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Table of Contents

	Acknowledgments	5
	Invited Participants	6
	Sponsors	8
Chapter 1	**The Relationship Between Rheumatoid Arthritis and Periodontitis** *P.M. Bartold (Australia)*	9
Chapter 2	**Comprehensive Investigations into Arterial Diseases and Periodontal Bacteria** *T. Iwai, I. Ishikawa, Y. Inoue (Japan)*	18
Chapter 3	**Diagnosis and Classification of Periodontal Diseases and Conditions: Current and Future Challenges** *G.C. Armitage (United States of America)*	21
Chapter 4	**The Role of Inflammation in Atherosclerosis** *J-J. Li (China)*	29
Chapter 5	**Options for Achieving High Predictability and Longevity in Periodontally Compromised Cases** *Y. Ono (Japan)*	42
Chapter 6	**Polymorphisms in Genes Coding for Enzymes Metabolizing Smoking-Derived Substances, Smoking and the Potential Risk of Periodontitis** *J. Zhang, S. Zhang, Y. Zhang (China)*	57
Chapter 7	**Prevalence of Systemic Diseases Among Periodontal Patients at the State University Clinic and Two Private Periodontal Practices in the Philippines** *N.V. Vergel de Dios, B.V. Murjani, M.C.U. Garcia (Philippines)*	69
Chapter 8	**Relationships Between Periodontal Diseases and Systemic Health in New Zealand: An Overview of Research at Otago University** *W. Duncan, M. Cullinan, H. English, E. Noor, P. Chou, D. Lam, W.M. Thomson, D. Holborow, J. Leichter, G. Seymour* (New Zealand)*	77

Chapter 9	**Bisphosphonate Associated Osteonecrosis of the Jaws: Implications for Periodontics and Implantology** *N. Surathu (India)*	83
Chapter 10	**Current Evidence of Periodontal Medicine in Thailand** *N. Laosrisin (Thailand)*	89
Chapter 11	**Relationship of Periodontal Disease to Pre-term Low Birth Weight Infants in a Selected Population in Malaysia: A Pilot Study** *T. Taiyeb Ali, K. Zainal Abidin, P.T. Lai, M.N Lau (Malaysia)*	99
Chapter 12	**Periodontal Infections, Systemic Inflammation and Cardiovascular Disease: Current Evidence and Perspectives** *L.J. Jin (Hong Kong SAR, China)*	114
Chapter 13	**A Perspective of Periodontal Systemic Relationships in Smokers in the Asian Pacific Region** *S.L.C. Masulili, S.W.A. Prayitno, B. Sutrisna, E.I. Auerkari (Indonesia)*	122
Chapter 14	**Dentistry in Cambodia** *S. Chea (Cambodia)*	129
Chapter 15	**Mucogingival Issues Today** *K.M. Chung (Singapore)*	134
Chapter 16	**Smoking and Genetics: Disease Modifiers in Periodontal and Systemic Diseases** *D.F. Kinane (United States of America)*	138
	Poster Presentations	149

Acknowledgements

The 7th International meeting of the Asian Pacific Society of Periodontology was held in Beijing, China from 21-22 September 2007, immediately preceding the 8th National Conference of the Chinese Society of Periodontology. Over 360 delegates attended this APSP meeting making it one of the largest meetings held to date which indicates the continuing interest in this group in the region.

At the Inauguration Ceremony presentations were made by the Vice-Director of Peking University Health Science Center, (Professor Weigang Fang), the President of the Chinese Stomatological Association (Dr Xing Wang), the Dean of Peking University School of Stomatology (Dr Guangyan Yu) and the President of the Chinese Society of Periodontology and Chair of the Organizing Committee of the 7th APSP Meting (Dr Zhifen Wu). Congratulatory letters were read from the Vice Chairman of the Standing Committee of the National People's Congress (Professor Qide Han) and from the Vice Minister of the Chinese Ministry of Health (Mr Xiaohong Chen). The meeting was then officially opened with a roll call of the delegates from the APSP member countries. The two-day programme was very full with 20 presentations from speakers from 13 different countries. In addition 160 posters were scheduled for presentation.

Many important topics in relation to periodontal systemic interrelationships were discussed including cardiovascular disease, diabetes, rheumatoid arthritis, smoking, pre-term low birth weight, bisphosphonate therapy and gene polymorphisms. The quality of speakers was, as usual, very high with representatives from Australia, Cambodia, China, India, Japan, Malaysia, New Zealand, The Philippines, Thailand and United States of America providing the major presentations. The poster sessions were very successful and in keeping with tradition from previous meetings three prizes were awarded for the posters judged to be the best on the day.

The generous support of our Diamond sponsors Procter & Gamble, Oral B and Sunstar Corporation in conjunction with Bronze Sponsors Johnson & Johnson, Colgate and GlaxoSmithKline is very gratefully acknowledged. Without this important financial support, the 7th APSP meeting and publication of these Proceedings would not have been possible. I also acknowledge the special support provided by my co-editors Professor Isao Ishikawa and Dr Jincai Zhang. As always I thank the presenters for providing their manuscripts for publication. Finally this publication would not have come to fruition had it not been for the outstanding production editing of Ms Catherine Offler.

P. Mark Bartold
April 2008

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
Edited by: PM Bartold, I Ishikawa, J Zhang

Chapter 1

The Relationship Between Rheumatoid Arthritis and Periodontitis

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Introduction

"In fact, adult periodontitis and rheumatoid arthritis have much in common, so much so that I have argued that they are really the same disease" (Greenwald, 1999).

This bold and challenging statement may, at first sight, seem to be stretching the boundaries of conventional thought too far. However, close inspection of two of the most common chronic diseases afflicting humans reveals remarkable similarities which warrant further investigation.

When one considers that the sum total of ulcerated periodontal tissue in an individual with 28 teeth and periodontal pockets of around 5-6 mm would have a total area of ulcerated tissue of about 75cm^2 (Page 1998) it is conceivable that such a large amount of chronic infection and inflammation would have the potential to influence inflammatory reactions elsewhere.

Periodontal diseases

Periodontal diseases range from the relatively benign form, gingivitis, to chronic and aggressive forms which are not only a threat to the dentition but may also be a threat to general health. Recent reports suggest an increased prevalence of diabetes, atherosclerosis, myocardial infarction and stroke in patients with periodontal disease (Kim and Amar 2006). The likelihood of periodontal disease being associated with, and influencing, systemic diseases is fast becoming established fact. All forms of inflammatory periodontal disease are associated with chronic inflammation (accumulation of B and T lymphocytes as well as monocytes and neutrophils) resulting in destruction of the periodontal ligament and bone. If left untreated, significant tissue damage occurs, the affected teeth can become loose and may be lost if the disease continues to be active. What is particularly important about this disease is the great variability in its presentation within the population. Because of the multifactorial nature of the disease, which is modified by systemic, environmental and microbiological factors, not all individuals are affected to the same degree despite the ubiquitous presence of dental plaque.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is also a chronic destructive inflammatory disease characterized by the accumulation and persistence of an inflammatory infiltrate in the synovial membrane that leads to synovitis and the destruction of the joint architecture

resulting in impaired function (Weyand *et al* 2000). As a systemic disease RA has extra-articular manifestations in the pulmonary, ocular, vascular systems and other organs or structures that may be affected by the inflammatory process. The current paradigm for RA includes an initiating event (possibly a microbial exposure or a putative autoantigen) leading to significant synovial inflammation and tissue destruction. As for periodontitis there is an accumulation of inflammatory cells (T and B lymphocytes, neutrophils and monocytes), tissue oedema, endothelial cell proliferation and matrix degradation. RA is also modified by systemic, genetic and environmental variables.

Is there a role for bacteria in the aetiology and pathogenesis of rheumatoid arthritis?

Data from a number of animal models demonstrate that arthritis can develop secondarily to several different stimuli and through several different effector pathways including exogenous infections. If the observations in animal models are also applicable to human rheumatoid arthritis, we might anticipate that different types of infections as well as other environmental exposures with capacity to induce excessive pro-inflammatory cytokines in genetically susceptible individuals may contribute to disease either together with some autoimmune reaction or by themselves.

Could periodontal pathogens provide an infective source for rheumatoid arthritis?

Many of the so-called periodontal pathogens exhibit similar characteristics to those microorganisms suspected to induce rheumatoid arthritis in a genetically susceptible host. Periodontal pathogens, which are organised in a biofilm with the other groups of bacteria, incite a chronic continuous infection within periodontal tissues and also serve as an abundant supply of lipopolysaccharide. The aetiological agents in periodontitis reasonably fulfil some of the requirements for a microorganism able to trigger the inflammatory cascade seen in rheumatoid arthritis. Thus, the possibility that an ongoing periodontitis can trigger, or exacerbate rheumatoid arthritis (and *vice-versa*) in genetically susceptible individuals is biologically possible. Recently two reports have indicated that rheumatoid arthritis patients have elevated IgG levels to a number of periodontal pathogens compared to non-rheumatoid arthritis controls (Yoshida *et al* 2001, Ogrendik *et al* 2005)

Rheumatoid factor in rheumatoid arthritis and periodontal disease

Rheumatoid arthritis is commonly associated with the presence of autoantibodies to antigenic determinants of the Fc receptor of IgG molecules, termed rheumatoid factors. Rheumatoid factors (RF) are found in more than two-thirds of adult patients with rheumatoid arthritis, but they are not specific to rheumatoid arthritis and are found in patients with a number of other chronic inflammatory conditions, including periodontitis (Hirsch *et al* 1989, The and Ebersole 1991). Rheumatoid factor seropositive periodontal patients have been shown to have elevated IgM and IgG antibodies to a number of oral bacteria including those of the *Capnocytophaga gingivalis, Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans* (The and Ebersole 1996, Hara *et al* 1996). While IgM-rheumatoid factor from rheumatoid arthritis patients reacts with these bacteria it is believed to be a non-specific reaction towards shared epitopes between these

bacteria and IgG (The and Ebersole 1996). The precise function, if any, of rheumatoid factors in periodontal disease is still unclear. Nonetheless, increases in IgM-rheumatoid factor seen in periodontitis seems to reflect a chronic antigenic stimulation by periodontopathic bacteria which have cross reactive epitopes which permit clearance of the IgG-coated bacteria (The and Ebersole 1996).

Citrullinated proteins and autoimmunity

Recognizing that rheumatoid factors are relatively non-specific markers for rheumatoid arthritis has led to investigations for more specific markers. One such group of markers/ antigens are found in the anti-cyclic citrullinated peptide (CPP) autoantibody system. In this cascade, proteins become antigenic through the conversion of arginine to citrulline via deimination enzymes such as peptidyl arginine deaminase (PAD) (Masson-Bessiere *et al* 2001). With the accumulation of proteins such as fibrin within the synovium and their prolonged and complex degradation, which includes citrullination, leads to exposure of new epitopes to immunocompetent cells within the synovium (Masson-Bessiere *et al* 2001). In this context, anti-CPP antibodies have been reported to have a high predictive value for rheumatoid arthritis onset several years before it is evident clinically and are also associated with more severe clinical outcomes (Kroot *et al* 2000). Moreover, the presence of both rheumatoid factor and anticyclic citrullinated peptide autoantibodies is highly predictive of severe and progressive rheumatoid arthritis (Rantapaa-Dahlqvist *et al* 2003). Recently, PAD enzymes have been reported to be synthesized by periodontopathic bacteria which have the capacity to citrullinate proteins within inflamed periodontal tissues (Travis *et al* 1997).

What is the evidence for a relationship between periodontitis and rheumatoid arthritis?

In recent years there have been a number of published studies reporting a significant association between rheumatoid arthritis and periodontal disease. Simple analyses of self reported illnesses have indicated the likely interrelationship between periodontitis and rheumatoid arthritis (Mercado *et al* 2000, Lagervall *et al* 2003, Georgiou *et al* 2004). A number of case/control studies have also reported a significantly higher incidence of tooth loss and alveolar bone loss in patients with RA (Malmström and Calonius 1975, Albander 1990, Kaßer *et al* 1997, Mercado *et al* 2000, Al-Shammari *et al* 2005, Bozkurt *et al* 2006). In addition, other studies which have addressed cytokine profiles, HLA-DR shared epitopes and antibody titres to periodontopathic bacteria have contributed to our body of knowledge strongly suggesting an interrelationship between periodontitis and rheumatoid arthritis (Bozkurt *et al* 2000, Moen *et al* 2003, Havemose-Poulsen *et al* 2005, Marotte *et al* 2005, Ogrendik *et al* 2005, Havemose-Poulsen *et al* 2006, Bozkurt *et al* 2006). Of particular interest have been several studies which have reported that periodontitis may serve as a risk factor or severity factor for rheumatoid arthritis (Mercado *et al* 2000, Riberio *et al* 2006) and that periodontal treatment might even have a beneficial effect on rheumatoid arthritis (Riberio *et al* 2005, Al-Katma *et al* 2007). From a recent laboratory study it was reported that following induction of adjuvant experimental arthritis in rats, there was subsequent evidence of periodontal breakdown characterized by alveolar bone loss and increased matrix metalloproteinase activity in adjacent gingival tissues (Ramamurthy *et al* 2005). Most

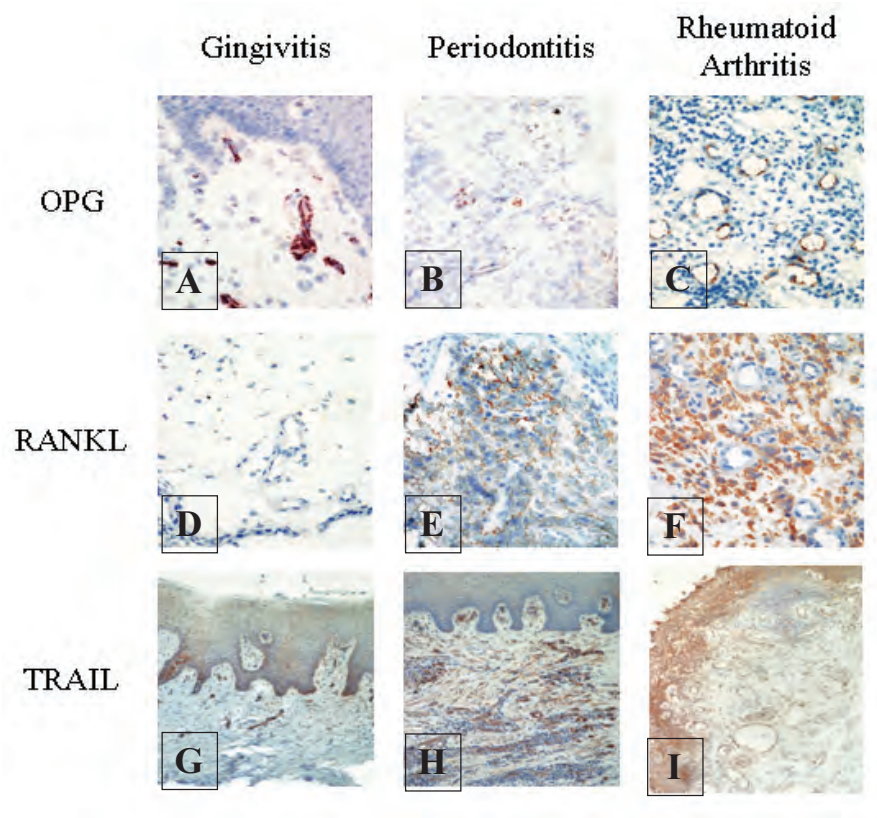

Figure 1. Staining of normal gingiva (A, D, G), inflamed tissue from periodontitis lesion (B, E, H) and inflamed rheumatoid synovium (C, F, I) stained for OPG (A, B, C), RANKL (D, E, F) and TRAIL (G, H, I). Note that OPG decreases with inflammation, RANKL increases with inflammation, and TRAIL increases with inflammation

recently yet another report, this time using data from the NHANES III survey, has demonstrated that rheumatoid arthritis may be associated with tooth loss and periodontitis (de Pablo *et al* 2007).

