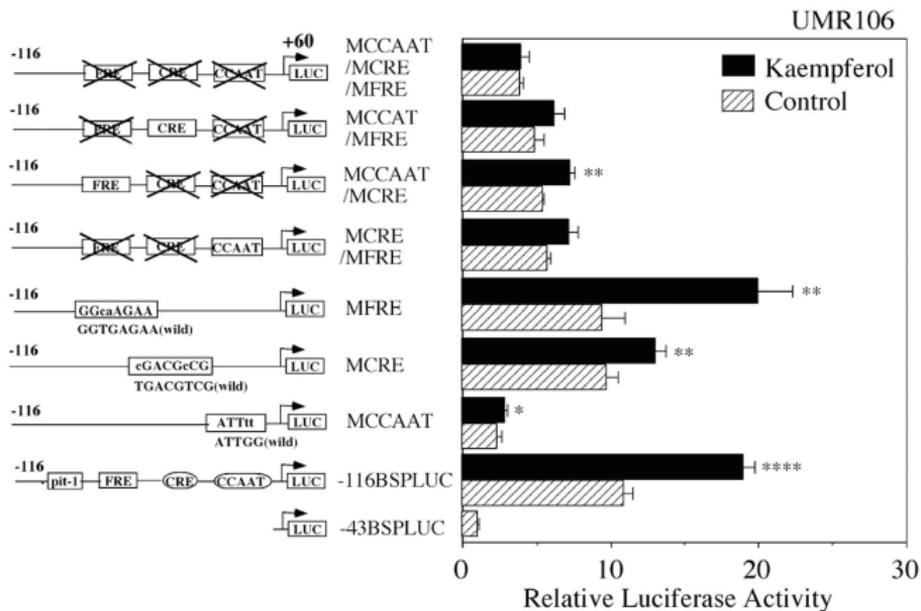


Figure 4. Site mutation analysis of luciferase activities. Dinucleotide substitutions were made within the context of the homologous -116 to +60 (pLUC3) BSP promoter fragments. M-CCAAT (ATTtt), M-CRE (cGACGcCG), M-FRE (GGcaAGAA), double and triple-mutated constructs were analyzed for relative promoter activity after transfection into UMR106 cells and examined for induction after treatment with kaempferol (5 μ M) for 12 hours. The results of transcriptional activity obtained from three separate transfections with constructs were combined and the values expressed with standard errors. Significant differences from control: *(P<0.1); **(P<0.05); ****(P<0.01).

(pLUC4, pLUC5 and pLUC6) (Figure 2a). Within the DNA sequence unique to the pLUC3 (-116 to -43) is an inverted CCAAT box (ATTGG; nts -50 and -46), a CRE (nts -75 and -68), a putative Runx2 binding site (nts -84 and -79), a FRE (nts -92 and -85), and a Pit-1 (nts -111 and -105), which is the target of parathyroid hormone (Figure 3). To determine more precisely the target sites in the BSP promoter through which the kaempferol effects were mediated, we prepared a series of 5' deletion constructs between nts -280 and -43. While the luciferase activities all of the constructs (-60, -84, -108, -116, -280BSPLUC) were increased by kaempferol (5 μ M, 12 hours), -84BSPLUC was induced by kaempferol maximum (Figure 2b). Next, we introduced 2 bp mutations in the putative response elements targeted by kaempferol within nts -116 to -43 of pLUC3 (MCCAAT, MCRE and MFRE) (Figure 4). The basal transcriptional activities of MCCAAT, MCRE and MFRE were lower than the basal level of pLUC3. Transcriptional stimulation by kaempferol was partially abrogated in these three single mutation constructs (Figure 4). To confirm the functional elements, we also performed double and triple mutation assays. When mutations were made in pairs of target elements (MCRE/MFRE, MCCAAT/MCRE, MCCAAT/MFRE), kaempferol-induced luciferase activities was partially abolished. In triple mutation (MCCAAT/MCRE/MFRE), kaempferol-induced luciferase activities were totally abrogated (Figure 4). We then used six signaling pathway inhibitors; protein kinase C inhibitor (H7), cAMP-dependent protein kinase inhibitor (H89), tyrosine kinase inhibitor herbimycin A (HA), ERK1/2 inhibitor (U0126), PI3-K inhibitor (LY294002) and antioxidant NAC, to determine the signaling pathway after kaempferol stimulation. Transcriptional stimulations by kaempferol were inhibited by H89, HA, U0126 and LY294002 (data not shown).

Gel mobility shift assay

To identify which transcription factors can bind to the promoter region of pLUC3, double-stranded oligonucleotides of CCAAT, CRE, FRE and Pit-1 elements were end-labeled and incubated with equal amounts (3 μ g) of nuclear proteins extracted from confluent UMR106 cells treated with or without kaempferol (5 μ M) for 3, 6 and 12 hours. When we used the inverted CCAAT as a probe, the DNA-NFY protein complex did not change after kaempferol treatment (Figure 5a, lanes 1-4) (Kim and Sodek 1999, Shimizu and Ogata 2002). With nuclear extracts from confluent control cultures of UMR106 cells, shifts of CRE-, FRE- and Pit-1 protein complexes were evident (Figure 5a, lanes 5, 9 and 13). After kaempferol stimulation for 3 and 6 h, CRE- and FRE-protein complexes formations were increased (Figure 5a, lanes 6 and 7, 10 and 11), and decreased again at 12 hours. On the other hand, Pit-1-protein complex formation did not change (Figure 5a, lanes 14-16). Further competition experiments in which a 20- and 40-fold molar excess of CCAAT, and 40-fold molar excess of CRE and FRE double-stranded oligonucleotides reduced the amount of complexes formation (Figure 5b, lanes 3 and 4; Figure 6a, lane 3; Figure 6b, lane 3). In contrast, mutation CCAAT (Figure 5b, lanes 5 and 6), mutation CRE (Figure 6a, lane 4) and mutation FRE (Figure 6b, lane 4) did not compete with complex formation. Interestingly, FRE-protein complex formation was in competition with a 40-fold molar excess of HOX (Figure 6b, lane 5), suggesting that FRE- and HOX-binding proteins were similar.

To further characterize the nuclear proteins in complex formation we used several antibodies. For inverted CCAAT box, NFYA antibody partially blocked CCAAT-protein complex formation (Figure 5b, lanes 8-10). CRE-binding proteins were supershifted by

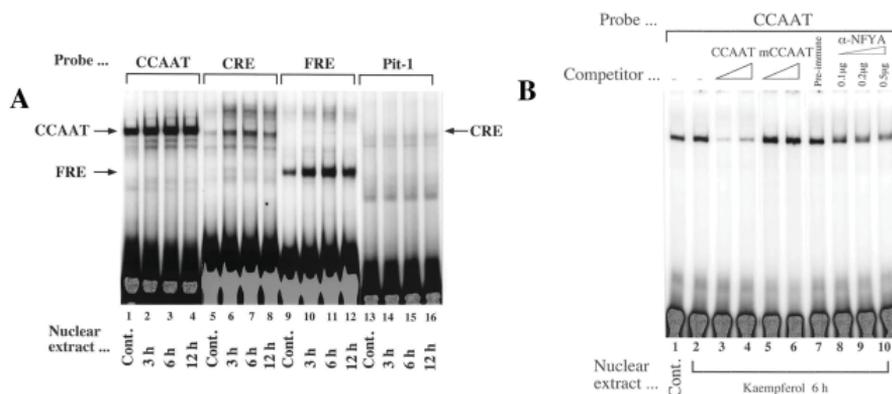


Figure 5. (A) Gel mobility shift analysis of inverted CCAAT, CRE, FRE and Pit-1 oligonucleotides. Radiolabeled double-stranded CCAAT (-61 CCGTGACCGTGATTGGCTGCTGAGA -37; lanes 1-4), CRE (-84 CCCACAGCCTGACGTCGCACCGGCCG -59; lanes 5-8), FRE (-98 TTTTCTGGTGAGAACCCACA -79; lanes 9-12) and Pit-1 (-115 CGTGTTGTAGTTACGGATT -96; lanes 13-16) were incubated with nuclear protein extracts (3 μ g) obtained from UMR106 cells stimulated without (lanes 1, 5, 9 and 13) or with kaempferol (5 μ M) for 3 hours (lanes 2, 6, 10 and 14), 6 hours ((lanes 3, 7, 11 and 15) and 12 hours (lanes 4, 8, 12 and 16). DNA-protein complexes were separated on 5% polyacrylamide gel in low-ionic-strength Tris-borate buffer, dried under a vacuum and exposed to an imaging plate for quantitation using image analysis. (B) Specific binding of nuclear protein to the inverted CCAAT. Radiolabeled double-stranded CCAAT (-61 CCGTGACCGTGATTGGCTGCTGAGA -37) was incubated with nuclear protein extracts (3 μ g) obtained from UMR106 cells treated without (lane 1) or with kaempferol (5 μ M) for 6 hours (lanes 2-10). Competition reactions were performed using a 20- and 40-fold molar excess of unlabeled CCAAT (lanes 3, 4) and mutation CCAAT (CCGTGACCGTGATTtCTGCTGAGA; lanes 5, 6). Supershift experiments were performed with 0.1, 0.2 and 0.5 μ g anti-NF-YA monoclonal antibody, which recognizes the A subunit of NF-Y (lanes 8-10).

CRE binding protein 1 (CREB) and phospho-CREB antibodies, and disrupted by c-Fos, c-Jun, JunD and Fra2 antibodies (Figure 6a, lanes 6-11). FRE-protein complexes formations were disrupted by Runx2, Dlx5 and Msx2 antibodies (Figure 6b, lanes 7-9).

Stimulatory effects of kaempferol on newborn rat calvaria

In order to further clarify the direct effects of kaempferol on bone formation, 5 μ l kaempferol (5 μ M) was injected into the parietal bone of newborn rats. Histologically, only osteoid matrix formation was seen in the control group 6 days after treatment, suggesting that calcification in the area of new bone formation was accelerated

by kaempferol treatment compared to the control. Immunohistochemical analyses were performed on the tissue sections of parietal bone which was harvested on day 6. Immunostaining with BSP antibody revealed staining of the bone matrix and osteoblastic cells on the superior surface of the calvariae. The increase in the thickness of mineralized bone matrix stained with BSP antibody was more pronounced in kaempferol group. Osteoblasts lining the bone surface, osteocytes, cells with fibroblastic morphology associated with the soft tissue stroma were stained with Runx2 and Osterix antibodies. This staining was accelerated by kaempferol treatment (data not shown).

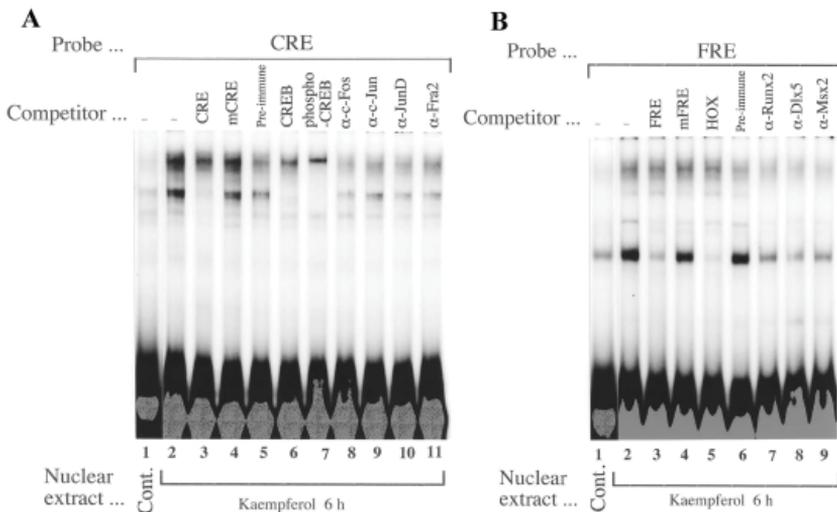


Figure 6. Specific binding of nuclear protein to the CRE and the FRE. Radiolabeled double-stranded CRE (-84 CCCACAGCCTGACGTGCACCGGCCG -59) and FRE (-98 TTTTCTGGTGAGAACCCACA -79) were incubated with nuclear protein extracts (3 μ g) obtained from UMR106 cells treated without (lane 1) or with kaempferol (5 μ M) for 6 hours (lane 2). Competition reactions were performed using unlabeled CRE and FRE (lane 3), and mutation CRE (CCCACAGCCcGACGcCGCACCGGCCG), mutation FRE (TTTTCTGGcaAGAACCCACA) (lane 4) and HOX (TCCTCAGCCTTCAATTAATCCCACA; B, line 5). Supershift experiments were performed using antibodies against CREB (A, lane 6), phospho-CREB (A, lane 7), c-Fos (A, lane 8), c-Jun (A, lane 9), JunD (A, lane 10) and Fra2 (A, lane 11), Runx2 (B, lane 7), Dlx5 (B, lane 8) and Msx2 (B, lane 9) added separately to each gel shift reaction.

Discussion

Flavonoids are widely found in vegetables and fruits, and four of the major subclasses are flavones, flavonols, isoflavones and flavanones, which have multiple biological and pharmacological activities owing to their anti-oxidant, anti-inflammatory and estrogenic effects (Dang and Lowik 2005, Gerritsen *et al* 1995, Hämäläinen *et al* 2007, Suh *et al* 2009). Quercetin exhibits anti-tumor effects, possibly through its ability to stimulate the immune system, alter the mitotic cycle, scavenge free radicals, modify gene expression, and block angiogenesis (Hayashi *et al* 2000). We previously reported that genistein increased BSP transcription via an inverted CCAAT box, and quercetin up-regulated BSP expression through inverted CCAAT and FRE elements (Kim *et al* 2007, Shimizu and Ogata 2002). These results suggested that the effects of

genistein (isoflavone) and quercetin (flavonol) were different. Quercetin inhibited RANK ligand (RANKL)-induced NF κ B and activator protein 1 (AP1) activation and decreased osteoclastic differentiation (Wattel *et al* 2004). Kaempferol belongs to the flavonol family, and could have more potent anti-osteoclastic activities than quercetin (Pang *et al* 2006). Additionally, kaempferol decreased osteoclastic bone resorption, by directly targeting the osteoclasts through a mechanism at least partially involving the ER (Rassi *et al* 2005, Tang *et al* 2008). Kaempferol increased ALP activity, and the effect was reduced by inhibitor of extracellular regulated kinase (ERK) and by an antagonist of ER (Prouillet *et al* 2004).

Sex steroid hormones have major beneficial effects on the development and maintenance of the skeleton (Syed and Khosla 2005). 17 β -estradiol does not upregulate BSP

transcription (data not shown) and there is no putative estrogen response element in the proximal promoter of the rat BSP gene (Figure 3), suggesting that the regulation of BSP gene expression by kaempferol is independent of its estrogenic activity.

In this study we have shown that kaempferol increased BSP transcription in osteoblast-like UMR106 cells through an inverted CCAAT box, CRE and FRE in the proximal promoter of the BSP gene. Kaempferol increased Runx2, Osterix and BSP mRNA levels in UMR106 cells (Figure 1a-d). Runx2 and Osterix deficient mice display absence of bone due to arrested osteoblast differentiation (Komori *et al* 1997, Nakashima *et al* 2002). Runx2 might involve the FRE binding transcription factors since a putative Runx2 binding site (CCCACA) is juxtaposed to FRE (GGTGAGAA) (Figure 3) and FRE-protein complex formations were disrupted by Runx2, Dlx5 and Msx2 antibodies. Dlx5 are activated after BMP2 addition to the mouse 2T3 osteoblast and primary fetal rat calvarial osteoblasts (Harris *et al* 2003). BMP2-induced Runx2 expression is mediated by Dlx5 (Lee *et al* 2003). Further, Dlx5 reverses Msx2 inhibition of osteocalcin promoter activation by FGF2/forskolin (Newberry *et al* 1998). Msx2 suppressed BSP transcription and the effect could be de-repressed by increasing Dlx5 levels (Barnes *et al* 2003).

From transient transfection assays using a series of 5' deletion constructs between nts -280 and -43, the luciferase activities of -60~-280BSPLUC were increased by kaempferol (5 μ M, 12 hours), and had a maximal effect on -84BSPLUC (Figure 2b). CRE and Runx2 binding sites are specific sequences in -84BSPLUC, suggesting that CRE and Runx2 binding transcription factors are crucial for kaempferol-induced BSP transcription. CRE-protein complexes were supershifted by CREB and phospho-CREB antibodies and disrupted by c-Fos, c-Jun,

JunD and Fra2 antibodies (Figure 6a). CREB and AP1 transcription factors JunD and Fra2 regulate BSP gene expression in breast cancer cells (Detry *et al* 2008). The results indicate that CREB, phospho-CREB, c-Fos, c-Jun, JunD and Fra2 might interact with CRE.

Transcription of the BSP gene is stimulated by v-Src through an inverted CCAAT box that is bound by a ubiquitous trimeric complex, NF-Y transcription factor (Kim and Sodek, 1999). This complex is composed of three conserved subunits, NF-YA, NF-YB and NF-YC, all of which are required for DNA binding (Caretto *et al* 1999, Li *et al* 1992). It is essential for expression of the class II genes of the major histocompatibility complex (MHC) and is likely involved in the regulation of albumin, α 1(I) and α 2(I) collagen genes (Caretto *et al* 1999, Maity *et al* 1992). NF-Y is also required for cyclin B1 transcription and that the switch-off of cyclin B1 expression in terminally differentiated skeletal muscle cells depends upon the loss of a functional NF-Y complex (Manni *et al* 2001). That the promoters of several cell cycle regulatory genes such as cyclin A, cyclin B1, cyclin B2, cdk1 and cdc25C contain CCAAT boxes suggests NF-Y is a key regulator of cell cycle (Manni *et al* 2001). However, the mechanism of NF-Y mediated gene transcriptional regulation is not well understood. In gel shift assays, we could not see any differences in the DNA-NF-Y protein complex formed with the inverted CCAAT sequence obtained from nuclear extracts of cells treated or not with kaempferol (Figure 5b). Therefore, it is likely that kaempferol treatment results in modifications of NF-Y that influence its transactivation properties, but these changes do not affect the binding of the DNA-NF-Y protein complex to the CCAAT element.

Twelve daily injections of 5 μ M kaempferol directly into the periosteum of parietal bones of newborn rats increased the number of osteoblasts and new bone formation. BSP,

Runx2 and Osterix protein expressions in parietal bone and osteoblasts lining the bone surface was also enhanced after kaempferol injection for 6 days. Therefore kaempferol could induce osteoblast activities at early stage of bone formation.

Conclusion

In conclusion, we have shown inverted CCAAT, CRE and FRE elements in the rat BSP proximal promoter through which the stimulatory effects of kaempferol on BSP gene transcription are mediated. Kaempferol stimulated new bone formation and increased BSP gene expression. Moreover, CREB1, phospho-CREB1, c-Fos, c-Jun, JunD, Fra2, Runx2, Dlx5 and Msx2 transcription factors appear to be key regulators of the kaempferol effects on BSP transcription and bone formation.

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Chapter 6

Current Research Activities of Postgraduate Periodontics Programs in Indonesia

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Background

Indonesia, the world's largest archipelago located in Southeast Asia, comprises five main islands: Java, Sumatera, Sulawesi, Kalimantan, and Papua. Indonesia has 26 dentistry faculties; 16 are located in Java, six in Sumatera, two in Sulawesi, one in Bali and one in Kalimantan. At present, out of 26 Faculties of Dentistry, only six offer postgraduate programs in clinical periodontics. Postgraduate programs in clinical periodontics were first offered by Universitas Indonesia, Jakarta in 1984. In the late 80's two other prominent state universities, Gadjah Mada University in Yogyakarta and Airlangga University in Surabaya, commenced their postgraduate programs. Following this, Padjadjaran University in Bandung, Sumatera Utara University in Medan and Hasanudin University in Makasar also started offering programs. Java offers four postgraduate periodontal programs while Sumatera and Sulawesi offer one each.

The Indonesian Medical Council stated there were about 131 registered periodontists responsible for caring for the 250 million inhabitants of Indonesia. Data from the Indonesian Dental Association said that most dentists, especially periodontists, worked in the big cities on Java, such as Jakarta, Surabaya, Yogyakarta, and Bandung, as

well as Medan and Makasar because dental faculties with postgraduate periodontal programs are located in those cities (Dwiati 2013).

Periodontal research, a requirement for postgraduate students, is on the rise. The following data shows periodontal studies carried out during the 2002-2012 period in some postgraduate periodontal programs. 42.5% of studies were on non-surgical therapy, 26.24% examined the correlation between periodontal disease and systemic disease, periodontal regeneration studies comprised 10%, surgical therapy 3.75%, biomolecular research 11.25%, and periodontal lasers were 3.75%.

Periodontal research

There has been a steady progress in periodontal research activities in Indonesia. The various studies in Indonesia universities have been focused on clinical studies relating to etiological factors, risk factors and treatment modalities.

Non-surgical research

Non-surgical research has examined clinical improvement after initial therapy, scaling and root-planing (SRP), use of chemotherapeutic agents and also the

relationship between systemic conditions and periodontal status. The chemotherapeutic agents used for the study are antimicrobial agents, such as tetracycline, minocyclin, doxycyclin, metronidazole, clindamycin, povidone-iodine, chlorhexidine, hyaluronic acid, aloe vera, morinda citrifolia (noni fruit), betel leaf and honey. These agents are used for sub-gingival irrigation, local application as gel or as mouthrinses. The aim of these studies is to evaluate the clinical achievement and improvement after periodontal treatment.

Sub-gingival irrigation with povidone-iodine and tetracycline both result in clinical improvement. Significant reductions in pocket depth (PD), clinical attachment loss (CAL) and papilla bleeding index (PBI) were observed in moderate chronic periodontitis but no significant improvements were seen in either severe chronic periodontitis or aggressive periodontitis (Ervina 2002, Natalina 2002).

Clinical studies of periodontal pocket treatment with gel have been undertaken by postgraduate students. They used gels containing metronidazole, tetracycline, doxycycline or betel leaf. These gels appeared to be effective in reducing PD, PBI and CAL. All subjects had moderate chronic periodontitis with 4 to 6 mm PD. A study with 25% metronidazole gel was carried out in 30 subjects divided into two groups (Suwandi 2003). The first group underwent SRP with application of metronidazole gel and the second group (control group) underwent SRP with 10% povidone-iodine. Bleeding on probing (BOP), PD and CAL were monitored, resulting in significant clinical improvement on day 35. No difference in improvement between groups before day 35 was noted. Gusriani (2005) reported significant improvements in PD, PBI and CAL following the use of 10% doxycycline gel after SRP in moderate chronic periodontitis. Rusminah (2005) reported the efficacy of betel

leaf gel as a conjunctive therapy in the control of clinical parameters (PD, PBI and CAL) in type 2 diabetic patients.

An evaluation of the difference in effectiveness between gingival application of 0.20% hyaluronic acid gel and gingival massage in 96 gingivitis patients indicated the gingival massage group showed better clinical improvement than the hyaluronic acid group (Restuning 2007).

A study to evaluate the efficacy of 25% and 50% aloe vera mouthrinses on periodontal status was undertaken by Napitupulu (2004). An improvement in dental plaque scores and papilla bleeding index following use of aloe vera was reported. The improvements shown in the 25% aloe vera rinse group were better than those in the 50% aloe vera group.

Anggraini (2004) examined a mouthrinse containing morinda citrifolia for improvements in dental plaque scores and papilla bleeding index without scaling and root-planing. This research concluded that scaling and root-planing is still better in reducing gingivitis than rinsing with morinda citrifolia alone when clinical parameters are examined.

Rosmelita (2003) evaluated the efficacy of 0.20% chlorhexidine and diluted 0.20% chlorhexidine 1:1 for gingivitis treatment. Clinical parameter improvement was evaluated after 7 days. Although both groups showed reduced gingivitis and tooth staining, a significant difference was not reported.

Rismanto (2004) evaluated the efficacy of progressive and flexible toothbrushes in reducing tooth staining from 0.2% chlorhexidine. They found that the progressive toothbrush was better than a flexible toothbrush in reducing tooth stain. Adisti (2005) showed the efficacy of a super tapered toothbrush in reducing dental plaque and gingivitis.

The effect of periodontal status on malodour and the reduction of volatile sulphur compounds (VSC) was analysed. Three studies were undertaken; the first evaluated

Authors	Study method	Findings
Suwandi 2002	Experimental clinic 30 subjects, contra lateral quadrant. PPD, BOP and CAL.	Significant clinical improvement on day 35. No difference in improvement between groups before day 35.
Gusriani 2005	Experimental clinic 108 sites. PD, PBI and CAL.	Significant improvement in PD, PBI and CAL following the use of 10% doxycycline gel after SRP in moderate chronic periodontitis.
Rusminah 2005	Experimental clinical study, 26 subjects, split-mouth. PD, PBI, and CAL in type-2 Diabetes mellitus.	Betel leaf gel effective in controlling clinical parameters (PD, PBI and CAL) in type 2 diabetic patients.
Restuning 2007	96 subjects with gingivitis. BOP.	Clinical improvement in two groups. Improvements in the gingival massage group were greater than the hyaluronic acid group.

Table 1. Non-surgical periodontal therapy.

Authors	Study method	Findings
Rismanto 2004	Experimental clinical. 60 subjects. Tooth staining.	Progressive toothbrush is better than flexible toothbrush in reducing tooth staining from 0.2% chlorhexidine rinse.
Adisti 2006	Experimental clinical. 20 subjects. Dental plaque and PBI.	Super tapered tooth brush significantly reduced dental plaque and PBI.
Wijayanti 2007	Experimental clinical. 47 males with chronic periodontitis. VSC rate.	VSC rates thirty minutes after SRP were reduced but not significantly.
Alibasyah 2008	Experimental clinical. 39 subjects. VSC rate.	VSC rate was reduced but not significantly, measured thirty minutes before and after tongue and tooth brushing.
Hartanto 2008	Experimental clinical. 27 subjects. VSC rate.	Reduces VSC rate but no difference in improvement between two groups (tongue brushing with two different dentifrices).

Table 2. Clinical studies: Tooth brushing and halitosis.

Authors	Study method	Findings
Ervina 2002	72 sites, contra lateral quadrant. Periodontal pocket depth (PPD), papilla bleeding index (PBI).	Significantly reduced pocket depth and papilla bleeding index in moderate chronic periodontitis (PD 6-7mm).
Natalina 2002	63 sites, contra lateral quadrant. PPD, CAL.	Significantly reduced pocket depth and clinical attachment loss in moderate chronic periodontitis, except for PD 6 mm .
Rosmelita 2003	99 subjects. BOP and tooth staining.	Correlation between chlorhexidine 0.2% and diluted chlorhexidine 0.2% 1:1 rinse and staining. Although two groups showed reduction in gingivitis and tooth staining, significant differences were not reported.
Napitupulu 2004	120 subjects with gingivitis in anterior teeth. Dental plaque scores and PBI.	Improvement in dental plaque scores and PBI. Improvements in 25% aloe vera rinse group better than 50% aloe vera group.
Anggraeni 2004	90 subjects with gingivitis. Dental plaque scores and PBI.	Improvement in the dental plaque scores and PBI without SRP. Clinical parameter improvement after SRP still better than rinsing with morinda citrifolia alone.

Table 3. Studies in antiseptic agents.

Authors	Study method	Findings
Mualim 2002	61 subjects. Glucose level, PPD, PBI, and CAL.	Periodontal debridement significant reduced PPD, PBI, CAL and glucose level in type 2 diabetes mellitus.
Andrena 2007	30 subjects with CHD. Dental plaque (PII) and dental calculus index (CI).	PII and CI in CHD subjects were higher than non-CHD.
Astuti 2009	60 subjects with DM, 53 subjects with DM and CHD.	Periodontal status diabetic patients were better than patients with DM and CHD.
Nasution 2012	16 subjects with CHD. <i>Streptococcus sanguinis</i> levels from dental plaque and saliva.	<i>S. sanguinis</i> levels in CHD group were higher than control group but not significant.

Table 4. Periodontal health status and systemic disease

the relationship between scaling and root-planing with malodour, the second examined the efficacy of tooth and tongue brushing in reducing malodour, and the third studied the efficacy of tongue brushing with two different dentifrices in reducing malodour.

Wijayanti (2007) analysed the VSC rates in 47 adult males with chronic periodontitis. VSC was observed 30 minutes after scaling and root planing. She found that VSC rate was reduced but not significantly.

The efficacy of tongue and tooth brushing in reducing malodour was examined by Alibasyah (2008). In a clinical trial comprising 39 subjects, measurement of VSC rate was performed 30 minutes before and after tongue and toothbrushing. She concluded that VSC rate was reduced but not significant. Hartanto (2008) performed a clinical trial to evaluate VSC rate after tongue brushing using toothpaste containing amiloglucosidase and toothpaste containing glucosidase enzyme. 25 subjects, aged 17 to 25 years old were divided into two groups. The first group performed tongue brushing using amiloglucosidase toothpaste and the second group performed tongue brushing using glucosidase enzyme. This study showed a reduction in VSC rate but no difference in improvement between the two groups.

Systemic disease and periodontal status research

Systemic disease as a modifying factor of periodontal disease has been established. Postgraduate studies have been undertaken on the association between periodontal disease and systemic diseases. Systemic diseases and systemic conditions examined by postgraduate included type 2 diabetes mellitus, coronary heart disease, human immunodeficiency virus/acquired immunodeficiency syndrome (AIDS), hypertension, gestational diabetic mellitus, postmenopausal women, pregnancy

and low birth weight infants. A case-control study of the effect of scaling and root planing on periodontal status and glucose levels in patients with type 2 diabetes mellitus showed that periodontal debridement significantly reduced papilla bleeding index, pocket depth, attachment loss and glucose levels in diabetic subjects (Mualim 2002). The subjects were 61 diabetic patients and clinical parameters and glucose levels were measured fourteen days following scaling and root planing.

The correlation of coronary heart disease (CHD) with dental plaque and dental calculus accumulation was studied. Andrena (2007) concluded that dental plaque and dental calculus index in CHD subjects were higher than non-CHD patients. Astuti (2011) performed a cross sectional study of 60 diabetes mellitus patients and 53 diabetes mellitus patients with coronary heart disease which showed that the periodontal status of diabetic patients was better than patients with diabetes and coronary disease. Aranti (2011) evaluated dental plaque and calculus accumulation in the same patients as the previous study however there was insufficient data for a definite conclusion. Sumali (2011) evaluated periodontal status in CHD patients with or without hypertension. The subjects were 26 males, aged 35 to 73 years old, suffering from CHD with or without a history of hypertension. The periodontal status between two groups showed no significant difference. Nasution (2012) observed the quantification of *Streptococcus sanguinis* from dental plaque and saliva from subjects with CHD. It showed that *S. sanguinis* levels in the CHD group were higher than in the control group but not significantly.

Research on periodontal status in postmenopausal woman was undertaken by two postgraduate students. Tadjoedin (2009) evaluated the relationship of blood calcium level with periodontal status in 59 postmenopausal women aged 45 to 70

Authors	Study method	Findings
Rachmawati 2010	86 subjects, 2nd and 3rd trimester with hypertension. PD and BOP.	Significantly different periodontal status in pregnant women with hypertension that those without hypertension
Korita 2010	34 subjects with gestational diabetes mellitus. PD and BOP.	Significantly worse periodontal status in GDM than without GDM.
Nasution 2010	70 subjects. Haemoglobin, erythrocyte and leukocyte levels.	Significantly reduced haemoglobin and erythrocyte in pregnant + gingivitis; higher leukocyte in pregnant + gingivitis.
Santana 2010	35 subjects. Levels of haemoglobin, erythrocyte, and leukocyte. CAL.	No significant correlation between haemoglobin, haematocrit and erythrocyte level and CAL in pregnant woman.
Komara 2006	Descriptive analytic.	Periodontitis as independent risk factor of LBW.
Emmanuel 2011	94 subjects. PPD, BOP, CAL.	Significant difference in periodontal health status between mothers of LBW and normal.

Table 5. Periodontal health status, pregnancy and low birth weight.

Authors	Study method	Findings
Rayanti 2008	Descriptive analytic. 30 subjects. Smoking history, bone loss and bone density . Digital radiograph.	No significant correlation between bone loss and bone density with smoking history, duration, quantity, and type of cigarette.
Adriani 2008	Descriptive analytic. 30 subjects. Smoking history, bone loss and bone density. Conventional radiograph.	No significant correlation between bone loss and bone density with smoking history, duration, quantity, and type of cigarette.
Putri 2011	Cross-sectional study. 45 subjects. Low-density lipoprotein (LDL), smoking habit and CAL.	No association between CAL and LDL; between CAL and smoking in CHD patient.

Table 6. Periodontal health status and smoking habit.

Authors	Study method	Findings
Koerniadi 2009	Experimental clinic. 35 sites. Bone level.	Significant increase of alveolar bone height after flap operation (FO) with demineralised freeze-dried bone allograft.
Hartanti 2009	Experimental clinic. 20 samples. FO + demineralized bovine bone powder; FO + Hydroxyapatite β tricalcium phosphate (H β TP); PPD, BOP, CAL, and crestal height of bone (CHB).	Significant differences clinical parameters before and after treatment, but not different between two groups.
Yunanthi 2009	Experimental clinic. 60 samples. FO + DFDBA; FO + demineralized freeze-dried bovine CAL, BOP and CHB.	Significantly improved periodontal parameters. CAL and CHB based at 3 and 6 bone xenograft (DFDBBX); PD, months differed between two groups; no different for PD and BOP.
Lestari 2010	Experimental clinic. 20 subjects. Dental recession, PD, CAL in coronal position, FO with or without acellular dermal matrix allograft (ADMA).	Significant reduction in recession in type 1 and 2 gingival thickness.

Table 7. Periodontal regeneration therapy.

years. This study showed no significant correlation between blood calcium levels and gingival index, periodontal pocket depth and attachment loss. Wulandari (2009) evaluated the periodontal status and oestrogen levels in the same cohort. There was no significant correlation between oestrogen level and gingival index, periodontal pocket depth or loss of attachment.

The correlation of pregnancy with periodontal status, gingival status, or anaemia status by blood test was studied by four postgraduate students.

Periodontal regeneration and biomolecular research

Biomolecular research in current postgraduate programs include examining MMP8 expression as a risk factor for aggressive periodontitis, RANKL (receptor activator-

kappa β ligand) expression in periodontal defect treatments with xenograft and PRF, and the correlation between periodontal inflamed surface and HbA1c levels in type 2 diabetes mellitus.

Discussion

At present some collaborative research with national and international institutions is occurring, although sometimes the funds available are not enough to cover advanced research. Materials are often difficult to obtain, because of the need to order from overseas and the high cost.