Recent studies investigating the relationship between rheumatoid arthritis and periodontitis

Studies have demonstrated that the prevalence of moderate to severe periodontitis is significantly elevated in individuals suffering from RA and also, the converse is also true in that periodontitis individuals have a higher prevalence of RA compared to the general population (Mercado *et al* 2000, Mercado *et al* 2001). Contrary to current dogma, RA patients do not have impaired oral hygiene (judged by plaque and bleeding scores). More recent studies from our laboratory have demonstrated that OPG and RANKL are highly expressed in biopsies of inflamed rheumatoid synovium and periodontitis lesions. We have noted that another ligand for OPG, TRAIL, is also expressed in the both types of tissue (Figure 1). With the development of inflammation OPG appears to decrease, while both RANKL and TRAIL increase with inflammation. These findings may be of considerable significance in light of OPG's ability to not only block the

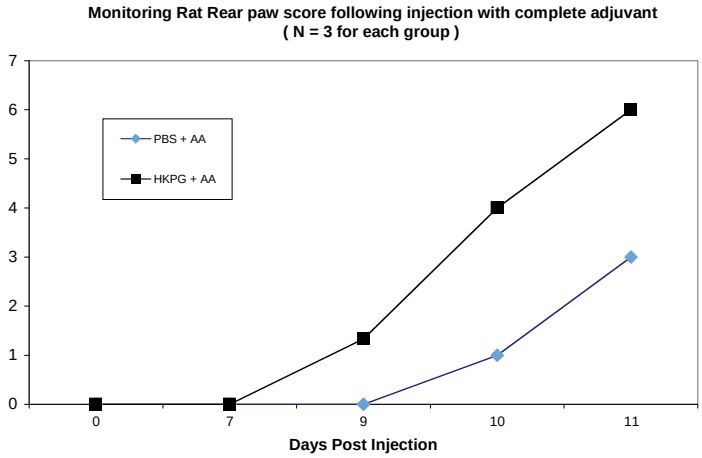

Figure 2. Effect of established chronic inflammation on development of experimental arthritis

activity of RANKL but also TRAIL and its anti-inflammatory properties (Crotti *et al* 2003, Haynes *et al* 2003).

A pilot study (unpublished) has demonstrated chronic inflammation associated with the *P. gingivalis* resulted in an earlier onset and more severe form of adjuvant arthritis in rats (Figure 2). We implanted heat killed *P. gingivalis* impregnated sponges implanted into rats and allowed 28 days for chronic inflammation to develop before induction of experimental arthritis (heat-killed TB in complete Freund's adjuvant). Figure 2 illustrates not only did the arthritis develop sooner but it developed faster and more severely in the animals with prior exposure to *P. gingivalis*-induced chronic inflammation than the control animals.

Current hypotheses for a relationship between rheumatoid arthritis and periodontitis

Local production of citrullinated proteins and anticyclic citrullinated peptide autoantibodies in periodontal tissues may be a source of extra-synovial autoantibodies

In a recent review, Rosenstein *et al* (2004) proposed a novel hypothesis for the development of rheumatoid arthritis via the humoral response to oral bacteria found in periodontitis (Figure 3). It is proposed that the development of autoimmune disease (eg. rheumatoid arthritis) can arise due to infectious agents (eg. periodontal pathogens) inducing immune responses to altered self-

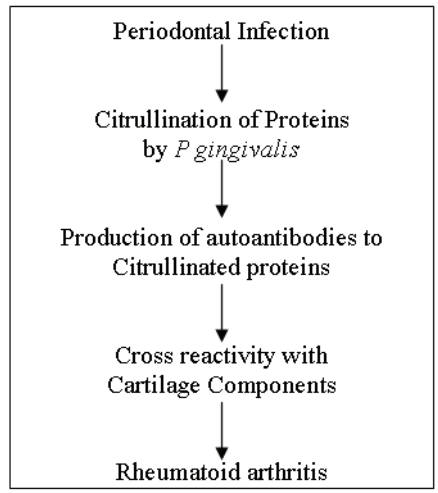

Figure 3. Proposed role of citrullinated proteins in rheumatoid arthritis

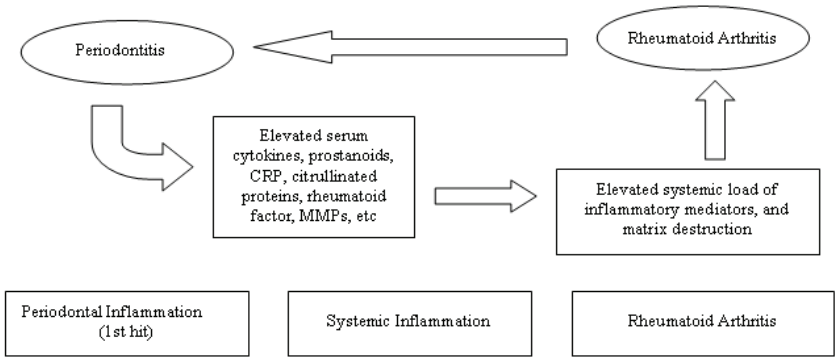

Figure 4. Schematic representation of "two-hit" model for development of rheumatoid arthritis and periodontitis

antigens in genetically susceptible individuals. This hypothesis recognises the importance of the production of rheumatoid factors and anticyclic citrullinated peptide autoantibodies in the development of rheumatoid arthritis. Since *P. gingivalis* produces deimination enzymes such as peptidyl arginine deaminase that can then induce autoantibodies, a link between periodontal infection and development of rheumatoid arthritis has been proposed.

Periodontitis and rheumatoid arthritis may be inter-related through a primed inflammatory response - "two-hit" model

This model seeks to explain how periodontitis and rheumatoid arthritis might be inter-related (Golub *et al* 2006). This model suggests that a primary "hit" of chronic inflammation via chronic periodontitis followed by an arthritogenic hit to induce rheumatoid arthritis can lead to an exacerbated response (Figure 4). Of course it may be that the converse could also hold true in that an initial hit of chronic inflammatory disease exacerbates the inflammatory response of developing periodontitis.

Reduction of periodontal inflammation may influence clinical parameters of chronic rheumatoid arthritis

Two reports have indicated that control of periodontal infection reduces the severity of active rheumatoid arthritis (Riberio *et al* 2005, Al-Katma *et al* 2007). These preliminary reports were based on a very small sample sizes and need to be extended and investigated in more detail. In the present proposal we plan to study a larger and better defined population of patients with both chronic periodontitis and chronic rheumatoid arthritis and monitor any responses through measuring a variety of clinical and laboratory markers for rheumatoid arthritis.

Concluding comments

For some time now it has been recognized that periodontitis and rheumatoid arthritis share many common pathologic features. Case control studies indicate that a strong relationship exists between disease severity and extent for individuals suffering from both rheumatoid arthritis and periodontitis. While causality between the two diseases is unlikely a number of possibilities exist which should be investigated such as the potential for

periodontal infection to lead to production of antibodies capable of reacting with auto-antigens from the synovial tissues leading either to initiation or modulation of the synovial tissue reaction seen in rheumatoid arthritis. Alternatively these diseases co-exist as a result of a generalized systemic dysregualtion of the immune and inflammatory responses and thus could be considered extensions or manifestations of the same disease process manifesting in different parts of the body. Whether this represents one in the same disease as suggested by some (Greenwald and Kirkwood 1999) remains to be established.

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Chapter 2

Comprehensive Investigations into Arterial Diseases and Periodontal Bacteria

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Introduction

Many types of infectious agents have been demonstrated as being associated with atherosclerotic lesions (Chiu 1999) or arterial plaque, including abdominal aortic aneurysms (Kurihara *et al* 2004). These agents include *Chlamydia pneumoniae,* cytomegarovirus (CMV), herpes virus, *Helicobacter pylori,* Hepatitis A and oral periodontopathic bacteria. Of these agents, the knowledge of *C. pneumoniae* has a long history and ample scientific evidence. In particular, it is known that *C. pneumoniae* and CMV are transported through monocytes in the blood without being killed by granulocytes. This translocation method assists us understand the process of bacterial invasion in the atherosclerotic area (Epstein *et al* 1999). However, few studies of other bacteria or methods of virus transportation have been undertaken. Under usual conditions, monocytes are phagocytic to bacteria except *C. pneumoniae* and CMV, so that due to strong adhesion activity *C. pneumoniae* safely reaches the damaged endothelial layer or completed athroma. In those which appeared in the literature, oral bacteria are the biggest group including 500 species and more than 2 billion instances even in healthy-looking individuals. Experimental and epidemiological studies suggested oral bacteria, especially periodontal bacteria, have an interesting behavior (Tonetti *et al* 2007).

Presence of oral bacteria

Chiu (1999) noted oral bacteria appeared from carotid plaque. Oral bacteria was also found in coronary arterial plaque, an abdominal aortic aneurysm and in lower leg atherosclerotic plaque. Some of this oral bacterium showed an adhesion effect to the endothelial cells (Nakamura *et al* 2007), or activation activity and aggregation of the platelets, and it is possibly that a bacterial delivery system by platelets is associated with increased infectious events to the aged endothelium, or surprisingly, no systemic effect by infection or even by bacteremia. This means that the oral bacterial must be classified as a kind of weak bacteria.

Bacterial transportation route from the periodontal area to the vein

This is still a controversial theory. However when we examine two papers detailing intra-mouth lymph flow and neck flow (Sato *et al* 2003, Haagensen *et al* 1972), it is reasonable that it originates from the gingival area down to the venous angle via the carotid bifurcation. From an anatomical point of view, they

suggested there may be a direct route from the mouth to the venous angle without passing the lymph nodes. Lymph vessels have valves in the lumen which accelerate the speed of the bacterial transportation. This route supports the bacterial appearance in the blood shortly after tooth brushing. Of course inflammation of the dental region may offer some kind of cytokine discharge into the vessels directly.

Clinical and experimental studies

A clinical study undertaken in 1995 suggested Buerger disease patients have severe periodontal conditions related to smoking (Iwai *et al* 2005), some HLA typing and a high bacterial serum titer compared to normal or control subjects (Chen *et al* 2007). Interestingly, countries with advanced levels of oral care, including Japan and South Korea showed a very low incidence of Buerger disease compared to countries such as India and Bangladesh, where oral conditions are affected by smoking of special cigarettes.

It has been shown that elderly persons are more likely to suffer from periodontal diseases. In addition, diabetic persons and pregnant women are also susceptible to periodontal diseases and arterial or venous trouble.

The results of many experimental studies are now available. Mice showed a small arterial thrombosis after continuous venous injection of oral bacteria (Kubota *et al* 2007) and dogs showed severe inflammation around the vein when the closed vein space was filled with bacteria (Umeda 2008). Li *et al* (2002) demonstrated the development of arterialsclerosis induced by *P. gingivalis* injection in the heterozygous apolipoprotein E-deficient murine.

Proposed mechanism of the vessel occlusion

Mechanisms of the occlusion or incompetent venous valve were easily explained from our and others' results. In Buerger disease, a large aggregated mass including bacteria causes embolic occlusion in the small arteries showing arterial spasms. Blood is contaminated with several tobacco substances, but endothelial function is almost normal. In contrast, atherosclerotic occlusion in atherosclerosis seems to be caused by endothelial surface adhesion in the large- or middle-sized arteries. Small-sized arterial occlusion may occur in diabetic patients in the same mechanism of Buerger disease. In varicosity, a severe periodontal condition during pregnancy may trigger venous valve incompetence (Kurihara *et al* 2007).

Conclusion

Oral bacteremia is a common phenomenon and individual susceptibility may be responsible for the development of arterial or venous lesions.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 3

Diagnosis and Classification of Periodontal Diseases and Conditions: Current and Future Challenges

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Introduction

In 1999 the "International Workshop for a Classification of Periodontal Diseases and Conditions" was held in order to revise and update the periodontal disease classification systems in use at that time (Armitage 1999). In attendance at that workshop were 60 invited participants from 18 different countries with significant representation from Europe (12/60 or 20%) and the Asian Pacific Region (10/60 or 16.7%). One of the challenges was to revise the widely used and popular classification system that emerged from the 1989 "World Workshop in Clinical Periodontics" (American Academy of Periodontology 1989). The need for revision had been recognized by many, including the Europeans who suggested some changes after the 1st European Workshop in Periodontology (Attström and van der Velden 1994).

The purposes of this paper are to briefly review the 1999 classification system with an emphasis on its strengths and weaknesses, discuss how diagnostic and classification systems serve different purposes, and summarize the current and future challenges posed by classification systems.

Classification systems of 1989 and 1999

Soon after the 1989 classification (Table 1) was published some of its numerous shortcomings were identified (Ranney 1991, Ranney 1993). The major problems included the following:

1. Did not include a gingivitis or gingival disease category
2. Periodontitis categories had non-validated age-dependent criteria
3. Extensive cross-over in rates of progression of the different periodontitis categories
4. Extensive overlap in clinical characteristics of the different periodontitis categories
5. "Refractory" periodontitis is not a single disease (extensive heterogeneity)
6. "Prepubertal" periodontitis is not a single disease (extensive heterogeneity)

The recommended solution for each of these problems was rather straightforward. A detailed Gingival Disease category was developed. Terms such as "adult" and "juvenile" presented troublesome questions such as "What validated biological criteria should be used to categorize an individual as a juvenile or an adult?" Therefore, it was suggested that "adult" and "juvenile" be eliminated as descriptors and replaced with Chronic and Aggressive periodontitis,

I.	ADULT PERIODONTITIS
II.	EARLY ONSET PERIODONTITIS
	A. Prepubertal Periodontitis
	1. Localized
	2. Generalized
	B. Juvenile Periodontitis
	1. Localized
	2. Generalized
	C. Rapidly Progressive Periodontitis
III.	PERIODONTITIS ASSOCIATED WITH SYSTEMIC DISEASE
IV.	NECROTIZING ULCERATIVE PERIODONTITIS
V.	REFRACTORY PERIODONTITIS

Table 1. Types of periodontitis suggested by the 1989 World Workshop on Clinical Periodontics (American Academy of Periodontology 1989).

respectively. Although rapid loss of clinical attachment (e.g. ≥2 mm in 3 months) can be observed, it occurs in different types of periodontitis. Since "Rapidly Progressive Periodontitis" was clearly a heterogeneous group, it was recommended the category be eliminated as a distinct or stand-alone disease entity. It was furthermore acknowledged that all forms of periodontitis can be non-responsive to therapy and cases designated as "Refractory Periodontitis" did not constitute a homogeneous group. Accordingly, this term was also eliminated as a separate disease category. Finally, the term "Prepubertal Periodontitis" was eliminated because of extensive heterogeneity within this group. Indeed, most of the reported cases of prepubertal periodontitis were actually periodontal manifestations of systemic diseases such as hypophosphatasia and leukocyte adherence deficiency (Armitage 2002, Armitage 2004a).

What emerged from the 1999 workshop was a slight modification of the previous classification (Table 2). It was not a revolutionary alteration of the way clinicians have traditionally grouped this complex group of diseases. Indeed, the classification still depends on clinical features or phenotypes of the various disease categories. Although the classification is clearly embedded in the infection/host response paradigm of periodontal disease (Armitage 2002), it is not firmly based on cause-related criteria.

Current challenges

Although the 1999 classification system was quickly adopted by many professional societies such as the American Dental Association, challenges or objections to the classification have been made. Some authors have voiced their objections since they believe that the system is not clinically useful (Meyer *et al* 2004, van der Velden 2000). For example, van der Velden (2005) believes that periodontal infections are so complex that any attempt to group diseases on the basis of their probable causes is a worthless exercise. He recommended that any classification of periodontal diseases should use a nominalistic (descriptive) approach since, "…the causal

I. GINGIVAL DISEASES
- A. Dental Plaque-Induced Gingival Diseases
  - 1. Gingivitis
  - 2. Modified by systemic factors
  - 3. Medications
  - 4. Malnutrition
- B. Non-Plaque Induced Gingival lesions
  - 1. Specific bacteria
  - 2. Viruses
  - 3. Fungi
  - 4. Genetic
  - 5. Manifestations of systemic diseases
  - 6. Traumatic injuries
  - 7. Foreign body reactions

II. CHRONIC PERIODONTITIS
- A. Localized
- B. Generalized

III. AGGRESSIVE PERIODONTITIS
- A. Localized
- B. Generalized

IV. PERIODONTAL MANIFESTATIONS OF SYSTEMIC DISEASES
- A. Associated hematological disorders
- B. Genetic disorders

V. NECROTIZING PERIODONTAL DISEASES
- A. Necrotizing ulcerative gingivitis
- B. Necrotizing ulcerative periodontitis

VI. ABSCESSES OF THE PERIODONTIUM
- A. Gingival
- B. Periodontal
- C. Pericoronal

VII. PERIODONTITIS ASSOCIATED WITH ENDODONTIC LESIONS

VIII. DEVELOPMENTAL OR ACQUIRED DEFORMITIES & CONDITIONS
- A. Tooth-related factors predisposing to plaque-induced diseases
- B. Mucogingival deformities (e.g. recession)
- C. Deformities on edentulous ridges
- D. Occlusal trauma

Table 2. Abridged outline of the classification of periodontal diseases and conditions that emerged from the 1999 International Workshop (Armitage 1999).

web for periodontitis is so complex and involves so many different constellations that a classification based on etiology is effectively precluded" (van der Velden 2005). The nominalistic classification suggested by him includes a combination of four major types of variables:

1. ***Extent of disease*** (i.e. incidental, localized, semi-generalized, generalized)
2. ***Severity per tooth*** (i.e. minor, moderate, severe)
3. ***Age of patient*** (Early Onset including prepubertal, postadolescent forms or Adult Periodontitis)
4. ***Clinical characteristics*** (Necrotizing, Rapidly Progressive, Refractory)

This approach retains most of the disease categories that were discarded by the 1999 classification. The reasons that these terms were abandoned have either been ignored or regarded as unimportant.

The nominalistic approach for the classification of diseases has other advocates besides van der Velden (Baelum and Lopez 2003). The descriptive approach can be useful since it involves the collection of information that might be important in developing a differential diagnosis for an individual patient's disease or condition. If little or nothing is known about the etiology of a complex groups of diseases, then the descriptive or nominalistic approach is a good first step toward organizing one's thoughts about the disease(s) being observed. Indeed, there was a period in the history of the classification of periodontal diseases in which these diseases were primarily grouped and classified according to their clinical characteristics. This method of classification, the "Clinical Characteristics Paradigm", was popular between1870-1920 (Armitage 2002). Based on the seminal work of many investigators over the past 50 years, it is now clear that the majority of periodontal diseases are infections (Löe *et al* 1965, Newman and Socransky 1977, Page *et al* 1997, Socransky and Haffajee 2002). This does not mean, however, that all afflictions of periodontal tissues are infections (Table 2).

One somewhat surprising challenge to the 1999 classification system has been made by authors who have developed major misconceptions about what the classification does, and does not, include. For example, Page and Sturdivant (2002) have incorrectly assumed that the classification only includes plaque-associated conditions. They proposed the existence of Noninflammatory Destructive Periodontal Disease (NDPD) on the basis of 2 case reports of patients with superb oral hygiene who experienced extensive loss of attachment and bone during follow-up periods of 10 to 13 years. These patients did not exhibit clinical signs of gingival inflammation nor did they develop deepened periodontal pockets over the observation periods. It is likely that the progressive gingival recession and loss of attachment was due to self-inflicted tissue damage since both individuals performed "aggressive daily oral hygiene" procedures. Periodontal damage caused by abusive oral hygiene practices has been well described in the literature (Hirschfeld 1931, Hirschfeld 1939). Under the 1999 classification (Table 2) these 2 cases could be placed in either of the following categories:

I. Gingival Diseases
  B. Non-plaque induced gingival lesions
    6. Traumatic lesions (accidental)
VIII. Acquired Deformities and Conditions
  B. Mucogingival deformities and conditions around teeth
    1. Gingival/soft tissue recession

A similar misconception was published by Hujoel *et al* (2005) in which they described cases of gingival recession and attachment loss in the absence of deepened periodontal pockets. They labeled these cases as examples of "periodontal atrophy" which they regarded as a distinct clinical phenotype. In this paper

they state, "Diagnostic classification systems for the past 30 years have been based on the premise that an individual with pocket-free gingival recession has a disease by the name of chronic periodontitis, and that both abnormally deep periodontal pocketing and pronounced gingival recession have one and the same etiology – plaque." This is simply not true. Unfortunately, no explanation is provided as to how or why they arrived at this highly questionable conclusion. Certainly the 1999 classification does not specify that all periodontal attachment loss must be caused by, and therefore categorized as, chronic periodontitis. Indeed, the 1999 classification does not rule out the possibility of having healthy gingival tissues on a reduced periodontium as might be found in a patient who has been successfully treated for periodontitis.

Classification systems and diagnoses serve different functions

Classification systems and diagnoses serve similar functions only when the cause of a disease is fully documented, such as the well-established etiological connection between *Mycobacterium leprae* and leprosy. However, when the group of diseases and conditions under consideration involve multifactorial and complex combinations of etiologic agents and risk factors, it is important to understand that classification systems and diagnoses serve different functions. In other words, when some of the links in the causative chain are missing or incompletely understood, classification systems and diagnoses serve different functions.

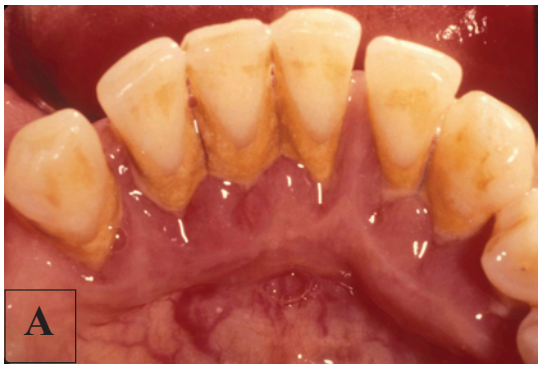
Classification systems group similar diseases and conditions into general categories and are useful in studying the full spectrum of diseases found in large populations of patients. These systems provide a general framework for studying the epidemiology, etiology, and pathogenesis of different categories of disease. They may be of value in evaluating optimal methods for treatment for a given group of diseases. Third-party (insurance) providers use classification systems as a guide or matrix for reimbursing healthcare providers for services rendered. Importantly, classification systems serve as a starting point for thinking about the periodontal diagnosis of an individual (i.e. the clinician's "first impression"). These systems do not serve as the only basis upon which a patient's periodontal disease is diagnosed.

Clinical diagnoses are derived from signs, symptoms, and laboratory findings (if applicable) of an individual patient. A diagnosis is a clinician's best guess as to what disease or condition the patient has and is the starting point for developing a patient-specific treatment plan. A periodontal diagnosis is an important label that clinicians place on a patient's periodontal condition or disease. It is primarily derived from information obtained from the patient's medical and dental histories combined with findings from a thorough oral examination (Armitage 2004b).

There are many ways clinicians can organize their thoughts when classifying and diagnosing a patient's periodontal condition. Some useful questions that might be asked in this clinically important thought process are:

1. How might this periodontal problem be classified?
2. What diagnosis might be assigned?
3. What are possible causes of the periodontal damage?
4. Are additional diagnostic tests advisable?
5. What treatment might be considered?

Figure 1A shows the lower anterior teeth of a 46 year old male who is a medically healthy nonsmoker with no history of periodontal therapy. Clinical findings at most sites included bleeding on probing (BOP), probing depths (PD) in the 5-6 mm range, and 5-9 mm of clinical attachment loss (CAL). The



Classification and Diagnosis

1. How might this periodontal problem be classified?

Chronic Periodontitis

2. What diagnosis might be assigned?

Generalized Severe Chronic Periodontitis

3. What are possible causes of the periodontal damage?

Poor oral hygiene; Plaque & Calculus

4. Are additional diagnostic tests advisable?

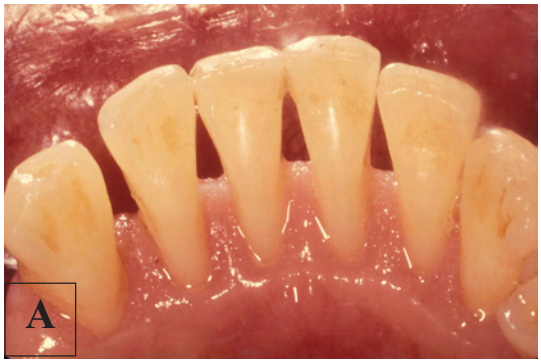
No

5. What treatment might be considered?

Oral Hygiene Instructions; Scaling and root planing; Surgery if needed for reduction of probing depths; Maintenance care.

B

Figure 1. (A) Lingual view of the lower anterior region in a 46 year old patient with extensive gingival inflammation and bleeding on probing, generalized probing depths in the 5-6 mm range, and 5-9 mm of clinical attachment loss. The patient is a medically healthy nonsmoker who has no history of periodontal therapy. (B) Classification and diagnosis of the condition.



Classification and Diagnosis

1. How might this periodontal problem be classified?

Acquired deformities; Gingival recession; Not a problem

2. What diagnosis might be assigned?

Periodontal Health on a Reduced Periodontium

3. What are possible causes of the periodontal damage?

History of periodontitis; Periodontal treatment

4. Are additional diagnostic tests advisable?

No

5. What treatment might be considered?

Continuation of periodontal maintenance care.

B

Figure 2. (A) Lingual view of the lower anterior region in the same patient shown in Figure 1 one year after nonsurgical periodontal treatment and maintenance care at 3 month intervals. There is no bleeding on probing, probing depths are in the 2-3 mm range, and there is 4-6 mm of clinical attachment loss. (B) Classification and diagnosis of the condition.

findings were generalized throughout the patient's remaining dentition. Figure 1B gives the answers to the five questions used when classifying and diagnosing this patient's periodontal problem. The patient's disease can be classified as a case of Chronic Periodontitis and assigned a diagnosis of Generalized Severe Chronic Periodontitis.

Figure 2A is the same region in the same patient as shown in Figure 1A except that it was 1 year after nonsurgical periodontal therapy (i.e. oral hygiene instructions plus scaling and root planing). The patient had been seen at 3-month intervals for periodontal maintenance care. Clinical findings at most sites included no BOP, PD in the 2-3 mm range, and 4-6 mm of CAL. Figure 2B gives the answers to the same five questions used in the initial diagnosis. The patient's condition can now be classified as a case of Acquired Deformities or Gingival Recession and assigned a diagnosis of Periodontal Health on a Reduced Periodontium.

The above example clearly demonstrates that treatment may change both the classification and diagnosis assigned to a patient. It should be noted that the classification and diagnosis are not necessarily interchangeable. In addition, the assigned classification and diagnosis are dynamic states that can be modified over time by changing environmental circumstances. The progression of untreated gingivitis into a case of chronic periodontitis is a common example of how one disease state can be transformed into another condition over time.

Future challenges

Any classification system for periodontal diseases and conditions should be viewed as a work in progress. As new information emerges about periodontal diseases, modifications in the classification system will be necessary. For example, in the original 1999 classification system, individuals with Localized Aggressive Periodontitis (LAP) were purported to exhibit a "robust serum antibody response to infecting agents," whereas those with Generalized Aggressive Periodontitis (GAP) were claimed to mount a "poor antibody response to infecting agents" (Lang *et al* 1999). Based on newer data it is now clear that the antibody responses of LAP and GAP patients are very similar and that it is not justified to include this feature as one of the distinguishing characteristics between these diseases (Albandar *et al* 2001, Picolos *et al* 2005).

Major shortcomings of the 1999 classification system for periodontitis include:

- It is quite likely that Chronic Periodontitis is a heterogeneous group (i.e. it is more than one disease). The same can also be said for each type of Aggressive Periodontitis (e.g. LAP is more than one disease; GAP is the phenotypic expression of multiple periodontal infections)
- The diseases are multifactorial (i.e. a large number of risk factors contribute to their overall pathogenesis)
- The diseases are polymicrobial and are associated with the endogenous oral microbiota
- Most of the diseases have similar clinical characteristics (i.e. they have few distinguishing features)
- The classification of these infections does not firmly rest on a foundation of validated and specific microbial agents

Among the important future challenges facing the classification of periodontitis is the need to determine the fundamental similarities and differences between chronic and aggressive forms of these infections. Much is already known, but additional studies of the comparative biology of chronic and aggressive periodontitis need to be conducted (Li *et al* 2004, Page *et al* 1997, Socransky and Haffajee 2002). These studies should coordinate all

meaningful components of this complex group of diseases such as their clinical features, histopathology, microbiology, immunology (innate and adaptive), risk factors (genetic and environmental), and response to treatment.

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Chapter 4

The Role of Inflammation in Atherosclerosis

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Introduction

Although atherosclerosis has been considered to be multi-factorial disease in which genetic, environmental, metabolic factors have been implicated, gaps remain in our knowledge of the etiopathogenesis of atherosclerosis. There is mounting evidence that inflammation plays an important role in the initiation, development and evolution of atherosclerosis, suggesting that atherosclerosis is an inflammatory disease (Ross 1999, Li 2004). Although triggers and pathways of inflammation are probably multiple and different in different clinical settings, the data from animals as well as humans, including our group, indicate that an inflammatory process is involved in all stages of atherosclerosis in different clinical entities.

Inflammation in silent myocardial ischemia

Silent myocardial ischemia (SMI) is a common phenomenon in patients with coronary artery disease. The phenomenon frequently occurs at rest, during daily life activities or after physical or emotional exertion (Pepine *et al* 1997). Although individual differences in pain threshold may partially explain the variability in pain perception, the mechanisms responsible for SMI are not well understood. A defective warning mechanism was proposed as the reason for the absence of pain, stressing that sensibility to pain differs from patient to patient (Droste and Roskamm 1983, Droste 1990). Differences in the central nervous system as well as alteration of peripheral nerve endings was also proposed (Falcone *et al* 1998a, Falcone *et al* 1998b).

Recent data, however, indicate that a significant increase of levels of anti-inflammatory cytokines, together with the decrease of leukocyte adhesion molecule expression, might identify one of the mechanisms for SMI. More recently, observations have shown that there is a particular biochemical pattern of inflammatory system activation (e.g. an increased production of inflammatory cytokines) that explains the lack of anginal symptoms in patients with SMI. That is, pain perception may result from microenviromental balance between pro-inflammatory and anti-inflammatory cytokines (Mazzone *et al* 2001).