Some of the topics of ongoing research about periodontal regeneration correlated with minocycline; effectiveness of minocycline on red bacterial complexes: *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythensis*; the relationship of smoking

to human β -defensin, IL-1, IL-4, and *Porphyromonas gingivalis* levels; bone morphogenetic protein in chronic and aggressive periodontitis; and periodontal health status in dental implants.

Conclusion

Current periodontal research in Indonesia focuses on non-surgical and surgical periodontal therapy, correlations between periodontal disease and systemic disease, biomolecular research, periodontal regeneration, periodontal epidemiology and the use of lasers in periodontics. Progress in periodontal research can be assisted by national and international funding.

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Chapter 7

Periodontal Medicine: Establishment of a New Frontier for Periodontology

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Introduction

The term “Periodontal Medicine” was first introduced by Offenbacher in 1996 and proposed to be a broad term defining a rapidly emerging branch of periodontology focusing on new data establishing a strong relationship between periodontal health and disease and systemic health or disease (Offenbacher 1997). Four years later the US Surgeon General's landmark publication titled “Oral Health in America. A Report”, stated clearly that oral health and general health should not necessarily be dissociated as they have in the past, and that in the interests of an holistic approach to patient care oral health must be considered as a critical issue for general well-being (US Department of Health and Human Services 2000).

A central hypothesis of periodontal medicine states that periodontal infection presents a chronic inflammatory burden at the systemic level (Williams and Offenbacher 2000). This level of inflammatory burden can best be understood if one were to consider the total ulcerated gingival sulcus area in a patient with 28 teeth and an average pocket depth of 5 mm. According to several sources this surface area would equate to an area of between 20 to 75 cm² (Hujuel *et al* 2001, Page *et al* 1998). This is depicted in Figure 1.

Therefore there are now two models for

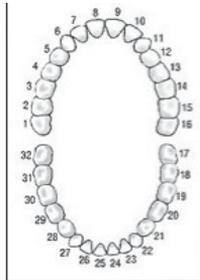
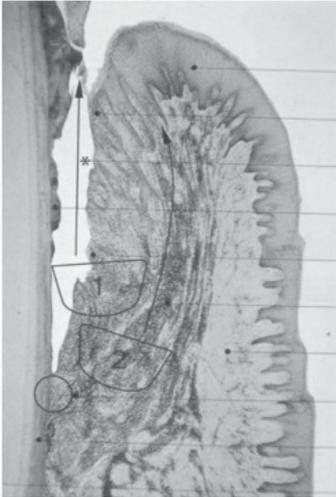
understanding periodontal disease. One is the conventional or accepted paradigm, in which a combination of susceptible host in the presence of periodontal pathogens and absence of beneficial bacteria together with a conducive local environment within the gingival sulcus all leads to the development of the clinical condition we know as periodontitis (Figure 2). However with the development of the concepts of periodontal medicine, we see an inversion of this paradigm in which periodontitis has the potential to impact on systemic conditions either through disease associations or disease causality (Page 1998) (Figure 3).

Periodontal medicine now allows us to consider periodontitis and systemic conditions as a “two way” relationship in which periodontitis can affect systemic health but also that systemic health can affect periodontitis. This new field now opens new vistas for periodontology with opportunities arising in new diagnostic strategies, new treatment strategies and new educational responsibilities for the profession.

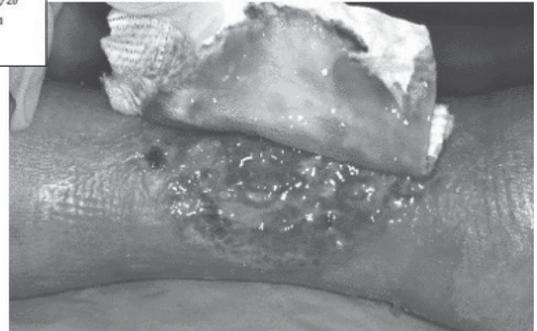
Oral health and systemic health interrelationships

The ways in which oral health (and in particular periodontal disease) and general health are inter-connected is very complex.

Ulcerated Gingival Sulcus
around 28 teeth with
pockets 5-6 mm



=



Klaus, et al. Color Atlas of Dental Medicine, Thieme Publishing, 1989

Figure 1. Comparative area of ulceration in a patient with average pocket depth of 5 mm on 28 teeth.

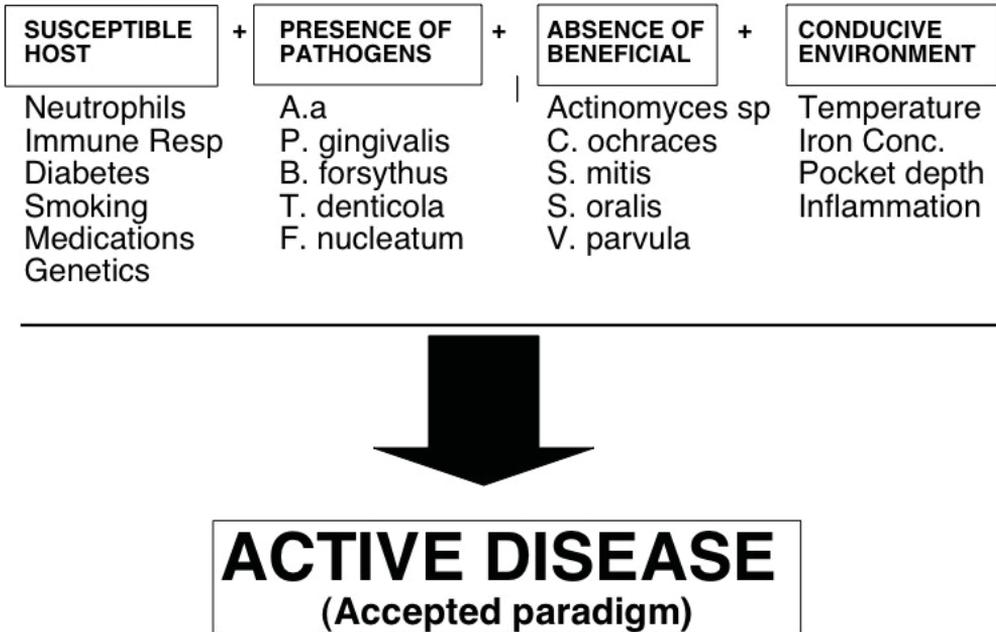


Figure 2. Conventional paradigm of periodontal disease.

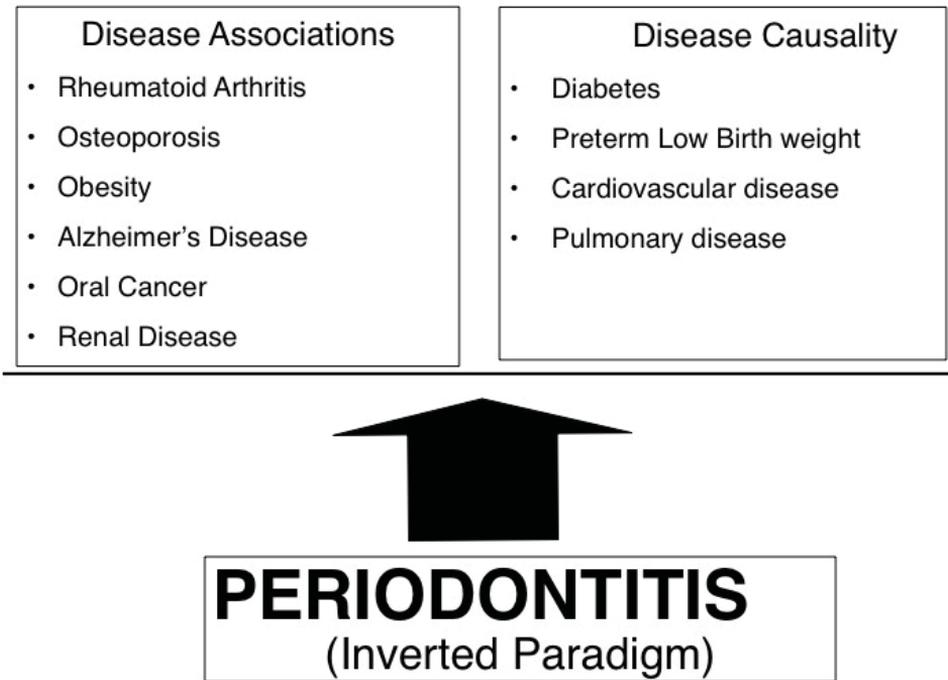


Figure 3. Inverted paradigm of periodontal disease.

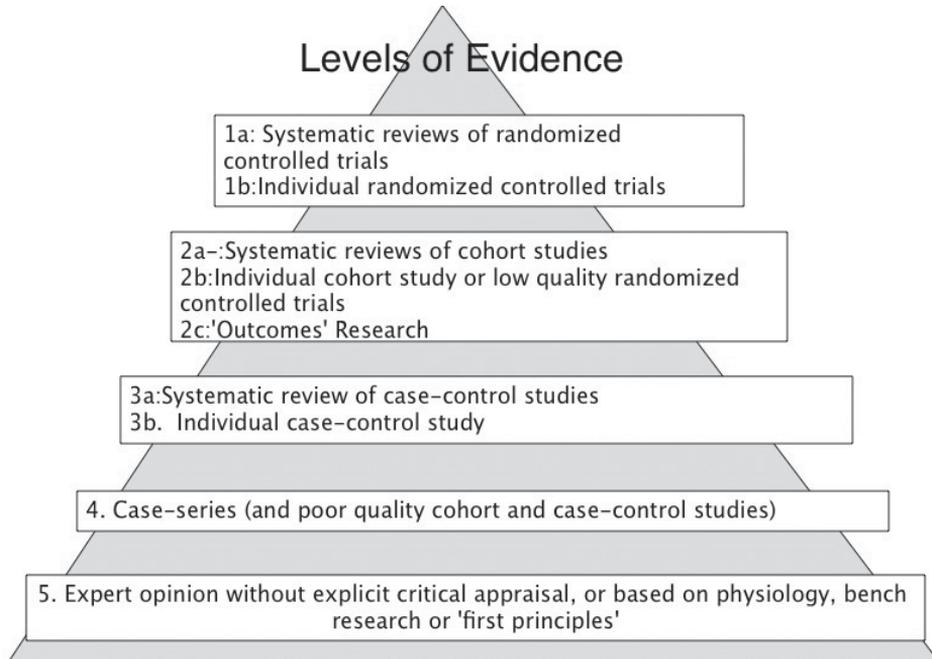


Figure 4. Levels of evidence for scientific conclusions.

Disease Association Check List	
	Systemic Condition
Biological Plausibility	???
Strength of Association	???
Effect of Periodontal treatment on disease condition	???

Figure 5. Disease association check list.

However there is emerging evidence to support significant associations, correlations and relationships. For example, the prevalence of systemic diseases in patients attending general dental practices and specialist practices has revealed that periodontal patients have a higher prevalence of systemic diseases compared to the general dental practice population (Georgiou *et al* 2004). Furthermore, from this study it was noted that for patients with advanced periodontitis, the most prevalent systemic conditions were bronchitis, hepatitis and rheumatoid arthritis. Patients with periodontitis also took more medications and were more likely to suffer from multiple conditions compared to the general dental population. Therefore, it was concluded that periodontal patients represent a generally greater proportion of “unwell” individuals than their periodontally healthy counterparts.

To explore these relationships further it is important to consider the various “levels of evidence” in the literature which allow us to make informed conclusions. In Figure 4, the levels of evidence are illustrated with expert opinion without explicit

clinical appraisal or based physiology, bench research or first principles as being the lowest form of evidence. On the other hand those studies which are published as systematic reviews of randomized controlled studies are considered the highest level of evidence and most likely to give us the best information available. In order to help us understand how oral health and systemic disease are related we have developed a simple “disease association check list” (Figure 5). When all the available data are assembled into such a table we can begin to see some patterns emerge as to how the evidence for various periodontal and systemic interrelationships measure up (Figure 6). Thus we can see that there is good evidence available to support significant relationships between periodontitis and diabetes, cardiovascular disease and rheumatoid arthritis, equivocal evidence for obesity and adverse pregnancy outcomes and negligible evidence for osteoporosis.

Diagnosis and risk assessment

With the development of periodontal medicine we see opportunities arising for the

Disease Association Check List

	Diabetes	Obesity	Adverse Pregnancy Outcomes	Cardiovascular Disease	Osteoporosis	Rheumatoid Arthritis
Biological Plausibility	Yes	Yes	Yes	Yes	No	Yes
Strength of Association	Yes	Poor	Debatable	Yes	No	Yes
Effect of Periodontal treatment on disease condition	Yes	Nil	Poor	Equivocal / None	No	Emerging

Figure 6. Evidence for various periodontal and systemic interrelationships.

diagnosis of periodontitis to take more of a medical basis towards diagnosis through the utilization of specific tests and approaches. For example cross sectional studies show that periodontitis elicits an elevation in markers of the acute phase response of inflammation including C-reactive protein, haptoglobin, alpha 1-antitrypsin and fibrinogen. Since many of these acute phase reactants are associated for being increased in conditions such as myocardial infarction, peripheral artery disease, diabetes and rheumatoid arthritis it would make sense that periodontal patients should undergo testing for levels of these components as part of their routine periodontal assessment. In doing so, this would allow the use of blood analyses to monitor periodontal patients with regards to risk for many systemic conditions, particularly those with a known systemic inflammatory component to them.

Within this model of taking a more medical approach to periodontal diagnosis and management, other new areas for future development would be in monitoring total systemic infectious and inflammatory burdens, monitoring pro-and anti-inflammatory

cytokine responses, investigating gene polymorphisms and measuring oxidative stress (Chapple *et al* 2007, Vaithilingam *et al* 2014).

A consistent underlying theme in such a medical approach is the important role that inflammation at both the local and systemic level plays in patients suffering from periodontitis. A number of methods have been devised to monitor periodontal inflammation but one recent development deserves some further consideration. In 2008 the Periodontal Inflamed Surfaces Area assessment tool was developed (Nesse *et al* 2008). In this model the surface area of inflamed epithelial surface of the periodontal pockets can be calculated as shown in Figure 7. Accordingly the PISA score can be equated to various periodontal conditions including periodontal health, severe localized aggressive periodontitis and severe generalized chronic periodontitis.

Translation to patient care

With the realization that significant relationships exist between periodontitis and many systemic conditions, an obvious

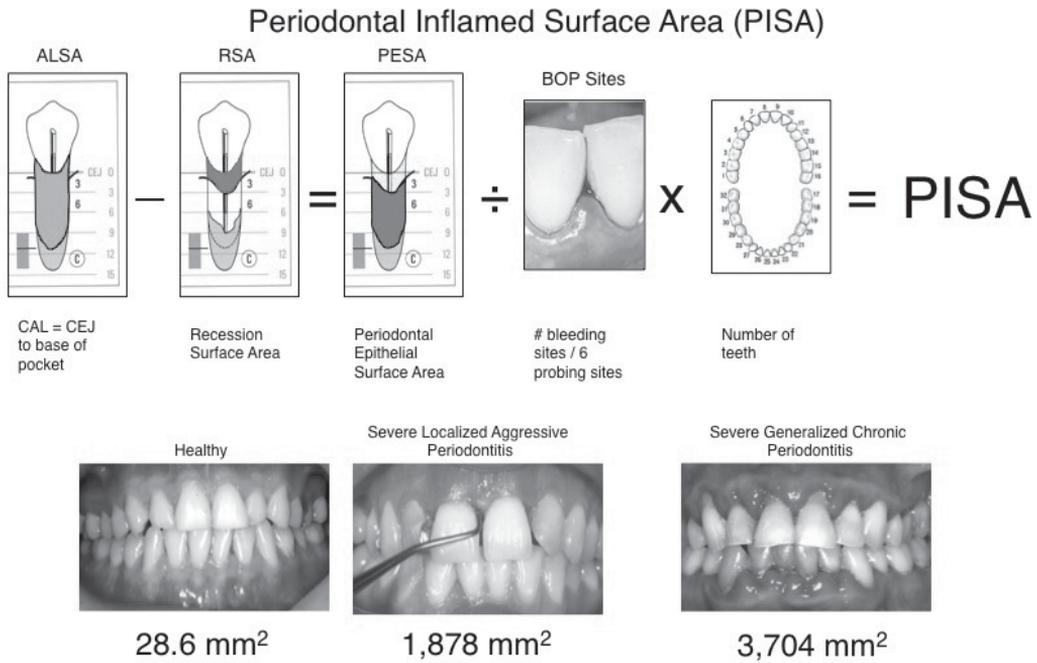


Figure 7. Periodontal Inflamed Surfaces Area assessment tool.

avenue of investigation has been to determine whether treatment of periodontitis (reduction of infection and inflammation) has any effect on any such related systemic conditions.

To date there is good evidence that periodontal treatment improves a number of biomarkers for cardiovascular disease. Specifically, leukocyte counts, fibrinogen, TNF-alpha, sE-selectin, C-reactive protein and endothelial cell function have all been shown to change significantly towards a more healthy profile following periodontal treatment (D'Auito *et al* 2013, Teeuw *et al* 2014). Similar findings have been reported for improvement of glycemic control in diabetics following periodontal treatment (Chapple *et al* 2013, Simpson *et al* 2010). From these studies it was concluded that there is some evidence of improvement in metabolic control in people with diabetes after treating periodontal disease. The randomized clinical trials appear to consistently demonstrate that mechanical periodontal therapy associates

with approximately a 0.4% reduction in HbA1C at 3 months, which is calculated to be a clinical impact equivalent to adding a second drug to a pharmacological regime for diabetes. Nonetheless, very recently a large-scale multicenter randomized intervention clinical trial has failed to show any clinical benefit with regards to diabetic outcome measures following periodontal treatment (Engebretson *et al* 2013). More recently there has been emerging evidence to demonstrate a beneficial effect of periodontal treatment on clinical outcome measures of disease activity in rheumatoid arthritis. These studies have shown that nonsurgical periodontal treatment may prove beneficial in reducing rheumatoid arthritis severity by reducing erythrocyte sedimentation rates, C-reactive protein, TNF-alpha and the DAS-28 score (Kaur *et al* 2014). However to date these studies have had very low sample sizes and larger scale intervention studies are required before a definitive statement regarding the effect of

Alzheimer's Disease	Leukemia
Alcohol Abuse	Leukoplakia
Anaemia	Low Birth Weight
Atherosclerosis	Lung Cancer
Autoimmune Disease	Lupus
Cancer	Metabolic Syndrome
Cardiac Dysrhythmia	Miscarriage
Chronic Obstructive Pulmonary Disease	Mouth Cancer
Depression	Multiple Sclerosis
Dyslipidemia	Myocardial Infarction
Colon Cancer	Obesity
Crohn's Disease	Obstructive Sleep Apnea
Death	Osteoporosis
Dementia	Pancreatic Cancer
Diabetes	Panic Disorder
Dry Mouth	Pneumonia
Endometriosis	Polycystic Ovaries
Erectile Dysfunction	Pre-eclampsia
Fatigue	Premature Birth
Fever	Psoriasis
Fibromyalgia	Renal Disease
Gastro Oesophageal Reflux Disease	Rheumatoid Arthritis
Hypertension	Sleep Apnea
Infertility	Stomach Ulcers
Inflammatory Bowel Disease	Stroke
Intellectual Function	Trigeminal Neuralgia

Table 1. Putative disease associations with periodontal disease.

periodontal treatment on rheumatoid arthritis can be made. To date the evidence for good outcomes for adverse pregnancy outcomes following periodontal treatment has been poor (Srinivas and Parry 2012).

Multidisciplinary opportunities and responsibilities

With the emerging field of periodontal medicine, special opportunities have arisen for the education of not only the dental profession but also our medical colleagues. To date there has been considerable effort made in the diabetes and cardiovascular fields to try to emphasize the clinical relevance of such interrelationships and the importance of early diagnosis and treatment of periodontal disease to help manage these conditions (Lockhart *et al* 2012, Mealey 2008). Recently two

publications of particular interest have been released. One is a monograph consisting of 18 chapters by selected experts in the field of periodontal and systemic inter-relationships which provides a comprehensive overview of the field (Genco and Williams 2010). A more recent publication has arisen from an international workshop on periodontitis and systemic diseases held in November 2012 and jointly sponsored by the European Federation of Periodontology and the American Academy of Periodontology (Tonetti and Van Dyke 2013a, Tonetti and Van Dyke 2013b). This supplement features papers from four working groups and consensus reports on cardiovascular disease and periodontitis, diabetes and periodontitis, adverse pregnancy outcomes and periodontitis, and other diseases including rheumatoid arthritis, pulmonary diseases and cancer and periodontitis.

Conclusion

There is no doubt that the field of periodontal medicine is a well-developed and important emerging sub-discipline of periodontology. The concept of a healthy mouth for a healthy body is the overarching thesis of this field and will be a very important part of clinical and scientific endeavors in the years to come. However, it must be noted that as with any new developments, care must be taken not to over interpret the evidence. Caution must be mandatory in the quest to understand the relationships between periodontal disease and systemic disease. Studies in this field must be based on sound principles and biologic rationale. The concern is that poorly conceived studies are being published with little or no thought as to the biologic plausibility of the relationships being studied. This is evidenced in Table 1 in which the disease associations studied to date are listed. This is a daunting list and is comprised of many poorly conceived and erroneous so-called relationships. This problem has been further highlighted in a recent publication detailing the spurious associations in oral epidemiological research (Hujoel *et al* 2006).

Notwithstanding the above, the development of the sub discipline of periodontal medicine has been an inversion of our current paradigms in periodontology. Traditionally treatment has been focused on preserving the structure, function and esthetics of the dentition. Now it is beginning to focus on preventing untoward effects on a patient's overall health. In light of current developments and considerable coverage in the public media patients may be more motivated towards seeking treatment. Furthermore, failure by oral health practitioners to screen for periodontal disease may have greater consequences in terms of litigation. In this context the question arises as to whether all patients with newly diagnosed diabetics, rheumatoid arthritis,

cardiovascular disease and pregnancy should undergo a mandatory periodontal assessment. Clearly there is now a need for much greater consultation and collaboration with the medical profession.

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Chapter 8

Modelling Bone Regeneration in *Ovis aries*

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Introduction

Many different techniques and materials may be used for bone augmentation prior to or contemporaneous with implant placement, including membrane exclusion, xenografts, allografts, and so-called “adult stem cells” (multi-potential cells, MPC) (Araújo *et al* 2008, Araújo *et al* 2009, Araújo *et al* 2010a, Araújo *et al* 2010b, Tognarini *et al* 2008). Development of these therapies involves a combination of bench-top biomechanical work, *in vitro* cell culture experiments and *in vivo* testing. The objective with *in vivo* testing is to progress from preclinical animal models to clinical trials in human participants.

There are many different animal models commonly used for the preclinical testing in the fields of periodontology and oral implantology, including the rabbit tibia, the rabbit metaphysis, the dog mandible, the non-human primate mandible and the long bones (tibia, femur and radii) of sheep (Becker *et al* 1990, Becker *et al* 1991, Carlsson *et al* 1989, Chappard *et al* 1999, Dahlin *et al* 1989, Fini *et al* 2002, Gotfredsen *et al* 1991, Lucchini *et al* 1996, Schenk *et al* 1994, Sennerby *et al* 1992). Animal models that use the long bones of the limbs (femur, tibia, radius, humerus) have an advantage in that only one operation is required, no teeth need be removed prior to implant placement. However, the reduction in morbidity and stress on the animal is

counterbalanced by the questionable relevance of using tibial bone as a substitute for mandibular bone (Frame 1980). Functional loading along the long axis of long bones, compared with cross-axis loading in the mandible, may influence patterns of implant osseointegration and differences in bone-implant contact to unloaded implants placed into different bones within the same animal have been demonstrated (Johansson and Morberg 1995, Rohner *et al* 2004, Wiskott and Belser 1999).

Sheep have been promoted as a useful large animal model for diverse fields of biomedical research because of their similarity to humans in size, weight and general physiology as well as their ease of handling and their robust recovery from anaesthesia and experimental surgery (An and Friedman 1999, Bosanquet and Goss 1987, Hollinger and Kleinschmidt 1990, Newman *et al* 1995). Of particular note are the sheep limb models used for orthopaedic research and the maxillary sinus grafting model (Fini *et al* 2002, Giavaresi *et al* 2003, Haas *et al* 1998). The use of domestic animals that commonly form part of the food chain for human consumption is also perceived as ethically more acceptable than the use of companion animals such as dogs. An and Friedman (1999) commented that “for most orthopaedic animal studies, there is no specific reason for dogs to be used when goats and sheep are also available”.

Development of sheep models at the University of Otago

The University of Otago Dental School has used sheep for periodontal and implant research since the mid 1980s. We have established or developed seven different models in three separate anatomical sites using the mandible, maxillary sinus and femurs of domestic New Zealand sheep (*Ovis aries*) (Table 1).

Initial work using the sheep mandibular premolar as an animal model was pioneered in New Zealand by Dr Angela Pack and her graduate students (Pack 1997). Danesh-Meyer *et al* (1995) developed an acute surgical defect model in the buccal furcation of the second mandibular premolar in sheep, and then investigated the effectiveness of expanded polytetrafluoroethylene membranes in promoting periodontal regeneration in this model. Whelan *et al* (1997) modified this into a model of chronic inflammation, and subsequent investigators then used the model to examine alloplastic grafting materials and transforming growth factor-beta (Cole 1999, Mohammed *et al* 1997). Recently we have returned to this model and have employed it to investigate the basic biological mechanisms of healing with respect to RANK, RANKL and osteoprogenin (OPG). We found that this model exhibited “distinctive temporal

and spatial expression patterns for RANK, RANKL and OPG proteins during healing of surgically created periodontal wounds” (Baharuddin 2010).

The conclusions drawn from the sheep premolar model are that the premolar furcation wounds exhibit a high degree of healing and regeneration without intervention, that converting this site to a chronically-inflamed site may yield more clinically relevant information, that access to the premolar region in sheep is technically challenging, and that this model shows promise for research into basic healing mechanisms. We have also noted that there seems to be a publishing bias in the dental literature with a preference for research conducted in other species such as dogs rather than sheep.

Animal model research at Otago was subsequently extended to include intraoral bone healing in critical size defects (CSD), in preparation for establishing a mandibular model for dental implantology in this species. The rationale for this was based on the comments of Martini *et al* (2001) that “strategies for selecting an experimental animal model also require clear understanding of spontaneous bone defect healing, to correlate experimental data obtained from humans. Interspecies differences may be overcome thanks to the knowledge of healing in critical size defects”. A CSD has been

Site	Location	Application
Mandible	Premolar furcation	Furcation healing
	Retro-canine diastema	Mandibular CSD
	Healed post-extraction ridge	Mandibular implants
	Premolars	Tooth extraction socket
Maxilla	Sinus	Sinus grafting ± implants
Femur	Epicondyle	Implants ± grafting
	Epicondyle	Titanium disc ± stem cells

Table 1. Seven models in three anatomical sites in sheep.

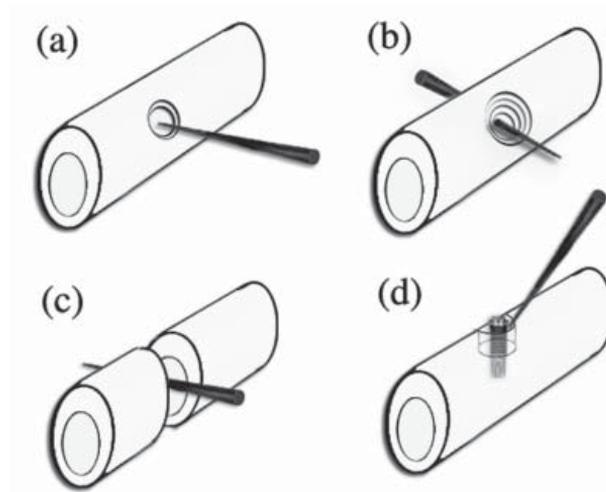


Figure 1. Diagram showing the four main kinds of critical defects. (A) Unicortical defect. (B) Bicortical or “through-and-through” defect. (C) Segmental defect. (D) Peri-implant defect. The pointer demonstrates the site from which bone has been removed to create a defect (Duncan 2005).

defined as “the smallest size intraosseous wound in a particular bone and species of an animal that will not heal spontaneously in the life time of the animal” (Schmitz and Hollinger 1986). Various kinds of critical size defects in bone have been described, including unicortical, bicortical, segmental and peri-implant defects (Figure 1). Our work focused initially on unicortical defects in the mandible, with subsequent work carried out on peri-implant defects.

A series of experiments were performed, establishing acute surgical 8 and 12 mm unilateral CSDs in the sheep mandibular edentulous diastema region. A follow on experiment then attempted to convert these acute defects into a chronic model (Duncan 2005, Marshall and Duncan 1997, Salmon and Duncan 1997). The conclusions from this work were again that the mandibular wounds showed a high rate of healing and that the unicortical CSD in the sheep mandible was >12 mm.

Further work was then conducted to develop a sheep mandibular model for dental implant research. Pilot experimental work employed the edentulous mandibular

diastema for implant placement; when this proved unsuccessful, histological analysis of the dimensions of the mandibular premolars was conducted, a protocol established for extraction of mandibular premolars, and a series of experiments conducted using 132 implants with seven different configurations in 57 sheep over three years. The implant surfaces that were examined included machined, titanium plasma-sprayed, hydroxyapatite (HA), resorbable-media blasted, and sandblasted and large-grit acid etched (SLA). Histomorphometric analysis after 12 weeks healing found percent bone-to-implant contact that ranged from 15% up to 85%. A clear rank order of bone-implant contact was established, with machined implants demonstrating the poorest response; the best results were found with HA and SLA surfaces. The overall survival rate for implants placed in the sheep model was 90.2% (Duncan 2006).

Three graduate students at Otago then performed experimental work to consider different types of wide-bodied, tapered implants that were either delayed- or immediately-loaded (Eggerath *et al* 2006,

Fitzgibbon *et al* 2006, Kim *et al* 2006). The results for these experiments were disappointing, with high failure rates of up to 60% for Southern Implants tapered, 80% for Biomet 3i Osseotite NT and 60% for Nobel TiUnite implants. Bone-to-implant contact ranged from 20% to 69% for the different surfaces. Surviving implants showed evidence of coronal alveolar bone loss that may have been caused by over-compression of the dense mandibular bone during insertion.

The next step in the use of sheep as an animal model at Otago involved the combination of three different surgical models in three distinct anatomical sites within the same animal. In collaboration with Professor Min-Ho Lee and his colleagues from Chonbuk University in Korea, three different implant systems (Megagen, Osstem and Neoss) were placed into two groups of 10 sheep. The resorbable-blasting media surfaces of the Osstem and Megagen implants were modified using a technique of anodic oxidation in calcium phosphate solution with hydrothermal annealing (Bai *et al* 2011a, Bai *et al* 2011b). Implants were placed into the epicondyle of the femur, into the maxillary sinus without grafting, and into the mandible using a submerged configuration, which was compared with a second group using a one-stage placement protocol and immediate fitting of healing abutments. Resonant frequency data was collected at placement and at euthanasia after one month of healing, and the implants were then examined radiomorphometrically using micro-computerised tomography, after which they were sectioned for histomorphometric measurements. Results showed encouraging increases in percent bone-to-implant contact after 4 weeks in the mandibular and femoral sites, however the ungrafted maxillary sinus sites showed comparatively low bone-to-implant contact with no statistically significant improvement gained through

surface enhancement of the implants. In the mandible, the unloaded, anodised-surface implants achieved percent bone-implant contact after one month that was equivalent to that achieved after three months healing by the best-performing surfaces (HA and SLA) in the previous sheep mandible experiments (Duncan *et al* 2008, Duncan *et al* 2010a, Duncan *et al* 2010b, Duncan *et al* 2012).

It has been suggested that animal model testing of dental implants becomes most relevant when linked to parallel experiments in human subjects (George *et al* 2011). A recent clinical trial undertaken by two graduate students at Otago University considered the outcomes of pure zirconia implants supporting maxillary and mandibular overdentures in human participants (Osman *et al* 2013, Siddiqi *et al* 2013). In order to further examine the response of bone to the zirconia implants at a histological level, one-piece ball-abutment zirconia and titanium implants were placed into 10 sheep, into both the mandible and the femur, for 12 weeks. Histomorphometric analysis showed similar results in the two anatomical sites, however the survival rate in the mandible was only 35%.

A similar experiment closely linked to current clinical practice was conducted in collaboration with Professor Patrick Schmidlin and Dr Alex Phillip of Zürich University. In this work, maxillary sinuses in 32 sheep were subjected to open-antral sinus lift with or without grafting with Straumann bone ceramic. Straumann SLA-surface and SLActive-surface implants were then placed into the sinuses in a submerged and unloaded configuration. Histomorphometry was conducted after 12 and 26 weeks healing. The principle conclusions from this study was that the activated surface made little difference to bone-to-implant contact compared to the conventional SLA surface, however there was some evidence supporting the use of the alloplastic graft after 26 weeks healing

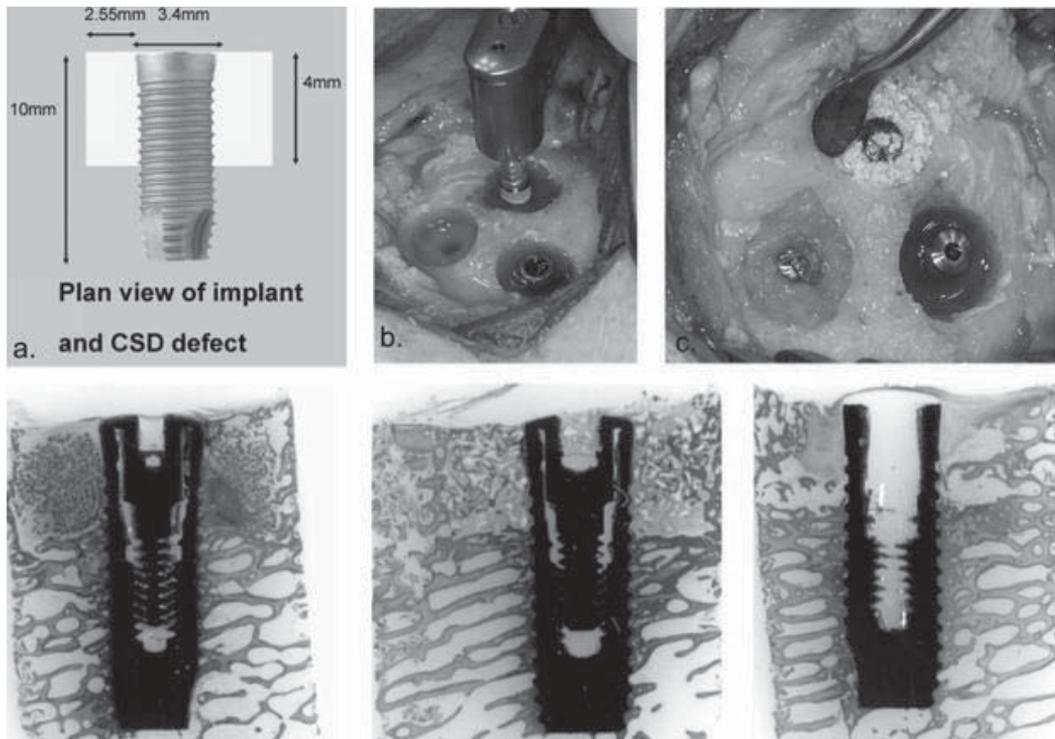


Figure 2. Establishment of a peri-implant defect model in the sheep femoral epicondyle. (A) Diagram of the peri-implant defect. (B) Establishing three defects per femur. (c) Implants placed and test materials grafted into defect. (D) Representative histology of the three different test conditions.