Previous data have shown that T-lymphocyte and monocyte infiltrates within atherosclerotic lesions may be responsible for plaque instability and coronary events. They may also be associated with pain perception. The T-lymphocyte responses can be

categorized as pro-inflammatory (Th1 type) or anti-inflammatory (Th2 type) (Liuzzo *et al* 2000). The Th1 type activation leads to pro-inflammatory cytokine release such as tissue necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8, whereas Th2 lymphocyte activation leads to endorphin and anti-inflammatory cytokine IL-4 and IL-10 release. The pro-inflammatory pattern activation seems to intensify nociception, whereas Th2 lymphocyte production seems to reduce the pain perception (Panerai and Sacerdote 1997). Several observations suggest that inflammatory cytokines orchestrate nociception and also suggest the role of pro-inflammatory and anti-inflammatory cytokines in the perception of pain. In particular, pro-inflammatory cytokines are known to induce the release of pain mediators, such as bradykinin and calcitonin gene-related peptide (CGRP) and to activate vagal afferents (Opree and Kress 2000).

Pain perception may result from a microenviromental balance between pro-inflammatory and anti-inflammatory cytokines. Amongst several cytokines, the IL-1β is a pro-inflammatory cytokine able to induce hyperalgesia. Data from experimental studies suggests that central administration of IL-1 enhances the spinal-cord-evoked release of substance P (SP) and CGRP (Lanza *et al* 2004). In contrast, the IL-1 receptor antagonist attenuated the IL-1β-induced hyperalgesia (Oka *et al* 1993). Other studies indicated that TNF-α induces the pain and hyperalgesia after injection; the effects are mediated by direct sensitization of primary afferent nociceptors and by upregulation of other pro-inflammatory proteins (Junger and Sorkin 2000). Both subcutaneous and intraplantar TNF-α injections contribute to hyperalgesia and inflammation; this evokes ongoing activity in type C nociceptors, causes significant and dose-related increases in skin vascular permeability, induces pro-inflammatory cytokine production (IL-1, IL-6, IL-8), and triggers the cyclooxygenase-dependent pathways to synthesize prostaglandins (Wordiczeck *et al* 2000). In experimental inflammation, pentoxifylline was shown to inhibit TNF-α release, with a consequent decrease of pain-related behavior.

However, administration of the anti-inflammatory cytokine IL-10 was shown to reverse the dynorphin-induced allodynia, which is a model of neuropathic pain in the mouse, thus suggesting that allodynia might be modulated by the inflammatory cytokine system (Laughlin *et al* 2000). IL-10 inhibits the positive inflammatory feed-forward loop by inhibiting the initial induction and the subsequent amplification of pro-inflammatory cytokines. A single peripheral administration of IL-10 was shown to decrease nerve injury-induced thermal hyperalgesia and intradermal endotoxin-induced hyperalgesia (Wagner *et al* 1998). Other data showed that IL-10 levels increased significantly in asymptomatic patients with coronary artery disease. Moreover, transforming growth factor-β (TGF-β) stimulates connective tissue growth and collagen formation, and it can virtually and strongly inhibit all the immune and hematopoietic function, especially if present before cell activation (Fontana *et al* 1992). It also has a role in mediating inflammation and cytotoxic reactions. TGF-β blocks the INF-γ induced cell activation, thus increasing the production of reactive oxidate stress, prostaglandins and nitric oxide. IL-4 has similar effects to that of TGF-β in limiting the inflammatory hyperalgesia.

Patients with coronary artery disease who have painful anginal attacks during a 24 hour period are likely to also have additional painless ischemic episodes, usually triggered by physical exertion or mental stress. Several recent studies suggested that acute mental stress in experimental animals as well as humans delayed increases in circulating

inflammatory cytokine levels, and suggested that individual differences in cytokine responses are associated with sympathetic reactivity (Maes 2001, Steptoe *et al* 2001). More recently, Mazzone *et al* (2001) investigated inflammatory cytokines in patients with transient myocardial ischemia to determine whether silent ischemia correlates with a particular pattern of cytokine production. Inflammatory system activation markers were detected in patients with symptomatic angina and in patients with silent myocardial ischemic episodes. The CD11b adhesion molecule expression was detected on phagocytes, and proinflammatory (IL-1β, TNF-α, IL-6 and INF-γ) and anti-inflammatory cytokines (IL-4, IL-10 and TGF-β) production was quantified in ischemic patients.

In addition, the CD11b/CD18 binds to the activated complement factor (C3a), enhancing the inflammatory process, and recognized fibrinogen-coated surface, leading to neutrophil-clot adhesion and fibrin digestion. Within the inflammatory site, the activated phagocytes release the cytokines interferon-γ (INF-γ), IL-4, IL-10 and TGF-β, which further enhance cellular migration. Other proinflammatory cytokines such as IL-1β, TNF-α and IL-6 are known to be released within inflamed tissue (Warkins *et al* 1994). Peripheral inflammation stimulates peripheral never endings, causing hyperalgesia due to enhanced localized inflammatory mediators; the local release of cytokines and endogenous opioids might be able to modulate the threshold for peripheral nerve-ending activation (Woolf 1997). Pain perception, therefore, may result from the microenviromental balance between proinflammmatory (IL-1β, TNF-α, IL-6, and IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines.

In brief, asymptomatic ischemia can be induced by physical or mental stress but may occur without any obvious trigger, and lack of chest pain did not exclude ischemic heart disease. The clinical significance of SMI is similar to that of symptomatic (painful) ischemia. Although the exact mechanisms responsible for SMI is not well-known, different patterns of myocardial ischemia attacks presenting as painful or silent may not only be related to a defective warning system, alteration of the central nervous system as well as peripheral nerve endings, but also as a different pattern of inflammatory response in patients with coronary artery disease.

Inflammation in cardiac syndrome X

Among patients undergoing coronary angiography due to angina typical enough to suggest coronary disease, 10-30% are found to have "normal" or "near normal" epicardial coronary arteries through angiography (Crea and Lanza 2004). These patients present with features of "cardiac syndrome X" (CSX). This syndrome is typically characterized by:

1. Predominantly effort-induced angina
2. ST segment depression suggestive of myocardial ischemia during spontaneous or provoked angina
3. Normal coronary arteries at angiography
4. Absence of spontaneous or provoked epicardiac coronary artery spasm
5. Absence of cardiac (for example, hypertrophic or dilated cardiomyopathy) or systemic (for example, hypertension, diabetes) diseases potentially associated with microvascular dysfunction (Dominguez-Rodriguez *et al* 2004).

CSX is diagnosed by typical angina pectoris, positive treadmill exercise test, negative intravenous ergonovine test and normal coronary angiography. The pathogenesis of CSX has been previously ascribed to myocardial ischemia that may be caused by microvascular dysfunction and increased sensitivity to intracardiac pain

(Dominguez-Rodriguez *et al* 2004, Maseri *et al* 1991, Cannon *et al* 1992). Despite extensive studies, the pathophysiological mechanisms in CSX, however, remain unclear. More recently, the data have suggested that chronic inflammation has been associated with CSX (Lanza *et al* 2004, Cosin-Sales *et al* 2003, Lin *et al* 2003, Tomai 2004, Arroyo-Espliguero *et al* 2003), with an increase in inflammatory markers associated with the disease activity in patients with CSX. Statin, a lipid-lowering as well as anti-inflammatory drug, has significantly modified the disease process in this special syndrome (Kayikcioglu 2003, Rosenson 2003, Pizzi *et al* 2004).

Recent studies suggest that low-grade inflammation might also play a pathogenetic role in the microvascular dysfunction of patients with CSX. The data suggest that inflammation is also involved in both coronary macro- and micro-circulation abnormalities observed in patients with coronary artery disease. Both endothelial cell activation and coronary endothelial dysfunction have been reported in these patients, which result in an increased release of constricting factors and the production of pro-inflammatory cytokines, cell adhesion molecules, and growth factors responsible, in turn, for microvascular dysfunction.

Firstly, recent findings have suggested that inflammation may contribute to endothelial dysfunction in CSX. Lanza *et al* (2004) reported that 2 indexes of systemic inflammation, C-reactive protein (CRP) and IL-1 receptor antagonist, increased in patients with CSX compared with well-matched healthy control subjects, suggesting that low-grade inflammation may play a pathogenetic role in at least a subgroup of such patients. CRP is a marker of chronic inflammation and a predicator of vascular disease, which has also been found to correlate with disease activity, electrocardiogram markers of myocardial ischemia, in patients with CSX. The possibility that inflammatory mechanisms might contribute to endothelial activation and dysfunction in CSX was first suggested by Tousoulis *et al* (2001), who found that increased blood levels of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), adhesion molecules that are synthesized by activated endothelial cells in response to inflammatory stimuli, in patients with CSX. Cosin-Sales *et al* (2003) found increased levels of CRP in patients with CSX. These patients also had evidence of more active disease, as indicated by more severe angina pectoris, more frequent episodes of ST-segment depression during Holter monitoring, and earlier and more evident ischemic ST-segment changes during an exercise stress test.

Furthermore, the article by Teragawa and colleagues (2004) provides new insights into the relation between inflammation and coronary microcirculatory dysfunction. They investigated the relation between CRP concentrations and coronary microvascular endothelial function in 46 Japanese patients with atypical chest pain and angiographically normal coronary arteries. They found that CRP values are inversely and independently correlated with acetylcholine-induced changes in coronary blood flow. In keeping with previous studies showing a close relation between inflammation and peripheral endothelial function in patients with CSX, this study further suggests a close relation between inflammatory mechanisms and coronary microvascular dysfunction (Lin *et al* 2003).

Despite the good prognosis of CSX, the chronic, frequent nature of persistent angina and reduced exercise tolerance can significantly impair quality of life. Besides their role in atherogenesis and in acute coronary syndromes (ACS), inflammatory mechanisms are likely to also play a role in coronary microvascular dysfunction. However, triggers and mechanisms of

inflammation in this setting are largely unknown and probably different to those involved in the two other pathophysiological conditions. Thus, lowering the inflammatory response, for example with the use of statin and/or aspirin, might improve coronary microvascular dysfunction. However, the validity of this approach is still unknown and deserves further investigation. Indeed, as mediators of inflammation are multiple, the strategy of identifying triggers and mechanisms of inflammation in each special clinical setting and directing treatment at the special triggers or to rate limiting steps in effector pathways appears more reasonable or a promising strategy.

Inflammation in acute coronary syndrome

An acceptable theory is that ACS is caused by rupture of the atherosclerotic plaque with superimposed thrombus formation, which is a complex process and involving a number of different stages. The data from animals as well as humans indicate that an inflammatory process is involved in all stages of atherosclerosis in different clinical settings (Sato *et al* 1995, Libby 1995, Tracy 1998, Fuster 1994, Lee and Libby 1997). Inflammation is one of the most important features of vulnerable plaque, and occurs in most vulnerable plaque, which is comprised of monocytes, macrophages, and lymphocytes in both the cap and in the adventitia (Libby 1995). Macrophages produce tissue's degrading enzymes; matrix metalloproteinases (MMPs) in response to T-lymphocyte stimulation. While macrophage-based MMPs degrade plaque collagen, T-lymphocytes produce interferon-gamma which inhibits collagen synthesis (Galis *et al* 1994, Schonbeck 1997). Inflammation leads to the localized recruitment of neutrophils and monocytes, and the presence of activated macrophages in the cap of the atherosclerotic plaque has led to suggestions that they secrete cytokines and other pro-inflammatory mediators and may contribute to plaque rupture through an effect on matrix metalloproteinase (Caliguri 1998). Patients with atherosclerosis involving coronary, carotid, or peripheral arteries often have elevated serum levels of these mediators. They may also have high levels of CRP, fibrinogen, and other acute-phase reactants produced by the liver in response to circulating cytokines (Li and Fang 2004, Omoda 2001, Angioi *et al* 2001).

Smooth muscle cells in plaque counterbalance the tissue degradative processes by producing interstitial collagen which strengthens the fibrous cap (Schonbeck *et al* 1997). Collagen synthesis and degradation thus constitute counterbalance forces in plaque architecture. Progressively thinning plaque fibrous caps suggest an equilibrium imbalance between collagen and matrix synthesis versus degradation. This equilibrium has yet to be fully characterized for atherosclerotic plaque, which is associated with an inflammatory process. Moreover, multiple cytokines and growth factors are also present at sites of inflammation, and each of these can potentially influence the nature of inflammatory response. Recent studies have shown that vascular inflammation can be attenuated by anti-inflammatory mechanism that maintain the integrity and homeostasis of the vascular wall, and that imbalance between anti-inflammatory mechanisms and pro-inflammatory factors, in favor of the pro-inflammatory factors, will result in rupture of atherosclerotic plaque (Libby 1995). Therefore, inflammation weakens plaque and is key to plaque rupture, and those inflammation vascular pathologies are assuming great importance in understanding their role in ACS.

Dynamic instability of a coronary

atherosclerotic plaque is now seen as the foundation for the development of the clinical syndromes we recognize as unstable angina and myocardial infarction. A complex intravascular inflammatory response is an integral component of this dynamic instability. Compared with patients who have chronic stable angina, patients with ACS have coronary plaques with more extensive macrophage-rich areas (Galis *et al* 1994). In addition, previous data showed that pro-inflammatory cytokines played an important role in acute coronary events, and that vulnerable plaques have also activated T cells that express proinflammatory cytokines such as IF-γ, TNF-α, IL-1 and IL-6, which affect extracellular matrix collage production and activate macrophages (Caliguri *et al* 1998). Previous studies have also demonstrated that decreased plasma concentration of anti-inflammatory cytokine was also associated with ACS (Li and Fang 2004, Omoda and Aoki 2001, Angioi *et al* 2001).

The concept that ruptures of atherosclerotic plaque are mainly triggered by inflammatory insults has stimulated the strategic development of vascular inflammation control. A well-known result is the development of statin therapy. Originally, reductions in cardiovascular disease events and mortality and overall improved outcomes were generally attributed to the cholesterol-lowering property of statins. However, an increasing number of *in vitro* and *in vivo* studies have revealed that statins reduce cardiovascular events to a greater extent than can be explained by their effect on lipids. Recently, several studies have attempted to elucidate the mechanism by which statins reduce cardiovascular risk, in particular through an anti-inflammatory effect. In our recent study, the results demonstrated that simvastatin significantly inhibits the inflammatory response in cultured monocytes induced by CRP as well as lipopolysaccharide in a dose-dependent manner (Li and Chan 2003). Data also suggested that treatment with a single high-dose or a short-term common dose of simvastatin could rapidly reduce the CRP level in patients with or without coronary artery disease (Li 2003, Li and Fang 2007). In fact, a large number of investigations have demonstrated that the administration of statin could modify CRP concentrations with a concurrent fall in cardiovascular events (Jackson 2000, Szucs 1998, Fairhurst and Huby 1998).