(Phillip *et al* 2013).

Most recently we have developed four new models in the sheep for testing novel materials, surfaces and therapeutic approaches. The first has involved the development of a sheep tooth extraction socket model for socket preservation experiments by Liu *et al* (2013). The three mandibular premolars on each side of the jaw in 18 sheep were extracted and the sockets grafted with a novel resorbable biomaterial developed by Professor Wendelin Stark of the Eidgenössische Technische Hochschule (ETH) Zürich, which was then compared to Endobon bovine xenograft (Biomet 3i). All sites were covered with an Osseoguard resorbable membrane (Biomet 3i), and the sockets were examined histometrically and histomorphometrically after eight and 16 weeks healing (Liu 2013). The principle findings from this study supported the utility

of the sheep tooth socket model for bone graft research and further noted that “the novel material ECWN did not impede bone ingrowth into sockets and showed evidence of material resorption”. In a parallel study in the same animals, Biomet 3i implants with Osseotite or Nanotite surfaces were placed into a peri-implant CSD defect model in the femoral epicondyle. The peri-implant defect was >2.5 mm circumferentially around the upper 40% of the implant, whilst the apical portion was inserted into trabecular bone and permitted to heal for eight or 16 weeks. At this stage histomorphometric analysis has yet to be completed, however preliminary observations suggest that the model has utility in distinguishing between different grafting materials when placed adjacent to implant surfaces (Figure 2).

The final two models have been developed

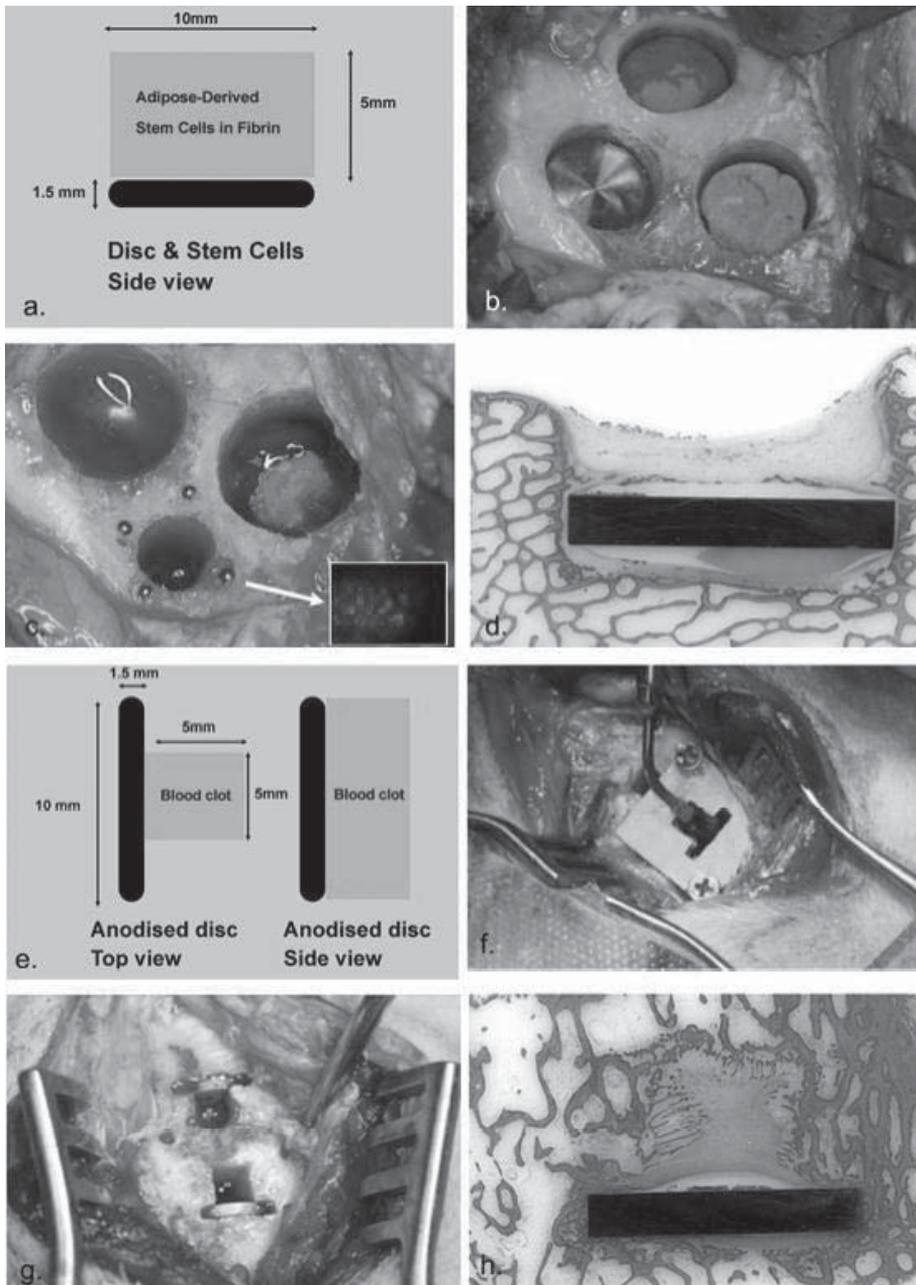


Figure 3. Two new models for bone healing in the sheep femur. (A) Diagram of the stem-cell-grafted defect above titanium disc. (B) Pilot animal showing three discs in the defects prior to stem cell grafting. (C) Sentinel defect with tantalum beads as markers; inset shows fluorescent microscopy image of the PKH-26-labelled adipose-derived multi-potential cells after one month healing. (d) Histology of pilot animal showing healed defect above implanted disc. (E) Diagram of the anodised-disc model. (F) Preparing the defect in the femur using a piezotome within a stainless-steel guide stent, fixed in place with screws. (F) Titanium discs placed into slots adjacent to the 5 mm x 5 mm x 10 mm peri-implant defect. (G) Histology of the healed defect (MacNeils Tetrachrome and toluidine blue stain).

to allow *in vivo* testing of titanium discs as a validation to *in vitro* bench top analysis. In the first model, a protocol has been developed that involves the harvesting of adipose-derived multi-potential cells (MPC, so-called “stem cells”) from sheep. Initial *in vitro* work has compared sheep-derived and human-derived adipose cells, suggesting that the sheep model is a valid model for preclinical testing. Zannicotti *et al* (2013) have also demonstrated the adipose-derived MPCs adhere, proliferate and transform into osteoblasts on titanium surfaces *in vitro*. The animal model involves placing the same titanium discs, with and without roughened surfaces, bilaterally into the femurs of 10 sheep, with a 5 mm x 10 mm diameter CSD superior to the discs (Figure 3). This defect has then been grafted with adipose-derived sheep MPCs, compared against an ungrafted control defect. To monitor the survival of the grafted cells, PKH-26 labelled cells will also be placed into a separate “sentinel” site; these are imaged immediately post-mortem using confocal laser scanning microscopy. To date the pilot study has shown encouraging results; the main study will commence shortly.

The final model involved a further extension of the anodisation protocols from Chonbuk University in Korea. Titanium and titanium-zirconia alloy discs have undergone nanomolecular surface enhancement and been examined *in vitro* using cell tissue culture. An animal model was then designed to test the enhanced discs *in vivo*. A stainless steel template was designed to ensure that the geometry of the defect was consistent, and fixed to the surface of the femur. A 1.5 mm x 10 mm slot was cut for the titanium disc, and a 5 mm x 5 mm x 10 mm deep defect created on one surface; this was then squared up using a piezotome. Test (anodised) and control discs of the two materials (titanium and titanium-zirconia alloy) were then tapped gently into the slots, the defects were covered

with resorbable gelatin sponge to prevent herniation of the overlying soft tissue into the defects, and the wounds were closed. The healing period for this experiment was 12 weeks; histomorphometric analysis is about to commence.

Conclusions

Multiple animal models have been established in sheep, with a variety of applications. This animal species is robust and tolerates surgery well, including surgery in multiple experimental sites, which is cost effective, allows healing under different conditions to be compared within the same animal, and is considered ethically acceptable. Sheep mandibular and femoral oral implant models distinguish between implants of different materials and different surfaces. The sheep maxillary sinus model distinguishes between different grafting materials and different implant surfaces. The sheep tooth socket model allows testing of socket preservation products, and shows potential for further development. Sheep femoral epicondyle models show promise for testing of novel bone regenerative approaches, including MPC “stem-cell” therapy.

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Chapter 9

The Global Type 2 Diabetes Epidemic and a Strategy to Fight It

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Introduction

We are living in a world of “the diabetes epidemic”. The International Diabetes Federation (IDF) has reported that the global prevalence of type 2 diabetes in 2012 was 8.3%, with 371 million people having diabetes globally (International Diabetes Federation 2013a) (Figure 1). The scope of the problem in the Western Pacific Region is particularly large for three reasons:

1. This region contains some of the most populated countries in the world approximately one third of the total number of diabetic patients live in WPR and 58% are undiagnosed.
2. The rapid change in lifestyle in this region suggests a large pool of people at substantial risk of developing diabetes.
3. Asians tend to develop diabetes at a lower bodyweight than Caucasians because of genetic differences.

The National Health and Nutrition Survey, conducted by the Ministry of Health, Welfare and Labor, Government of Japan, reported that the crude number of people with diabetes increased from 6.9 to 8.9 million between 1997 and 2007, however age-adjusted values have not increased significantly (Japanese Ministry of Health, Wealth and Labour 2013) (Figure 2). The major problem is that only 40% and 36% of 30 to 49 year old diabetic

men and women, respectively have been managing their diabetes continuously.

Pathophysiology of type 2 diabetes in Japan

To reduce the population’s risk of developing type 2 diabetes, and its complications including periodontal diseases, physicians should always take into account the pathophysiology of diabetes. In Japan, and perhaps in many other East Asian countries, the pathophysiology and clinical phenotype of type 2 diabetes is somewhat different from that seen in Caucasians. Japanese type 2 diabetes can be summarized as:

1. Primary phenomenon is reduced beta-cell function represented by low insulinogenic index ($\Delta I/\Delta G30$).
2. Elevation of fasting glucose appears later.
3. Post-meal hyperglycaemia is a key diagnostic feature.
4. Treatment of post-meal glucose is central to the management due to our very low capacity of pancreatic beta-cell function.

These characteristics are clearly reflected in DECODE and DECODA data (DECODE study group 1998, Qiao et al 2000) (Figure 3).

Management of HbA1c

The IDF guidelines, and other guidelines

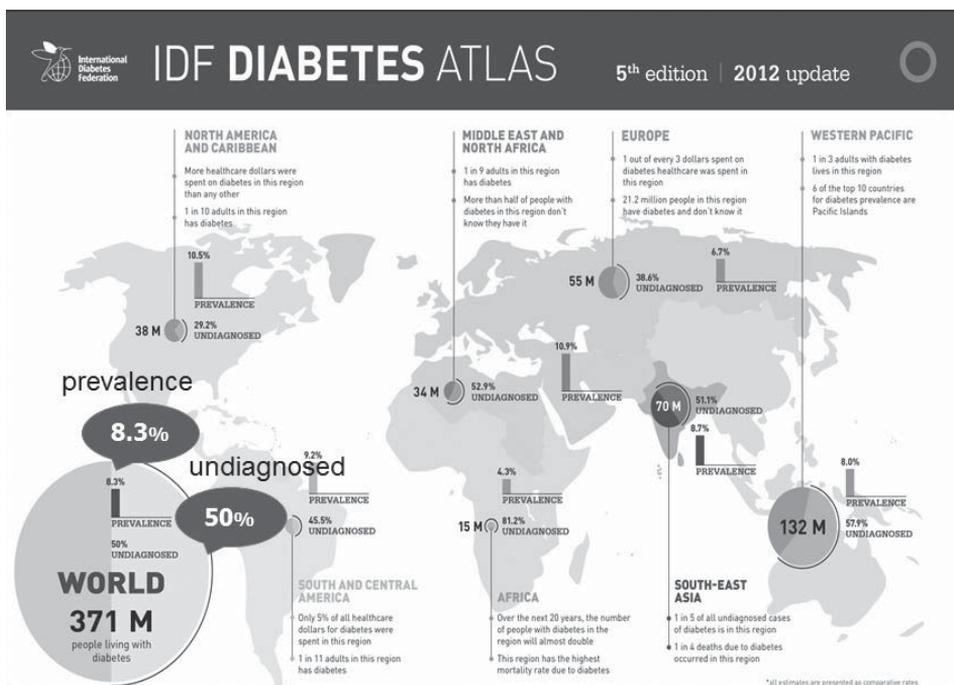


Figure 1. Prevalence and number of people with type 2 diabetes mellitus worldwide.

Numbers of people with diabetes in Japan 1997-2007

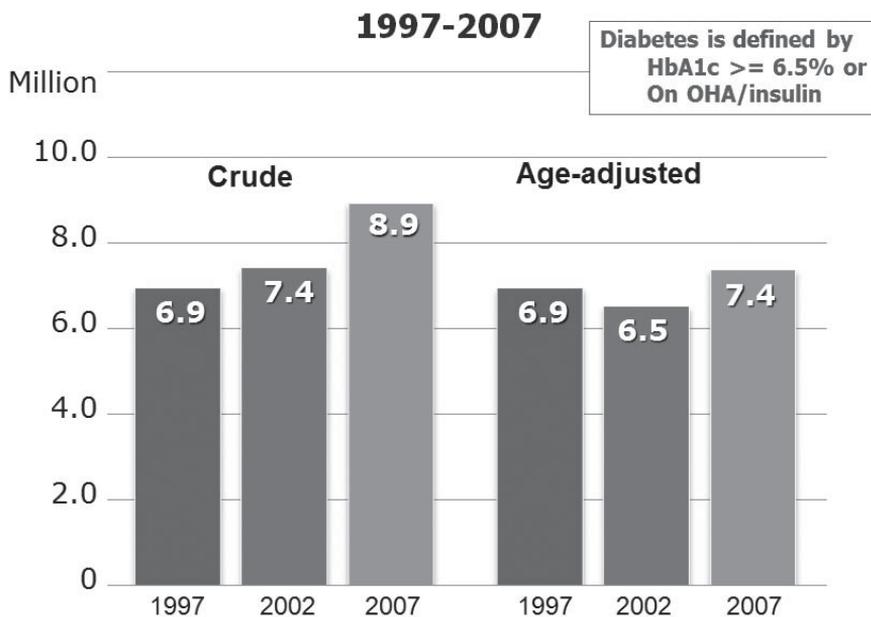


Figure 2. Millions of people with diabetes (defined as HbA1c \geq 6.5% or on OHA/insulin) in Japan 1997 to 2007).

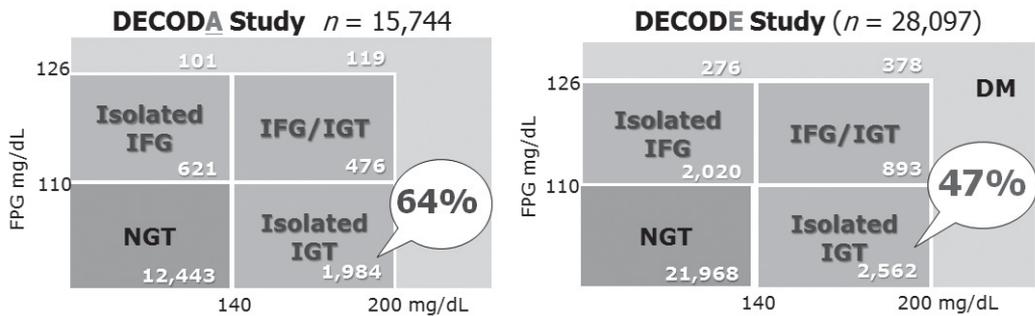


Figure 3. Results of DECODA and DECODE studies indicating a high percentage of isolated IGT in the DECODA cohort. (IFG = impaired fasting glucose, PG = plasma glucose)

including ours, highly recommend maintaining an HbA1c level of less than 7% without hypoglycemia and weight gain. However, the treatment target should be personalized by age, duration of diabetes, history of cardiovascular diseases, and other factors. For instance, for people with diabetes older than 70 years old, with diagnosed diabetes of more than 5 years and in the presence of cardiovascular disease(s), HbA1c would better be set at 7 to 8%.

Guidelines for maintaining a HbA1c target <7% can be summarized as:

- Should be achieved safely and easily to prevent diabetic complications.
- Should be individualized by age, duration of diabetes, complications, comorbidities, and risk of hypoglycaemia.
- A lower HbA1c level (e.g. <6.0%) should be considered in people with newly diagnosed diabetes, simple treatment regimen, no cardiovascular disease.
- Higher target (e.g. <8.0%) in people with long standing diabetes, recurrent hypoglycaemia, cardiovascular disease, complicated treatment regimens, comorbidities, and limited life expectancy.
- Individual target HbA1c should be reviewed regularly.

The IDF treatment algorithm outlines a global approach to the management of type 2 diabetes, providing guidance on how the

algorithm can be personalized and how therapeutic choices can be refined based on the patient's level of diabetes control and individual patterns of glycaemia (International Diabetes Federation 2013b). It should be also emphasized that at every therapeutic decision point, patients should be reminded of the importance of physical activity and adherence to diet control (Figure 4).

Association between diabetes and periodontitis

The Japan Diabetes Society (JDS) published "Evidence-based Clinical Guidelines 2013" and listed four statements in the chapter "Diabetes and Periodontitis" regarding the association between diabetes and periodontitis. (Japan Diabetes Society 2013):

1. Diabetes and periodontitis are complex disorders with a bidirectional relationship, therefore, diabetic patients should visit dentists periodically to have their mouth and gums checked.
2. Hyperglycaemia in diabetes is associated with adverse periodontal outcomes.
3. People with periodontal disease are more likely to develop diabetes and if it develops, their glycaemic control would be more difficult.
4. Periodontal therapies may result in a

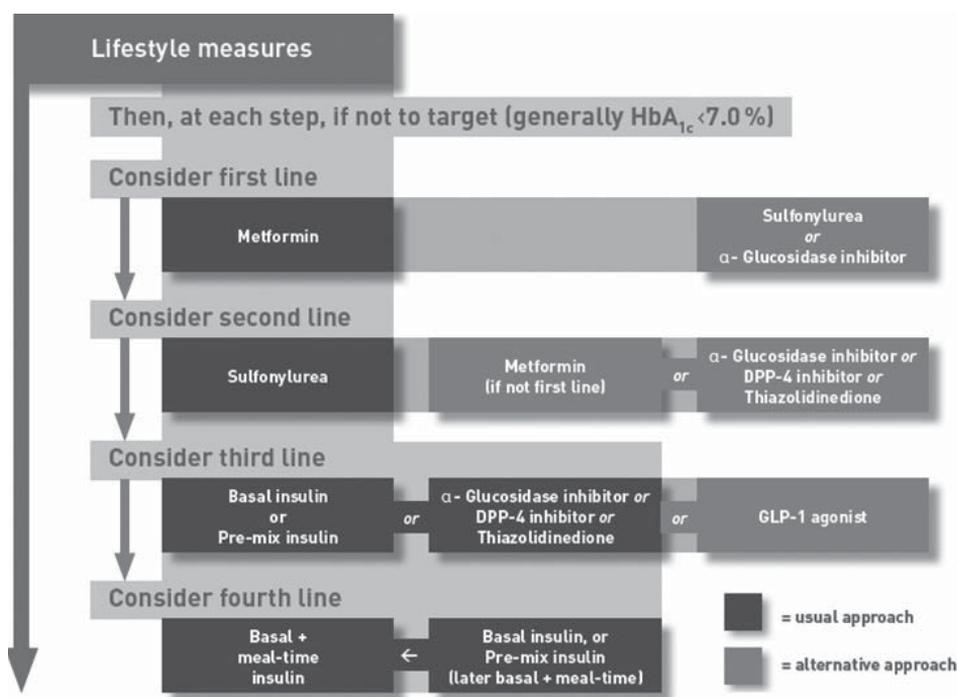


Figure 4. Personalizing IDF treatment algorithm.

Projects/Activities for the Prevention of Diabetes and its Complications since 2000: Japan

2000	Health Japan 21 (2000-2012)
2004	A 5-year Strategic Plan for the Prevention of Diabetes (JDS)
2005	Establishment of the Japan Council for the Promotion of Countermeasures Against Diabetes (JDS, JADEC, JMA) Japan Diabetes Outcome Intervention Trials (J-DOIT) 1,2,3
2006	Japan Diabetes Complications and Prevention Prospective (JDCP) Study (JDS) A New Strategic Plan for the Health Frontier
2008	Health Check-up and Health Guidance Program
2010	A launch of action plan 2010 "DREAMS" (JDS) <i>Dagnosis and Care, Research and Cure, Evidence for optimum Care, Alliance for Diabetes, Mentoring Program for Prevention, Stop the DM</i>
2013	Health Japan 21: second term (2013-)

Figure 5. Projects/activities for the prevention of diabetes and its complications in Japan since 2000.

reduction in HbA1c levels.

Strategies for managing and reducing diabetes in Japan

In Japan, under the vision of a national plan “Health Japan 21”, several nationwide projects for the primary, secondary, and tertiary prevention of diabetes planned by the joint efforts of the Japan Diabetes Society, Japanese Association for Diabetes Education and Care and Japan Medical Association are in progress. In 2010, a new plan called “DREAMS” was launched by the JDS. In 2013, the second term of Health Japan 21 was launched (Figure 6). Therefore, we should continue to do our very best in improving diagnosis and care, in promoting research aimed at curing diabetes, creating and establishing evidence for optimum care, constructing an international alliance for diabetes, mentoring programs for the prevention of diabetes, and finally stop the morbidity and mortality associated with diabetes by 2015. We should continue to do our very best to win the war against diabetes.

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Chapter 10

Re-evaluation of the Significance of Attached Gingiva in Orthodontics

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Introduction

Along with an expanding demand and interest in aesthetics and health, there are currently an increasing number of orthodontic cases in adults. This has enabled us to achieve excellent therapeutic results that can satisfy both patients and dentists in those cases that were originally considered to have some limitation in results without orthodontic intervention (Figure 1).

However, specific problems have been encountered in adult orthodontics, one of which is gingival recession. The incidence rate of gingival recession after orthodontic therapy with or without tooth extraction is 7% immediately after treatment, 20% two years after and increased to 38% after five years. This is considered to be one of the most frequent complications of orthodontic

treatment (Figure 2).

Many cases can be treated by grafting the connective tissue which will provide sufficient results. However, in cases with not only gingival recession but with a mobile tooth and/or spontaneous pain, retaining the tooth will be compromised (Figure 3).

To prevent post-orthodontic treatment complications, careful examination and diagnosis before onset of treatment is crucial. Ono *et al* (1986) indicated the critical points of examination pre-, mid- and post-orthodontic therapy from a periodontal point of view.

Critical periodontal problems to be considered before orthodontic treatment are:

- Plaque control levels
- Deep periodontal pockets
- Problems with the gingivae and mucosa
- Lesion of furcation involvement
- Morphologic abnormality of bone



Figure 1. A case with aesthetic improvement through orthodontic therapy. (A) Pre-treatment. Patient requested aesthetic improvement. Limited results if only prosthodontic treatment was performed. (B) After orthodontic therapy. Esthetic cervical line was acquired by correcting tooth position. (C) After restorative treatment with laminated veneers. Better aesthetic results were achieved through gum line adjustment by orthodontic treatment.



Figure 2. A case of gingival recession after orthodontic therapy. (A) Patient required correction of gingival recession after orthodontic therapy. (B) Connective tissue graft was performed to cover the root surfaces. (C) After treatment. Exposed root surfaces were covered and attached gingiva increased in thickness.

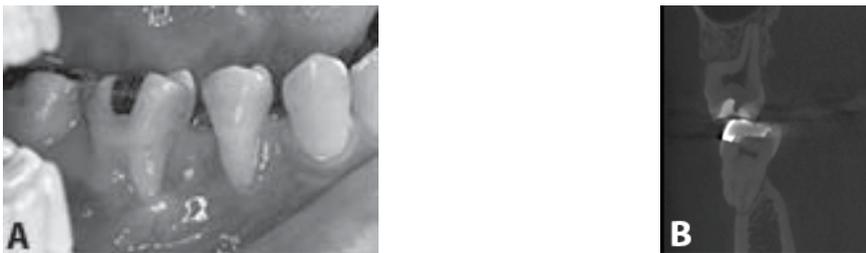


Figure 3. A case with problems after orthodontic therapy. (A) Patient complained of tooth mobility and spontaneous pain after orthodontic treatment. (B) Bucco-lingual cross-sectional image of mesial root of mandibular right first molar. More than half of the root and its apex were exposed from the bone.

- Tooth mobility
- Ill-fitting restorations (margins, contact points, etc)

Recently, muco-gingival tissue has been considered to be of importance in pre-restorative examinations. To commence safer orthodontic treatment by ensuring no gingival recession and a thick and wide attached gingiva is favorable, although these conditions are not essential for all cases. In this report, we will focus on the muco-gingival soft tissue and consider its relation to orthodontic therapy.

For further evaluation of this issue, factors in examining muco-gingival problems are:

- Width and thickness of attached gingiva
- With or without gingival recession
- Degree of root protrusion
- Direction of tooth movement

Width and thickness of attached gingiva

The requirement for gingival attachment around natural teeth has been widely

discussed. In some circumstances, healthy teeth can be retained without any problem even with a small width of attached gingiva, however attached gingiva is considered to be necessary for patients with history of periodontal disease and when restorative treatment with a sub-gingival margin is planned. In particular, keratinized gingiva is regarded to be necessary around implants. In addition, alveolar bone and soft tissue can move according to the movement of attached gingiva and periodontal ligament following tooth movement by appropriate orthodontic force as gingiva is not only attached to the teeth but to the alveolar bone (Coatoam *et al* 1981, Maynard 1987, Stoner and Mazdyasna S 1980) (Figure 4). Post-orthodontic gingival recession tends to occur in cases with smaller widths of attached gingiva (<1 mm), but can be prevented by augmenting attached gingiva in advance of orthodontic therapy (Wilcko *et al* 2001, Wilcko *et al* 2003, Wilcko *et al* 2005) (Figure 5).



Figure 4. A case of attached gingiva retention during movement of teeth. (A) Infralabioversion of canine but ample attached gingiva around tooth. (B) Post orthodontic treatment. Attached gingiva is preserved during tooth movement.



Figure 5. A case with reduced attached gingiva before orthodontic therapy. (A) Before orthodontic therapy. Thickness of attached gingiva of bilateral mandibular canines is less than 1 mm. Gingival recession could occur if orthodontic therapy is performed, so connective tissue grafting was performed to enlarge width and thickness of attached gingiva. (B) Post orthodontic therapy, no gingival recession is observed.

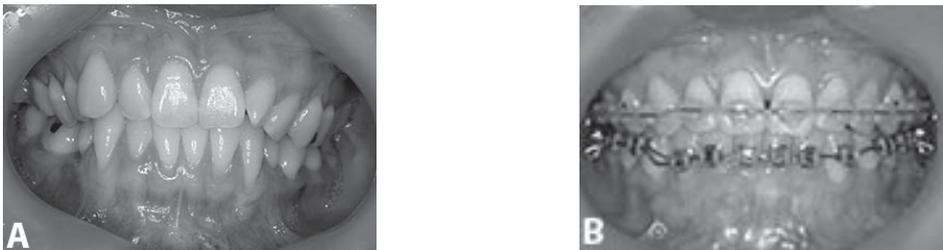


Figure 6. A case with gingival recession prior to orthodontic therapy. (A) Before orthodontic treatment. Marked gingival recession is noted in the mandible. (B) Root coverage prior to orthodontic therapy prevented further gingival recession.



Figure 7. A case with marked tooth root protrusion. (A) The patient requested orthodontic therapy, yet marked gingival recession was noted on maxillary central incisors and canines that are extremely protruded from alveolar bone. (B) Exposed roots were covered by a connective tissue graft with an adequately released and elevated flap. (C) Condition before orthodontic therapy. Complete coverage of exposed roots was not achieved, but attached gingiva achieved thickness. Careful observation is required to prevent losing this attachment during orthodontic treatment.



Figure 8. Total root coverage was difficult, but augmented attached gingiva made orthodontic treatment safe. (A) Photo at first visit. Mandibular bilateral canines protruded on labial side and had gingival recession. Attached gingiva is narrow and thin. (B) Complete root coverage cannot be expected, but a connective tissue graft was performed to augment the width of attached gingiva. (C) Four years after orthodontic treatment was completed. Movement of teeth into bone housing was helpful to cover previously exposed roots.

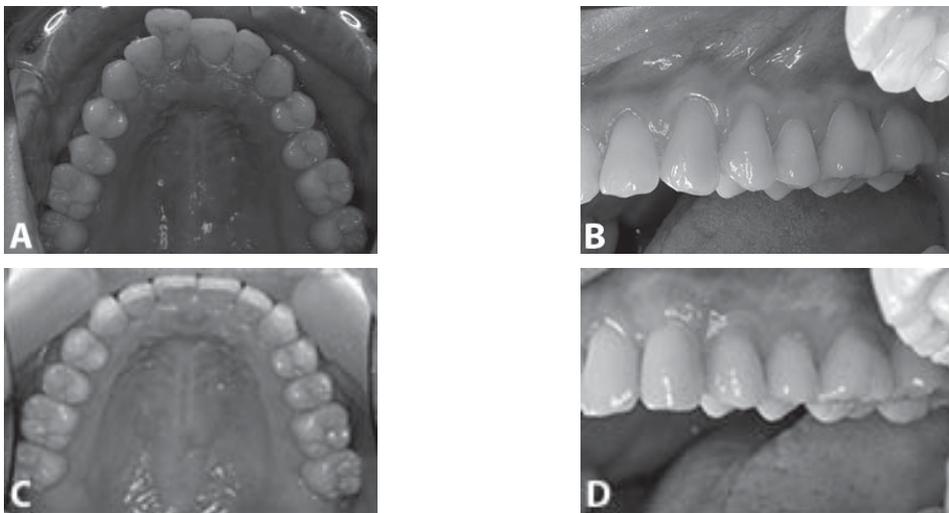


Figure 9. (A&B) Maxillary occlusal and left lateral photos of pre-orthodontic treatment. Correction of crowding by expanding the dentition is planned. Although the buccal side of lateral teeth retained wide gingival attachment, gingival recession is already noted. In preparation for the planned further movement of teeth to the buccal side, connective tissue grafting was performed before the onset of orthodontic treatment. (C&D) No gingival attachment problems were noted on the expanded lateral teeth.

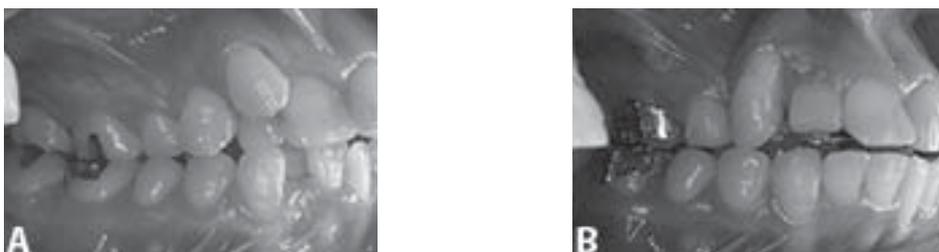


Figure 10. A case of attachment loss resulting from orthodontic treatment. (A) Pre-orthodontic treatment condition to improve crowding. No gingival recession was noted around canines and no significant periodontal problems. (B) Gingival recession has occurred during the orthodontic treatment. It is supposed that the root of the canine was needed to move largely to the buccal side.

With or without gingival recession

The risk of gingival recession progressing during orthodontic treatment is very high for cases with previous gingival recession. Because anatomical and/or required factors which induce gingival recession are already present, these conditions should be addressed. Orthodontic forces result in loss of tooth attachment, however root coverage by connective tissue grafting can increase attached gingiva width and may inhibit further recession (Figure 6).

Root protrusion

Many teeth protruding from the alveolar bone on either the labial or buccal side tend to have narrow attached gingiva and/or already have gingival recession. When an orthodontic force is loaded on the cervical area in such situations, attachment will easily move apically. As previously mentioned, improvement of the condition prior to orthodontic treatment leads to a more satisfactory outcome. However, protruded roots are difficult to perform root coverage procedures on (Figure 7).

Even though the root surface is not completely covered, connective tissue grafting can result in thick attached gingiva at the root apex, which can prevent further gingival recession by orthodontic movement (Figure 8).

Direction of tooth movement

Wennstroem *et al* (1987) reported that recession of gingiva may have a high incidence of occurring when tooth with previously narrow gingiva attachment was moved labially or lingually. Therefore, more careful evaluation of the periodontium is necessary when moving teeth into the bone housing, especially when expanding the dentition or

movement of teeth on the labial or buccal side is planned. Currently, the number of orthodontic patients who do not want teeth extracted is increasing therefore the need to evaluate the periodontium will increase (Figure 9).

Some cases with sufficient width of attached gingiva and movement of teeth to the outer side of alveolar bone planned may experience marked gingival recession (Figure 10).

We discovered that attachment loss can easily occur even in cases without any problems apparent in pre-treatment evaluation, depending on the direction of tooth movement. Inflammation is often experienced after loading orthodontic force on a tooth with gingival recession, which may lead to the additional loss of supporting tissue. Therefore, intensive examination of oral conditions is essential when the “orthodontic movement” of the tooth is performed.

Conclusion

In this report, the concept of attached gingiva in orthodontic therapy has been summarized. Several critical points have become clear by considering the relationship of attached gingiva with orthodontic therapy. Orthodontic therapy increases the forces on teeth and at the same time reduces the ability for the patient to perform adequate oral hygiene. Therefore the condition must be carefully evaluated. In particular, if gingival recession already exists or the width of attached gingiva is less than 1 mm when tooth movement commences, it is preferable to improve the conditions prior to treatment, because a large load will concentrate on a fulcrum at cervical area. Some dentists may hesitate to cover exposed root surfaces surgically because it is very difficult, but other procedures to improve the exposed root conditions such as widening the attached

gingiva or small surgical procedures after moving teeth into final position should be attempted. In addition to meeting increasing orthodontic demands, greater esthetic, safe and stable outcomes can be obtained by utilizing periodontal concepts.

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Chapter 11

The Close Relationship Between Periodontitis and Diabetes and the Diagnosis of Diabetes-Associated Periodontitis

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Introduction

Epidemiological studies have revealed that diabetes is a major risk factor for periodontitis (Chapple *et al* 2013). The susceptibility for periodontitis is increased approximately threefold in people with diabetes (Nelson *et al* 1990). There is a clear relationship between the degree of hyperglycemia and severity of periodontitis, and between periodontal inflammation and glycemic control (Khader *et al* 2006, Preshaw *et al* 2012). Treatment of periodontitis is associated with a reduction in hemoglobin A1c (HbA1c) levels of approximately 0.4% (Teeuw *et al* 2010). In this chapter, we confirm the close relationship between periodontitis and diabetes by describing some clinical cases and presenting our findings concerning the clinical markers of diabetes-associated periodontitis (DM-P).

Close relationship between periodontitis and diabetes

In DM-P, severe inflammation and destruction of periodontal tissues are frequently observed. Typical clinical cases of DM-P are shown in Figure 1. Two patients with type 2 diabetes had severe periodontitis with marked

gingival inflammation, marked alveolar bone resorption, and periodontal abscesses around anterior teeth. In general, severe gingival inflammation, deep periodontal pockets, rapid bone loss and frequent periodontal abscesses often occur in diabetic patients with poor oral hygiene (Klokkevold *et al* 2002).

The periodontal condition tends to be worse in poorly-controlled diabetic patients. In the case of diabetic nephropathy, we found that patients undergoing dialysis may have severe periodontitis (Figure 2). Panoramic x-ray examination of two dialysis patients with diabetic nephropathy revealed considerable alveolar bone resorption and tooth loss. This destruction of periodontal tissues may be due to protracted diabetes and a bone disorder resulting from renal dysfunction. In a study with collaborators from Kawashima Hospital (Tokushima, Japan) which investigated 3 groups of patients consisting of those with only diabetes (DM), dialysis patients with kidney disease (HD), and dialysis patients with diabetic nephropathy (DM+HD), the rate of alveolar bone loss and the number of teeth lost were highest in patients with diabetic nephropathy (DM+HD) (Figure 3). The mean rate of alveolar bone loss was 23.0, % 20.7% and 27.0% in DM, HD, and DM+HD groups, respectively. The mean number of tooth loss

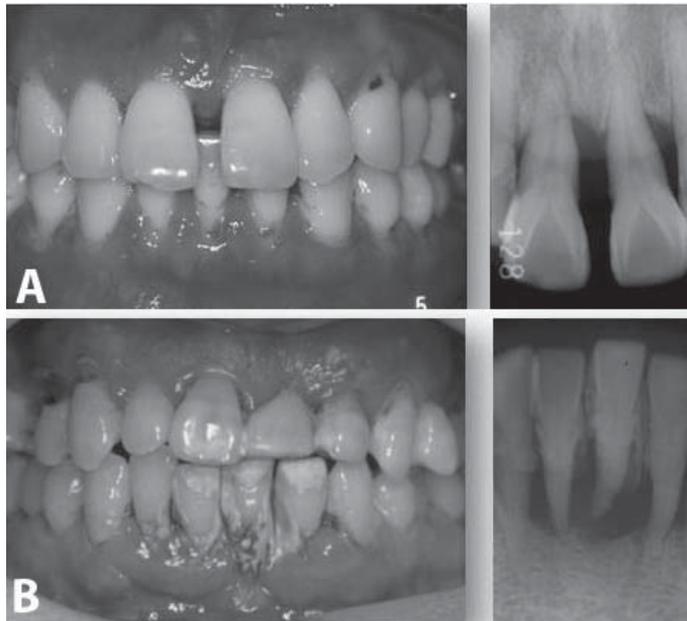


Figure 1. DM-associated periodontitis. (A) 42 year old male with type 2 diabetes. (B) A 50 year old male with type 2 diabetes.

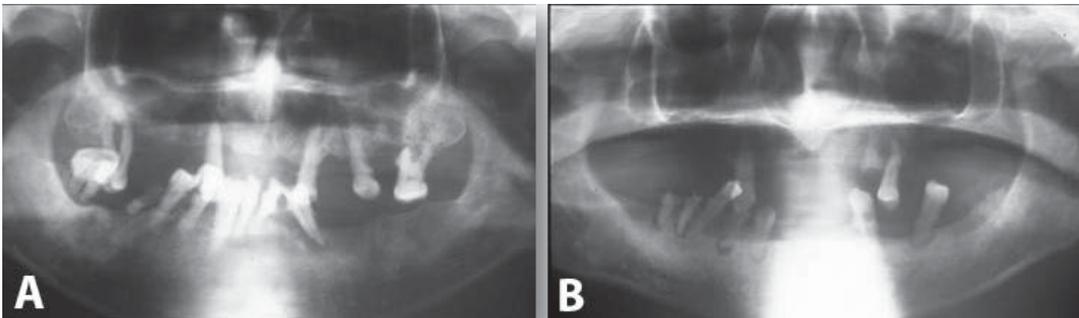


Figure 2. OPGs of patients with diabetic nephropathy. (A) 68 year old male, 3.5 years on dialysis. (B) 68 year old male, 1 year on dialysis.

was 7.6, 6.8, and 11.4 teeth in DM, HD, and DM+HD groups, respectively. These data suggest that diabetic nephropathy may exacerbate periodontitis. Chronic kidney disease has been reported to affect the teeth, periodontium, bone and salivary glands, and several reports have demonstrated higher rates of oral pathology in dialysis patients (Akar *et al* 2011).

From systemic reviews it has been demonstrated that a modest reduction in HbA1c is observed as a result of periodontal

treatment in subjects with type 2 diabetes (Engebretson *et al* 2013, Teeuw *et al* 2010). Such reductions in HbA1c level of diabetes patients have been noted in our daily clinical work. Figures 4 and 5 show a clinical case exhibiting a marked reduction of HbA1c level through the treatment of periodontitis. The patient was a 66 year old female with diabetes and severe periodontitis. Aggressive initial therapy including tooth extraction was performed (Figure 4). After the initial therapy, marked reduction of not only the

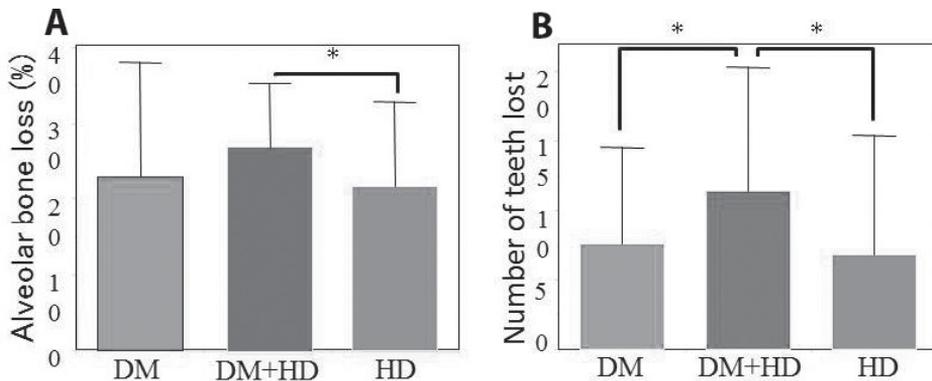


Figure 3. Alveolar bone loss and tooth loss in patients with diabetic nephropathy. (A) Rate of alveolar bone loss. (B) Number of teeth lost. DM: outpatients with diabetes (n=51), HD: dialysis patients with renal diseases (n=84), and DM+HD: dialysis patients with diabetic nephropathy (n=32). Data express mean \pm SE. *p < 0.05

rate of pockets over 4 mm and bleeding on probing, but also HbA1c level were obtained (Figure 5). Continued periodontal treatment for 2 years successfully decreased HbA1c levels in this patient. The reduction was from 6.45% to 5.45% (-1.07%). A meta-analysis by Teeuw *et al* (2010) reported a weighed mean difference of HbA1c of -0.4% before and after therapy, favoring periodontal intervention in type 2 diabetic patients. The case presented in Figures 4 and 5 showed a marked decrease of HbA1c level following periodontal treatment.

Diagnosis of DM-associated periodontitis

Gingival crevicular fluid (GCF) is an exudate containing many biological components that have been reported as clinical markers for periodontitis. We have been studying the significance of calprotectin in GCF as a marker in periodontitis (Kido *et al* 1998, Kido *et al* 1999, Kido *et al* 2012a, Kido *et al* 2012b, Nakamura *et al* 2000). In particular, we reported that calprotectin was a potent marker reflecting the inflammatory condition in periodontal tissues. We also demonstrated that calprotectin was a sensitive and stable molecule and its production

was induced by *Porphyromonas gingivalis* lipopolysaccharide, tumor necrosis factor- α (TNF- α), and interleukin-1 β in human monocytes (Suryono *et al* 2005).

Accurate diagnosis of DM-P is required not only for the treatment of periodontitis, but also for the confirmation of diabetes. We recently commenced investigating novel DM-associated biomarkers using GCF from diabetes patients. Patients with diabetes (DM), periodontitis (P), DM-P, and healthy subjects were selected for this study. As shown in Figure 6, GCF samples were collected using paper strips (Harco Electronics, Winnipeg, Canada) and the volume absorbed in each strip was determined using a Periotron 8000® (Harco Electronics). The GCF-permeated strip was placed in Tris-HCl buffer (pH 7.4) and then GCF-extracted solution was obtained by a centrifugal method as described by Griffiths *et al* (1988). Glycoalbumin, calprotectin, resistin, adiponectin, TNF- α , and pentosidine were selected for DM-associated biomarkers, and calprotectin as a periodontitis-associated marker. These molecules were measured using an ELISA kit.

Our results are summarized in Table 1. The GCF determination demonstrated that glycoalbumin levels in GCF from patients



Figure 4. Periodontal treatment in a 66 year old female with type 2 diabetes. Right and left upper molar teeth were extracted due the progression of severe periodontitis. After initial therapy, satisfactory oral conditions were achieved.

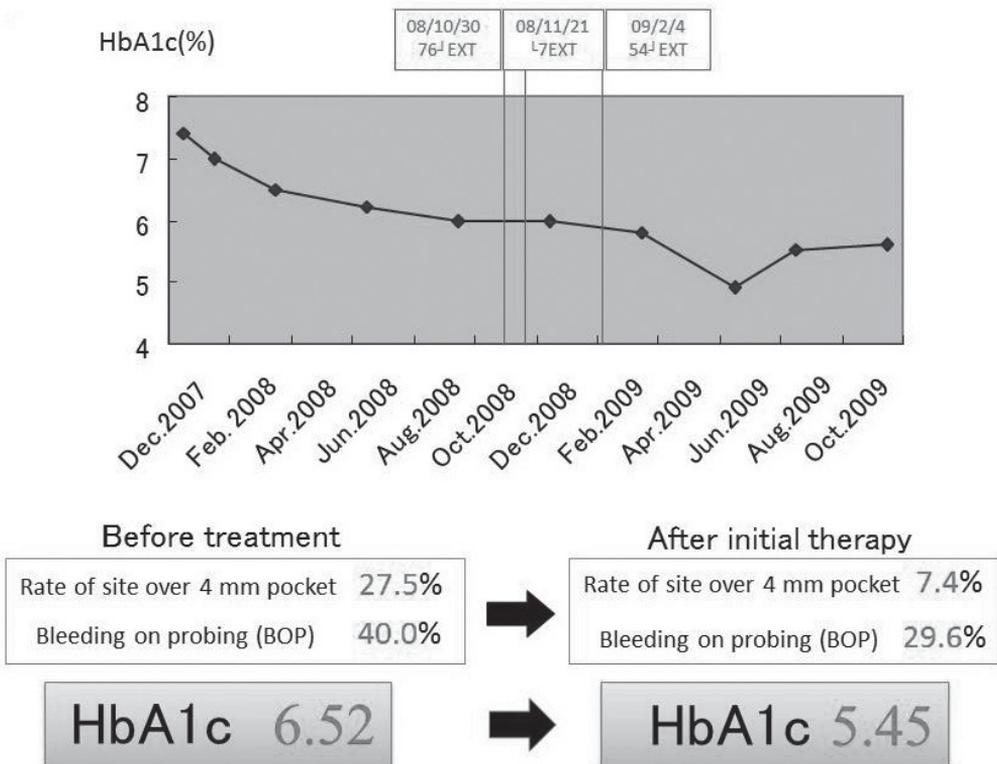


Figure 5. Temporal change of HbA1c level in patients with type 2 diabetes who received periodontal treatment for 2 years.

Marker	DM	P	DM-P
Glycoalbumin	++	0	++
Calprotectin	+	++	++
Resistin		+	+
YKL-40	0	+	+
Pentosidine		-	-
TNF- α		0	0
Adiponectin		0	0

Table 1. Changes in GCF markers of patients with DM, P and DM-P compared to healthy controls. (0 = no change, + = slight increase, ++ = marked increase, - = decrease)

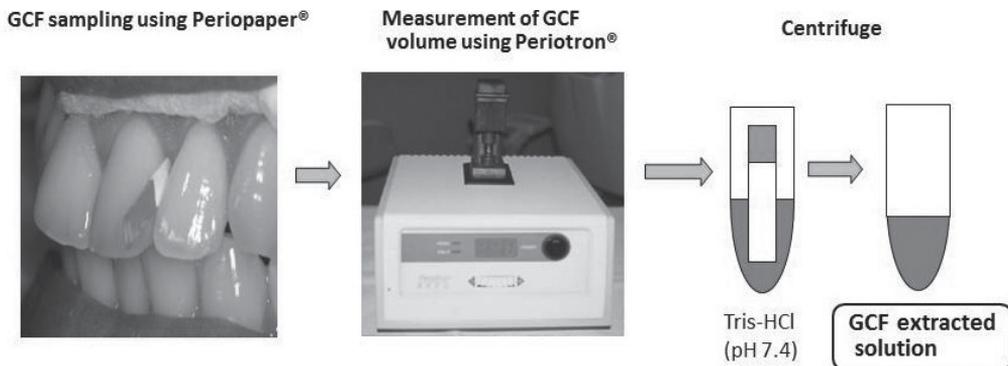


Figure 6. Collection of GCF samples from subjects with periodontitis.

with DM and DM-P was significantly higher than in patients with periodontitis or healthy subjects. This finding indicates that glycoalbumin may be useful as a marker of glucose levels in GCF. Interestingly, when glycoalbumin levels in GCF and HbA1c levels in blood from each patient were statistically analyzed, a positive correlation was found between glycoalbumin and HbA1c levels (unpublished data). These results suggest that the glycoalbumin level in GCF may reflect the level of blood glucose and diagnosis of diabetes may be possible by measuring GCF glycoalbumin. On the other hand, calprotectin level was significantly higher in patients with DM, P and DM-P than in healthy subjects.

Marked increase of GCF calprotectin was observed in patients with P and DM-P and slight increase was noted in patients with DM. Since, as described in our previous study, GCF calprotectin increases in periodontitis patients it is reasonable that calprotectin level was high in P and DM-P patients (Kido *et al* 1998, Kido *et al* 1999). However, the reason for the increase of GCF calprotectin level in DM patients without P is not clear. Some diabetic conditions such as insulin resistance and obesity may be associated with calprotectin increase in GCF, because serum and urinary concentrations of calprotectin are linked to chronic low-grade inflammation and insulin resistance and plasma calprotectin is known

to be a marker of obesity (Ortega *et al* 2012, Mortensen *et al* 2009).

Although we could not obtain complete data from this study for resistin, YKL-40, pentosidine, TNF- α , and adiponectin, these markers did not show any DM-P-identified results in the present study. However, GCF levels of resistin and YKL-40 were high in both patients with P and DM-P, indicating that both markers may reflect an inflammatory condition in periodontal tissues. We reported, for the first time, the presence of resistin in GCF (Hiroshima *et al* 2012). The level of pentosidine, a molecule of advanced glycation end-products (AGEs), was low in patients with P and DM-P and there was no change in the level of TNF- α and adiponectin. Further investigation is necessary to clarify the association level of these molecules in GCF with progression of periodontitis and diabetes.

Discussion

In this chapter, we have demonstrated the close relationship between periodontitis and diabetes by presenting clinical cases of severe periodontitis patients with diabetes. It was confirmed that severe periodontitis was observed in type 2 diabetes and that alveolar bone loss and tooth loss progressed in periodontitis patients with diabetic nephropathy. A clinical case showing the improvement of HbA1c level through periodontal treatment was also presented. Although our case presentations demonstrated the close relationship between periodontitis and diabetes, there are also clinical cases that indicate a two-way relationship between periodontitis and diabetes.

In the GCF assays, the present data confirmed that glycoalbumin and calprotectin increased in GCF from patients with DM-P. Since glycoalbumin was also present in GCF from diabetes patients without periodontitis, glycoalbumin in GCF might be recommended

as a marker of diabetes. It seems that GCF calprotectin may reflect the presence of diabetes and periodontal inflammation. Although further study is necessary, we suggest that two methods of determination of glycoalbumin and calprotectin in GCF may be useful for the diagnosis of DM-P.

Conclusion

There is a two-way relationship between periodontitis and diabetes. Diabetes affects occurrence and severity of periodontitis and periodontal treatment may improve glucose level in type 2 diabetes patients. Assessment of glycoalbumin and calprotectin in GCF may be useful for the diagnosis of diabetes-associated periodontitis.

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Chapter 12

Implant Surface Development for Regeneration of Bone Defects

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Introduction

Since the introduction of the Branemark system in 1971, there has been a steady increase in the number of implant procedures performed across the world. Implants are considered to be a predictable modality of treatment with a high success rate of around 90 to 95%, owing to the development of implant materials and technology (Pye *et al* 2009). Albrektson (1981) outlined six important elements of a successful implant treatment: implant material, implant design, implant surface, status of bone, surgical technique and implant loading condition. Among these, implant surface has a direct effect on bone metabolism and rough surfaces are known to accelerate the differentiation, growth, attachment and mineralization of osteocytes (Cochran 1999, Ivanovski 2010). In a review by Cooper (2000), increases in implant surface roughness were reported to increase osteoconduction and osteogenesis, thereby enhancing osseointegration. A review by Le Guehennec (2007) also reported that, although the exact mechanism is unknown, rough surface implants enhance osseointegration. Many studies have shown that rough surface implants give better osseointegration than

smooth, and therefore various methods have been developed to create a rough surface, such as acid etching, sand blasting and coating with biocompatible materials (Junker *et al* 2009, Le Guehennec *et al* 2007, Shalabi *et al* 2006).

A recent trend in research involves coating of the implant surface at both the micro and macro scale to alter the surface characteristics and improve osseointegration, thus providing a favorable environment for healing (Mendoca *et al* 2008). Nano-structured surfaces are considered to have an influence on the early interaction between the implant and surrounding tissues, and nano-scale changes have been shown to induce significant increases in the formation of the fibrin clot (Lavenus *et al* 2010). Clinically, the benefits from nano-scale changes are expected to lead to improved prognosis, even in patients and sites in poor condition. The increasingly ageing population, often suffering from systemic illnesses such as osteoporosis, has a greater susceptibility to inadequate bone to implant (BIC) contact and compromised healing, which could lead to an increased risk of implant failure. In addition, the posterior maxillary region with its poor bone quality can be a challenging area for many clinicians. Development of implant surface coating

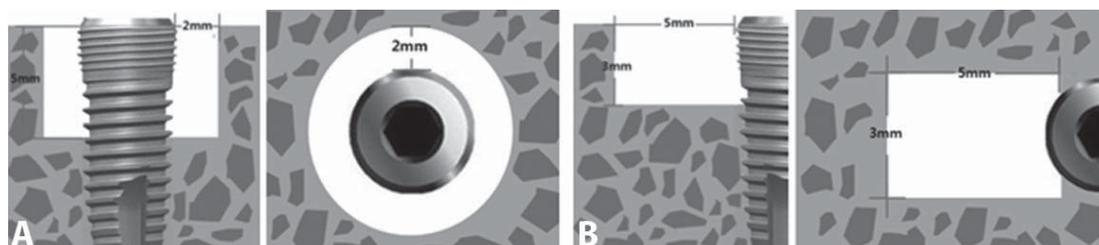


Figure 1. Critical sized defects unlikely to heal naturally. (A) Circumferential defect 2 mm in width, 5 mm in depth. (B) Three walled intrabony defect size 3 mm x 3 mm x 5 mm.

techniques may assist the prognosis of implant treatment despite unfavorable circumstances and may reduce healing time and allow earlier loading.

The implant surface coating which has received the most attention is calcium phosphate (CaP). It is biocompatible and possesses a similar mineral chemistry to that of human bone (Han *et al* 1984). It is also known to speed up bone formation when applied to implant surfaces (Davis 2003). Despite these advantages, clinical complications have arisen from the difference in solubility of amorphous and crystalline forms of CaP, as well as detachment of the coating layer from titanium (Hanisch *et al* 1997, Liao *et al* 1997). These failures could be due to lack of development of suitable coating techniques.

This review article will deal with the ionized-beam-assisted-deposition (IBAD) technique as a solution to overcome the drawbacks of existing CaP coating methods, based on a series of research carried out at the Yonsei University, Department of Periodontics.

Materials and methods

Preparation of CaP coated implants using IBAD method

A number of implant coating methods were used by Choi *et al* (2000) for research purposes. Ion-beam assisted deposition (IBAD) technique was utilized for the

deposition of thin hydroxyapatite layers on the metal substrate. A cryopump (OB-10, Helix Technology, Mansfield, MA, USA) was used to evacuate the chamber to a pressure of 10⁻⁷ Torr. Argas (P¹⁰-4 Torr) was then introduced into the chamber. Whilst an electron beam (Telemark, Fremont, CA, USA) at 8.5 kV and about 0.1 A was evaporating the target, the end-hall type ion gun (Mark II, Commonwealth Scientific, Alexandria, VA, USA) was applied to the metal substrate surface to assist the deposition. The voltage was fixed at 130 V and the current level was gradually increased up to 1.0 A. In order to improve uniformity of the coating layer, the substrate was rotated at 8 rpm during deposition.

Evaluation of CaP coated implants using IBAD method

A sequence of studies was carried out to investigate the conditions that may alter the characteristics of the implant surface coating, including post heat treatment, coating thickness, underlying situation and bone graft materials (Table 1).

Critical size defects determined through studies performed at the current institution include gap defects of 2 mm width, 5 mm depth and three walled defects sized 3 mm x 3 mm x 5 mm (buccolingual x coronal x mesiodistal), selected for the evaluation of the effects of implant surface coating (Choi *et al* 2010, Yoon *et al* 2008) (Figure 1).

Author	Defect model	Healing Time	Study design
Chae <i>et al</i> (2008)	Gap defect	12 weeks	<ul style="list-style-type: none"> • Anodized surface • Anodized surface/CaP coating (150 nm)/430° heat treatment • Anodized surface/CaP coating (300 nm)/430° heat treatment • Anodized surface/CaP coating (150 nm) • Anodized surface/bone graft • Anodized surface/CaP coating (150 nm)/430° heat treatment/bone graft
Song <i>et al</i> (2009)			<ul style="list-style-type: none"> • Anodized surface • Anodized surface/CaP coating (500 nm)/350° heat treatment • SLA surface/anodizing/CaP coating (500 nm)/350° heat treatment
Um <i>et al</i> (2009)	Gap defect	12 weeks	<ul style="list-style-type: none"> • Anodized surface • Anodized surface/Bone graft • Anodized surface/CaP coating (150 nm) • Anodized surface/CaP coating (150 nm)/350° heat treatment
Kim <i>et al</i> (2009)	Gap defect	8 weeks	<ul style="list-style-type: none"> • SLA surface • SLA surface/CaP coating (200 nm) • SLA surface/CaP coating (500 nm)
Yoon <i>et al</i> (2009)	Gap defect	12 weeks	<ul style="list-style-type: none"> • SLA surface • SLA surface/CaP coating (500 nm)/350° heat treatment • SLA surface/CaP coating (500 nm)/450° heat treatment
Lee <i>et al</i> (2012)	Gap defect	4/8 weeks	<ul style="list-style-type: none"> • Machined surface/CaP coating (500 nm) • SLA surface/CaP coating (500 nm)
Kim <i>et al</i> (2011a)	Gap defect	8/16 weeks	<ul style="list-style-type: none"> • SLA surface • SLA surface/bone graft (CaP:0.6)
Kim <i>et al</i> (2011b)	Gap defect	8/16 weeks	<ul style="list-style-type: none"> • SLA surface • SLA surface/bone graft (70% HA, 30% β-TCP)
Jung <i>et al</i> (2005)	Intrabony defect	8 weeks	<ul style="list-style-type: none"> • SLA surface • SLA surface/bone graft (non-crystalline calcium phosphate glass) • SLA surface/bone graft (xDBM) • SLA surface/bone graft (β-TCP)
Choi <i>et al</i> (2010)	Intrabony defect	12 weeks	<ul style="list-style-type: none"> • SLA surface/CaP coating (500 nm) • SLA surface/CaP coating (500 nm)/bone graft (ACP) • SLA surface/CaP coating (500 nm)/bone graft (MBCP) • SLA surface/CaP coating (500 nm)/bone graft (FDBA)

Table 1. List of studies undertaken at Yonsei University, Seoul, South Korea on various implant surface coating methods.

Result and discussion

CaP coating by IBAD method

In order to allow the CaP on the coated implant surface to be available for bone formation, it must be attached strongly enough to resist the frictional force during placement (Wang *et al* 1996). CaP also has to be released continuously over the prolonged period of time required to induce bone formation. The plasma spray technique has been the most commonly used CaP coating method until now, as it is cheap and easy to manufacture. However its drawbacks include uneven density and a thick and highly porous coating layer with weak bond strength to titanium. As a result it has yet to produce a long term successful clinical outcome (Albrektsson 1998, Hayashi *et al* 1993, Klein *et al* 1991).

To overcome these problems, other methods have been suggested, such as pulsed laser deposition (PLD), sputter coating and ion beam-assisted deposition (IBAD) (Ong *et al* 1992, Singh *et al* 1994, Yoshinari *et al* 1994, van Dijk *et al* 1996). Among these, when compared to the previous methods, IBAD enhances the bond strength between the coating and titanium and reduces its solubility. This has the effect of a controlled CaP release over a prolonged period of time and allows for the formation of a uniform coating layer (Choi *et al* 2000). Choi *et al* (2000) also reported an increase in the bond strength between the coating layer and titanium from 35MPa to 70MPa according to the intensity of the ion beam current during IBAD coating. This is a very high bond strength considering that the average value is about 7MPa.

Hwang and co-workers (unpublished data) also demonstrated high bond strength by evaluating the surface of CaP coated machined implants by IBAD. The machined surface implants were immediately removed after installation and inspected with an energy

dispersive spectrometer (EDS). The authors observed that CaP was well-maintained on the surface, which suggests a strong bond strength to titanium which can resist the frictional force during placement.

CaP coatings may promote osteoinductive activity around dental implants resulting in conditions favorable for osseointegration. Kim *et al* (2007) examined IBAD CaP coatings based on an anodized surface and observed more active contact osteogenesis on a CaP coated anodized surface than an anodized surface without additional surface treatment. The effectiveness of CaP coating has also been demonstrated in machined surfaced implants. Lee *et al* (2002) have compared removal torque and bone-to-implant contact in IBAD CaP coated machined surface implants to uncoated machined surface and rough surfaced implants. Each of the three groups was placed into the femur of rabbits and then analyzed after 12 weeks of healing. The result showed that CaP coated machined surface implants displayed greater removal torque and BIC than the other non-coated surfaces (Figure 2). Furthermore, CaP coating led to successful osseointegration histologically in the spinning model implants which did not have primary stability in the early healing period (Kim *et al* 2013). Considering that a smooth surfaced implant on its own has low BIC, weak attachment to bone and a low success rate, CaP coating can be deemed to provide an advantageous environment for cell adhesion and bone formation.

The advantages of the IBAD method were demonstrated in areas previously considered as limitations for other coating methods. Firm binding of the CaP coating to the implant surface during placement combined with the later release of CaP is known to promote early osseointegration. However, the optimum requirements of the CaP coating by IBAD for implant stability and clinical performance are still areas requiring further investigation.

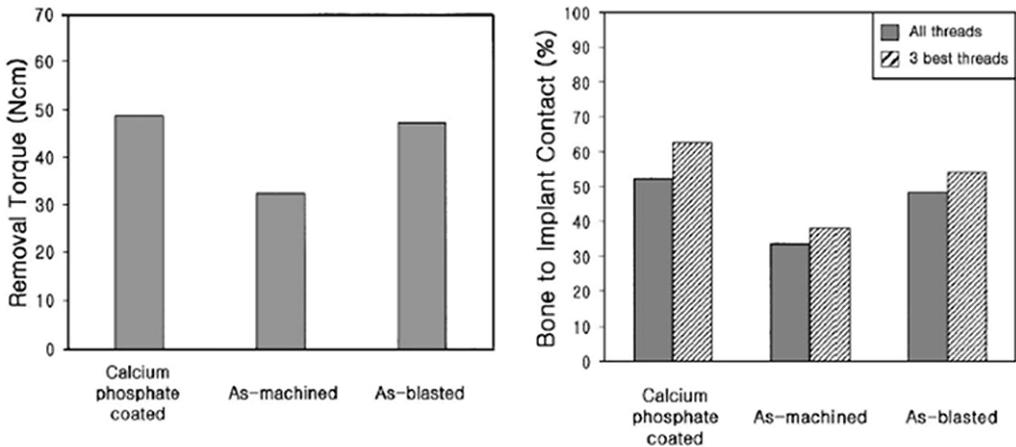


Figure 2. CaP coated machined surface, machined surface and rough surfaced implants placed inside rabbit femur in order to test for removal torque and BIC. The highest values were obtained from the calcium phosphate coated machined surface group (reproduced from Lee *et al* 2002 with permission).

The quoted studies in the following sections were performed at Yonsei University (Seoul, Korea).

Coating thickness

The thickness of the CaP coating is an important factor in bone formation around an implant. If the coating layer is too thin its stability may become compromised due to the brittleness of the CaP's crystalline structure. If too thick, there is an increased risk of delamination of the coating's external layer. The coating layer may also block the micropores on the implant surface and reduce its roughness. Furthermore, the thicker the coating layer, the longer required for it to become substituted by bone during osseointegration.

The ideal thickness of hydroxyapatite of a plasma sprayed CaP surface has been determined in many studies to be approximately 50 μm (de Groot *et al* 1987, Geesink *et al* 1987). The IBAD method can be used to reduce this coating thickness to a nano-size (Narayanan *et al* 2008). A certain nano-scale thickness can promote adsorption of protein molecules and attachment of the osteocytes

thereby speeding up osseointegration (Brett *et al* 2004). Another advantage in reducing the coating thickness is that delamination of the coating layer can be avoided. The optimum nano-scale thickness is as of yet undetermined.

Chae *et al* (2008) used anodized surface implants coated with 150 or 300 nm HA prior to heat treatment at 430°C. When placed in critical size gap defects, the HA coated implants showed enhanced bone to implant contact and bone density compared to the control group without the coating. Song *et al* (2009) carried out experiments on the same anodized surface with 500 nm CaP coating and 350°C heat treatment, which produced greater bone formation than the control.

When the two experiments described above were compared, the 150 nm coating (Bone to implant contact (BIC): 49.6 \pm 11.2%, Bone density (BD): 59.9 \pm 11.6%) showed the most favorable bone formation, and 500 nm coating (BIC: 32.7 \pm 8.0%, BD: 31.3 \pm 4.6%) and 300 nm coating (BIC: 36.8 \pm 10.8%, BD: 43.1 \pm 12.9%) produced more positive results than control (BIC: 28.8 \pm 9.9%, BD: 33.8 \pm 12.4%) (Table 2). Furthermore, Kim *et al* (2013) demonstrated that a 500 nm CaP

	BIC (%)	Bone density (%)
Anodized surface	28.8±9.9	33.8±12.4
Anodized surface with HA coating (150 nm), 350°C heat treatment	49.6±11.2	59.9±11.6
Anodized surface with HA coating (300 nm), 350°C heat treatment	36.8±10.8	43.1±12.9
Anodized surface with HA coating (150 nm)	27.6±10.6	30.6±15.2

Table 2. Bone to implant contact percentage (%) and bone density percentage (%) according to the thickness of HA coating with or without heat treatment.

coated implant with heat treatment could successfully integrate without initial stability. There was an increasing tendency of bone to implant contact and bone density throughout the healing period and no statistical difference was found between the group without initial stability and the control.

On the basis of the studies above, it is possible to create a uniform CaP coating layer that is several nanometers thick using the IBAD method. The coating thickness of 150 to 500 nm appears to accelerate bone formation and reduce healing time.

Post heat treatment

Regardless of the surface coating method, the generalized formation of amorphous CaP coating layer results in high level of solubility. Solubility of the amorphous layer can be reduced by heat treatment which crystallizes the layer structure (Chen *et al* 1997, Yang *et al* 2003a). However, formation of crack lines as a result of heat treatment may lower the bond strength between the coating and the implant surface, which could lead to failure of the implant upon placement (Chen *et al* 1994, Chen *et al* 1997). According to Choi *et al* (2000), heat treatment was revealed to increase crack formation even in the case of IBAD, but the strong bond strength may be

able to maintain the stability of the coating layer.

Um *et al* (2009) compared heat treated (350°C) and non-heat treated HA coatings (150 nm) on anodized surface implants placed in gap defects. After 12 weeks of healing, increased bone formation was seen on heat treated surfaces and histometric findings also showed greater bone to implant contact and bone density compared to the non-heat treated group. The outcomes for non-heat treated and HA coated anodized implants were similar to those for anodized implants with no surface modification (Figure 3).

Through the application of heat treatment as mentioned above, the amorphous coating layer becomes crystalline, which promotes cell attachment and accelerates bone formation (Yang *et al* 2003b). Thus, heat treatment can be used to alter the surface characteristics of the CaP coating layer by reducing solubility and increasing roughness, consequently enhancing osseointegration and bone formation around an implant.