In contrast to complex clinical trials, animal models allow the separation of the potential direct anti-inflammatory activity of statins from their lipid-lowering action. Lefer *et al* (1999) were among the first to report on the anti-inflammatory action of statins in an *in vitro* model of acute myocardial ischemia-reperfusion. Since this initial study of statin therapy in acute myocardial infarction *in vitro*, there have been a number of *in vivo* reports demonstrating highly potent anti-inflammatory and cardioprotective actions of statins in acute myocardial infarction in normocholesterolemic, hypercholesterolemic, and diabetic animal models. A study performed by Diomede *et al* (2001) showed that lovastatin inhibits leukocyte recruitment in an animal model of acute inflammation (air-pouch model) at oral doses of 5-10 mg/kg. The short treatment schedule used in this model inhibits hepatic HMG-CoA reductase activity but does not affect blood cholesterol levels. The effect on leukocyte migration was associated with the downregulation of the chemokines monocyte chemotactic protein-1 (MCP-1) and RANTES (regulated on activation, normal T-cell expressed and secreted), and the cytokine IL-6. Consistent with these results, Kimura *et al* (1997) showed that fluvastatin treatment (6 mg/kg) of hypercholesterolemic rats reduced the number of leukocytes that adhered to postcapillary venules in response to platelet-activating

factor or leukotriene B4. As observed in the air-pouch model, the protective action occurred independently of the cholesterol-lowering effect of the drug. Stalker *et al* (2001) found that systemic administration of rosuvastatin (0.5-1.25 mg/kg) attenuates thrombin-induced leukocyte rolling, adhesion and transmigration in normocholesterolemic rats. In this model, rosuvastatin treatment enhanced the release of nitric oxide (NO) from the endothelium and downregulated endothelial P-selectin. Further evidence for a direct anti-inflammatory effect of statins is provided by Sparrow *et al* (2001) using an established model of acute inflammation (carrageenan-induced foot pad edema). These authors demonstrated a significant reduction in edema formation following oral simvastatin treatment (3-10 mg/kg). Moreover, they show that simvastatin administered orally (100 mg/kg) exhibits anti-atherosclerotic activity in apolipoprotein-E-deficient mice on a high-fat diet. Maggard *et al* (1998) found that pravastatin (5-10 mg/kg) prevents the development of coronary vasculopathy in a rat cardiac transplant model. This effect is associated with reduced infiltration of macrophages to the graft. Finally, a study by Stanislaus *et al* (2001) showed that intraperitoneal administarstion of lovastatin (2 mg/kg) has beneficial effects in experimental allergic encephalomyelitis in rats. The authors found that lovastatin decreased the transmigration of mononuclear cells into the spine cord, possibly by inhibiting the expression of the β2 integrin lymphocyte function associated antigen 1.

It has also been demonstrated that a reduction in CRP levels decreased coronary events in patients with ACS. Numerous trials have indicated the efficacy of statins in ACS, and aggressive lipid lowering, by either diet or combination of diet and statins, can reduce angina and coronary events after revascularization. However, several previous clinical trials have been designed to investigate the early effect of statins in ACS, in which the statins were given up to 48-96 hours after admission, and designed to as long-term trials focused in the majority of case on clinical outcomes or lipid profile. The rapid reduction of serum CRP levels with 8 weeks cerivastatin treatment in patients with primary hypercholesterolemia has been demonstrated recently (Ridker *et al* 2001). More recently, Plenge *et al* (2002) and our data (Li 2003) have demonstrated that simvastatin induced significant reductions in median CRP levels and in mean CRP levels on day 14 without a dose-dependent manner in patients with primary hypercholesterolemia. In our very recent study, data indicated that a rapid reduction of CRP in patients with coronary artery disease could even be achieved within 24 hours if a statin was given immediately after admission. The statin also positively influenced their short-term follow-up clinical outcomes (Li and Fang 2007). These data demonstrated that statin should be given as early as possible in patients with ACS, like aspirin, and rapid reduction of inflammatory marker may produce early benefit to coronary endothelium in this high-risk subgroup of coronary artery disease.

According to the theory that imbalance between pro- and anti-inflammatory cytokines plays an important role in the development of vulnerable plaque, we hypothesized that enhanced anti-inflammatory cytokines may be beneficial for ACS. Our recent data have shown that statins possess multiple anti-inflammatory actions, including increasing an anti-inflammatory cytokine IL-10 in patients with coronary artery disease (Li *et al* 2005, Li *et al* 2006). This novel finding may be of a great interest, because statin therapy may translate to a balance in the inflammatory response in patients with coronary artery disease. This may also provide a new theory or therapeutic strategy for ACS.

Inflammation in percutaneous coronary intervention

A systemic inflammatory response to coronary angioplasty has also been reported after balloon angioplasty or stent implantation (Sha 2003, Aggarwal *et al* 2004, Blum *et al* 2004, Li *et al* 2004a). Data from different catheter laboratories has shown that percutaneous coronary intervention (PCI) could trigger or initiate the inflammatory process in vascular lesions. Inflammatory cells are activated promptly after vascular injury and recruited to the site of injury. These cells are capable of releasing mediators, for example, pro-inflammatory cytokines, that facilitate smooth muscle cell migration and proliferation.

PCI could induce an early inflammatory action. Recently, Aggarwal *et al* (2004) performed a prospective study evaluating the early increase in markers of inflammation after coronary stenting. They studied seventy-five patients undergoing PCI, and evaluated plasma CRP, IL-6, IL-1 receptor antagonist as well as soluble CD 14 ligand (sCD14L) before and 10 minutes after PCI. They found that a systemic inflammatory response is detectable 10 minutes after coronary stent placement and there is also a very early increase in sCD14L, indicating a possible role for this marker in the initiation of inflammation after coronary stenting. Blum *et al* (2004) observed postprocedure endothelial dysfunction and levels of pre and postprocedure cytokines in 30 consecutive patients who underwent coronary stenting. All patients underwent brachial artery testing with responses to high-shear stress (flow-mediated-dependent dilation) and to nitroglycerin (flow-mediated-independent dilation) 18-24 hours after PCI established. The data showed that patients undergoing PCI exhibit a significant dysfunction of both endothelium-dependent and –independent dilation 24 hours after the procedure. They also found a significant association between concentrations of CRP and the severity of endothelial dysfunction after PCI. These findings identify a potential mechanistic association between inflammation and adverse events after PCI mediated by increased systemic endothelial dysfunction. Our recent data have indicated that renal artery stenting could trigger an inflammatory response as evidenced by an increase in plasma levels of CRP and IL-6. IL-6, however, was an early initiator of inflammatory cytokine, and CRP was a later marker of systemic inflammatory response to renal artery stenting (Li *et al* 2004a). These observations indicate that systemic inflammation characterizes the response to vascular injury after PCI.

Moreover, inflammatory markers after PCI are associated with cardiovascular events, and magnitude of the systemic inflammation is linked to adverse late clinical outcome. Several studies have shown that greater concentrations of CRP before and after PCI is associated with an increased risk of post procedure complications (Drachman and Simon 2005, Gogo *et al* 2005, Angioi *et al* 2001). A clinical investigation demonstrated that patients with diabetes mellitus undergoing PCI have significantly higher concentrations before intervention, which is consistent with their heightened risk of complications after PCI. Chew *et al* (2001) has reported that CRP could increase the prognostic value among established markers of risk in PCI. Their data suggests that patients undergoing PCI may be risk stratified according to an increase in concentration of cytokines before PCI as well as an increase in their concentrations afterward. In other words, the strong association of inflammation with adverse events after PCI, therefore, suggests that modulation of inflammatory cytokines is a promising therapeutic target.

In addition, inflammatory markers are

associated with restenosis after PCI. Kubica *et al* (2005) reported that increased preprocedural TNF-α and CRP levels and elevated CRP concentrations evaluated 24 hours after the PCI were significant predictors of both clinical restenosis and major adverse cardiac events, while high serum amyloid A values at 24 hours accurately predicated clinical restenosis. Interestingly, recent data show that an inflammatory response to PCI appears similar in those treated with drug-eluting stent and bare mental stent. Accordingly, the reduction in restenosis after drug-eluting stent is likely not mediated by attenuation of the systemic markers, such as CRP and IL-6 (Gogo *et al* 2005). In human studies, the severity of post-angioplasty luminal loss has been found to correlate with activation of circulating leukocytes, and restenosis in patients undergoing directional atherectomy has been determined to be correlated with the percentage of macrophages in retrieved tissue at the time of angioplasty.

In brief, restenosis following coronary stenting has long been attributed to neointimal proliferation, thrombosis, and negative remodeling. More recently, the important role of inflammation in vascular healing has also been increasingly well understood. From animal models and clinical experience, we know that endothelial injury, platelet and leukocyte interactions, and subcellular chemoattractant and inflammatory mediators are pivotal in the development of inflammatory response following implantation. By examining the specific mechanisms governing the inflammatory response to PCI, we may gain insight into potential therapeutic targets and strategies to prevent restenosis in clinical practice.

Atherosclerosis: a more rational term

The term 'atheroma', a Latin word meaning 'tumor filled with gruel-like pus', was first used in 1755 by Albrecht von Halles to designate the plaque deposited on the innermost layer of systemic artery walls, which resembles an abscess or cyst containing pus-like material. In 1940, however, Félix Marchand suggested the word 'atherosclerosis' should be better instead of 'atheroma', which is derived from two Greek roots: athéré means gruel or porridge and sclerosis meaning hardening. It is an obvious improvement over the older designation arteriosclerosis, which recognizes the calcific hardening of advanced disease but overlooks the fatty debris of active plaques. The term atherosclerosis is still used to date because it describes the two components of plaque: the lipid-filled core of atheroma encased in a shell of sclerosis or fibrosis, which presents the feature of atherosclerotic structure (Capron 1996).

Inflammation leads to the localized recruitment of neutrophils and monocytes and the presence of activated macrophages in the cap of the atherosclerotic plaque has led to suggestions that they may contribute to plaque rupture through effects on matrix metalloproteinase, secrete cytokines and other pro-inflammatory mediators. Patients with atherosclerosis involving coronary, carotid, or peripheral arteries often have elevated serum levels of these mediators. They may also have high levels of CRP, fibrinogen, and other acute-phase reactants produced by the liver in response to circulating cytokines. Our data have shown that increased levels of CRP in patients with unstable angina are associated with short-term clinical outcomes, the response the conventional therapy, and activation of nuclear factor-kappa B (NF-κB), but it is not correlated to coronary artery stenosis as well as lipid profiles (Li *et al* 2004b, Li *et al* 2002). More recently, accumulating evidence suggest that CRP may have direct proinflammatory effects associated with all stages of atherosclerosis. In our recent

study, the results demonstrate that monocytes exhibit an enhanced production of IL-6 in response to CRP, and this response is significantly inhibited by simvastatin in a dose-dependent manner (Li and Wang 2004). This may be of important interest in the connection between atherosclerosis and CRP (Li and Fang 2004).

Chronic inflammation is a hallmark of atherosclerosis and inflammatory reactions associated with vascular alterations seem to play a major role in the development of atheromatous plaque. In addition, the role of infection and autoimmunity has been implicated based on microbiological, biochemical, and immunological evidence from experimental animal model and from humans. Coronary heart disease was found to be associated with chronic dental infections, helicobacter pylori and chlamydia pneumonia infections (Rugonfalvi-Kiss *et al* 2002). Furthermore, inflammation is also of major importance to the restenotic process. Inflammatory cells are activated promptly after vascular injury and recruited to the site of injury. These cells are capable of releasing mediators that facilitate smooth muscle cells migration and proliferation.

Therefore, increasing evidence shows that the development of atherosclerosis is associated with inflammation. Prospective epidemiologic studies have consistently shown that markers of inflammation are independent predictors of cardiovascular events. Increased levels of inflammatory markers have been documented in various settings of coronary artery disease, especially in ACS. Clinical studies in patients with ACS found highly increased levels of inflammatory markers on admission and a strong association with clinical outcome. Patients with chronic and stable coronary artery disease have shown clear evidence of a low-grade inflammation, which is independent of traditional cardiovascular risk factors. A systemic inflammatory response to coronary angioplasty after balloon angioplasty and after stent implantation has also been reported.

Based on this evidence and in light of the new understanding that inflammation is an intrinsic part of the process, further change of nomenclature, calling the disease atherosclerosis, should be considered.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 5

Options for Achieving High Predictability and Longevity in Periodontally Compromised Cases

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Introduction

Patients generally present at dental clinics with functional or esthetic problems such as missing teeth, vertical or horizontal alveolar bone loss, mobile teeth, swelling or pain. However, periodontal disease is known as a silent disease and many patients do not visit a dentist until the progress of the disease causes difficulty in chewing.

Over 80% of the cases of periodontal disease seen in our daily practice are chronic periodontitis. Most periodontal problems can be resolved by treating local factors.

Periodontal disease can be observed as various pathological phenomena in periodontal tissues such as swelling or bleeding, deep periodontal pockets, lack of attached gingival and gingival recession in the gingiva. Biofilms on teeth, furcation involvement or root caries can also be seen. Pathological conditions can also cause bone loss. Therefore, one should treat loss of attachment of the marginal gingiva to bone to create an environment in which teeth can be cleaned easily.

There are many treatment options now available (Table 1). If there is swelling gingival bleeding, one should try to reduce the inflammation by brushing or scaling and root planning (SC/RP). When there are deep periodontal pockets, one should try to reduce or eliminate the deep pockets by use of a modified Widman flap (MWF), apically positioned flap (APF) or free gingival graft (FGG) depending on the condition.

If a patient has difficulty brushing due to a lack of attached gingiva, one can regain the attached gingiva by APF, FGG or connective tissue graft (CTG), so that a patient can then brush easily. If gingival recession occurs, root coverage can be achieved with CTG dependent on the degree of the recession.

Mechanical debridement and root conditioning can be applied to remove the biofilm (Table 2). If root caries exists beneath the marginal gingiva, osseous surgery can be conducted in order to retain the biologic width. One of the most difficult areas to treat in periodontics is a furcation region, so regeneration is an ideal treatment for a tooth with class 1 or 2 furcation involvement. However, if a tooth has class 2 or 3 furcation involvement, the aim should be to make cleaning easier by tooth sectioning or extraction.

There are two types of bone loss; horizontal and vertical. In cases of horizontal bone loss, soft tissue is treated to eliminate pockets, but if the teeth are mobile consideration should be given to splinting the teeth together to stabilize the occlusion. In

Problem list	Objective	Procedure
Swelling, bleeding	Reduce inflammation	Brushing, SC/RP
Deep pocket	Reduction, elimination	MWF, APF, FGG
Lack of attached gingiva	Gain of attached gingiva	APF, FGG, CTG
Gingival recession	Root coverage	CTG

Table 1. Treatment options for gingival conditions

Problem list	Objective	Procedure
Plaque/calculus	Removal of biofilm	Mechanical debridement Root conditioning with chemical agent
Root caries	Biological width	Osseous surgery & APF, FGG Extrusion & osseous surgery & APF, FGG
Furcation involvement	Elimination	Root resection Tooth sectioning
	Regeneration	GTR Bone graft Tissue engineering

Table 2. Management options for root surface conditions

Problem list	Objective	Procedure
Vertical bone loss	Bone leveling	Osseous surgery Extraction Extrusion, OS Regenerative Surgery, OS

Table 3. Management options for vertical bone loss

Category	Procedure options
Periodontal plastic surgery	Root coverage, ridge augmentation & preservation, crown lengthening, papilla preservation and reconstruction
Resective procedure	Apically positioned flap, tooth sectioning, osseous surgery, extraction
Tissue attachment procedure	Modified Widman flap, open flap curettage
Regenerative procedure	Guided tissue regeneration, EMD, bone graft, tissue engineering (BMP, PDGF, FGF, others)
Implant therapy	Ridge augmentation, sinus augmentation

Table 4. Periodontal surgery options

cases of vertical bone loss, there are four treatment options available depending on the condition (Table 3). Osseous surgery (OS) to create an even bone level, extraction, osseous surgery after extrusion of the tooth, or osseous surgery after regenerative surgery are all treatment options for bone loss

There are many periodontal surgery options now available (Table 4). No single option can correct all of the problems seen in patients; thus, consideration of all the various available options will provide beneficial results for patients.

Many patients with bone loss due to severe periodontal disease are likely to want esthetic and functional restoration. Such patients wish to know if treatment is painful, how much time is needed per visit and in total, and how much it costs. The final question that most patients ask is how long the treatment result will last. In other words most patients want long- term stability following the treatment. Therefore, the periodontist should consider predictability and longevity.

To achieve long term stability, the intra-oral environment should be enhanced for easy self-care by the patient. This means that periodontal treatment should achieve not only the elimination of causative factors of the disease but also improve ease of cleaning, which will result in longevity of the treatment result.