Multi-treatment

The various combinations of implant surface treatment methods do not always produce positive outcomes. Song *et al* (2009) carried out both SLA and anodization

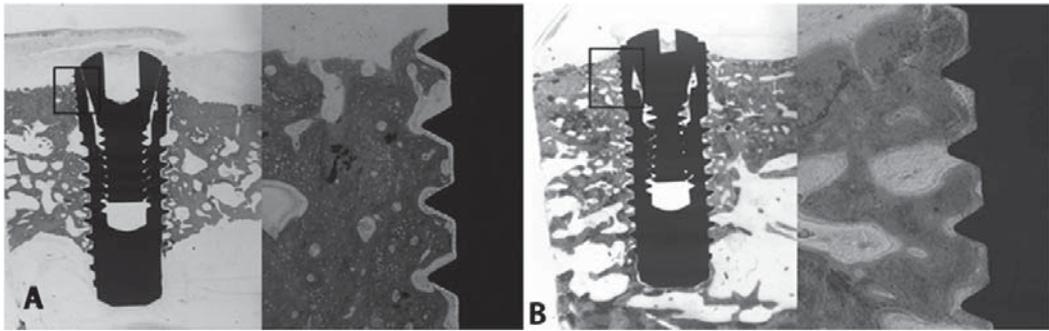


Figure 3. Histologic view of the heat treated group (A) shows greater osseointegration and bone formation than the group without heat treatment (B) (reproduced from Um *et al* 2009 with permission).

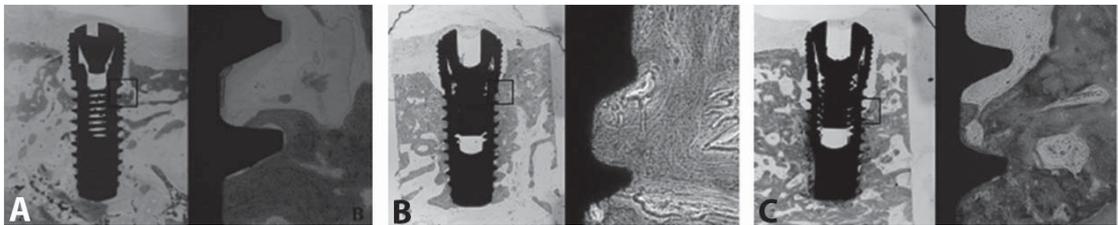


Figure 4. Histologic view of implants with anodized surface (A), anodized surface + HA 500 nm coating/IBAD 350°C (B), SLA + anodizing + HA 500 nm coating/IBAD 350°C (C) in circumferential defects. Anodized surface and HA coating showed a synergistic effect whereas anodized SLA surface and HA coating showed an offset effect (reproduced from Song *et al* 2009 with permission).

on a surface prior to CaP coating and heat treatment. After 12 weeks of healing, CaP coated anodized surfaces produced the highest BIC and bone density whereas the surfaces which received SLA, anodizing and CaP coating together showed the lowest values (Figure 4). In light of such results it could be suggested that a CaP coating applied by IBAD on an anodized surface can create a synergistic effect for osseointegration but a combination of both SLA and anodization in conjunction with CaP coating may generate an offset effect.

Implant surface coatings

As mentioned above, various methods are available to roughen the implant surface, with each method producing a surface with distinctive roughness and characteristics. Therefore, a series of studies have been

undertaken with the aim of finding the most effective combination of implant surface and CaP coating technique for the treatment of bony defects.

SLA surface

SLA surface is one of the most commonly used implant surfaces. It possesses macro and micro roughness as a result of sand blasting and acid etching respectively (Le Guehennec *et al* 2007). Kim *et al* (2009) applied a CaP coating onto SLA surfaces using IBAD method. SLA surface implants with coating thicknesses of 200 and 500 nm were placed in critical sized gap defects and compared with uncoated SLA surface implants after 8 weeks of healing. No significant differences were demonstrated between the groups, however heat treatment for increasing crystallinity was not carried out after the CaP coating and the

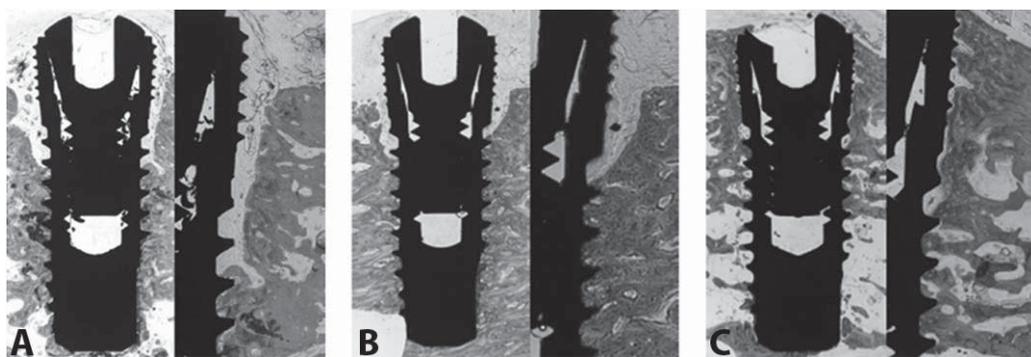


Figure 5. Histologic view of implants with SLA surface only (A), SLA surface and CaP coating applied by IBAD after 350°C heat treatment (B) and SLA surface and CaP coating applied by IBAD after 450°C heat treatment (C) in circumferential defects. CaP coated SLA surfaces showed greater osteogenic potential (reproduced from Yoon *et al* 2009 with permission).

observational period was limited.

On the other hand, Yoon *et al* (2009) reported a dissimilar outcome from a comparable experimental model. SLA surface implants were coated with 500 nm thick CaP and heat-treated at 350°C and 450°C. After 12 weeks of healing, the CaP coated implants showed higher BIC and bone density than the uncoated implants (Figure 5). Therefore, CaP coating followed by heat treatment has a potential to convert the conventional SLA surfaces into more osteoinductive surfaces.

Anodized surface

Implant titanium surfaces form an oxide layer as soon as they become exposed to the atmosphere. Implementation of thermal and electrochemical oxidation increases its thickness and roughness which improves reactivity with the bone; anodization of an implant surface is based on such principles. It has been demonstrated that CaP coating on an anodized surface could improve osteoinductivity and osseointegration of the implant (Le Guehennec *et al* 2007). Choi *et al* (2000) reported that application of CaP coating by IBAD on an anodized implant surface increases bond strength of the coating layer and reduces its solubility. Also according

to the study by Kim *et al* (2007) the same implant surface treatment has enhanced bioactivity and produced a synergistic effect on osseointegration. Chae *et al* (2008), in a gap defect model, compared anodized surfaces with 150 nm and 300 nm thick heat-treated CaP coating and an anodized implant surface with no CaP coating. After 12 weeks of healing, the CaP coated implants showed greater BIC and new bone formation than the non-coated implants. As a result, CaP coating of an anodized surface can be suggested to improve osteoinductivity and osseointegration of the implant.

Machined surface

Rough surfaces promote early healing and increased BIC but may provide a niche for bacterial proliferation and endotoxins, which can accelerate the progression of disease and complicate treatment in the case of peri-implantitis (Lang *et al* 2011, Renvert *et al* 2011). In contrast, machined surfaces are more hygienic than rough surfaces once exposed to the oral environment due to reduced bacterial colonization, and therefore are easier to manage. If successful osseointegration can be achieved by applying CaP coating onto a machined surface, it would be possible

to produce an implant that shows improved resistance to peri-implantitis. According to a study by Lee *et al* (2002), placement of a CaP coated machined surface implant into a completely healed ridge showed similar results to that of a rough surfaced implant, which means efficacy of the machined surface may be re-visited. However, these results have been inconsistent and the research is in its infancy. Lee *et al* (unpublished data) placed CaP coated and heat-treated machined surface and SLA surface implants in a gap defect model. The authors demonstrated no significant differences in bone regeneration or impact on the arrangement of collagen fibers after 8 weeks of healing.

Additional studies are required in regard to the improvement of machined surfaces using CaP coating. If such a treatment modality produces a superior regenerative capacity to the other surfaces, it may help to enhance the prognosis, maintenance and management of implant treatment.

Combination with bone substitute materials

Type of bone substitute material as well as the implant surface is an important element in the treatment of bony defects. As described previously, CaP shares comparable characteristics with alveolar bone, hence its utilization as a bone graft material. Among CaPs, hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP) have been shown to produce good osteoconductive capacity in many studies, and can be used separately or together in various ratios (Friedmann *et al* 2009, Nery *et al* 1992, Lindgren *et al* 2012).

Kim *et al* (2011a) placed SLA implants in a gap defect model in combination with CaP bone graft with a Ca/P ratio of 0.6. After 16 weeks of healing, bone substitute material was completely resorbed, and no significant difference was demonstrated compared with

the control group in terms of BIC and residual defect depth.

Conversely, an experiment using biphasic calcium phosphate (BCP) composed of 70% HA and 30% β -TCP in the same defect model demonstrated significant reduction of the residual defect depth and unresorbed bone graft after 16 weeks of healing, but there was no difference in BIC between the group with or without bone grafting (Kim *et al* 2011b). Jung *et al* (2005) compared three types of bone substitute materials (CaP, β -TCP and xenogenic DBM putty) in a three wall defect. The size of defects was reduced, but no significant difference in BIC was reported between the groups including the control. Therefore, bone grafting materials may maintain space and reduce residual defects depending on the type of the material, but the material itself may be limited in its ability to increase bone to implant contact.

In the previous studies, CaP coated implants demonstrated favorable defect filling and bone formation in defect models. However, large defects exceeding the critical limit might require adjunct regenerative procedures. Choi *et al* (2010) grafted various bone substitute materials in a critical sized intrabony defect model around CaP coated implants. Slightly different results were obtained according to the type of bone substitute, but all experimental groups displayed greater osseointegration and new bone formation than the control, which indicates that adjunct bone substitute can enhance bone regeneration with CaP coated implants (Figure 6). Likewise, Chae *et al* (2008) acquired successful results from the application of BCP bone substitute in defects surrounding CaP coated implants.

Conclusion

CaP possesses adequate characteristics for an implant surface coating material, but the search for the most appropriate coating

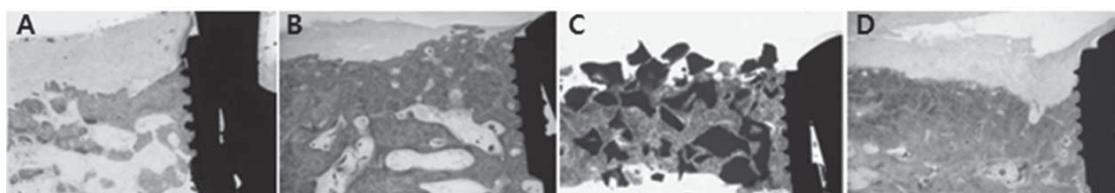


Figure 6. Ground section of a structured surface with CaP nanocoated SLA implant. (A) Control group with a surgically created three-wall defect at 12 weeks. (B) Amorphous CaP group. (C) Micro-macro porous-biphasic CaP group. (D) Freeze-dried bone allograft group. The remaining defect depth, BIC and bone area within the defect of the implant of the experimental groups showed significant differences compared to the control but the differences between the three different materials were insignificant (reproduced from Choi *et al* 2010 with permission).

method is ongoing. Numerous methods have been studied in order to overcome the limitations of the previous systems. One example is the ionized beam assisted deposition (IBAD) method which is able to create a thinly uniform coating layer with high bond strength to titanium and low solubility. However, its properties may vary according to other factors including coating thickness, post heat treatment and the underlying situation. A series of studies have been performed in this regard, and the following conditions can be suggested to enhance osseointegration and bone regeneration:

1. CaP coating thickness ranging from 150 to 500 nm is the best for successful osseointegration.
2. Heat treatment of the coating layer increases osseointegration and bone formation.
3. Multiple treatments of a surface including SLA, anodizing and CaP coating in conjunction may create an offset effect.
4. Additional studies are required to investigate the effects of CaP coating on the machined surface.
5. Synthetic bone substitutes can be effectively used in large critical sized defects.

On the basis of the current evidence, the CaP coated implant using the IBAD method produced successful implant stability, osseointegration and bone regeneration. Within the limitations of the suggested list of

studies, the potentials of the IBAD method could be substantiated through further investigation.

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Chapter 13

Periodontic Specialist Services in the Public Sector: The Malaysian Experience

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Introduction

Malaysia's healthcare providers can be either public or private sector. The government heavily funds the public healthcare sector, which is open to all Malaysians, with a nominal payment required for treatment. Fees for private healthcare services are fully paid by the patients themselves, their employers or by insurance companies. The number of health facilities such as private hospitals, clinics, and dental surgeries has increased tremendously in Malaysia over the last decade and both private and public healthcare sectors are still expanding.

In the public sector, the Ministry of Health (MoH) is the main government agency responsible for providing healthcare services in the country. Other ministries that also provide healthcare services include the Ministry of Higher Education and the Ministry of Women, Family, and Community Development. Oral Health Services are also provided by these agencies, with the MoH as the main provider, covering the whole country, both rural and urban.

In the MoH, the Oral Health Division looks after the oral health services for the population, which encompass primary and specialist care. Periodontic Specialist Services is one of the specialties provided to the population within the Oral Health Services in the MoH.

Malaysia - Facts and figures

Malaysia has an estimated population of 29,179,952 as of July 2012, with a diverse ethnicity. It is a multicultural society. It has an area of 329,995 km² (127,355 miles²), with 4,675 km (2,905 miles) of coastline. It is further subdivided into Peninsular Malaysia and East Malaysia, separated by the South China Sea.

As of 2012 there were 4,558 active dental practitioners with 2,664 in the public sector and the remaining 1,894 in the private sector. In 2012, 613 new dentists registered with the Malaysian Dental Council, 399 of them were trained locally, while the rest (214) were trained abroad. The majority of registered dentists are females (63%), however the majority of dentists practicing in the private sector are males (53%) (MDC 2013). The number of registered periodontists is fewer than 100, with most being employed in the MoH and the Universities. Others are employed in the army and private practice.

Malaysian Public Healthcare System

There are a few features that make the Malaysian public healthcare system unique.

Heavily subsidised

The Malaysian public health sector is highly subsidised by the government and provides a strong health care infrastructure thus making it universally accessible by its residents. Due to the high government subsidies, public health care is affordable for the majority of the population. Moreover, the Ministry of Health provides free health services to civil servants, pensioners and the needy. This also includes services provided by periodontists.

Quite extensive coverage

The healthcare system is accessible to many around the country, both in rural and urban areas, especially in the Peninsular region. Apart from static facilities, there are mobile teams which service special groups or remote areas. Periodontists, along with other oral health specialists, are located mainly in major towns but some do provide services to smaller towns and rural areas by travelling to these areas regularly. The scope of services provided is comprehensive, from basic care to more complicated cases and conditions.

Health delivery system in MoH

There are three tiers of service. Primary care is for basic health services with some secondary care given at some health centres. For general health, there are family health physicians available to attend to the needs of the population. Periodontists, orthodontists and restorative specialists are located at the primary care facilities, providing secondary care to the population. There are also hospitals where inpatients and more complex cases are treated.

The value of Malaysia's healthcare industry is estimated at around RM8.4 billion (US\$2.5 billion), with total expenditure on healthcare

estimated at 4.75% of gross domestic product (GDP). Government and private funding currently account for around 55% and 45% of total health expenditure in Malaysia, respectively (Inside Malaysia 2012).

Dental specialties in MoH

Oral health specialist services are part of the oral health services of the MoH (Table 1). Periodontics is one of the specialties listed under MoH and the National Specialist Register (NSR). Any practitioner planning to practice as a specialist in Malaysia is required to register with the NSR. Only qualified individuals with formal training in the relevant specialties will be admitted and the NSR covers not only MoH but also other health providers such as the university, army and the private sectors.

MoH commenced the provision of periodontic specialist services in 1986 in Kuala Lumpur, when an officer returned from specialist training in the UK. The number has now increased to 24, covering all the states in the country. They are trained locally and abroad. Locally there are 3 universities providing specialist training with a duration of 4 years. Overseas training has traditionally been undertaken in the UK, with new training areas include Australia and New Zealand. Some are still in training in Singapore and Hong Kong. This diversity of training grounds improves the specialty as there are a range of approaches in dealing with periodontal disease in the community.

The workloads of each specialist are collected daily and later compiled into monthly and yearly reports through the Health Information Management System. This recorded workload is used to assess the productivity of each operator and more importantly is used for planning future expansion and treatment coverage (Selvaraju 2006).

Discipline	2000	2004	2011
Oral Surgery	32	34	57
Orthodontics	28	31	44
Periodontology	8	10	21
Paediatric Dentistry	8	13	31
Oral Pathology and Oral Medicine	2	5	9
Restorative Dentistry	-	2	18
Dental Public Health	-	124	123

Table 1. Oral health specialists employed by MoH Malaysia (OHD Annual Report 2006, 2011).

Periodontal Status	1990 (%)	2000 (%)
Subjects examined having periodontal diseases	92.8	90.2
Gingivitis (CPITN 1)	4.6	4.2
Calculus (CPITN 2)	65.1	56.9
Moderate Pocketing (CPITN 3)	17	20.8
Deep Pocketing (CPITN 4)	6	5.5

Table 2. Periodontal status in Malaysia (Dental Services Division, MoH Malaysia 1993 and NOHSA 2000).

For clinical recording of cases, the MoH have piloted a program which is planned to be implemented nationwide in all oral health service facilities including the periodontics specialist services, called the Oral Health Clinical Information System. The development has been challenging from the aspects of content development and the physical hardware needed.

With the emphasis now on a multi-disciplinary approach to complex treatment, combined clinics have been established in a few locations to deal with this issue. With the combined effort of the different specialties, patients will be provided with the best option from each specialty.

Periodontal health status of Malaysians

Surveys on oral health and related issues in adults are conducted every 10 years under the National Oral Health Survey on Adults (NOHSA) scheme. For periodontal disease, the Community Periodontal Index was used in the last three surveys undertaken in 1990, 2000 and 2010. Results from the 1990 and 2000 surveys have already been published (Table 2). The 2010 survey has been completed and will be published soon.

From this, it is noted that the level of periodontal problems is high, with around 6% of those surveyed having an advanced stage of periodontal disease and complex treatment

Treatment Needs	1990 (%)	2000 (%)
OHI (TN1)	92.8	90.2
Scaling/Subgingival Debridement (TN2)	88.1	83.2
Complex treatment (TN3)	6	5.5

Table 3. Treatment Needs of Malaysians (Dental Services Division, MoH Malaysia 1993 and NOHSA 2000).

	1960	1986	1996	2006	2011
Percentage of population	2.0	6.3	8.3	14.9	20.8

Table 4. Prevalence of diabetes mellitus in Malaysia, <30 years old (Malaysian NHMS I, II, III and IV).

needs (Table 3). With the small number of practicing periodontists, both public and private, and 70% of the 29 million population being adults, the task ahead of treating this population is enormous. There are plans to address this problem, especially in the MoH, by such methods as increasing the number of periodontists and providing more training places in the local universities. There are also plans for prevention, where more promotional and other motivational activities were planned and implemented.

Challenges ahead

The periodontics specialist service is still developing and will face many challenges ahead. The greatest challenges could be those that affect the health system as a whole:

1. The rise in lifestyle diseases.
2. An ageing population.

And those that are more specific to periodontics include:

3. Awareness of periodontal health.
4. The training of specialists.

The rise in lifestyle diseases

The rise in lifestyle diseases such as diabetes and cardiovascular conditions is due to diet, sedentary lifestyles and environmental changes.

Diabetes mellitus

The increase in the rate of diabetes prevalence is of concern. In results from the National Health and Morbidity Surveys the incidence of diabetes increased from 8% of the population in 1996, to more than 14% in 2006. In the fourth survey in 2011, the rate of diabetes was an alarming 20.8% (Table 4).

It is well established that diabetes and periodontal disease are interrelated and the increase in diabetes will also have an effect on the prevalence of periodontal disease. As reported in the Malaysian NHMS II (1996), the known rate of diabetes was found to be 5.7% and the 2 hour-post-glucose load tests was 2.5%, making the total figure 8.3%.

Year	<15 yrs (%)	15-64 yrs (%)	>64 yrs (%)	Pop (million)
2001	32.7	63.4	3.9	24.12
2002	31.9	64.1	4.0	24.72
2004	30.4	65.5	4.1	25.91
2005	29.7	66.1	4.2	26.48
2006	29.2	66.5	4.3	26.83
2007	28.7	66.9	4.4	27.18
2008	28.2	67.3	4.5	27.54
2009	27.7	67.7	4.6	27.90
2010	27.2	68.1	4.7	28.25

Table 5. Population age distribution trends for 2001 to 2010 (Department of Statistics Malaysia 2010).

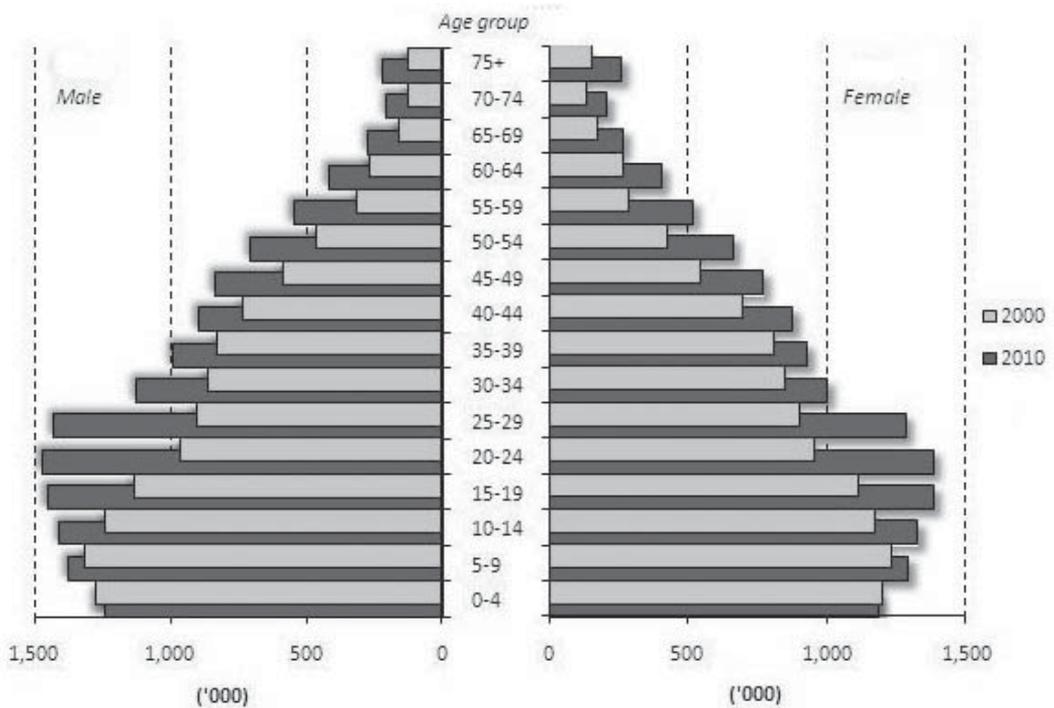


Figure 1. Age pyramid of Malaysian population in 2000 and 2010.

Age Group	Examined Dentates		% Subject Coded				
			Healthy (0)	Bleeding (1)	Calculus (2)	Shallow (3)	Deep (4)
15-19	1,639	1,639	25.9	10.1	60.6	3.2	0.1
20-24	1,040	1,040	14.8	6.4	68.8	8.9	1.0
25-29	958	958	8.6	4.1	68.8	16.2	2.1
30-34	1,064	1,061	7.0	3.3	62.0	22.8	4.6
35-44	2,329	2,258	5.2	2.8	54.5	29.0	7.5
45-54	1,806	1,619	4.8	1.9	49.9	28.8	10.6
55-64	1,159	849	3.9	1.3	44.6	32.3	9.2
65-74	664	392	2.8	1.8	44.6	26.5	9.4
75+	232	116	1.7	0.9	44.0	19.8	6.9
All	10,891	9,932	9.8	4.2	56.9	20.8	5.5

Table 6. Prevalence of periodontitis in Malaysia by age group (NOHSA 2000).

Coronary heart disease and cardiovascular disease

Malaysians admitted to hospital for Acute Coronary Syndrome had an average age of 59 years, 7 years below the average in other countries (NHMS IV 2011). In Malaysia, cardiovascular diseases are the leading cause of death in individuals aged over 40 years. The prevalence of hypertension amongst adults aged 30 years and above has increased from 32.9% in 1996 to 40.5% in 2004 and to 42.6% in 2006. The overall prevalence of pre-hypertension and hypertension was 11.1% and 11.6% respectively among children aged 13 to 17 years. This prevalence increased significantly with age (Rampal *et al* 2008).

Periodontal disease has a proven relationship with these two systemic diseases, either in the form of risk factors or in a bidirectional relationship (Iacopino 2001). The role periodontists can play in the management

of patients with these conditions can be significant, through prevention, intervention and holistic management of these patients.

There are plans for periodontal specialist services to become involved in the holistic management of these conditions. Awareness of this relationship need to be further enhanced within the medical fraternity in order for them to accept this relationship and involve periodontists in the management of patients with these diseases.

An ageing population

The percentage of the population of Malaysia below the age of 15 years decreased to 27.2% compared to 32.1% in 2001. In contrast, the percentage of working age population (15 to 64 years) increased to 68.1% in 2010, from 63.4% in 2001. The proportion of population aged 65 years and over also increased to 4.7% as compared with 3.9% in

2001 (Table 5, Figure 1).

As noted, the prevalence of periodontitis increases as age increases, therefore a more aged population would also mean that there will be more people with periodontitis requiring treatment (Table 6).

The outcome of periodontal disease treatment will be more favorable if the condition is detected and treated early. Efforts are being made to ensure this occurs. The Oral Health Division of the Ministry of Health Malaysia has in place a School Dental Programme that provides care to children aged 7 to 18 years attending school. After this age oral health care is the individual's responsibility. As shown in Table 6, the highest percentage of moderate to severe periodontitis is in the age group 30 to 64 years old. Therefore, more programs need to be focused on the 20 to 30 year age group, so that the good outcomes achieved during the Incremental Dental Care Programme can be maintained in these individuals into older age. This will in turn provide the adults in the older age groups with better periodontal health. With less disease to treat, more time can be focused on prevention and early detection.

Awareness of periodontal health

The awareness of Malaysians towards periodontal health still needs to be improved, as is also the case in other countries. In the NOHSA survey in 2000, less than half of subjects reported making a dental visit within the last 2 years, and >50% only did so because they had dental problems. Only 60% of those with problems of the oral cavity perceived a need to see a dentist and 50% of those with problems of the oral cavity had not made a dental visit within the last two years (NOHSA 2000).

The Periodontics Specialist Services, under the Oral Health Division, Ministry of Health Malaysia, have made several

attempts to increase the awareness towards periodontal health amongst the population, through joint efforts with the Dental Public Health Specialists in the MoH itself and professional bodies like the Malaysian Society of Periodontology. Activities have included Periodontal Health Awareness Week, and setting up a program of dental staff nurses with enhanced training in periodontology and placing them in the community services. More efforts are planned and these will help improve this problem of awareness amongst the public.

The training of specialists

The number of specialists available to attend to the needs of the population is still far from ideal. With increased awareness, the demand for services will lead to the need for more manpower in specialists and the necessary auxiliaries. As local training places are very limited, more places for training abroad are needed. This is not as easy as it seems, as it will involve some administrative adjustment in terms of manpower planning and financial implications.

At present only three training institutes are available in Malaysia with the intake of candidates ranging from 8 to 12 every year. There will be one or two more training institutes in the near future, with an increase of 4 to 8 more places.

More places abroad are planned to be included for training of dental officers in the MoH Malaysia, in addition to the traditional areas of UK, Australia and New Zealand. This training abroad is quite expensive and proper planning is necessary to optimize the resources available at present.

With a population of more than 29 million and the present number of less than 100 trained periodontists (no official figures available), there is a need to increase the number of trained periodontists. As all other

institutions that provide periodontic specialist services are located in urban and specific areas only, the MoH will play a big role in ensuring these particular specialist services are available to all categories of the population and in all locations of its citizens.

Conclusion

As a key player in the provision of periodontic specialist services in Malaysia, the MoH must attend to these challenges and develop new strategies in providing new avenues for improvement. As heavily subsidized public health services increased the burden on the government, new solutions should be planned so the provision of healthcare will still be equitable and affordable to all Malaysians. These challenges, increasing lifestyle diseases, ageing population and low awareness of periodontal health amongst the population, although difficult and complex, can be managed. It must be addressed by all stakeholders including the Periodontic Specialist Services, MoH.

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Chapter 14

The Effect of Chronic Periodontitis on Diabetic Kidney Disease and its Possible Mechanisms in OLETF Rats

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Introduction

There is currently overwhelming evidence to support the concept that periodontitis contributes to a chronic inflammatory burden at the systemic level (D'Aiuto *et al* 2004, Craig *et al* 2003, Loos 2005). It has been reported that periodontitis and type 2 diabetes mellitus (T2DM) are independent risk factors for each other (Preshaw *et al* 2012). The greatest hazards of T2DM are long-term chronic complications. One such complication is diabetic nephropathy (DN) which is the leading cause of death of patients with T2DM. The main pathological changes of DN are the excessive accumulation of renal extracellular matrix-type IV collagen, resulting in glomerulosclerosis and renal interstitial fibrosis (Mason and Wahab 2003, Nerlich *et al* 1991, Ziyadeh 1993). Mechanisms leading to kidney lesions are very complex, involving several key cytokines and proteases (Liu 2011). TGF- β 1 plays a key role in renal interstitial fibrosis and glomerulosclerosis (Ziyadeh 2004). Matrix metalloproteinase MMP-2 (gelatinase A), MMP-9 (gelatinase B), and inhibitor TIMP-1 were found to be the key factors to regulate renal extracellular matrix balance (Tan and Liu 2012).

The effect of chronic periodontitis on chronic kidney disease has received attention in recent years (Ariyamuthu *et al* 2013). However, there have been few studies investigating the effect of periodontitis on DN (Shultis *et al* 2007). The aim of the current study was to test the effect of chronic periodontitis on renal function and renal structure in a diabetic animal model, and to explore the underlying mechanisms further.

Materials and methods

Establishing animal models

30 Otsuka Long Evans Tokushima Fatty (OLETF) rats, 4-week-old, male, spontaneously type 2 diabetic, were used as the test group (Test) and 20 Long Evans Tokushima Otsuka (LETO) rats, male, with the same germline and the same age but having normal glucose tolerance, were used as the control group (Control). Every 4 weeks, oral glucose tolerance was determined. Diagnosis of diabetes was based on the results of the oral glucose tolerance test, with a blood glucose peak >16.7 mmol/L and blood glucose >11.1 mmol/L in 120 minutes as the criteria. Meeting either one of the above criteria was recognized as indicating impaired glucose

tolerance (IGT).

At 36 weeks old, 26 OLETF rats, (IGT state), and 20 LETO rats were randomly divided into two parts respectively; Test CP(+) and Test CP(-), Control CP(+) and Control CP(-). The rats in Test CP(+) and Control CP(+) were ligatured bilaterally on the maxillary first and second molars with 3/0 silk sutures soaked in periodontal pathogens to induce experimental periodontitis for a period of 20 weeks, the remaining animals were left unligated as controls.

OGTT was conducted and body weight was measured every four weeks. Tail vein blood samples were obtained at 36 weeks and 46 weeks of age to evaluate the dynamic changes of serum insulin levels and HOMA-IR score. At 56 weeks of age, rats were sacrificed and samples were collected for further treatment and investigations.

Observing renal function and pathological changes of the tissues

Serum levels of albumin (ALB), total

protein (TP), urea (UREA) and creatinine (Cr) were analyzed. Renal pathological changes were investigated through routine HE staining and special staining of PAS, MASSON and PASM.

Glomerulosclerosis index (GSI) was calculated by analysing PAS stained sections (Takamitsu *et al* 2003). According to the percentage of sclerosis lesion area after PAS staining in each glomerulus, the score was recorded, and all the glomeruli on the slide were examined.

The criteria for scoring were as follows: normal = 0; $\leq 25\%$ = 1; 26%-50% = 2; 51%-75% = 3; $>76\%$ = 4. $GSI = (1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4) / (N0 + N1 + N2 + N3 + N4)$

MASSON staining was used to calculate the renal interstitial fibrosis index (RIFI). According to the percentage of interstitial lesion area after MASSON staining, the score was recorded, 10 different view scopes were examined and the average scores were taken.

The criteria for scoring were: normal = 0; mild ($\leq 25\%$) = 1; moderate (26%-

	Primer Set	Sequence	Product Length
GAPDH	Sense Antisense	5'-AGCCGCATCTTCTTGTGCAGTG-3' 5'-TGGTAACCAGGCGTCCGATACG-3'	96bp
MMP2	Sense Antisense	5'-CCCAAGTGGGACAAGAATCA-3' 5'-AAGCGTAGTGGAGTTACGTC-3'	128bp
MMP9	Sense Antisense	5'-GCTGTATGGCTTCTGTCCTAC-3' 5'-CGCCGGTACAGGTAGAGTA-3'	119bp
TGF- β 1	Sense Antisense	5'-TCAACTGTGGAGCAACACG-3' 5'-AGCCACTCAGGCGTATCA-3'	104bp
IVa3	Sense Antisense	5'-GTTTACCAGGATGCAATGGCT-3' 5'-TTCTCCGTCTTCTCCTTGAGC-3'	137bp
TIMP-1	Sense Antisense	5'-CGCTAGAGCAGATAACCACGA-3' 5'-GGCACAGCTACAGGCTTTAC-3'	100bp

Table 1. Primer Sequences of TGF- β 1, MMP-2, MMP-9, TIMP-1, type IV α 3 and GAPDH.

50%) = 2; severe (>50%) = 3. RIFI = $(1 \times N1 + 2 \times N2 + 3 \times N3) / (N0 + N1 + N2 + N3)$

Expression patterns of TGF-β1, MMP-2, MMP-9, TIMP-1, type IV collagen in kidney tissues

The expression of TGF-β1, MMP-2, MMP-9, TIMP-1, type IV collagen in the kidney tissue of four groups of rats were detected through immunohistochemistry and determined with ENVISION two-step semi-quantitative analysis. The levels of mRNAs of type IV collagen, TGF-β1, MMP-2, MMP-9, and TIMP-1 were tested with SYBR Green real-time quantitative PCR. Primer sequences of TGF-β1, MMP-2, MMP-9, TIMP-1, type IV α3 and GAPDH are shown in Table 1.

Results

Spontaneously diabetic and experimental periodontitis animal model established

OLETF reached the IGT period at 36

weeks age, showing typical clinical features of T2DM, such as polyphagia, polydipsia, polyuria and rapid weight gain. The AUCs (area under curve) of OGTT in Diabetic group were significantly higher than in Control group at various time points. Serum levels of fasting insulin (FI) and HOMA-IR in Diabetic group was significantly higher than that in Non-diabetic group. Diabetic CP(-) group and Diabetic CP(+) group had significantly increased HOMA-IR values with increasing age.

After ligation for 20 weeks, the rats showed typical periodontitis lesions, such as swollen gingivae, alveolar bone resorption and periodontal pockets. The rats in Test CP(+) group displayed more severe alveolar bone loss than the rats in Control CP(+) group as assessed by micro-CT three-dimensional reconstruction of the rat alveolar bone (Table 2).

Renal function and pathological changes

The levels of serum TP and ALB decreased

	CP(+)	CP(-)	P value
Control	14.340±1.064	9.663±0.845	0.004
Test	16.357±1.062	11.993±1.402	0.013
P value	0.041	0.039	

Table 2. Volume percentages of alveolar bone resorption.

	CP(+)	CP(-)	P value
Control	0.221±0.024	0.203±0.012	0.076
Test	2.261±0.169	1.692±0.057	0.000
P value	0.000	0.000	

Table 3. Glomerulosclerosis index of four groups.

	CP(+)	CP(-)	P value
Control	0.226±0.014	0.211±0.012	0.296
Test	2.272±0.047	1.783±0.035	0.000
P value	0.000	0.000	

Table 4. Renal interstitial fibrosis index of four groups.

	CP(+)	CP(-)	P value
Control	0.061±0.004	0.057±0.003	0.062
Test	0.118±0.003	0.096±0.004	0.000
P value	0.000	0.000	

Table 5. Expression of type IV collagen in four groups.

significantly in Test group over those in the group Control ($P < 0.01$). Consistently, serum levels of ALB and TP decreased significantly in Test CP(+) group than in Test CP(-) ($P < 0.05$), while in the Control groups, there was no significant difference with or without CP. Serum creatinine and urea levels were significantly higher in Test groups than in Non-diabetic groups ($P < 0.01$).

The histological features in Control CP(+) group were close to Control CP(-) group showing normal renal histological features. The kidney tissues of OLETF rats showed various pathological changes, including:

- The glomerular diameter increased, cortex narrowed and medulla widened, Bowman's capsule wall significantly thickened.
- Moderate to severe hyperplasia in mesangial matrix diffusively or nodularly, basement membrane thickening of glomerular capillary, focal glomerulosclerosis in some areas, glomerular capillary plexus lobulation, and vascular wall thickening of arteries in and out of glomerular surrounding.
- Hyaloid and vacuolar degeneration in

cortical tubular epithelial cells, dilatation of some tubular, protein-like substances or protein in lumen.

- Tubular epithelial cell desquamation, tubular degeneration and atrophy in some areas, scattered or gathering infiltration of lymphocytes and mononuclear cells and fibrosis in interstitial areas.

GSI and RIFI in OLETF rats was significantly higher than LETO rats ($P < 0.01$), and was slightly higher in the Control CP(+) group than in the Control CP(-) group (without significance), however, they were significantly higher in Test CP(+) rats than in Test CP(-) rats ($P < 0.01$) (Table 3, Table 4).

Expression of TGF- β 1, MMP-2, MMP-9, TIMP-1, type IV collagen in the kidney tissues

Type IV collagen was found in the glomerular basement membrane and mesangial matrix, blood vessel walls, tubular basement membrane, etc. The expressions of type IV collagen in four groups were semi-quantitatively analyzed and shown in Table 5.

	CP(+)	CP(-)	P value
Control	0.163±0.006	0.135±0.006	0.000
Test	0.265±0.006	0.227±0.006	0.000
P value	0.000	0.000	

Table 6. Expression of TGF-β1 in four groups.

	CP(+)	CP(-)	P value
Control	0.103±0.004	0.122±0.003	0.000
Test	0.053±0.002	0.055±0.003	0.085
P value	0.000	0.000	

Table 7. Expression of MMP-9 in four groups.

	CP(+)	CP(-)	P value
Control	0.187±0.007	0.223±0.009	0.000
Test	0.054±0.003	0.106±0.005	0.000
P value	0.000	0.000	

Table 8. Expression of MMP-2 in four groups.

	CP(+)	CP(-)	P value
Control	0.061±0.004	0.059±0.004	0.234
Test	0.137±0.006	0.115±0.004	0.000
P value	0.000	0.000	

Table 9. Expression of TIMP-1 in four groups.

TGF- β 1 was expressed in proximal convoluted tubules and distal convoluted tubules and the expression was weak in renal glomerulus. The expressions of TGF- β 1 in four groups were semi-quantitatively analysed and shown in Table 6.

MMP-9 protein was found to be mainly expressed in the proximal tubule and distal convoluted tubule epithelial cells and a small amount of expression was found in mesangial cells. The expression of MMP-9 in four groups were semi-quantitatively analyzed and shown in Table 7.

MMP-2 protein was expressed in the proximal tubule, distal convoluted tubule and glomerular visible trace. Reduced expression was also found in the area of inflammatory infiltration of inflammatory cells. The expressions of MMP-2 in four groups were semi-quantitatively analyzed and shown in Table 8.

The expression of TIMP-1 protein was found in proximal tubules, distal convoluted tubule, and renal interstitial and glomerular matrix regions. The expression of TIMP-1 in four groups were semi-quantitatively analyzed and shown in Table 9.

After real-time quantitative PCR analysis, it was shown that the expression trends of TGF- β 1, and collagen type IV, TIMP-1 and MMP-2 mRNAs in the four groups were consistent with the protein expression patterns respectively.

Discussion

Diabetic nephropathy is the most common cause of end-stage renal disease worldwide. Various theories have been proposed concerning the pathogenesis of diabetic nephropathy, including proteinuria, genetics, race, hypoxia, ischemia, and inflammation. Individually, the theories proposed thus far may not be able to explain the progression of diabetic nephropathy. Among these theories,

inflammation appears to be the critical pathway for the development and progression of diabetic nephropathy (Lim and Tesch 2012).

Diabetic nephropathy is characterized pathologically by mesangial expansion, glomerulosclerosis, tubular atrophy, and interstitial fibrosis. The importance of inflammation in the development and progression of renal fibrosis has been well documented (Kansaki *et al* 2012). Inflammation is regulated by the complex interactions of various factors, involving cytokines, chemokines, and adhesion molecules (Lee and Kalluri 2010). Renal inflammation is characterized by glomerular and tubule interstitial infiltration by inflammatory cells. The recruitment and activation of inflammatory cells, such as macrophages, play an important role in the production of inflammatory cytokines and profibrotic cytokines. The local accumulation of profibrotic cytokines in the microenvironment following kidney injury leads to the activation of extracellular matrix producing cells, which are essential for renal fibrogenesis.

Periodontitis is one of the most common inflammatory diseases worldwide, characterised pathologically by an inflammatory process extending from gingival margin to the deeper connective tissues inducing alveolar bone loss and periodontal ligament destruction. The connective tissue adjacent to the periodontal pockets is heavily infiltrated with a dense cellular infiltration of PMN, macrophages, B and T lymphocytes. It has been estimated that in patients with moderate to severe periodontitis and pocket depths of 6 to 7 mm, the surface area of inflammation and infection ranges from 8 to 20 mm² (Hujoel *et al* 2001). Thus, the inflamed and infected periodontal pockets containing subgingival biofilms can serve as a large reservoir from which the bloodstream is permanently flooded with bacteria, bacterial

products such as LPS and pro-inflammatory cytokines that could reach all parts of the body (Hayashi *et al* 2010). Periodontitis may expose the host to higher inflammatory challenges.

The OLETF rat has been established by selective breeding based on impaired glucose tolerance from a spontaneously diabetic rat with polyuria, polydipsia and mild obesity (Kawano *et al* 1992). The OLETF rat develops late onset hyperglycemia and has been considered as one of the best models for human T2DM. In this study with the OLETF rat model, it was shown that periodontitis promotes the degree and progress of insulin resistance in T2DM, thereby deteriorating the T2DM state, and conversely T2DM aggravates periodontal tissue destruction.

The results of our study indicate that chronic periodontitis may not cause significant changes in kidney function and histopathology in normal rats, however chronic periodontitis may be significantly associated with an increase in the renal interstitial inflammatory cell infiltration risk in T2DM. Moreover, chronic periodontitis may promote kidney glomerulosclerosis and renal interstitial fibrosis in T2DM, aggravating kidney disease and thereby deteriorating kidney function. The proposed underlying mechanism is that in the presence of T2DM, periodontitis stimulates fibrogenic core factor TGF- β 1, TIMP-1 expression and inhibits expression of extracellular matrix degrading enzymes MMP-2 and MMP-9, resulting in increased type IV collagen deposition in kidney tissue.

The results of this study suggest that the metabolic balance of the extracellular matrix in the kidney has been damaged by the increase of collagen production and the decrease of collagen degradation which occurs in T2DM. This imbalance is exacerbated in the presence of periodontitis, resulting in the accelerated glomerulosclerosis and renal interstitial fibrosis.

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Chapter 15

Periodontics in the Philippines: Then, Now and Beyond

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Introduction

The clinical practice of periodontics has seen many changes in the last two decades. The Asian Pacific Society of Periodontology (APSP) has been present throughout the many developments that periodontology has experienced and adopted through the years. Through its member countries, which have shared experiences and have opened avenues for networking, periodontology and the clinical practice of periodontics have become more appreciated in the region.

The Philippines, as a member country since the APSP's inception in 1993, has participated in all of its meetings, even hosting the 5th APSP Meeting in Cebu City in 2003. APSP member countries, whilst united with the common goal of uplifting the study and clinical practice of our specialty, experience diverse situations and conditions that by and large, highlight the great divide between the developed and underdeveloped/developing member countries. Adverse social, economic and political factors and even acts of nature have influenced the conditions in member countries, thereby producing differences in the evolution of periodontology in the region.

Our counterparts in Japan have made tremendous progress in the area of periodontal

regeneration using cell sheet engineering and stem cell biology (Ishikawa *et al* 2010, Ishikawa *et al* 2012). In Thailand, there have been studies carried out in the field of immunology and the same is true in Indonesia (Maduratna *et al* 2012, Mahanonda *et al* 2012). Although sporadic research showed that periodontal disease remains a concern both in clinical practice and in community health programmes, the Philippines has not been able to progress in periodontal research due to a lack of adequate facilities, trained and skilled staff and most of all, because of the absence of research grants and financial assistance (Monse and Yanga-Mabunga 2007, Yanga-Mabunga and Serrao 2003).

Periodontology in the Philippines

The Philippine Society of Periodontology has increased its membership from a group of five members in 1993, to 20 active members at present. In 1993 there were only two members with postgraduate training of two years and one who had completed a one year fellowship training in Japan. Presently, we have two members with PhDs in microbiology from Japan, six members with Masters degrees in periodontics, two members with certificate courses in periodontology from Denmark

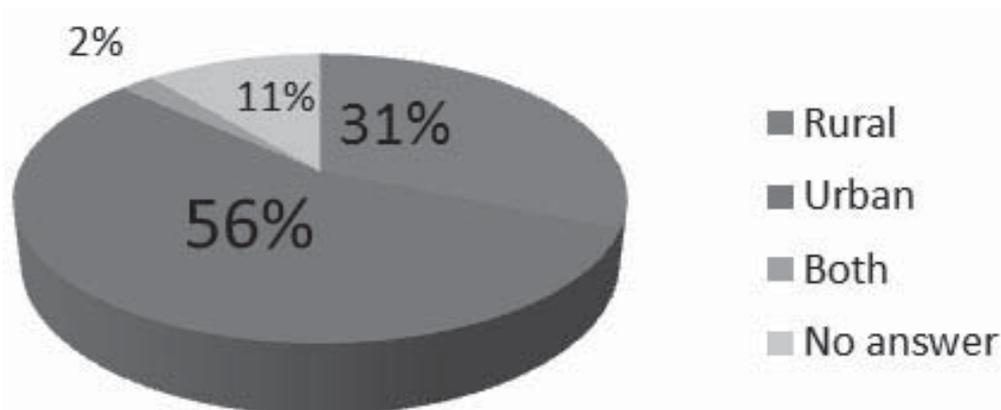


Figure 1. Distribution of practicing dentists in the Philippines by area.

and the USA, five members with training in periodontics of one year duration or less from Australia, Japan, France and Germany and six others with local training in periodontics. Most PSP members have clinical practices in Metro Manila, with only one member practicing outside of Metro Manila, specifically in Cebu City. Based on our last survey, an increase in the number of practicing periodontists, albeit slight and confined to Metro Manila, has been observed (Vergel de Dios *et al* 2010).

Given the number of dentists trained in periodontics and the growing population of the country, it would be logical to assume that the majority of patients in the Philippines who need periodontal care will be managed by general dental practitioners (GDPs). Periodontal disease is an emerging risk in general dental practice and it thus becomes necessary that GDPs should be updated on current clinical management philosophies as well as evidence-based dental procedures, in order to ensure the successful management of periodontal diseases and the maintenance of periodontal health (Jin 2010).

Changing trends of clinical periodontal practice?

The Philippine Society of Periodontology (PSP) recently conducted a survey, similar to

one undertaken in 2010, to assess the progress of periodontal practice in the country (Vergel de Dios *et al* 2010). The purpose of conducting the study was to provide information to the organization and related agencies so that data gathered can serve as guiding tools in creating programs that would translate to better dental service in the field of periodontics. The other aim was to determine the current status of the practice of periodontics and provide intervention measures based on the assessment of the results. It was observed that when members of the different dental specialty organizations in the country discuss their cases and research, and analyze product updates, it appears that the country is on par with others in the region in terms of progress in periodontics. Advancements in dental treatment procedures are already being implemented in clinical practice. The true picture of health progress however, is the degree by which patients with the disease are being serviced throughout the country.

A study conducted by the PSP during the nationwide dental convention held last April 2013 revealed that only a small percentage of Filipino dentists value participating in dental surveys. From the reported 7,293 attendees, only 415 (5.69%) participated, thus resulting in a very small sample size. Nonetheless, the results could still provide us with a

Area	Number of Practitioners	Percentage
Urban	230	55.42%
Rural	130	31.33%
Both area	9	2.17%
No response	46	11.08%
Total	415	100.00%

Table 1. Distribution of dentists in the Philippines by area of practice.

Perform periodontal treatment	Actual count	Percentage
Yes	321	77.35%
No	69	16.63%
No response	25	6.02%
Total	415	100.0%

Table 2. Percentage of dentists in the Philippines who perform periodontal treatment.

description of the Philippine scenario in terms of periodontal practice. As anticipated, the majority of dentists who participated in the survey have clinical practices located in urban areas (Figure 1). This urban bias is reflected by the figures which showed that 56% of respondents practice in the cities and urban centers (Table 1).

An interesting observation noted was that the practice of periodontics had seemingly remained unchanged over the last twenty years despite the greater awareness and the opening of two postgraduate programs in periodontology by two private dental universities. A large percentage of GDPs (77.35%) claim to perform periodontal treatment procedures. These however, were mainly scaling and prophylaxis and non-surgical debridement procedures (Tables 2 & 3).

The reasons cited for not performing periodontal surgical procedures were basically the same as seen in previous years (Vergel de Dios *et al* 2000, Vergel de Dios *et al* 2010). Personal choice and the lack of training and skill as well as the lack of proper instruments are among the main reasons given by the present respondents (Table 4).

Implant therapy in the Philippines

Implant therapy has gained greater acceptance amongst dental practitioners in the Philippines. This is evidenced by the proliferation of implant training courses offered by dental implant companies in the country. This may be part of a sales and marketing strategy to introduce their products to the profession. Pros-Apac Company®, a local distributor of Straumann™ dental

Type of procedure	Number of Practitioners	Percentage
Scaling and prophylaxis (SP) only	147	45.79%
SP and non-surgical root planing (NSRP)	135	42.06%
SP, NSRP, periodontal surgery (PS)	15	4.67%
All procedures including regenerative procedures	12	3.74%
SP and periodontal surgery	5	1.56%
All except periodontal surgery	4	1.25%
Non-surgical root planing only	2	0.62
SP and regenerative procedures	1	0.31%
Total	321	100.00%

Table 3. Distribution of periodontal treatment procedures performed by practitioners in the Philippines.

Reason	Frequency	Percentage
Personal choice	19	27.54%
All of the reasons	14	20.29%
Lack of skill and training	10	14.49%
Lack of adequate instruments	9	13.43%
Lack of both instruments and skill	5	7.25%
Lack of adequate knowledge	4	5.80%
Lack of adequate knowledge, skill, instruments	4	5.80%
Other combination of reasons	4	5.80%
Total	69	100.00%

Table 4. Reasons for not including periodontal surgical procedures in clinical practice.

implants, has had a total of 200 participants in their training programs. Another implant company, Alpha-biotec®, has conducted training sessions with 150 attendees. DENTSPLY® introduced its implant system Ankylos™ in the Philippines in 2009 through training sessions as well. To date, there have

been 32 attendees with four more currently attending training seminars.

The cost of implant therapy, being a major consideration in most countries in Asia, saw the entry of implant systems developed and fabricated in Korea and Israel. Training sessions were conducted by these groups as

Performs implant therapy	Number of practitioners	Percentage
No	378	91.1%
Yes	29	7.0%
No response	8	1.9%
Total	415	100.00%

Table 5. Percentage of dentists in the Philippines performing implant therapy.

Institution	Number of practitioners	Percentage
Centro Escolar University	107	25.78%
University of the East	54	13.01%
University of the Philippines	51	12.29%
De Ocampo Memorial College	28	6.75%
Manila Central University	24	5.78%
South Western University	26	6.27%
University of Baguio	14	3.37%
National University	13	3.13%
Unciano	11	2.65%
Cebu Doctor’s College	6	1.45%
Iloilo Doctor’s College	6	1.45%
Others	22	5.30%

Table 6. Institution where DMD degree was earned.

well. Neobio-tech™ has had a total of 62 participants in the nine training cohorts run to date. MIS™ dental implants, also distributed locally by Pros-Apac Company®, on the other hand, have had 40 participants in the past four series of training sessions.

The College of Dentistry at the state university, University of the Philippines Manila, has conducted the only university-based basic dental implantology course in the country. Only two training programs

have been conducted so far with seventeen participants completing the program. A third program, with six attendees is ongoing. A private university, Centro-Escolar University, through its College of Dentistry, has previously conducted a training program in coordination with the 3i™ implant system but has recently discontinued the program.

The interest in dental implants has prompted the formation of two organizations for members practicing dental implantology.

The Philippine College of Implant Dentistry claims to have at least 30 plus members. They are also recognized as the Philippine Chapter of the Asia Pacific Association of Implant Dentistry. Another group called the Philippine Association of Implant Dentistry claims to have at least 200 members. Comparing these with the number of dentists claiming to do procedures beyond scaling (Table 3), certainly one can deduce that periodontal procedures are not important aspects of the GDP's dental treatment regimen compared to dentists claiming to have participated in implant training courses and inserting dental implants. Our recent survey showed that 7% of respondents perform implant therapy (Table 5) as compared with only 4.67% claiming to perform periodontal treatment procedures beyond scaling and prophylaxis (Table 3).

A probable reason for the observation of low interest in performing periodontal procedures is the reality that in all of the private dental colleges, where most of our respondents graduated from, periodontics as an undergraduate course is only a single semester offering (Table 6). Only the University of the Philippines, the only state university in the country, has two semesters of undergraduate periodontics.

Only two private dental colleges currently offer postgraduate training courses in periodontics; Centro-Escolar University College of Dentistry and University of the East College of Dentistry. We can thus infer that there is an obvious dearth of training programs for undergraduate students of dentistry and even practicing general dentists in the Philippines who may be interested in pursuing postgraduate periodontics.

Looking beyond to the future

What can be done? It is obvious that the Philippines needs to exert a tremendous effort and mobilize resources to uplift the present

status of periodontology in the country. The PSP needs to set the trend and enable its leaders to lobby for educational reforms in the dental schools. It is a large job, the existence of which was recognized even in the PSP's early years. While it seems to be a daunting task, small steps can surely help so that the periodontist's primary role of disease control can be addressed (Corbet 2010).

Seminar workshops in periodontics have been regularly offered by the Philippine Society of Periodontology since 1993. About 25 seminar workshops have been conducted so far, with a total of approximately 200 participants to date. The University of the Philippines College of Dentistry started its short course offering in 2009 and has had a total of 50 participants. We believe that this has helped in increasing GDPs recognition of the importance of incorporating periodontics in their clinical practices. We likewise believe that such training programs have allowed general dentists to better service their patients by increasing their awareness of the disease process, enhancing proper diagnosis, strengthening treatment protocols and referral systems. Much more however needs to be done.

The changes that the Association of South East Asian Nations member countries are currently discussing with regards to practice of dentistry within the region provides a good opportunity for the Philippine Society of Periodontology to gather and motivate academic staff members of dental faculties and GDPs on the practice of periodontics. Discussions can also occur with the Philippine Dental Association, as well as its local chapter and affiliate societies, regarding the standard of care in periodontal diagnosis and treatment. Implementation should be considered as a priority by the leadership of dental organizations. This measure could then help revive the interest of GDPs to update themselves in the specialty field and possibly

even gain additional training.

In addition to this, the members of the society could actively seek opportunities to lecture in various dental groups nationwide, re-emphasizing the basic concepts of periodontology and providing evidence on current treatment trends and advances. Integrating these with lectures on implants, which are currently much sought after, could be a good strategy to reach a greater percentage of dentists in this country.

Conclusion

Conducting surveys such as this one provides important information and enforces the PSP's committed goal of addressing the oral health needs of the country and making a difference. It may mean going beyond the comforts of private practice and stepping out to the countryside to be able to see the improvements which are so desperately needed.

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Chapter 16

Dental Implants - Maintenance Free?

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Introduction

Dental implants have been indicated for our patients since being proven as a viable and reliable replacement for missing teeth. The American Academy of Implant Dentistry reported that 5.5 million dental implants had been placed in the United States by 2006. Implant survival rates have been recorded to be as high as 95.2% for implant supported single crowns and thus many dentists will be inclined to place implants in their patients in the future (Jung *et al* 2012). This appears to be the global trend, as dental implants become more popular with both dentists and patients.

However, as more implants are being placed, there is a concomitant rise in the number of complications, both biological and mechanical. Are these prostheses maintenance-free or do they require regular maintenance long-term? Whilst dental caries will not be an issue with a dental implant, instead there is peri-implant disease, for which we must be prepared to meet the challenge of prevention, diagnosis and management. This

article presents an overview of the potential biological complications that may follow implant therapy over time.

Biofilms on dental implants

Biofilms readily develop on implant fixtures and prostheses. Microorganisms in these biofilms have the potential to cause tissue inflammation, thereby resulting in peri-implant mucositis which subsequently has the potential to develop into peri-implantitis.

Elter and co-workers (2008) analyzed 15 Nobel Biocare healing abutments placed in 11 patients for 14 days (Table 1). The investigators concluded that biofilm was detected on all abutments regardless of surface type. Rough surfaces had more surface area covered by the biofilm. They found that 17.3 +/- 23.1% of supragingival surfaces and only 0.8 +/- 1% of subgingival surfaces was covered with biofilm.

Fürst and co-workers (2007) sampled bacterial plaque from implants and neighboring teeth taken at different time

Surface	Supragingival	Subgingival
Sandblasted (Ra 0.9µm)	47.4% +/- 32.4%	1.3% +/- 1.2%
Ground (Ra 0.4µm)	18.6% +/- 31.4%	0.6% +/- 0.8%
Acid-etched (Ra 0.3µm)	15.6% +/- 18.7%	0.6% +/- 1.3%
Untreated (Ra 0.2µm)	5.7% +/- 14.4%	0.6% +/- 1.2%

Table 1. Biofilm levels found on Nobel Biocare healing abutments (Elter *et al* 2008).

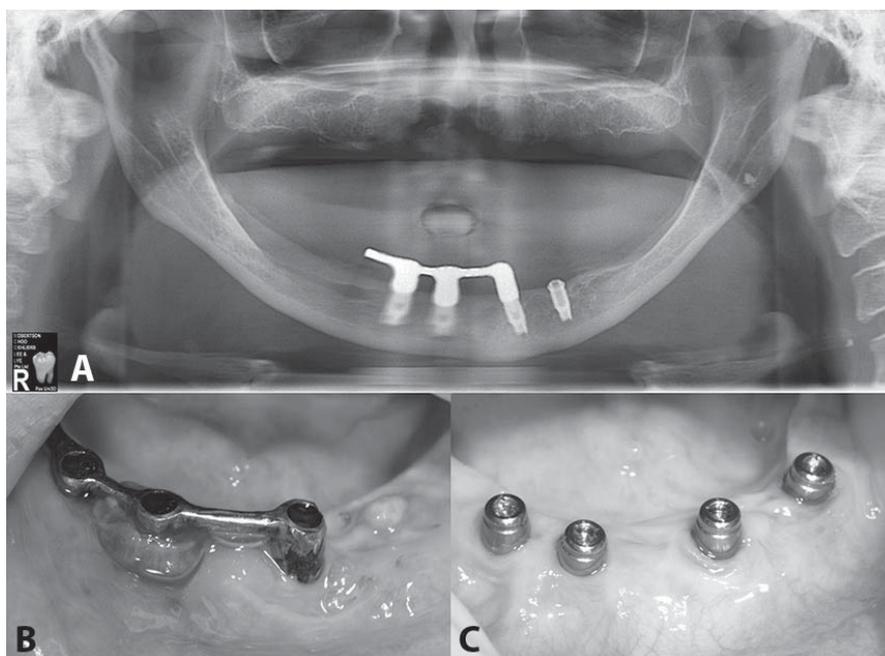


Figure 1. Peri-implantitis around multiple implants. (A) Radiograph prior to treatment. (B) Sites prior to treatment. (C) Sites following treatment.

points: before the surgery, 30 minutes post-surgery, and at 1, 2, 4, 8 and 12 weeks after surgery. The subgingival plaque samples were analyzed using checkerboard DNA-DNA hybridization. The investigators found that levels of pathogens found at implant sites were half those around teeth. They discovered that biofilms began forming as early as 30 minutes after insertion of the fixtures. The early colonizing bacteria species consisted of *S. aureus*, *A. actinomycetemcomitans*, *P. micros* and other Gram negative facultative cocci. 29 of the 40 species that can be identified by the checkerboard method were more common around implants than teeth, although higher amounts of bacteria were found around teeth. At 12 weeks, the bacteria around implants included *P. gingivalis*, *T. forsythia* and *T. denticola*. The colonization patterns differed between tooth and implant surfaces and the latter consisted of fewer Gram-negative anaerobic and facultative rods. In healthy sites, the bacterial composition appears stable.

The key important finding in these studies is that putative periodontal pathogens can be present from the time of insertion and hence one cannot assume that it would take a long time for these pathogens to begin to colonize the implant surfaces. Once periopathogenic bacteria establish their presence in biofilms around peri-implant surfaces it has the potential to induce an inflammatory response around the peri-implant soft tissue, leading to peri-implant disease. There are two forms of peri-implant disease; peri-implant mucositis and peri-implantitis, both of which are characterized by an inflammatory reaction in the tissues surrounding an implant.

Peri-implant mucositis

Peri-implant mucositis is described as inflammation confined to the soft tissue surrounding a dental implant, with no signs of loss of supporting bone following initial bone remodeling during healing (Figure 1).

Pontoriero *et al* (1994) conducted a study in which 20 partially dentate patients were treated for moderate to advanced periodontitis with oral hygiene instruction, scaling and root planing and periodontal surgery. IMZ implants were then placed in the posterior regions and three months following surgery the prosthetic abutments were installed. These patients were then monitored for another two months and baseline data were obtained. The patients were then asked to refrain from oral hygiene practices for 21 days. After this test period, periodontal parameters (Plaque Index, Gingival Index, Sulcus Bleeding Index, Pocket Probing Depths and Recession) for both implants and teeth were obtained and submucosal/subgingival plaque samples were taken. Optimal oral hygiene was reinstated and the patients were reviewed three to six months thereafter. There was no statistical difference found between implants and teeth with regards to the clinical parameters at any of the time points. During the period of no oral hygiene, a cause-effect relationship between plaque accumulation and inflammation of the mucosal margins around implants and also the gingival margins around teeth was observed. This study demonstrated that the development of plaque-induced peri-implant inflammation can be reversed following plaque removal and good oral hygiene. Clinically, it would appear that gingivitis and peri-implant mucositis are similar.

Lang and co-workers (2011a) studied the differences between peri-implant mucositis and gingivitis. Not surprisingly, host responses to biofilm do not differ on either teeth or implants. The two diseases are not fundamentally different from both a pathogenesis and diagnosis point of view too. Since these diseases are pre-cursors of the more detrimental pathological conditions of periodontitis and peri-implantitis, it is important that peri-implant mucositis should be diagnosed and managed appropriately so

as to reduce its potential progression to peri-implantitis.

Salvi *et al* (2012) conducted a study on 15 subjects who were placed on no oral hygiene for three weeks, after which they were placed on optimal plaque control for another three weeks. Periodontal parameters along with bacterial samples and inflammatory cytokines were also studied during this period. Implant sites showed significantly higher Gingival Index scores and crevicular fluid levels of matrix-metalloproteinase-8 (MMP-8) were significantly higher than at tooth sites.

They found that peri-implant soft tissues had a stronger inflammatory response to biofilms compared to their gingival counterparts. Even when optimal plaque control was reinstated, the gingival and peri-implant mucosal tissues did not return to healthy pre-experimental levels by the end of the study. They concluded that the peri-implant tissues may take more than three weeks to heal and recover from peri-implant inflammation.

Results indicate that peri-implant mucositis is an apparently reversible disease and clinically similar to gingivitis. Hence, when oral hygiene is lacking the clinician can expect to observe plaque and calculus accumulation around the implant prosthesis and abutment causing peri-implant inflammation, bleeding on probing and possible slight recession of mucosal tissue. However, there are no signs of radiographic bone loss around the affected implants in peri-implant mucositis.

Peri-implantitis

Peri-implantitis is characterized by changes in the levels of crestal bone, in conjunction with bleeding on probing with or without concomitant deepening of peri-implant pockets with presence of pus being a common finding (Lang and Berglundh 2011a) (Figure 2).

An experiment on the pathogenesis of peri-

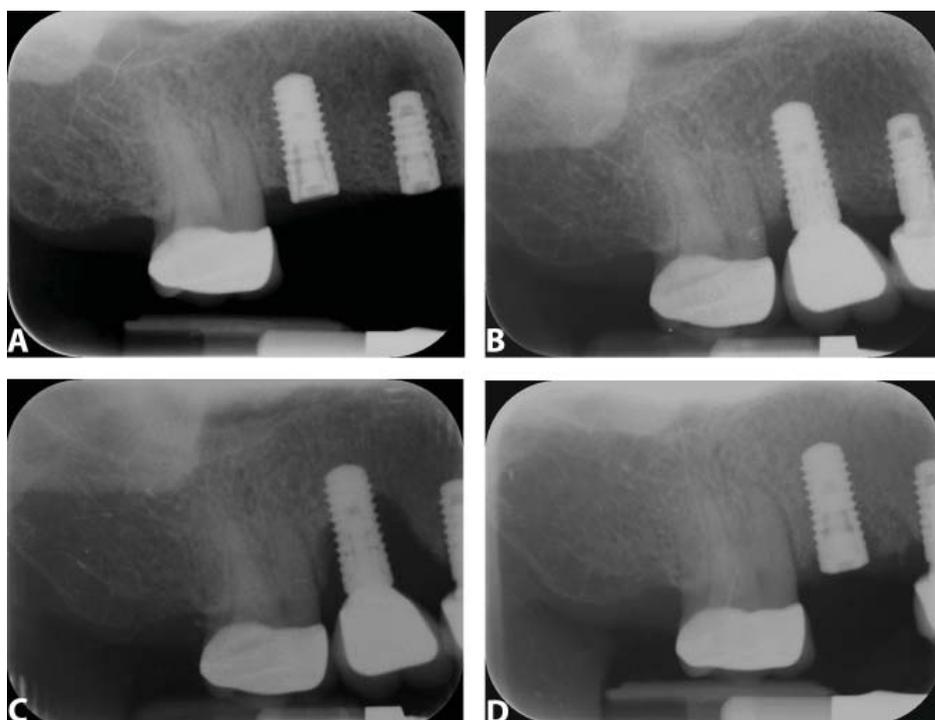


Figure 2. Treatment of peri-implantitis using GBR. (A) Implant before restoration. (B) Implant 2 years after restoration. (C) Peri-implantitis 3 years after restoration. (D) Peri-implantitis treated with GBR.

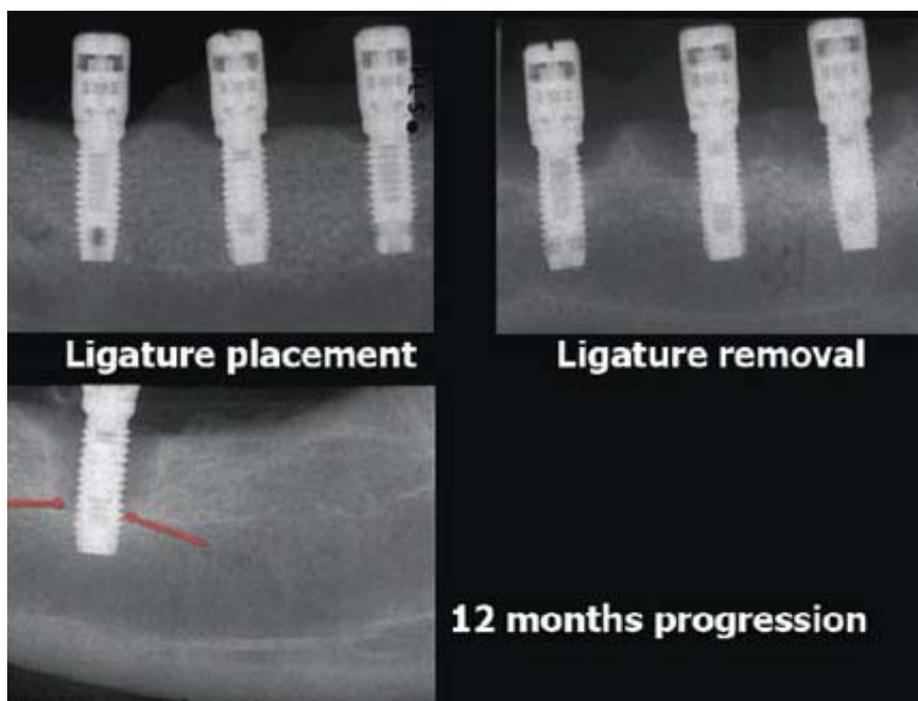


Figure 3. Experimental ligature placement around implants (Zitzmann *et al* 2004).

implantitis was conducted by Lang and co-workers (1993) on Cynomolgous monkeys. 90 days after extraction of teeth, implants were placed. Following their osseointegration (60 days later), ligatures were placed around these implants for eight months after which the animals were sacrificed. Plaque Index, Gingival Index, Clinical Attachment Level data were collected. Subtraction radiography, microbial analysis and histologic analysis were conducted. The investigators found similar findings between ligated teeth and ligated implants. The ligature induced plaque accumulation caused disease which resulted in tissue inflammation and bone loss around the teeth and dental implants.