Over the last 40 years the concepts of periodontal treatment have evolved (Table 5) Before the 1970s periodontal-prosthesis was the main treatment modality in periodontics. In the 1980s, the treatment rationale changed significantly with the advent of regenerative therapy and implants. The indications for application of implants were thus extended.

1970s	Periodontal prosthesis
1980s	Guided tissue regeneration
1980s	Implant prosthesis
1990s	Guided bone regeneration
1990s	Sinus augmentation
2000s	Tissue engineering

Table 5. Changing concepts of periodontal treatment

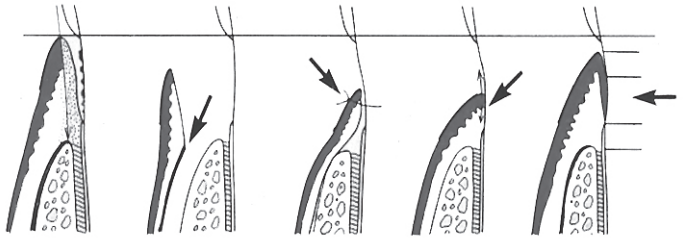


Figure 1A. Apically positioned flap with full thickness

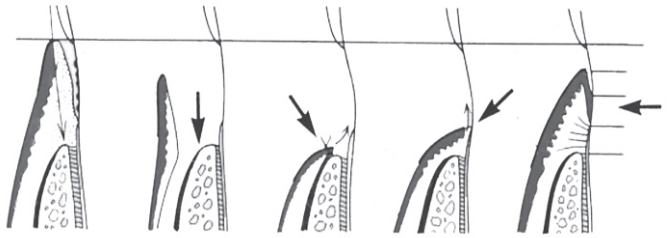


Figure 1B. Apically positioned flap with partial thickness

Guided bone regeneration and sinus lift procedures were introduced to clinical practice in the 1990s. Since 2000, progress in tissue engineering has further increased the expected outcomes of regenerative therapy. However, no matter how much change takes place and how much progress is achieved in treatment modalities, the basic concept of periodontology will not change so long as patients come to us asking for longevity and stability of treatment results.

The findings of many articles can be applied to clinical practice in order to treat periodontal disease predictably.

Kramer offered a biologic rationale for mucogingival surgery that suggests that the topography of the periodontium can preclude or deter the inception and progression of the inflammatory process. The gingival fibre connection to the tooth offers an impediment to the spread of inflammation and further destruction of the periodontium. Goldman postulated that the destruction of these fibres is necessary before the epithelium can migrate apically along the root. Gingiva has more connective tissue fibres and less vasculature and thus is proposed to be a better deterrent to the initiation and progression of the inflammatory process.

Many articles support the effectiveness of an apically positioned flap, especially the clinical study performed by Levy *et al* (2002). Machtei and Ben-Yehouda (1994) reported that in those cases where minimal probing depth is desired following periodontal flap surgery the flap be secured to the underlying structures at or slightly coronally to the bone crest.

Levy *et al* (2002) showed that a reduction in pocket depth by surgical means, and the associated decrease in reservoirs of periodontal pathogens, may be important for achieving sustained periodontal stability.

There are two types of apically positioned flap (APF); a full thickness flap or partial thickness flap (Figure 1). The concept of APF with a partial thickness flap is totally different from that of APF with a full thickness flap because APF with a partial thickness flap can obtain lasting stable biologic width. The tissue healing process following APF with a full thickness flap is the same as that with the

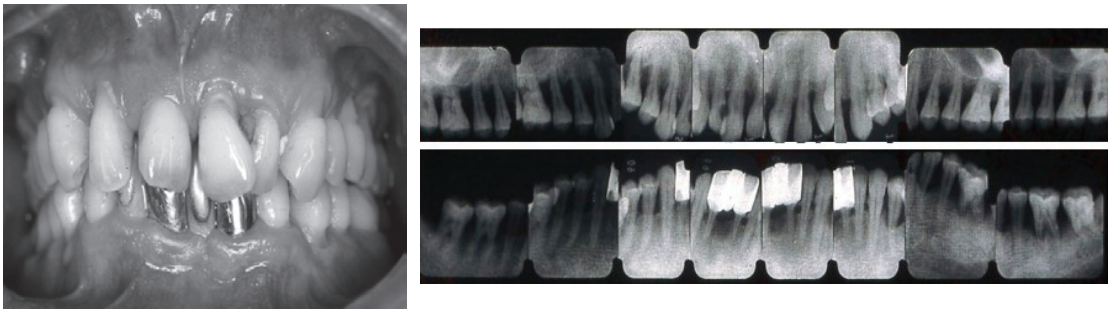


Figure 2. Case 1 - Initial appearance before treatment

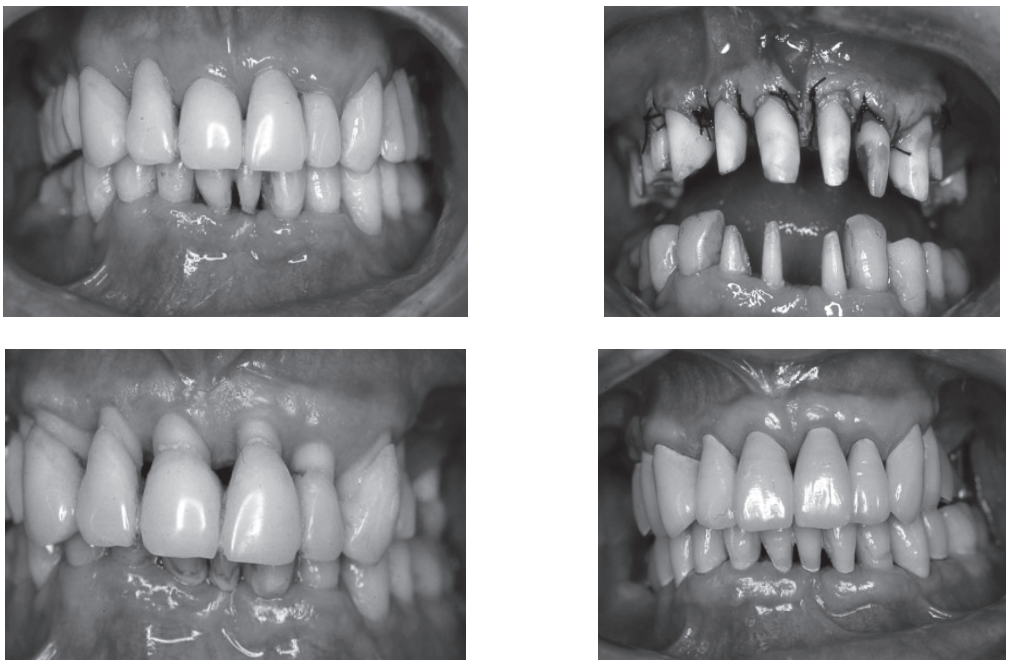


Figure 3. Case 1 - Treatment phases

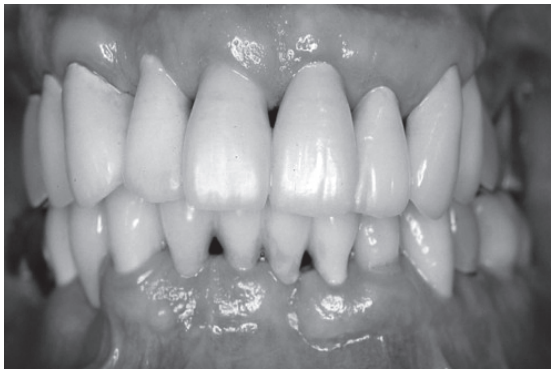


Figure 4. Case 1 - Appearance 17 years after treatment

modified Widman flap, which heals with a long epithelial attachment and deep sulcus. Therefore, in the case of a patient who has severe bone loss due to periodontal disease, one must create an oral environment that can be easily cleaned by the patient.

Case 1. Advanced periodontitis

A 37 year old patient desired restoration of function even though he had severe bone loss (Figure 2). The teeth were stabilized with provisional restorations, then deep pockets were eliminated using a apically positioned flaps (Figure 3). The tissues were allowed to heal for 5 months before the final restoration. The marginal gingivae position and occlusion have been stable for 17 years (Figure 4).

Maynard and Wilson (1979) suggested that 5 mm of keratinized tissue is the appropriate dimension required for intracrevicular restorative dentistry. This would satisfy the requirement of 2 mm of free gingival (1 mm for the crevicular epithelium and 1 mm for the junctional epithelium) and 3 mm of attached gingival. With less than 3 mm of keratinized gingiva, there is probably very little actual attachment to deter an inflammatory lesion and withstand the trauma from restorative procedures.

Nevins (1986) reported that, if there is less than 3 mm of keratinized attached gingiva, there is little actual attachment to defer an inflammatory lesion when planning an intrasulcular margin for a restoration.

These studies show the effectiveness and importance of attached gingiva and show relevant clinical cases.

Kramer (1995) stated that ostectomy is an effective and predictable technique for preventing the progressive loss of attachment in selected sites of patients with periodontitis.

Case 2. Periodontal plastic surgery

A female patient (33 years old) presented stating that she was afraid that the marginal tissue recession would continue. The initial appearance of her gingival tissues and bone levels are shown in Figure 5. Teeth 10, 11 and 12 were all Miller Class I and were treated by the modified procedure of Langer (Figure 6). The bone loss is apparent. The donor tissue has to be at least three times as large as the exposed root surface. The results at 4 months after surgery are shown in Figure 7. The previously exposed root surface is now completely covered up to the CEJ.

Case Report 3. Management of recession using the tunneling technique

Patient presented with recession of teeth # 7, # 8 and # 9 which was treated using the tunneling technique (Figure 8). This procedure does not utilize a lateral incision that might result in scar tissue after healing. Root conditioning was performed with tetracycline in order to remove the smear layer. The suturing was completed after the connective tissue graft was inserted into the inside of the flap. After the root coverage procedures were completed, the patient proceeded to have orthodontic therapy to make the incisal line even (Figure 9). Tooth 8 was successfully intruded. She then wished to proceed with teeth whitening. The final appearance and outcome of treatment is shown in Figure 10.

Case 4. Periodontal prosthesis case

In this case a 45 year old male with significant bone loss resulting from severe periodontal disease was treated with a periodontal prosthesis and long term follow-up. The initial clinical features are shown in Figure 11. This patient had observed friends

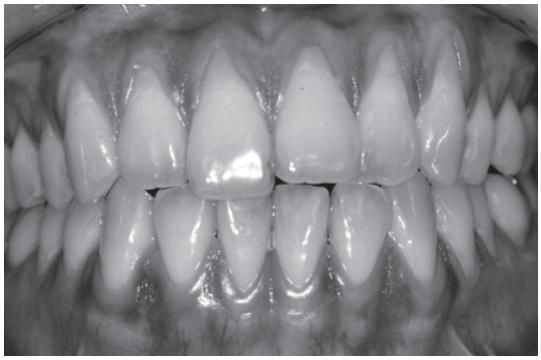
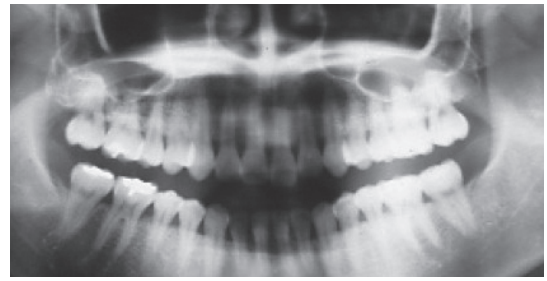



Figure 5. Case 2 - Initial appearance of periodontal tissues

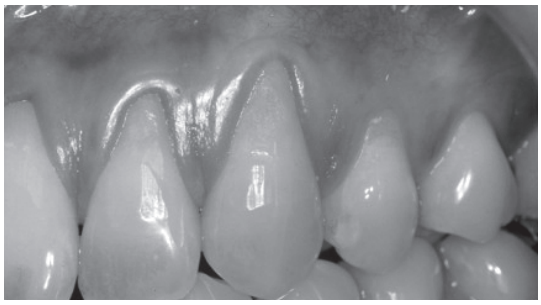
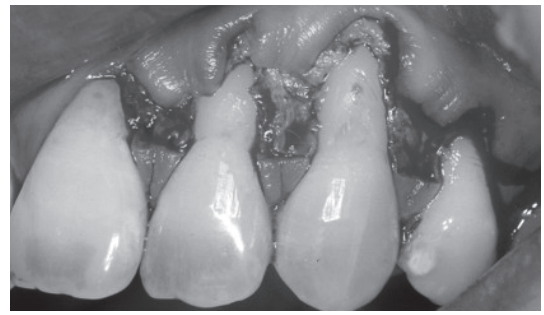



Figure 6. Case 2 - Miller Class I recession and surgical approach

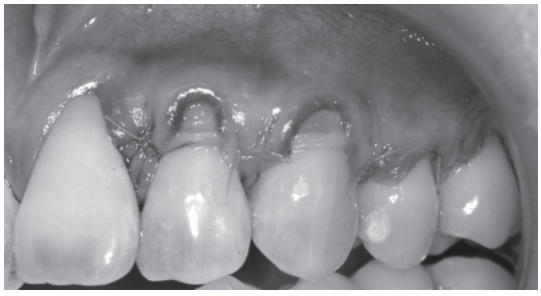
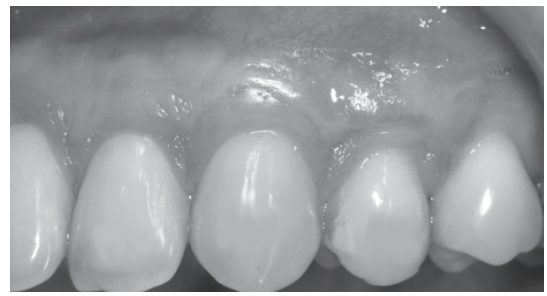