Another animal study was conducted by Zitzmann and co-workers (2004) in which ligatures were placed around implants in five dogs after abutment connection at four months post-surgery. After five months, experimental peri-implantitis was induced which caused 30 to 40% bone loss. The ligatures were then removed and plaque formation was allowed to continue for 12 months. Radiographs were taken before and after the experiment (Figure 3). Biopsies were obtained for histometric and morphometric examinations. It was clearly demonstrated that ligature induced peri-implantitis may continue to progress after removal of ligatures. From the histological findings, some sites displayed encapsulation of the inflammatory lesion in the peri-implant tissue after ligature removal. The majority of sites however, exhibited additional loss of supporting bone during the 12 months of plaque accumulation.

As dental implants have developed the surface design of implants has changed from the relatively smooth machined type to highly textured roughened surfaces preferred by both dentists and manufacturers. By the turn of the century, nearly all implant design had roughened surfaces so as to increase bone to implant contact for better osseointegration.

This design trend towards a roughened surface is now revealed to be a double-edged sword. In cases of spontaneous progression of peri-implantitis, it was found to be more pronounced in implants with roughened surfaces compared to smooth surface implants (Berglundh *et al* 2007).

Patients at risk of peri-implant disease

Are patients with a history of periodontal disease at risk of peri-implant diseases? Ong and co-workers (2008) conducted a systematic review to assess the outcomes of such patients. They looked at studies up to 2006 and included nine studies in the review although they had a medium to high risk of bias. This review concluded that patients who have been treated for periodontitis may experience more complications around implants like peri-implant mucositis and peri-implantitis and implant loss.

Karoussis and co-workers (2003) conducted a prospective cohort study, which showed that implants placed in periodontitis and non-periodontitis patients had a survival rate of 90.5% and 96.5% respectively after ten years. This tends to suggest that periodontitis patients may have an increased risk of implant loss.

A retrospective study on peri-implantitis was conducted by Roos-Jansäker and co-workers (2006). Here 294 patients had implants in function for 9 to 14 years. They found that progressive bone loss was observed in 16% of patients who had no periodontitis, while patients treated for periodontitis had a prevalence of 22%. This study seems to suggest that patients with prior experience of periodontitis had a higher risk of developing peri-implantitis.

Mengel *et al* (2007) followed five patients treated for generalized aggressive periodontitis monitored for 10 years after dental implant placement. Implant survival

rate was 83% in this group when compared to five periodontally healthy patients who had a 100% survival rate. The periodontal patients also displayed more bone loss in the first and subsequent nine years.

Taken together these findings suggest that periodontal patients are at higher risk of developing peri-implant disease and/or loss of dental implants. However, it may not be entirely true for implant patients with a history of periodontitis to have increased risk of implant complications. A paper by Pjetursson and co-workers (2012) studied 70 patients with 165 dental implants on supportive periodontal therapy with follow up periods ranging from 3 to 23 years (mean of 7.9 years). 58 of the patients had supportive periodontal therapy at the University while 12 had their maintenance in a private clinic. Only six implants were lost out of the 165 implants, giving a cumulative implant survival of 95.8%. They concluded that those with residual pockets of >5 mm at the completion of active periodontal therapy (periodontitis susceptible), had a significant risk for development of peri-implantitis and implant loss. However, they observed that the prevalence of peri-implantitis was lower in the group with well-organized supportive periodontal therapy.

A more recently published study by Marrone *et al* (2013) looked at 103 Belgian patients with 266 implants of a minimum of five years in function (mean of 8.5 years). They examined the prevalence and risk factors for peri-implant disease. They observed a prevalence rate at the patient level of 31% for peri-implant mucositis and 37% for peri-implantitis. Their results demonstrated that subjects older than 65 years had OR of 1.39 and those with active periodontitis had OR of 1.98 while more significantly, fully edentulous patients had OR of 5.56 for peri-implantitis. The authors commented that considering more and more implants are placed every year, this information warns us that we would likely

have to deal with greater incidences of peri-implantitis in the coming years.

Other factors in peri implantitis (excess cement)

Wilson (2009) used a dental endoscope (DentalView) to study 39 consecutive patients displaying signs of peri-implant disease. There were 20 control implants and 42 test implants with signs of peri-implant disease. He observed that in 34 implants there was excess cement was found, while no excess cement was observed in all 20 control implants. The excess cement was removed from the 34 affected implants and in all sites except for one, excess cement implant site was evaluated 30 days later. No clinical or endoscopic signs of inflammation were observed in 25 of the 33 excess cement implant sites. The author noted that it was surprising that initial signs of inflammation and/or peri-implant disease were observed from as early as four months to more than nine years after implant restorations was cemented. The study concluded that excess cement was associated with signs of peri-implant disease in 81% of the cases. Resolution of clinical signs of inflammation was observed in 74% of the implants with excess cement 30 days after the excess cement was removed. This study is particularly relevant today when more clinicians prefer to use a cement-retained implant prosthesis (Figure 4).

Dental implant maintenance

It is vital to assess the risk profile of our patients before commencement of dental implant therapy. The following evaluation will be helpful to ascertain the long term risks for peri-implant disease:

1. A detailed history of the patient's periodontal disease and current status should be considered.

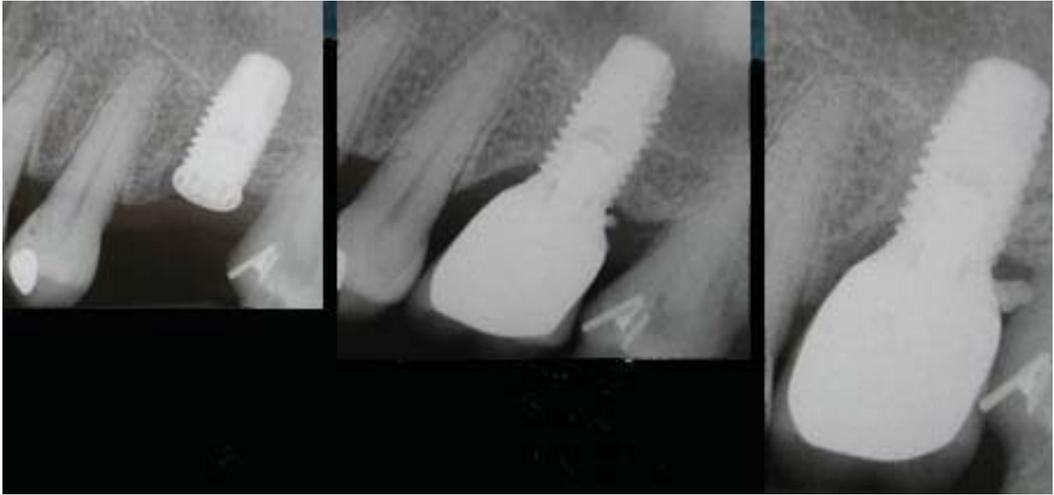


Figure 4. Excess cement around implant fixtures.

2. Age, since older patients may have difficulty in maintaining oral hygiene plus the likelihood of interrelated impacts of systemic conditions and side-effects of medications.
3. Edentulousness.
4. The choice in implant types, abutment profiles and final prosthesis design facilitates cleaning of the implant abutment and prevention of plaque accumulation.

In addition, the level of compliance in maintaining good oral hygiene and preventive maintenance visits are a good indication of how well implants will perform after they are inserted.

Maintenance protocol

- Regular screening for peri-implant mucositis and implantitis. Look for signs and symptoms with evaluation of peri-implant pocket charting, bleeding upon probing, plaque and calculus accumulation.
- Review of home care and patients' compliance of oral hygiene measures.
- Prophylaxis tailored specifically for implants and teeth.
- Periodic (annual or bi-annual) radiographic

assessment.

Regular supportive peri-implant care has to be carried out by the dentist and patients should be instructed on how care should be carried out at home, since implants and the prosthesis would be different in their morphology and profile as compared to their natural teeth. The management of the peri-implant tissues, specifically the keratinized gingiva of the neighboring teeth and the mucosal tissue around the implants, has to be emphasized. Cleaning of the implant abutments is important and the use of appropriate instruments is needed to properly perform such work without damaging the implant surfaces and surrounding tissues. It is important to stress that preventive maintenance is vital for long-term success of dental implants.

Conclusion

Dental implants have been proven to be a viable and reliable treatment option in replacing missing teeth. However, in the last two decades, research has revealed that all implants appear to be susceptible to peri-implant mucositis and patients with past

history of treated periodontitis are at a higher risk that is comparable those who have active periodontal disease (AAP 2013).

1. Primary prevention needs to be emphasized to both the patient and clinician so as to avoid inflammation and infection. This can be done with steps to prevent plaque buildup.
2. Cause-related therapy has to be carried out to remove bacterial deposits, noting that peri-implant mucositis can be reversed.
3. Alteration of local microbial ecology such that it is difficult or impossible for the growth of potential pathogens is critical to prevent disease recurrence.

With proper maintenance and risk profiling, appropriate long term management strategies can be developed by dentists to help patients with implants remain in function without complications. Thus for dental implants, maintenance is a key factor to long term success.

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Chapter 17

The Application of *Andrographis Paniculata* Gel as an Adjunct in the Treatment of Chronic Periodontitis: Clinical Effect

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Introduction

Periodontitis is an infection occurring in the tissues which support teeth. The presence of subgingival plaque represents the principal etiologic factor involved in the initiation and progression of inflammatory periodontitis (Offenbacher 1996, Zambon 1996). The treatment of various forms of periodontitis is based on thorough debridement of the root surfaces to remove calculus, plaque and subgingival microorganisms. Scaling and root planing is also used for controlling the progression of periodontal diseases. However, this technique requires access to, and visibility in, the areas to be treated. In some cases, complete subgingival plaque and calculus removal is not achieved and is sometimes ineffective (Rateitschak *et al* 1992). There are limiting factors such as deep pockets, furcation areas and biofilms on the cementum and exposed dentin (Adriaens *et al* 1987, Caffesse *et al* 1986, DeSanctis and Murphy 2000, Fleischer *et al* 1989, Loos *et al* 1988, Rabbani *et al* 1981, Waerhaug 1978). In addition, it may not be possible to eradicate bacterial species that can reach epithelial cells and subepithelial connective tissues of the periodontium (Adriaens *et al* 1987, Danser *et*

al 1996). In order to eliminate the remaining bacteria, systemically or locally administered antimicrobial agents are used as an adjunctive treatment to improve the management of periodontitis (Kornman 1993, Mombelli and Samaranayake 2004).

Controlled-release local drug delivery systems are designed to slowly release a drug in the treatment site for prolonged drug availability and extended drug action. Recently a local drug delivery system containing an antimicrobial traditional herb, *Andrographis paniculata*, has been developed as an adjunct to scaling and root planing (Komwatchara 1996, Narakorn 1999). *Andrographis paniculata* gel (AP gel) has been shown to reduce probing depth and improve coronal radiopaque fill in the AP gel-treated sites at three and six months (Sirirat and Rojanapanthu 2003). Moreover, the proportion of black-pigmented anaerobes was significantly reduced in the AP gel-treated sites, but not in the metronidazole gel-treated sites (Atsawasuwan *et al* 1998). AP gel also demonstrated a more consistent increase of cocci and decrease in percentage of motile rods when compared to 2% minocycline gel during a period of three to four months (Boonchaipanichwatana 2001). A clinical

study investigating the concentration of AP gel in gingival crevicular fluid showed that andrographolide could be detected in the periodontal pocket 24 hours after application in only one tooth from 15 teeth at a concentration of 201.964 $\mu\text{g/ml}$ (Kuphasuk *et al* 2004). Kuphasuk *et al* (2008) examined the concentration of andrographolide in gingival crevicular fluid, saliva and blood plasma after application of AP gel into the periodontal pocket following treatment. They found that andrographolide could be detected in gingival crevicular fluid in the first hour, however at 24 hours only two cases still had andrographolide at a concentration of $0.969 \pm 2.9638 \mu\text{g/ml}$. Andrographolide in saliva was found up to 1 and a half hours after application at the concentration of $0.2740 \pm 0.5354 \mu\text{g/ml}$. It could not be detected in the blood plasma. The study therefore concluded that AP gel did not remain in the periodontal pocket for very long.

This study was developed to evaluate the effectiveness of AP gel as an adjunct to scaling and root planing. The clinical parameters, namely probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI) and bleeding on probing (BOP), were assessed after treatment with scaling and root planning (SRP) plus AP gel, compared to SRP applied with gel base and SRP only.

Materials and methods

Patient selection

32 patients diagnosed with chronic periodontitis aged 30 to 65 years participated in this study. Patients included in the study were in good health with no systemic diseases and were required to have at least three single-rooted teeth of the same tooth type in different quadrants. Initial probing pocket depth was required to be 5 mm or more, bleeding on probing had to be present and there needed to be radiographic evidence of bone loss.

Pocket depths among the teeth should not have differed by more than 1 mm. Patients were excluded from the study if they had received periodontal therapy or antibiotics within 6 months prior to the study. Pregnancy, smoking and antibiotic treatment was to be avoided during the study. All volunteer patients signed the informed consent form after receiving detailed information about the clinical trial. Ethical approval was obtained from the Committee on Human Rights Related to Human Experimentation, Mahidol University.

Clinical procedures

Prior to the study, baseline data were collected from all patients on day 0, including clinical and microbiological parameters. Using randomized controlled trials, the experimental teeth in each patient were randomly assigned into three treatment groups. The first treatment group received scaling and root planing with AP gel. The second and third treatment groups received scaling and root planing with gel base and scaling and root planing only, respectively. Scaling and root planing was performed with hand instruments (Gracey curettes, HuFriedy®, Chicago, IL, USA) and ultrasonic scalers (Sonicflex, KaVo, Biberach, Germany) until all supra and subgingival root surfaces felt hard and smooth. The pockets were then gently irrigated with 2 ml of 0.9% sterile normal saline solution in a syringe with a 21 gauge blunt needle. The AP gel or gel base was gently applied subgingivally into the pocket around the teeth until the pocket was filled to the gingival margin. The excess gel was removed by sterile cotton pellets and a suction device. The teeth receiving scaling and root planing only served as the controls. Patients were asked to avoid rinsing, drinking and eating for 1 hour after gel application and were recalled for the same treatment at 1, 2 and 3 weeks. The parameters were assessed at 1 and 3 months after treatment. The patients

received oral hygiene instructions, full mouth scaling, root planing and oral prophylaxis. All treatments were performed by the same periodontist and the clinical parameters were collected by another periodontist.

Four clinical parameters were studied. The probing pocket depth was measured to the nearest millimeter of the distance from the gingival margin to the base of the pocket with a standard periodontal probe (UNC 15, Hu-Friedy®, Chicago, IL, USA). The clinical attachment level was measured as the distance from the cemento-enamel junction to the base of the pocket with the same periodontal probe. Bleeding on probing was assessed by gentle probing into the sulcus of the gingival crevice using the same periodontal probe. If bleeding occurred within 10 seconds a positive result for BOP was recorded. All four tooth surfaces were recorded as + or – for BOP. Gingival index, indicating gingival inflammation, was scored from 0 to 3 on the facial, lingual and mesial surfaces of all teeth.

Statistical analysis

Variables were the scores for probing pocket depth, clinical attachment level, bleeding on probing and gingival index. The data were analyzed using the statistical package SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). The different clinical evaluation of the three treatments were compared by Kruskal-Wallis test and further analysis was undertaken using Multiple Comparison for nonparametric statistic, except bleeding on probing which was analyzed by Fisher's Exact test. For within comparison of each treatment, the difference between baseline and each follow up visit was analyzed by Friedman test and further analyzed by Multiple Comparison for pairwise comparison. However, Cochran'Q test was used to assess the significant difference of bleeding on probing over time within

treatment and McNemar test was used for pairwise comparison. The level of statistical significance was considered at $p < 0.05$ in all tests.

Results

32 systemically healthy and non-smoking subjects with chronic periodontitis, comprising 7 males and 25 females aged 34 to 66 years (mean age 50.31 ± 8.09 years) were recruited in the study. These subjects were employees of Prasat Neurological Institute. No subjects were excluded during the study. No side effects occurred in any of the subjects and no complaints were received about the bitter taste after AP gel application.

Clinical evaluation

Four clinical parameters were collected to evaluate and compare the clinical changes of each treatment group. These were probing pocket depth, clinical attachment level, gingival index and bleeding on probing.

Probing pocket depth (PPD)

Mean PPD values at baseline, 1 month and 3 months from the treated sites of three treatments are shown in Table 1. Mean PPD at baseline of all treatments (SRP plus AP gel, SRP plus gel base, and SRP only) were not significantly different, with a mean range of 5.41 to 5.50 mm. However, significant differences of PPD between treatments at 1 month and 3 months were found ($p < 0.05$). Multiple comparison was used to show the significant difference of PPD between SRP plus AP gel and SRP plus gel base and SRP plus AP gel and SRP only at 1 and 3 months ($p < 0.05$) as shown in Table 1. After treatment, all groups showed the significant reductions of PPD at 1 month and 3 months compared to baseline ($p < 0.05$) (Figure 1).

Treatment	Baseline	1 month	3 months
SRP+AP gel	5.47+0.72	3.09+0.89	2.78+0.71
SRP+gel base	5.50+0.76	3.63+1.09	3.47+1.11
SRP only	5.41+0.71	3.53+1.02	3.53+0.88

Table 1. Mean and standard deviation of probing pocket depth (mm) of all treatments at different time intervals. *Statistical significance of difference between the treatments ($p < 0.05$) (SRP = scaling and root planing, AP gel = *Andrographis paniculata* gel)

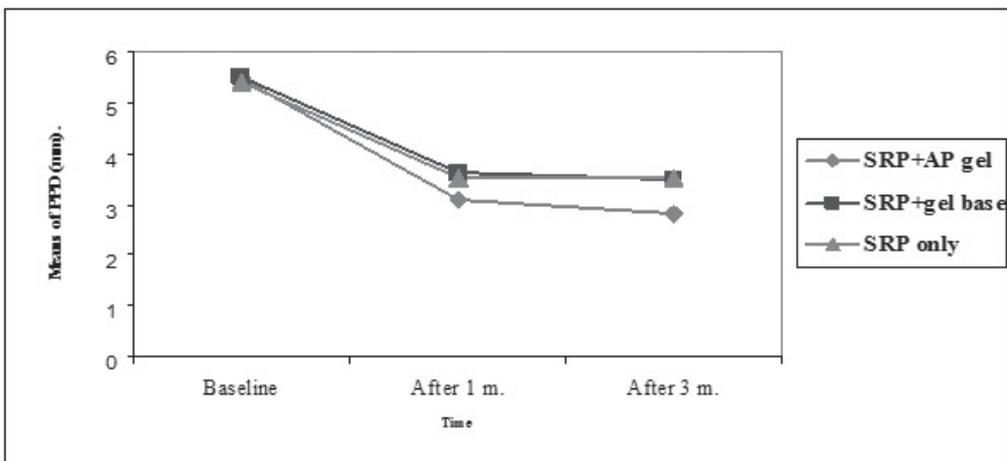


Figure 1. Mean probing pocket depth of 3 treatments at baseline, 1 month and 3 months after treatment.

Clinical attachment level (CAL)

Mean of clinical attachment levels at baseline, 1 month and 3 months from the treated sites of the three groups are shown in Table 2. CAL at baseline of all treatments were not significantly different with a means range of 5.41 to 5.88 mm. In addition, when compared between treatments using Multiple comparison for pairwise comparison, the significant difference of CAL was found between SRP plus AP gel and SRP plus gel base, and SRP plus AP gel and SRP only at 3 months after treatment ($p < 0.05$) as shown in Table 2. At 1 and 3 months after treatment, the CAL of all groups showed significant reduction compared to baseline level ($p < 0.05$) (Figure 2).

Gingival index (GI)

Means of gingival index at baseline, 1 month and 3 months from the treated sites of the three groups are shown in Table 3. GI of all groups at baseline were not significantly different, with means ranging from 1.84 to 1.91. Comparison between treatments using Multiple Comparisons showed a significant difference in gingival index between of SRP with AP gel and SRP with gel base, and SRP with AP gel and SRP only at 3 months ($p < 0.05$) as shown in Table 3. A significant reduction in gingival index at 1 month and 3 months compared to baseline data were observed in all treatment groups ($p < 0.05$), as shown in Figure 3.

Treatment	Baseline	1 month	3 months
SRP+AP gel	5.53+0.72	3.63+1.24	3.22+0.97
SRP+gel base	5.88+1.04	4.25+1.67	4.19+1.55
SRP only	5.41+0.84	4.16+1.72	3.88+1.41

Table 2. Mean and standard deviation of clinical attachment level (mm) of all treatments at different time intervals.

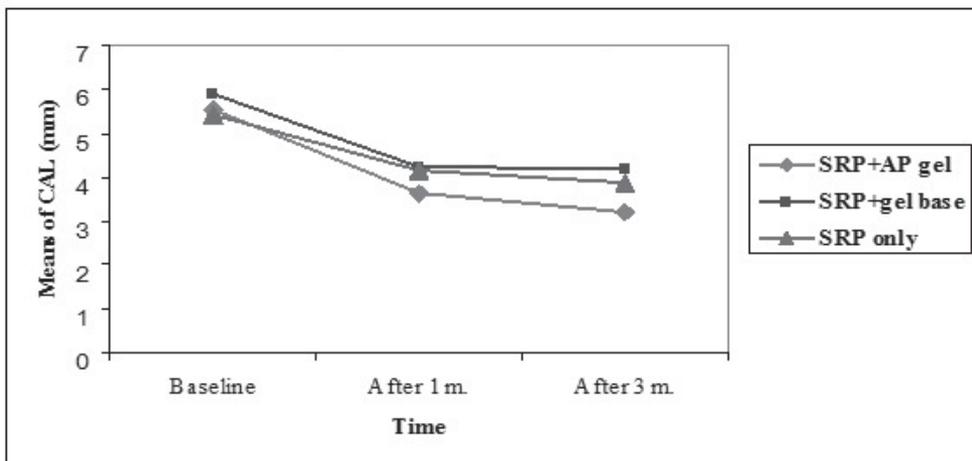


Figure 2. Mean clinical attachment level of 3 treatments at baseline, 1 month and 3 months after treatment.

Treatment	Baseline	1 month	3 months
SRP+AP gel	1.88+0.34	1.22+0.49	1.00+0.25
SRP+gel base	1.84+0.45	1.53+0.51	1.22+0.42
SRP only	1.91+0.39	1.34+0.48	1.28+0.46

Table 3. Mean and standard deviation of gingival index of all treatments at different time intervals.

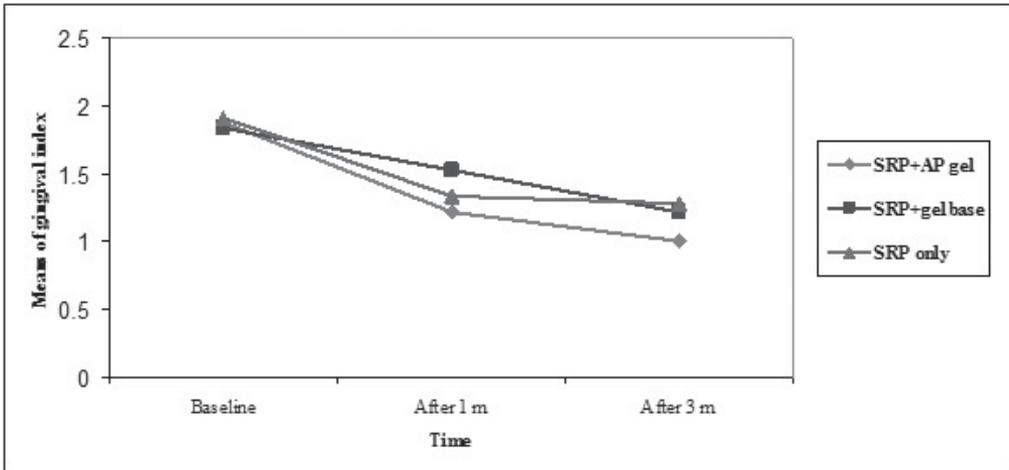


Figure 3. Mean gingival index of 3 treatments at baseline, 1 month and 3 months after treatment.

Treatment	Baseline	1 month	3 months
SRP+AP gel	100.00	53.13	46.88
SRP+gel base	93.75	68.75	81.25
SRP only	96.88	75.00	78.13

Table 4. Percentage bleeding on probing of all treatments at different time intervals.

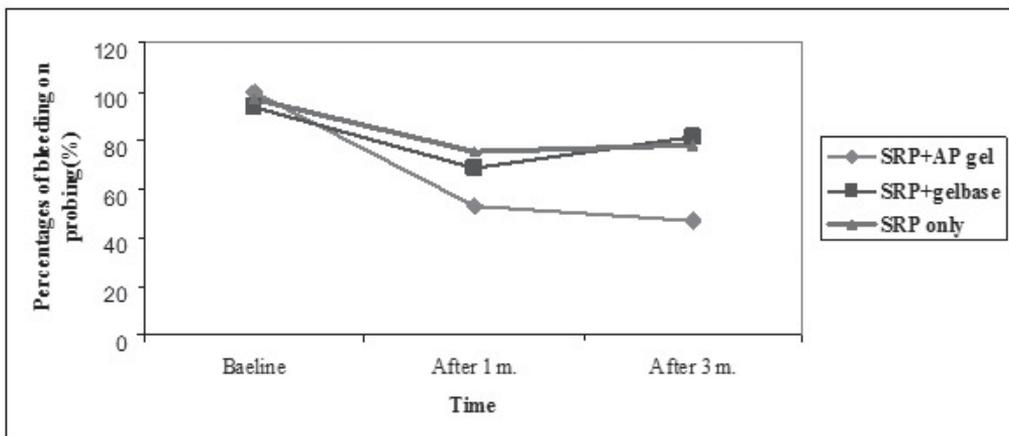


Figure 4. Percentage bleeding on probing of 3 treatments at baseline, 1 month and 3 months after treatment.

Bleeding on probing (BOP)

Percentage of bleeding on probing at baseline, 1 month and 3 months from all treatment groups are shown in Table 4. Bleeding on probing of all groups at baseline was not significantly different, with a range from 93.75 to 100%. When treatment groups were compared, the sites treated with SRP plus AP gel showed significant decrease in percent BOP compared to the other two treatment groups at 1 and 3 months after treatment ($p < 0.05$), as shown in Table 4.

Using McNemar Test, percentage BOP of the sites treated with SRP plus AP gel and SRP only 1 month and 3 month post treatment were significantly decreased when compared to baseline ($p < 0.05$). However, percentages of BOP of the sites treated with SRP plus gel base was significantly decreased only at 1 month after treatment as shown in Figure 4.

Discussion

It is generally accepted that scaling and root planing is the treatment of choice for managing periodontitis. In some cases treatment failure occurs due to the incomplete removal of subgingival plaque and calculus and therefore antimicrobial agents may be given as an adjunct. Recently an AP gel containing *Andrographis paniculata*, a medicinal plant with antimicrobial activity, has been formulated and used locally in the periodontal pocket (Narakorn 1999). However, at least one clinical study failed to show the significant benefit of AP gel (Rassameemasuang *et al* 1998). This might be due to the low viscosity of the gel, enabling rapid diffusion of the active ingredient below therapeutic levels. A new formulation of AP gel with increased viscosity was been developed and used in this study. The efficiency of AP gel was evaluated by assessing clinical and microbiological parameters at disease sites.

A randomized single blinded controlled trial protocol was used in this study. Three non-adjacent sites of each periodontitis patient were randomly assigned to one of the following treatments; 1) SRP with AP gel, 2) SRP with gel base, 3) SRP only. The parameters were monitored at day 0 (baseline), 1 and 3 months after treatment. Results showed that clinical parameters, including PPD, CAL, GI and BOP, were improved in all treatments. The results showed that all treatments were effective methods of therapy for treatment of periodontal disease, due to the removal of microbial products, calcified deposits and contamination from the root surface which reduced gingival inflammation, pocket depth and improved or maintained attachment levels (Hughes and Caffesse 1978, Lisgarten *et al* 1978, Mousques *et al* 1980, Axelsson and Lindhe 1978). Comparison of the three treatments showed that the clinical outcomes of the group treated with gel base as an adjunct to SRP were similar to those treated with SRP only. Thus, it might be concluded that this gel base formulation did not have an effect on decreasing inflammation or healing promotion. However, the clinical parameters of the group treated by SRP with AP gel were found to be significantly improved when compared with the other groups. It showed that the treatment using AP gel as an adjunct to scaling and root planing improved periodontal condition.

There are other studies that have shown the advantage of using AP gel as an adjunct in periodontal treatment. Amornchat *et al* (1991) demonstrated that extracted *Andrographis paniculata* with 95% ethanol exhibited inhibitory activity against *P. gingivalis*. Atsawasuwan *et al* (1998) also found that concentrations of black-pigmented anaerobes were significantly reduced in the pockets treated with AP gel but not in those treated with metronidazole gel. The study by Boonchaipanichwatana (2001) found that AP

gel resulted in a more consistent increase in percentage of cocci and decrease in percentage of motile rods over the period of three to four months compared to 2% minocycline gel. The effect of AP gel on repairing periodontium was demonstrated by Noppamassiri (2009). They found AP gel and AP extract can enhance alkaline phosphatase activity and the induction of mineralized nodule formation in gingival tissues at AP gel treated sites. These results suggest that AP gel and AP extract can promote the differentiation of human PDL cells into bone-forming cells. In addition, Sirirat and Rojanapanthu (2003) found that AP gel can improve radiopaque fill in periodontal defects at 3 and 6 months after treatment with AP gel. The results of previous and present studies clearly showed that AP gel as an adjunct to scaling and root planing improved the clinical parameters of periodontitis when compared to SRP only. These suggest AP gel has benefit as an adjunct to scaling and root planing for the treatment periodontal disease.

Conventional periodontal therapy consists of mechanical debridement to disrupt the subgingival biofilms and appropriate home care oral hygiene. However, to achieve consistent success, it demands the compliance of patients in oral hygiene. The classical experimental gingivitis studies (Loe and Theilade 1965) showed that clinical symptoms of gingivitis developed in students with clinically healthy gingivae within two to three weeks if dental plaque was allowed to accumulate. When adequate oral hygiene measures were resumed, the gingival inflammation subsided within a week.

Conclusion

This study presents clinical results after treating disease sites with *Andrographis paniculata* (AP) gel as an adjunct to scaling and root planing, comparing to those treated with SRP plus gel base and SRP only. The

results showed that clinical changes at the disease sites were significantly improved after all three treatments. When compared, the sites treated with AP gel showed a significantly better improvement compared to the sites treated with gel base and SRP only. In conclusion, the results indicate the benefit of local application of AP gel as an adjunct to SRP in the treatment of chronic periodontitis.

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Chapter 18

Regulation of Osteoblast Differentiation by Mechanical Strain and Parathyroid Hormone: Role of Interleukin (IL)-11

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Introduction

Osteoblasts undergo differentiation from their progenitor cells of mesenchymal origin, and exert their osteogenic functions in a differentiation-dependent sequential manner. Osteoblast differentiation is tightly regulated by various systemic and local factors. Among these systemic factors, parathyroid hormone (PTH) positively regulates, and glucocorticoids negatively regulate osteoblast differentiation. Bone morphogenetic proteins (BMPs) are important local factors to enhance osteoblast differentiation. Other than these soluble factors, mechanical strain is one of the most important stimulators of osteoblast differentiation. In addition, aging causes refractoriness to most of the osteogenic stimuli and suppresses bone formation. Thus, mechanical strain and PTH are two important stimuli for osteoblast differentiation and bone formation.

We have demonstrated that both mechanical loading and PTH enhance interleukin (IL)-11 gene expression in mice, that both anti-IL-11 antibody and IL-11 siRNA block mechanical strain- and PTH-induced enhancement of osteoblast differentiation. Here, we summarize the role of IL-11 in mechanical strain and PTH-induced stimulation of

osteoblast differentiation.

Mechanical stress signaling and bone formation

Mechanical stress to bone plays an important role in the maintenance of bone homeostasis (Figure 1). Mechanical unloading through prolonged bed rest, immobilization, or microgravity in space causes a marked loss of bone, due to an imbalance between bone formation and resorption. Whilst enhanced bone resorption in the endosteal surface is a major feature of unloading-induced bone loss in mature animals and humans, the impairment of bone formation in the periosteal surface constitutes an important mechanism for unloading-induced bone loss especially in the growing stage (Jaworski *et al* 1980, Robling *et al* 2006).

Mechanical stress on bone causes a rapid flow of interstitial fluids in the canaliculi of the osteocyte network. Fluid flow along cell surfaces produces fluid shear stress (FSS) and stress-generated electric potential, but cells appear to be more sensitive to FSS than to electric potential. FSS opens the stress-activated cation channel (SA-Cat), which causes an influx of extracellular Ca^{2+} to increase intracellular Ca^{2+} . Stretching of cell surfaces

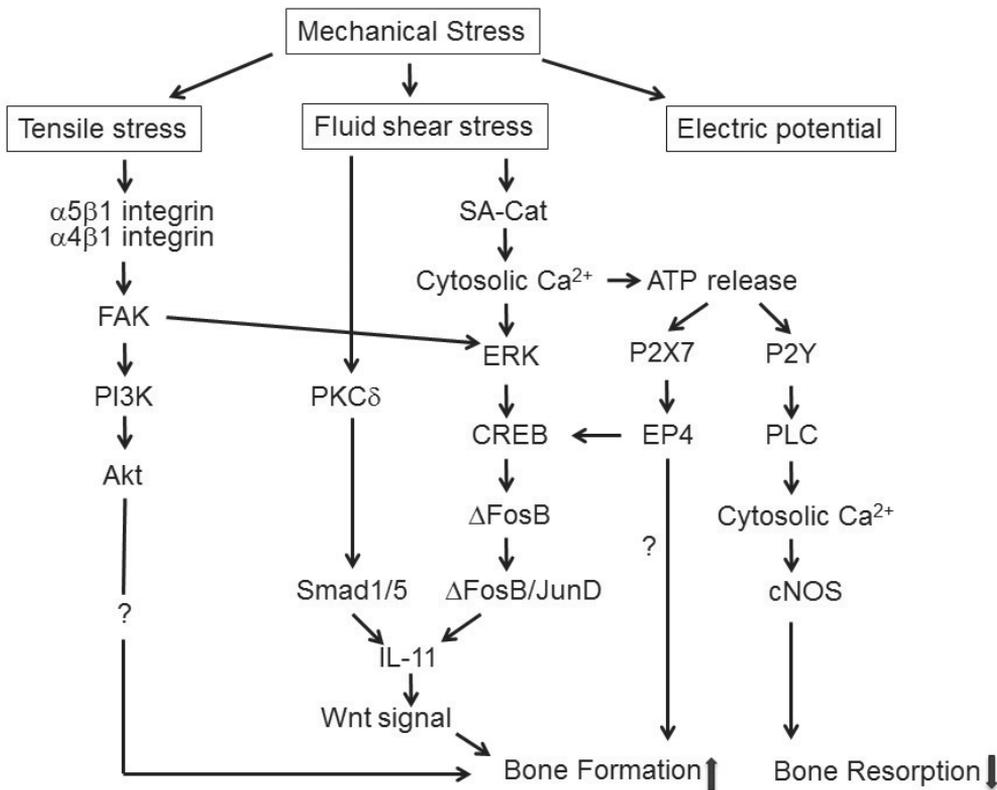


Figure 1. Signals that mediate mechanical stress to bone formation.

also produces tensile stress, causing changes in integrins and cytoskeletal proteins (Marie 2013). Among them, FSS is one of the most important signal transduction mechanisms to enhance osteoblast differentiation and bone formation in response to mechanical loading to bone (Burr 2002, Knothe Tate 2003).