Figure 7. Case 2 - Clinical results of periodontal plastic surgery

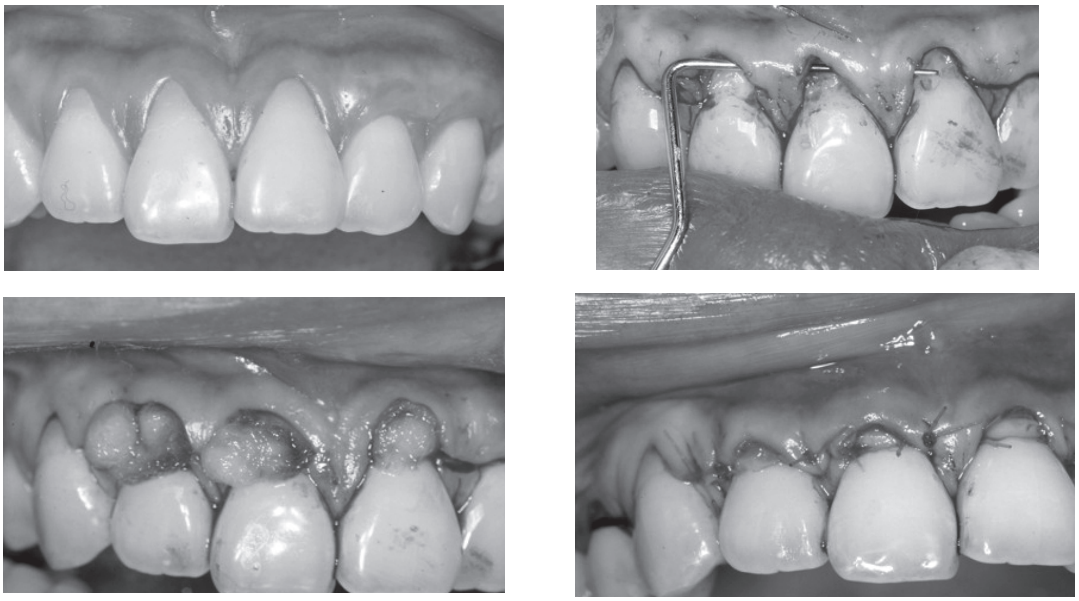


Figure 8. Case 3 - Initial appearance and surgical technique

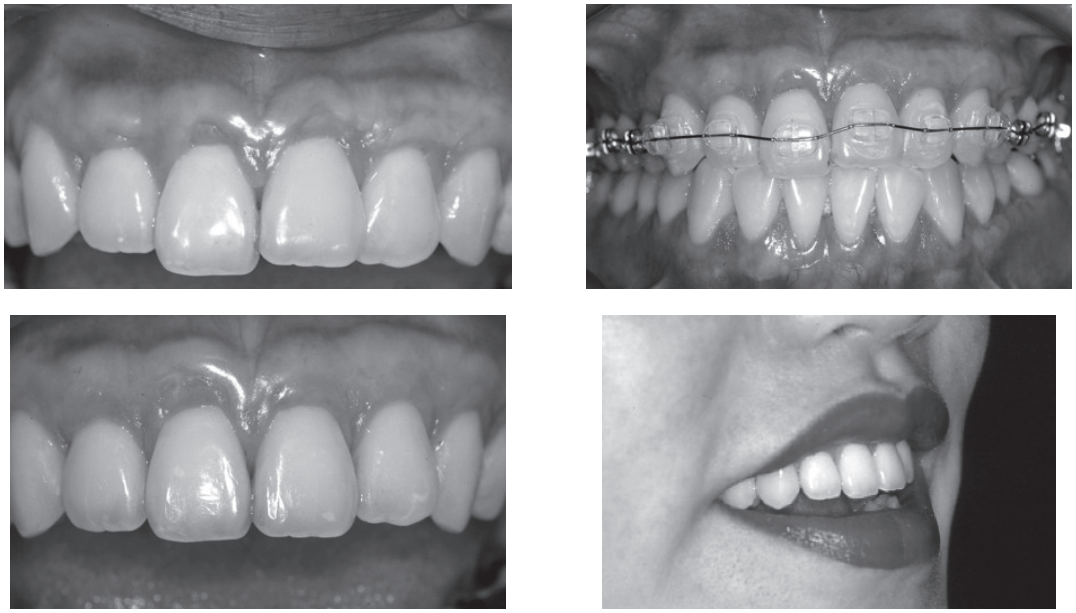


Figure 9. Case 3 - Orthodontic and tooth whitening procedures

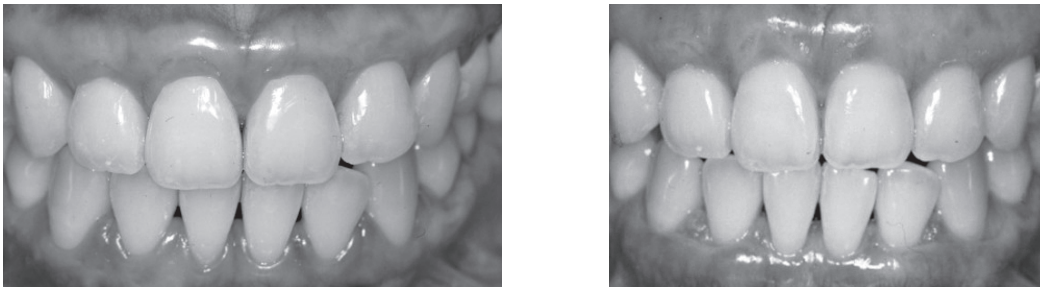


Figure 10. Case 3 - Appearance 2 and 6 years following treatment

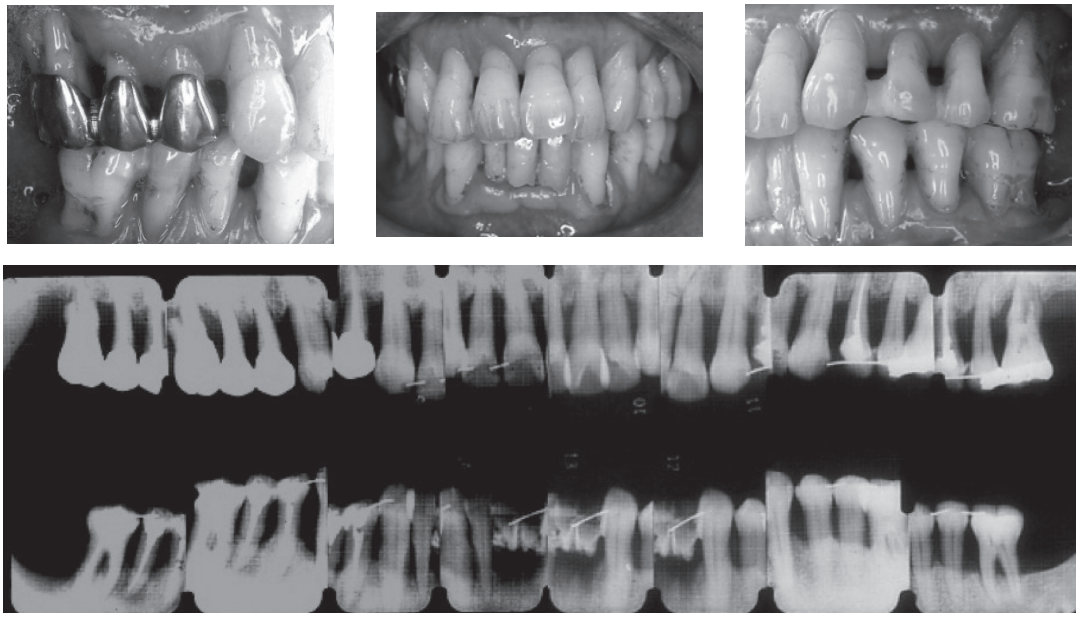


Figure 11. Case 4 - Initial appearance

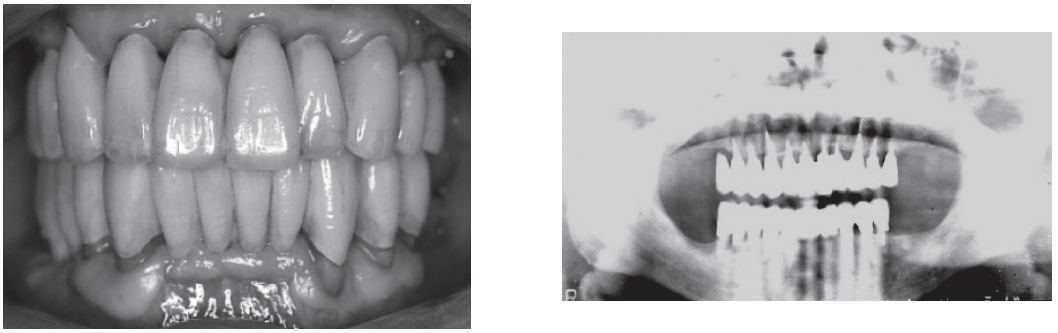
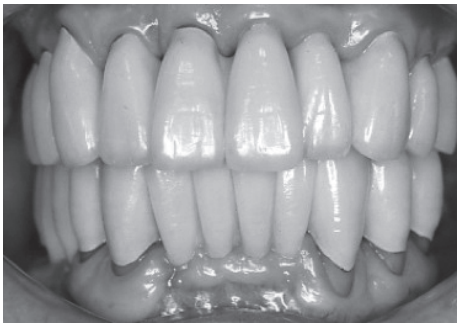


Figure 12. Case 4 - Appearance immediately following treatment



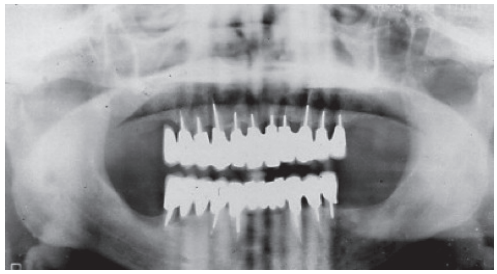


Figure 13. Case 4 - Appearance 16 years following treatment

suffering from implant failure, so he was determined not to have implants himself. He asked if the remaining teeth could be saved.

In Phase 1 of treatment hopeless teeth were extracted and a provisional restoration was performed, including both secure and questionable teeth. After the mobile teeth were stabilized, scaling and root planing were performed.

In Phase 2, definitive periodontal surgeries were performed to eliminate pockets, flatten the bone, and gain attached gingiva for longevity of the treatment result. At the time of reassessment it was confirmed that suitable conditions to move into the maintenance phase had been achieved and the definitive prosthesis was made.

The appearance at initial completion of treatment is shown in Figure 12 and the final appearance at 16 years after treatment is shown in Figure 13.

Case 5. Guided tissue regeneration

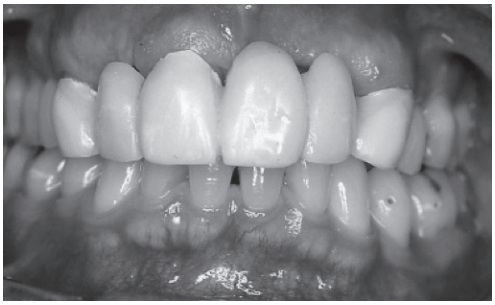
Guided tissue regeneration was developed in the mid-1980s and has been applied to cases that could not be treated with resective therapy. In this case a patient with deep pockets throughout the mouth presented for treatment (Figure 14). After scaling/root planing, regenerative therapy was performed in the posterior teeth. Prior to surgery quite a large amount of bone loss can be seen (Figure 15).

A full thickness flap was reflected and bone grafting using demineralized freeze-dried bone allograft was applied into the bony defects and a guided tissue regeneration membrane placed over the graft material (Figure 16). Even though regenerative surgery was successful there are still some remaining bone defects and osseous surgery was necessary in order to achieve long-term stability of the treatment results. 1 year following the regenerative surgery, definitive periodontal surgery was performed to eliminate the remaining periodontal pockets and to gain attached gingiva (Figure 17). The radiographic and clinical results at 2 years and 13 years are shown in Figure 18.

Case 6. Advanced periodontal disease

A 50 year old female presented complaining of difficulty with chewing. The clinical and radiographic at the first appointment are shown in Figure 19. During the course of treatment particular attention was paid to upper right canine (#3), which is a key tooth, so that following debridement, regenerative therapy was performed using bone graft material, Emdogain and a non-resorbable membrane (Figure 20). 8 months after the regenerative surgery only 1 mm could be probed, there was a shallow vestibule and no keratinized tissue was found.

Following 8 months of healing there was



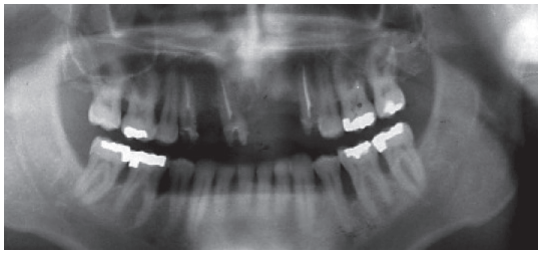
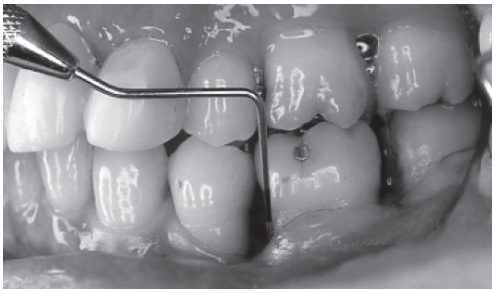


Figure 14. Case 5 - Initial appearance



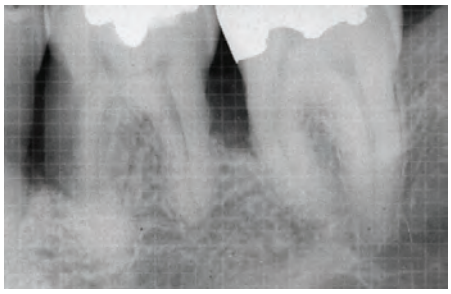
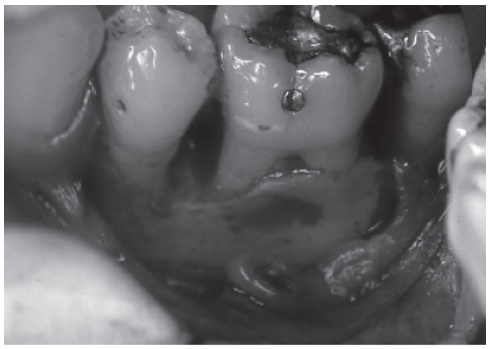
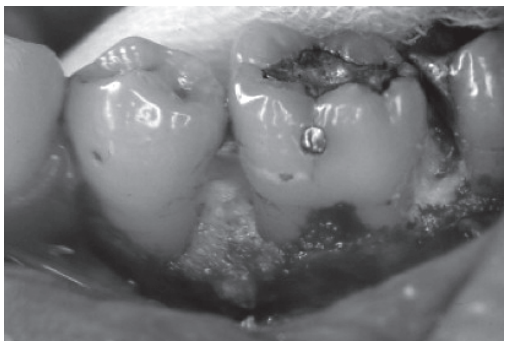
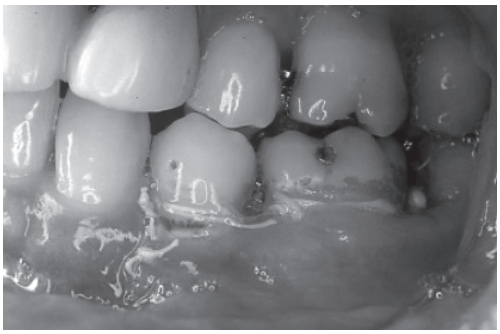


Figure 15. Case 5 - Preoperative appearance of defect on lower first molar







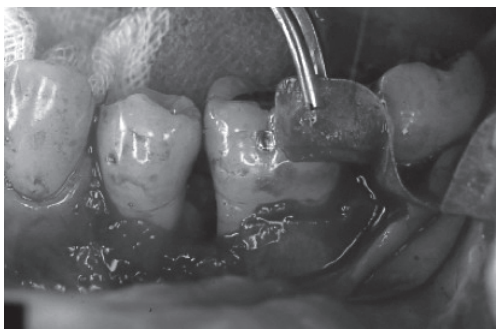


Figure 16. Case 5 - Surgical management of bony defect demonstrating flap elevation, placement of DFDBA, flap coverage and subsequent membrane removal

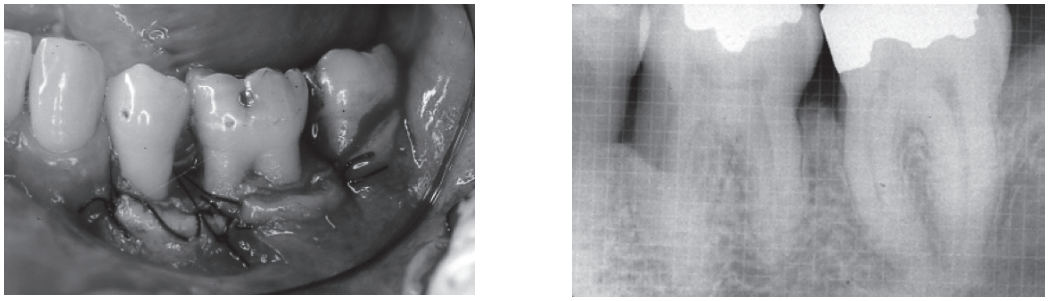


Figure 17. Case 5 - Periodontal surgery following GTR procedure

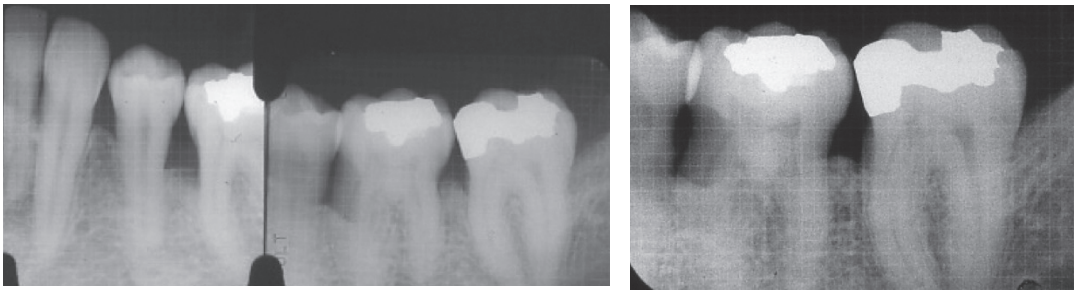


Figure 18. Case 5 - Radiographs taken 2 and 13 years post-treatment

a 7 mm attachment gain on the distal side of the canine. Further definitive periodontal surgery was conducted for pocket elimination and to obtain gingival attachment (Figure 21). Sinus augmentation and placement of 3 implants was then performed to restore function (Figure 22). This case has been followed for 5 years and the appearance after this time is shown in Figure 23.