Activation of SA-Cat by FSS causes an increase in Ca^{2+} influx, which activates extracellular signal-regulated kinase (ERK) that phosphorylates and activates cyclic AMP response element-binding protein (CREB) (Liu *et al* 2008, Kido *et al* 2009). Phosphorylated CREB binds to CRE region of the fosB gene promoter, and rapidly enhances fosB gene transcription (Inoue *et al* 2004). Alternative splicing of fosB gene produces splice variants, including FosB, Δ FosB and $\Delta 2\Delta$ FosB. Among them, Δ FosB heterodimerizes with JunD on the IL-11

gene promoter, and enhances IL-11 gene transcription. Among Fos family transcription factors, overexpression of Δ FosB, Fra-1 and Fra-2 is shown to enhance bone formation, while FosB overexpression does not enhance bone formation and c-Fos overexpression results in osteosarcoma (Jochum *et al* 2000, Sabatakos *et al* 2000).

Increased intracellular Ca^{2+} also enhances adenosine triphosphate (ATP) release from cells, which activates G protein-coupled ATP receptors, ionotropic P2X7 and metabotropic P2Y receptors. P2X7 stimulates prostaglandin E_2 (PGE_2) synthesis, and PGE_2 signaling via EP4 receptor is shown to enhance bone formation (Yoshida *et al* 2002). Because EP4-mediated signals also activate CREB signaling, this signal may merge with Ca^{2+} -ERK-CREB-FosB/ Δ FosB signal cascade. In contrast, P2Y activates phospholipase C

(PLC), which induces inositol triphosphate (IP₃) release to stimulate Ca²⁺ efflux from intracellular stores and increases cytosolic Ca²⁺ (Fujino *et al* 2005, Genetos *et al* 2005). The increase in cytosolic Ca²⁺ can activate constitutive nitric oxide synthase (cNOS) to increase NO production. NO is an inhibitor of bone resorption and is reported to suppress the expression of receptor activator of nuclear factor- κ B ligand (RANKL) and increase osteoprotegerin (OPG) expression in bone marrow stromal cells (Fan *et al* 2004). Therefore, the increase in NO production may be important for the suppression of bone resorption by mechanical stress (Figure 1).

PTH and bone formation

Another important stimulator of bone formation is PTH. Although a continuous elevation of PTH predominantly enhances bone resorption, intermittent elevation of PTH stimulates osteoblast differentiation and bone formation with less stimulation of osteoclastic bone resorption. Thus, daily subcutaneous injections of PTH dramatically increase new bone formation along with bone resorption, resulting in an increase in spine, hip and total body bone mass with an increase in bone strength in animals and in osteoporotic patients (Ma *et al* 2006, Neer *et al* 2001, Sato *et al* 2004).

Regarding the mechanism of enhancement of bone formation by PTH, O'Brien *et al* (2008) demonstrated that mice expressing a constitutively active PTH receptor in osteocytes exhibited increased bone mass and bone remodeling, reduced expression of Wnt antagonist, sclerostin, with enhanced Wnt signaling. Interestingly, deletion of low-density lipoprotein receptor-related protein 5 (LRP5) attenuates the increase in bone mineral density (BMD), but does not affect the increase in bone remodeling by the transgene. These observations suggest that the anabolic

effect of PTH appears to be mediated via a suppression of the expression of canonical Wnt signal inhibitor, sclerostin, in osteocytes, which causes an activation of canonical Wnt signaling. In contrast, the effect of PTH on bone remodeling is independent of sclerostin-LRP5 pathway in osteocytes.

PTH also suppresses Dickkopf 1 (Dkk1) expression, another Wnt signal inhibitor, in osteoblasts. Guo *et al* (2010) created mice overexpressing Dkk1 in osteoblasts, and examined whether the suppression of Dkk1 in osteoblasts is essential for PTH-induced activation of Wnt signaling and bone formation. The results demonstrated that PTH can still activate Wnt signaling despite overexpression of Dkk1. Thus, although PTH-induced activation of canonical Wnt signaling plays an important role in the anabolic effect of PTH, the relationship between osteocyte-mediated and osteoblast-mediated effects of PTH, as well as that between the inhibition of osteocyte-derived sclerostin and of other Wnt inhibitors from osteoblasts and osteocytes, remains to be clarified.

Regulation of IL-11 expression by mechanical stress and PTH

Mechanical unloading causes a marked reduction in the expression of IL-11 mRNA in the unloaded bone, and mechanical loading causes a rapid and robust increase in IL-11 mRNA expression in the loaded bone (Kido *et al* 2009). Recently, we found that not only mechanical stress but also PTH enhances the expression of IL-11 in osteoblasts both *in vitro* and *in vivo* (Kuriwaka-Kido *et al* 2013). These observations are consistent with the notion that enhanced IL-11 expression may mediate the stimulation of bone formation in response to mechanical stress and PTH.

One of the earliest responding factors induced by mechanical loading in bone cells is an AP-1 family transcription factor, c-Fos

(Lean *et al* 1996). However, because ubiquitous overexpression of c-Fos in transgenic mice develops osteosarcoma without evidence for increased bone formation, c-Fos is unlikely to be a factor mediating mechanical stress to bone formation (Grigoriadis 1993). In addition to mechanical loading, PTH stimulation to bone *in vivo* or *in vitro* also induces fosB gene transcription with a similar time course to that in c-fos gene transcription. The increase in fosB gene transcription is mediated via the Ca²⁺-ERK-CREB signaling pathway as mentioned previously, and activated CREB stimulates fosB gene transcription through binding to a putative CRE site in fosB gene promoter (Inoue *et al* 2004). Enhanced fosB gene transcription by mechanical stress or PTH causes an accumulation of mainly Δ FosB protein, a short splice variant of FosB lacking C-terminal transactivation domain. Because Δ FosB protein has a long half-life which enables this protein to accumulate after transient stimulations, Δ FosB can be a suitable mediator of intermittent mechanical loading or PTH signal to sustained bone formation signal (Chen *et al* 1997).

IL-11 gene promoter contains two tandem AP-1 sites located upstream TATA box which confer transcriptional activation by transforming growth factor (TGF)- β and other stimuli. Site-directed mutagenesis revealed that, of the two AP-1 sites, 5' upstream AP-1 site confers the transcriptional activation of IL-11 gene by mechanical stress and PTH. Mechanical loading and PTH enhances the expression of Δ FosB, and the upregulated Δ FosB forms heterodimers with JunD on the 5'AP-1 site of the IL-11 gene promoter. JunD is bound to the 5'AP-1 site regardless of mechanical stimuli. Binding of the Δ FosB/JunD heterodimer to the 5'AP-1 site of IL-11 gene promoter causes an enhanced transcription of IL-11 gene (Kido *et al* 2009). Thus, down-regulation of Δ FosB/JunD expression by siRNA reduces

and overexpression of Δ FosB/JunD enhances IL-11 gene promoter activity in osteoblasts (Kido *et al* 2009).

BMPs play pivotal roles in the regulation of osteoblast differentiation and bone formation. However, the role of BMPs in mediating mechanical stress or PTH signal to osteogenic signal is controversial. Although compressive forces to osteoblastic cell cultures or tensile forces to cranial suture of neonatal calvaria in culture caused an elevation of BMP-4 expression in osteoblasts, FSS to SaOS-2 cells in culture was reported to reduce BMP-4 expression (Ikegame *et al* 2001, McCormick *et al* 2006, Rath *et al* 2004). In addition, although FSS to osteoblasts in culture or mechanical loading to bone *in vivo* does not increase BMP-2 expression, BMP-2 stimulates IL-11 expression in osteoblastic cells (Kido *et al* 2010).

BMP signals are transduced via phosphorylation by type I BMP receptor of BMP-specific receptor-regulated Smads (BR-Smads), Smad1, 5 and 8. Phosphorylated BR-Smads then form a heteromeric complex with Smad4, a common Smad, and translocate into the nucleus, where they regulate transcription of various target genes (Miyazono *et al* 2005). However, it is unknown whether mechanical stress to bone or PTH stimulation activates BR-Smad signaling, and whether BR-Smad signaling is also involved in the enhancement of osteoblast differentiation by mechanical stress and PTH. We found that not only FSS but also PTH activates protein kinase C (PKC), which induces phosphorylation of Smad1/5 but not of Smad2/3 in osteoblasts (Kido *et al* 2010).

Mouse IL-11 gene promoter contains a putative Smad-binding element (SBE) along with the AP-1 sites. Studies with site-directed mutagenesis at the putative SBE and 5'AP-1 sites revealed that both SBE and AP-1 sites were required for full activation of IL-11 gene promoter activity by FSS or PTH.

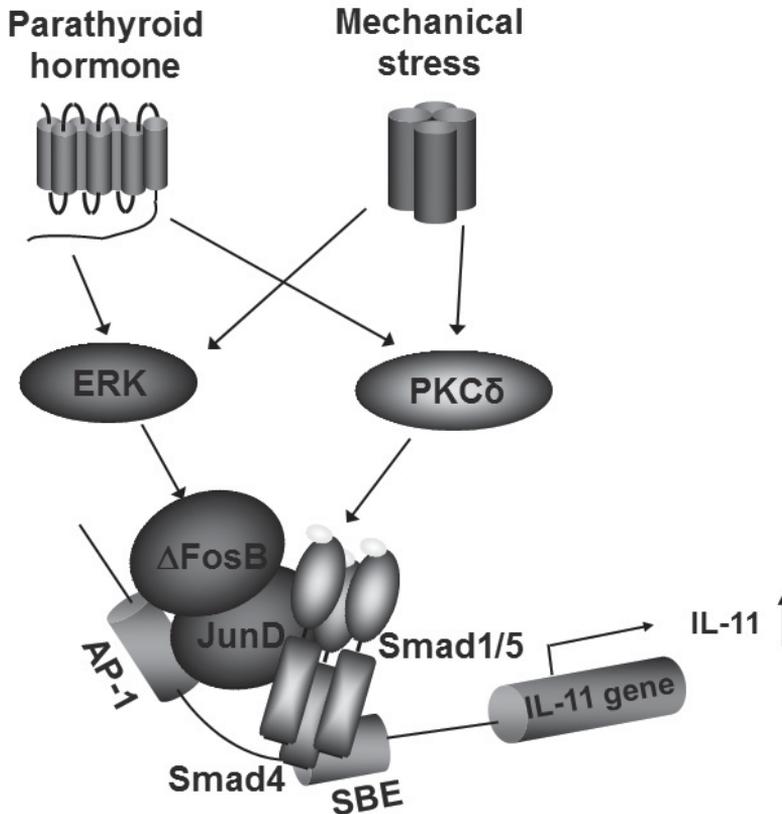


Figure 2. Mechanical stress and PTH enhance IL-11 gene transcription by stimulating fosB gene transcription and Smad1/5 phosphorylation.

FSS- or PTH-activated Smad1 is bound to SBE and forms a complex with FosB/JunD heterodimer, which is bound to the 5'AP-1 site on the IL-11 gene promoter (Kido *et al* 2010) (Figure 2). These observations demonstrate that Ca^{2+} -ERK-CREB- Δ FosB signaling and PKC δ -Smad1/5 signaling pathways merge on the IL-11 gene promoter, and that AP-1 and Smad signaling cooperatively stimulate IL-11 gene transcription in response to mechanical stress and PTH.

Wnt signaling and IL-11

Wnt/ β -catenin signaling pathway plays an important role in the regulation of bone formation (Baron and Kneissel 2013). LRP5 and LRP6 are co-receptors for Wnt with the

frizzled family of receptors, and are involved in signaling through the canonical Wnt/ β -catenin pathway. Inactivating mutations in LRP5 results in osteoporosis pseudoglioma syndrome, and gain of function mutations in LRP5 gene gives rise to a high bone mass phenotype in humans (Boyden *et al* 2002, Gong *et al* 2001, Little *et al* 2002, Van Wesenbeeck *et al* 2003). As to the mechanism of the stimulation of bone formation by Wnt/ β -catenin signaling, canonical Wnt signaling shifts cell fate of mesenchymal precursor cells toward the osteoblast lineage by induction of the osteoblastogenic transcription factors such as Runx2 and osterix, and suppression of the adipogenic transcription factors CCAAT enhancer binding protein α (C/EBP α) and peroxisome proliferator-activated receptor γ

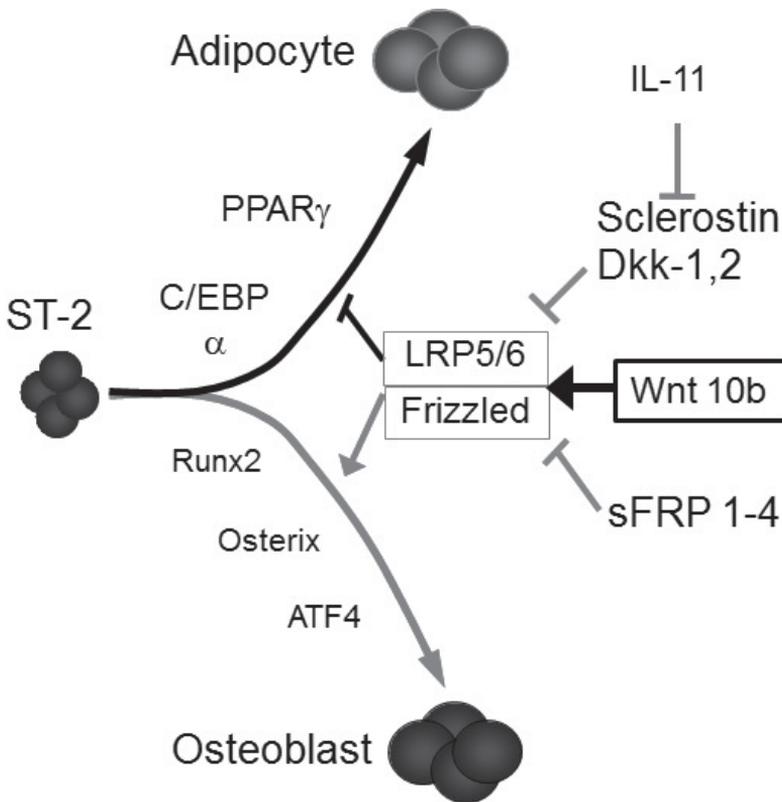


Figure 3. Regulation of osteoblast & adipocyte differentiation by IL-11 and canonical Wnt signaling.

(PPAR γ) (Bennett *et al* 2005).

Mechanical loading suppresses and mechanical unloading enhances the expression of sclerostin in osteocytes (Robling *et al* 2005). As mentioned earlier, PTH exerts its anabolic effect by enhancing Wnt/ β -catenin signaling via a suppression of sclerostin expression in osteocytes. However, the upstream signal that reduces sclerostin expression in response to mechanical stress or PTH remains unknown. Wnt/ β -catenin signaling can be inhibited by not only sclerostin but also Dkk1/2. Using murine primary osteoblasts that do not express sclerostin but express Dkk1 and 2, we demonstrated that not only FSS but also PTH enhances Wnt/ β -catenin signaling by suppressing Dkk1 and 2 expression (Kido *et al* 2009). The reduction of Dkk1 and 2 is mediated via the increased expression of IL-11, and knockdown of IL-11 expression

by siRNA enhances and overexpression of IL-11 suppresses Dkk1 and 2. These observations demonstrate that stimulation of Wnt/ β -catenin signaling is a major pathway mediating mechanical stress and PTH signals to osteogenic signal, and that enhanced IL-11 expression by mechanical stress and PTH is at least one of the major upstream signals to enhance Wnt/ β -catenin signaling (Figure 3).

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Poster Presentations

The following is a record of the posters presented at the
10th Meeting of the Asian Pacific Society of Periodontology

Vitamin D Decreases the *Porphyromonas gingivalis* LPS-Induced Expression of IL-8 in Human Periodontal Ligament Cells

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Background: Vitamin D has been reported to regulate immune and inflammatory processes. The present study aimed to explore the effect of *P. gingivalis* LPS, an important virulence factor of *P. gingivalis*, on the expression of IL-6 and IL-8 in human periodontal ligament cells (hPDLCs) and the effects of 1 α ,25-dihydroxyvitamin D₃ (1,25D) on the above processes.

Materials and methods: Primary cultures of hPDLCs were obtained from the extracted premolars of 10 patients undergoing orthodontic treatment. The samples of hPDLCs at the fourth passage were individually treated with 1,25D, LPS, or 1,25D combined with LPS. 24 and 48 hours later the protein levels of IL-6 and IL-8 were detected using the ELISA method.

Results: 25 μ g/ml LPS exerted the highest promotion effect on the expression levels of IL-6 by 6.19-folds at 24 hours ($P=0.000$) and on IL-8 by 1.015-folds at 48 hours ($P=0.000$) individually. 1,25D down-regulated the IL-8 level in a dose- and time- dependent manner. The highest inhibition effect reached 2.00-fold ($P=0.000$) by 10⁻⁸M 1,25D at 48 hours. 1,25D significantly decreased LPS-induced IL-8 production. At 24 hours, 10⁻⁸M 1,25D combined with LPS significantly down regulated the IL-8 levels in hPDLCs compared with those in cells treated by LPS alone (1840.61 pg/ml versus 2044.37 pg/ml, $P=0.015$). At 48 hours, both 10⁻¹⁰ M (1943.80 pg/ml, $P=0.016$) and 10⁻⁸M (1916.43 pg/ml, $P=0.004$) 1,25D combined with LPS significantly down regulated the IL-8 levels in hPDLCs compared with LPS treated alone (2046.80 pg/ml). 1,25 D showed no significant effect on the expression of IL-6 in hPDLCs.

Conclusion: Our results suggest that vitamin D may partially suppress the periodontal inflammation induced by *P. gingivalis* LPS by inhibiting IL-8 expression in hPDLCs.

**Recipient of Poster Presentation Award*

Genetic Polymorphisms of Th2 Cell Regulatory and Effector Molecules in Non-Smokers With or Without Periodontitis

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Objective: The T helper 2 (Th2) cell has been reported to be related to periodontitis. A recent genome-wide association study (GWAS) of aggressive periodontitis indicated Th2 cell may play important roles in the pathogenesis of periodontitis. The present pilot study investigated the association between periodontitis and single nucleotide polymorphisms (SNP) of genes known to control or regulate Th2 cell differentiation (C028, CTLA4, IL4R, GATA3, STAT6) and Th2 cell effector molecules (IL4, IL13) among Hong Kong Chinese non-smokers with or without chronic periodontitis.

Methods: Genomic DNA was extracted from whole blood samples of 41 periodontitis patients and 41 periodontitis-free controls (39 females; mean 43.6 years, range 36-60 years), were analyzed. SNPs of seven Th-2 cell regulatory or effector molecules genes (CD28, CTLA4, IL4, IL 13, IL4R, GATA3, STAT6 and rs1537415; total 116 SNPs) were selected after a comprehensive genetic screening approach and were genotyped using Sequenom iPiex assays.

Results: 90 SNPs met the inclusion criteria and were evaluated. After adjustment for age and gender, a tagging SNP rs3024619 located in the region of the seventh intron of IL4R was significantly associated with chronic periodontitis ($p < 0.05$). rs1537415, previously reported in the GWAS to be an aggressive periodontitis associated SNP, was found to be not related to chronic periodontitis ($p = 0.052$).

Conclusion: Within the limitations of this study, polymorphisms of Th2 cell regulatory gene IL4R appeared to be associated with non-smokers with chronic periodontitis. A larger scale study and follow-up fine mapping however, are needed to confirm the current observation that such Th2 cell-related genetic variation would be genuine risk indicators for chronic periodontitis.

**Recipient of Poster Presentation Award*

One Visit Subgingival Ultrasonic Debridement in Well- and Poorly-Controlled Type 2 Diabetes Mellitus Periodontitis Patients

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Introduction: Increased prevalence, progression and severity of periodontitis are commonly found in diabetic patients, especially poorly-controlled diabetics. It has also been found that long term periodontitis may affect glycaemic control. Thus, successful periodontal treatment may influence both periodontitis and diabetes mellitus.

Objectives: This study evaluated the effects of one visit subgingival debridement with a piezoelectric ultrasonic device on improvement of periodontal status and glyceemic control between well-controlled and poorly-controlled diabetes mellitus type 2 patients with chronic periodontitis

Methods: 37 subjects with periodontitis and diabetes mellitus (DM) received periodontal treatment from the Royal Mobile Dental Units of the Faculty of Dentistry, Srinakharinwirot University consisting of one visit subgingival ultrasonic debridement without antibiotics. The study consisted of 17 well-controlled DM patients (HbA1c <8%) and 20 poorly-controlled DM patients (HbA1c <8%) with moderate to severe chronic periodontitis were enrolled. Clinical parameters, including visible plaque index (VPI), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), and HbA1c level, were evaluated before and at 1 and 3 months following periodontal treatment.

Results: All periodontal parameters were statistically significantly decreased ($p < 0.01$) in both well-controlled and poorly-controlled diabetic patients after receiving periodontal treatment but no significantly differences were observed between groups. Only the poorly-controlled diabetes group had a significant reduction in HbA1c level at 3 months after periodontal treatment ($p < 0.05$).

Conclusion: Periodontal treatment consisting of one visit subgingival ultrasonic debridement improved periodontal status in DM patients with periodontitis, and also contributed to enhanced glycaemic control in poorly-controlled DM patients.

**Recipient of Poster Presentation Award*

The Association Between Width of Attached Gingiva and the Health Status of the Supporting Tissue Around Dental Implants

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The presence of a competent seal surrounding dental implants or abutments has an important role in achieve long term stability of the dental implant restoration. The attached gingiva provides resistance to external injury, contributes to the stabilisation of the gingival margin and aids in dissipating physiological forces exerted by the muscular fibres of the alveolar mucosa on the gingival tissue.

Objective: This cross-sectional study was performed to determine the association between the width of attached gingiva and the health of implant-supporting tissue.

Materials and Methods: Data on 50 implants were collected. Periodontal parameters measured included plaque index, gingival index, width of attached gingiva, thickness of gingiva, radiographic bone level, and bleeding on probing. Statistical analysis was performed with the t test, Wilcoxon rank sum test. Significance was established when P results were less than 0.05.

Results: The mean plaque index score, gingival index score, and radiographic bone loss were significantly higher for those implants with a narrow zone (<2 mm) of attached gingiva.

Conclusion: The dimension of the attached gingiva to the implant surface was considered important for the maintenance of peri-implant tissue health.

**Recipient of Poster Presentation Award*

ROCK inhibitor Y-27632 maintains the proliferation of confluent human mesenchymal stem cells

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Background and Objective: The transplantation of cell-sheets of mesenchymal stem cells (MSCs) is expected to be the next generation of periodontal regenerative therapy. An adequate method of multi-layering MSCs has yet to be established. When cell sheets proliferate, they usually contract and detach from culture dishes and then the proliferation of cells in the contracted areas is arrested. ROCK-mediated contractile force causes cell contraction. Although multilayer formation medium (MFM) stimulated the proliferation of growth-arrested confluent MSCs, MSCs detached from the culture dish. Therefore, we investigated the effects of ROCK inhibitor Y-27632 on the proliferation and detachment of confluent MSCs, and examined the ability of differentiation of the cells contained in the cell sheets.

Materials and Methods: Confluent MSCs were cultured in MFM containing transforming growth factor-131, ascorbic acid, and fetal bovine serum either with or without Y-27632. Cell proliferation was examined by BrdU incorporation assays and total DNA measurement. Sheet contractions were examined by light microscopy and stereomicroscopy. Multilayer formations and focal adhesion assembly were observed with confocal microscopy. The characteristics of cells were examined by flow cytometric analysis. Osteoblastic lineage differentiation was observed with ALP and alizarin red S staining. Adipocytic lineage differentiation was observed with oil red O staining.

Results: The addition of Y-27632 to MFM prevented the cell sheets from detaching and did not inhibit MSC growth. The cell numbers in sheets cultured with MFM/Y-27632 were significantly higher than obtained with MFM-only on day 4. Cell sheets detached from the culture dish on day 4 and the number of BrdU-positive cells in the detached area decreased. The cells in the cell sheets had similar characteristics to primary MSCs, and differentiated into osteoblastic and adipocytic lineages.

Conclusion: Y-27632 both prevented the MSC sheets from detaching and maintained the multilayered proliferation of confluent MSCs by MFM. Cells in the sheets had differentiation potency.

**Recipient of Poster Presentation Award*

Effects of Adenovirus Expressing Bone Morphogenetic Protein-4 on Different Cell Types of Osteoblastic Differentiation

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Objective: Bone morphogenetic proteins (BMPs) play a pivotal role in inducing undifferentiated cells into osteoblastic cells which induce bone formation. In the present study, we investigated the effect of gene delivery of adenovirus expressing BMP-4 on osteoblastic differentiation of different cell types.

Materials and methods: An adenovirus expressing BMP-4 (AdSBMP4) was constructed by *in vivo* homologous recombination and transduced MC3T3-E1 (mouse osteoblastic cell line), C2C12 (mouse myoblastic cell line), NHDF (normal human dermal fibroblast adult) to induce cell transformation. We assessed BMP-4 expression by ELISA for evaluation of gene expression activity of Ad5BMP4. MTT assay was performed at 3, 7 and 15 days to assess proliferation of cells transduced with Ad5BMP4. Alkaline phosphatase activity was measured at 3, 7 and 15 days to assess osteoblastic differentiation of cells. The effect of Ad5BMP4 on mineralization was determined using alizarin red S staining after 14 days.

Results: At 3 days, all three different cell types that transduced with Ad5BMP4 were observed to have BMP-4 expression increased in proportion to the increase of concentration of Ad5BMP4. Up to 14 days levels of BMP-4 was maintained on MC3T3-E1, but remarkably decreased on C2C12 and NHDF compared with 7 days. When transduced with Ad5BMP4, the cell proliferation was not inhibited on MC3T3-E1, but significantly decreased on C2C12 compared with not transduced cells at 14 days. Cell proliferation of NHDF that transduced with 6.2 pfu/cell concentration of Ad5BMP4 was similar to that of non-transduced NHDF, but decreased after 7 days. To observe the osteogenic abilities of cells that transduced with Ad5BMP4, we investigated alkaline phosphatase (ALPase) activity and mineralization formation. ALPase activities were significantly increased after 7 days on MC3T3-E1 and C2C12 and after 14 days on NHDF. Mineralization formation was observed on MC3T3-E1, but the others were not observed at 14 days.

Conclusion: The present study demonstrated that gene delivery using adenovirus expressing BMP-4 facilitated osteoblastic differentiation of MC3T3-E1, C2C12, NHDF cells. Also we confirmed the possibility of *ex vivo* BMP-4 gene delivery that be able to apply to effective technique for regeneration of bone defects.

**Recipient of Poster Presentation Award*

Occlusal trauma accelerates attachment loss at the onset of experimental periodontitis in rats

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Progress of Alzheimer's disease enhanced by experimental periodontitis in transgenic mice

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The effect of non-cultured human adipose-derived stromal cells for periodontal tissue regeneration

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Ridge augmentation as a correction for anterior maxillary ridge defect pre bridgework placement - Case report

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Effect of bone formation on TGF- β 2 immobilized titanium

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Fanconi's anaemia: Periodontal destruction and rehabilitation with Straumann SLActive® implants - Case report

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Influence of the distance from the contact point to the alveolar crest on the presence of anterior interdental papilla

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Diagnosis of diabetes-associated periodontitis using glycoalbumin and calprotectin in gingival crevicular fluid

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Periodontal health in treated young periodontitis patients responsible for their own supportive care

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Efficiency of a newly-developed salivary multi-test system for examination of periodontal disease

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Tooth loss and mild memory impairment in the elderly: The Fujiwara-kyo study

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Point of care system for detecting salivary *Porphyromonas gingivalis*

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Prevalence of periodontal disease and oral health related quality of life in the Malaysian obese population: A preliminary study

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The association between periodontal condition and preterm birth in Japanese pregnant women

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Cross sectional study on the relationship between serum biochemical examination and periodontal parameters

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FoxO4 expression in gingival tissues of chronic periodontitis patients

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A major neurodegenerative disease protein TDP-43 enhances TNF- α transcriptional regulation in human monocyte-derived macrophage-like cells

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Effect of hypoxia on gene expression in human oral keratinocytes

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The effect of SAA on atherosclerogenesis in ApoE deficient mice

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Inflammasome expression in ligature-induced mouse periodontitis lesion

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Interleukin-6 gene promoter methylation in periodontal tissues

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Tumor necrosis factor-alpha gene promoter methylation in chronic periodontitis

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HMGB1 stimulates proinflammatory cytokine synthesis in human gingival and PDL fibroblasts

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Periostin stimulates inflammatory response of gingival fibroblasts in an autocrine manner

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Effects of hypoxia on inflammatory responses of gingival epithelial cells
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Outer membrane protein 29 from *Aggregatibacter actinomycetemcomitans* induce gingival epithelial cells apoptosis via TGF-13 type 1 receptor-smad2-caspase3/9 signaling pathway

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Oral *Porphyromonas gingivalis* translocates to liver and regulates hepatic glycogen metabolisms by attenuating insulin signaling

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The influence of water resources to periodontal diseases

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Periopathogenic bacteria in oral plaque, saliva and placenta from preterm low birth weight cases

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The expressions of TIMP and iNOS in chronic periodontitis with type 2 diabetes mellitus

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***Porphyromonas gingivalis* infection affects the exacerbation of rheumatoid arthritis in a mouse model**

Yamakawa M, Ouhara K, Kajiya M, Kittaka M, Fujita T, Shiba H, Kurihara H

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Localization of tissue stem cells in periodontal ligament tissue

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Gingival hyperplasia in hypertension patients at the endocrine clinic of Dr Wahidin Sudirohusodo Hospital, Makassar

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Effect of interleukin-6 receptor inhibition therapy on periodontal condition in patients with rheumatoid arthritis and periodontitis

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Acceleration of bone regeneration with β -TCP and b-FGF around dental implant

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Effect of superporous hydroxyapatite for the treatment of periodontal defects: 2-year results

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Bone regeneration of biphasic calcium phosphate with varying methods of material preparation in rabbit calvarial defect

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Improvement of dental pulp stem Cells and 13-tricalcium phosphate graft on periodontal bone defect

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An exploratory study on the efficacy of rat dedifferentiated fat cells with polylactic-co-glycolic acid/hydroxylapatite composite for bone formation in a rat calvarial defect model

Shirakata Y, Shinohara Y, Taniyama K, Nakamura T, Sakoda K, Yoshimoto T, Noguchi K

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Comparison of BCP with or without collagen matrix as rhBMP-2 carrier on rabbit calvarial defect

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Formulation and evaluation of a new biodegradable periodontal chip containing thymoquinone in a chitosan base for the management of chronic periodontitis

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Inhibition of Spry2 decreased EGF receptors in gingival epithelial cells

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Clinical and microbiological evaluation of the cause-related therapy combined with administration of macrolide antibiotics for severe generalized periodontitis patients

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Application of cavitating jet for cleaning oral biofilm from titanium surface
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Regeneration of calvarial defects using dental pulp and periodontal ligament derived cells in nano hydroxyapatite silk scaffolds in rabbits

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Grp78 mediates endocytosis of amelogenin

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Identification of novel amelogenin-binding proteins by proteomics analysis

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Sprouty2 controls cell proliferation and differentiation of periodontal ligament cells

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Effects of periodontal treatment on antibodies to *Porphyromonas gingivalis* and citrulline levels and rheumatoid arthritis condition

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A synthetic oligopeptide derived from enamel matrix derivative promotes the differentiation of human bone marrow stem cells into osteoblast-like cells with increased mineralization

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Effect of non-surgical supportive therapy by piezoelectric sub-gingival debridement on *Porphyromonas gingivalis* intensity

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Comparative study between piezoelectric ultrasonic device and hand curette for supportive treatment

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Adjunctive effects of passive immunization with supportive periodontal therapy

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The effectiveness of light activated disinfection in periodontal surgical procedures

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The use of silversol treatment in non-surgical periodontics

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Induction of BMP-2 by extracellular Ca²⁺ and Pi as well as nano-hydroxyapatite in periodontal ligament cells

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Effectiveness of coconut oil pulling to anaerobic bacterial reduction in oral cavity

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Exophytic bone formation in rabbit calvaria: comparative effect among synthetic bone graft materials

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Periodontal tissues regeneration after demineralized freeze dried bone treatment

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Cholhexidine solution used in fixed orthodontic appliance patients

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The management of hyperplasia gingival on patient with orthodontic treatment: Case report

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Effect of streptococcus asper on ratio of RANKL/OPG in primary human bone cells induced by sonicated *Porphyromonas gingivalis*

Pradoemdee A, Taweekhaisupapong S, Pungchanchaikil P, Chayasodom A, Rattanaphan P

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Effects of fibroblast growth factor-2 on the periodontal healing in streptozotocin-induced diabetic rats

Bizenjima T, Takeuchi T, Ishii Y, Okubo N, Seshima F, Fujita T, Kinumatsu T, Ota M, Saito A

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Influence of nicotine and *Porphyromonas gingivalis* lipopolysaccharide on HUVECs

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Comparison of enamel and ameloblasts structure in amelotin-overexpressed and deficient mice

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Inhibition of osteoblast cell-related transcription factors induces de-differentiation of osteoblasts

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TLRs and NODs expression in human periodontium

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Preliminary user evaluation on a six degree-freedom haptic simulation of pathological changes in periodontal operations

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Inaccuracy of gingival biotype assessment using the transparency of the periodontal probe: verification of actual gingival thickness with an ultrasonic device

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Junctional epithelium formed by the eruption of reconstructed tooth germ is originated from odontogenic epithelium

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Different tufting profiles of toothbrushes for the delivery and retention of toothpaste

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Relationship between periodontal condition and metabolic syndrome in Thai patients

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Controlled stemness of human periodontal ligament stem cells by c-kit
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Biodurability and bone regenerative capacity of D-ribose cross-linked collagen membrane in the rabbit calvarium

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Effect of multiple wave electrical stimulation on human periodontal fibroblasts

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In vitro analysis of human periodontal microvascular endothelial cells

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Oral biofilm inhibition effect and the cytotoxicity test of codium fragile extract

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