Treatment goals

The following is a list of conditions which are our treatment goals, which we always aim to achieve upon completion of active therapy, so that the patient can go into the maintenance phase with desirable conditions.

- Shallow sulcus
- No vertical bone loss & flattened bone level
- No furcation involvement
- No mucogingival problem
- Stabilized occlusion

Conclusion

The concept of periodontal prosthesis, even implant prosthesis, is still effective for the treatment of periodontal disease.

There are three keys to the longevity of treatment results in cases of high susceptibility to periodontal disease. These are pocket elimination, osseous surgery to make the bone flat and gain attached gingival so that the oral environment can be cleaned easily.

The periodontist should try to retain the natural dentition as much as possible in order to preserve proprioceptors, which implants do not have. With more options available for periodontal treatment, our patient's problems can be solved more predictably. Fewer complications and failures will occur as a result of increased knowledge and skill. It can be noted that the collection of treatments available for periodontal management can be applied to implant therapy in order to provide good and reasonably predictable results for patients.

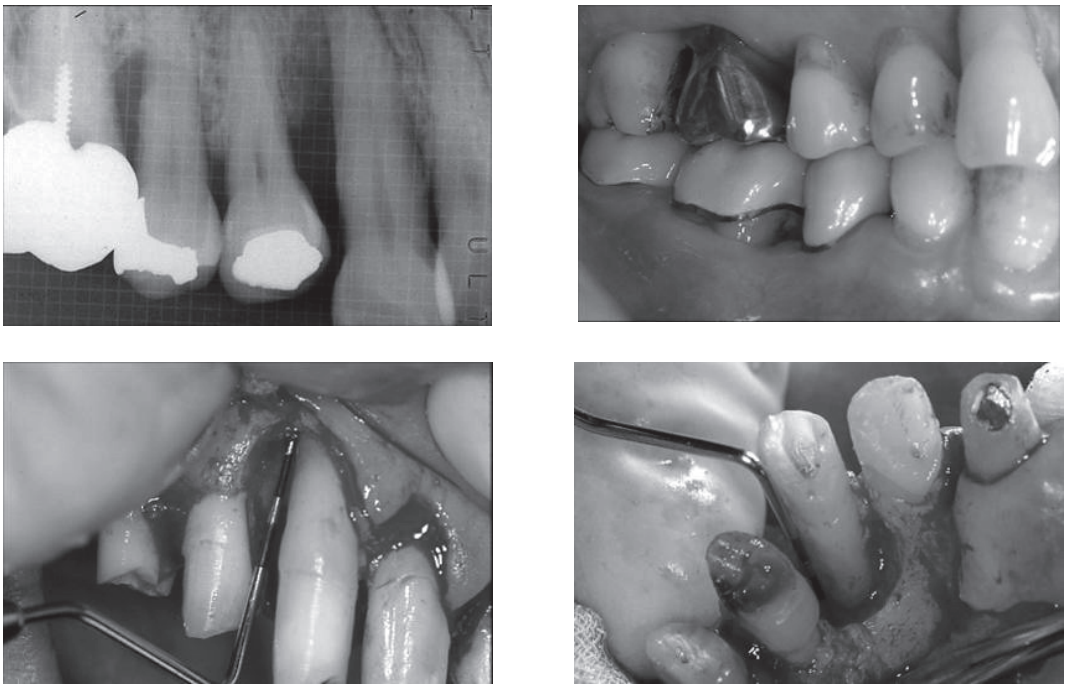


Figure 19. Case 6 - Initial appearance

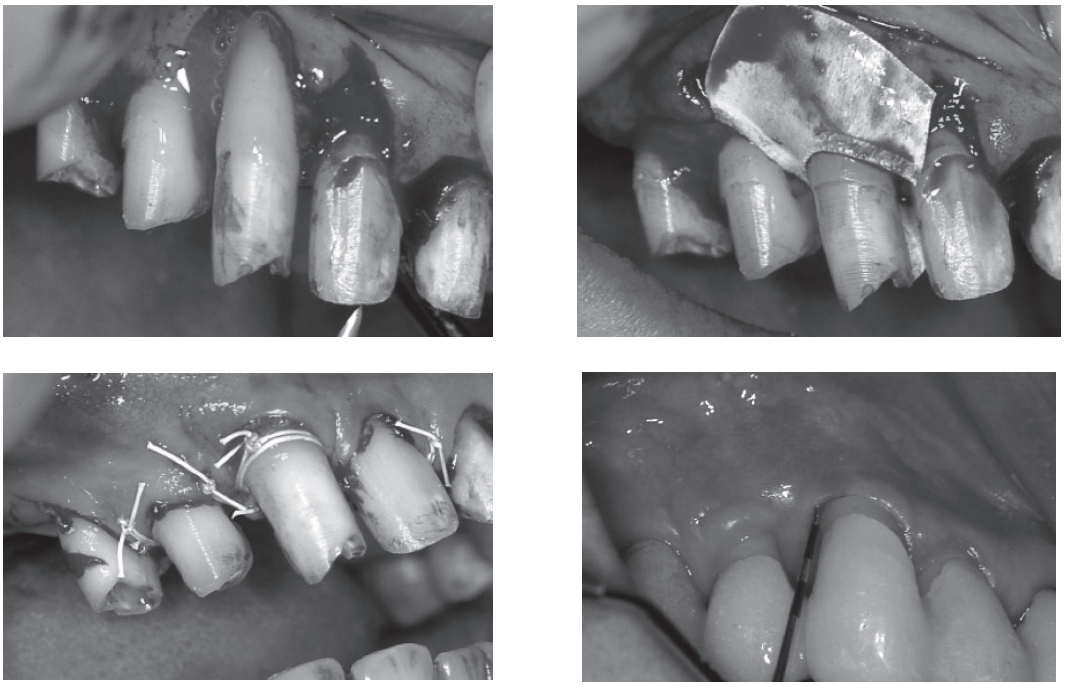


Figure 20. Case 6 - Regenerative procedure on upper right canine

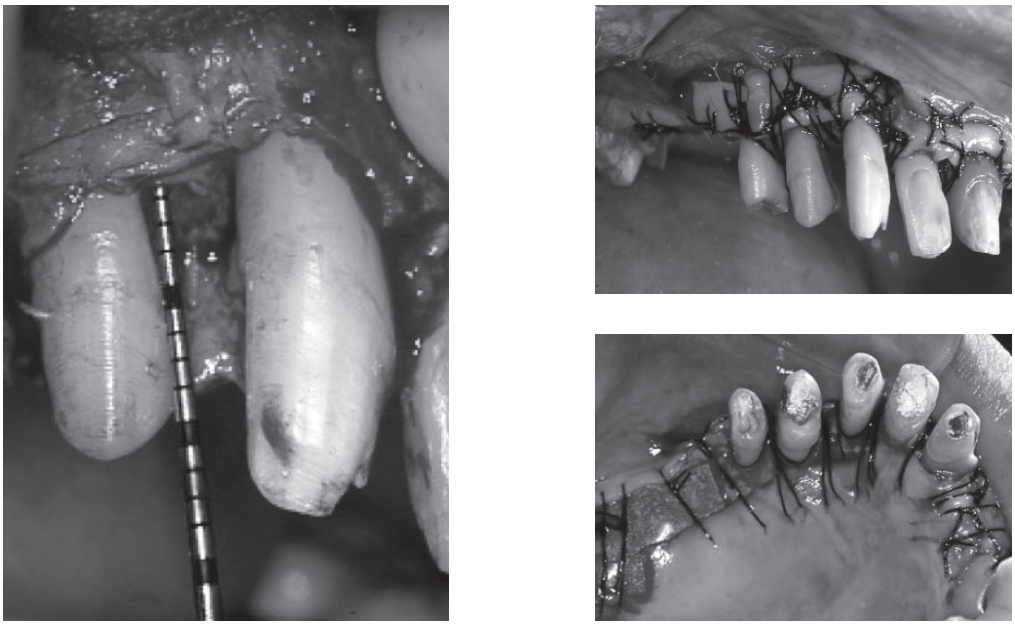


Figure 21. Case 6 - Periodontal sugery on upper left canine following regenerative surgery

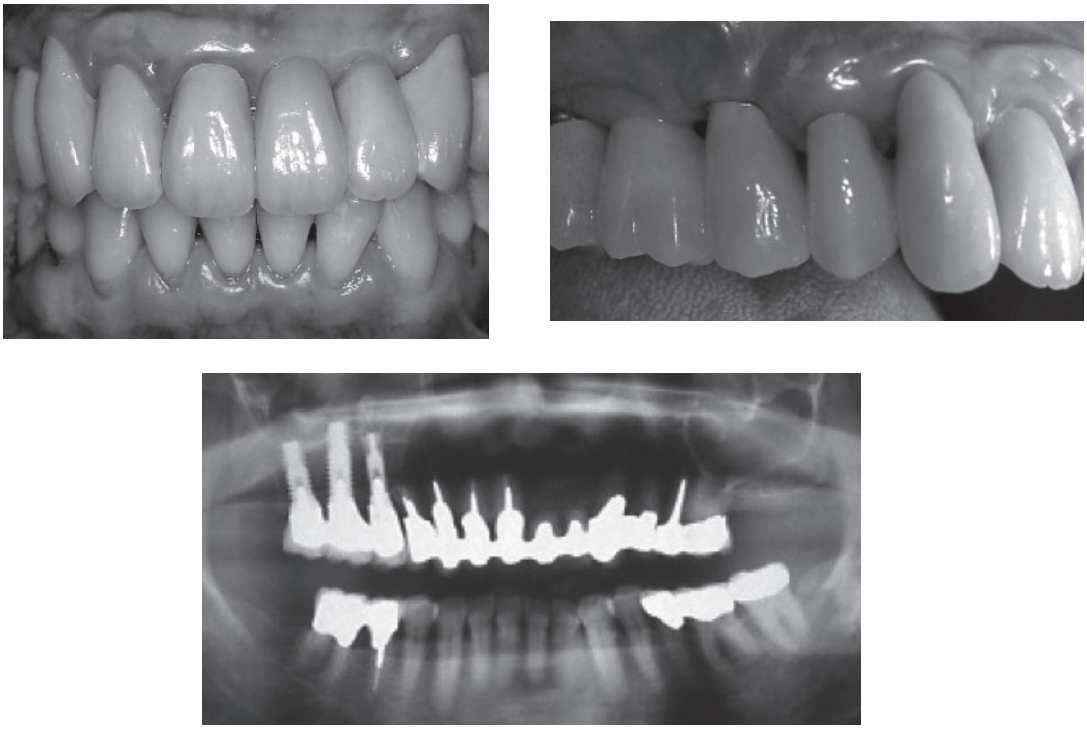


Figure 22. Case 6 - Final appearance and radiograph

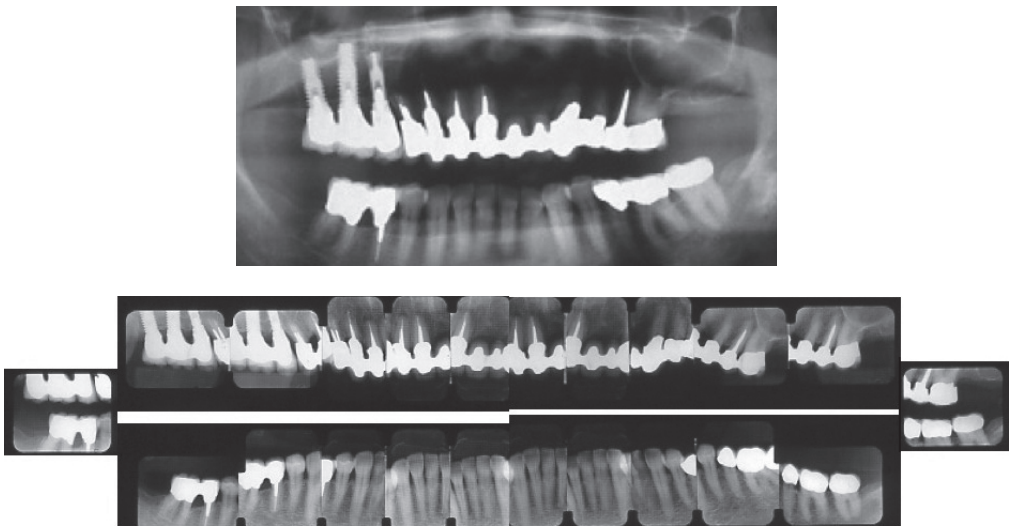


Figure 23. Case 6 - Radiographs 5 years after completion of treatment

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 6

Polymorphisms in Genes Coding for Enzymes Metabolizing Smoking-Derived Substances, Smoking and the Potential Risk of Periodontitis

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Introduction

Periodontitis is an inflammatory condition of the periodontium that is initiated by bacterial plaque on the tooth surface adjacent to the gingiva. Microbial factors are essential for the initiation of periodontitis. However, according to recent epidemiological studies, a positive correlation between the quantity of bacterial plaque and the severity of periodontitis is yet to be found (Leo *et al* 1986). The specific bacterial component accounts for a relatively small proportion (about 9-16%) of the variance in periodontitis expression (Offenbacher 1996) which suggests that not everyone is equally susceptible to periodontitis, which is significantly modified by the host response to bacterial plaque. Studies on periodontal status in adult twins showed that between 38-82% of the population variance for periodontal status may be attributed to genetic factors (Michalowicz *et al* 1991a, Michalowicz *et al* 1991b). Genetic factors may determine the susceptibility to periodontitis by modifying the type and intensity of host inflammatory response. Numerous studies have shown that certain genetic polymorphisms, such as those of IL-1α, IL-1β, IL-1Ra, TNF-α, FcγRIIA, FcγRIIIBand HLA II genotypes were associated with the susceptibility of periodontitis (Nares 2003). Studies on different genes are being undertaken.

In recent years, numerous epidemiological studies in different populations have shown that smoking is a high risk factor for periodontal disease (Meisel *et al* 2000). The mechanisms through which cigarette smoke modulate periodontitis are not well defined. Smoking produces more than 4000 kinds of noxious substances such as carbon monoxide, nicotine, dimethyl-nitrosamine, arylamines, aromatic hydrocarbon and benzopyrene. These substances may have a direct or indirect effect on periodontitis. Aside from the xenobiotic substances in tobacco, other exogenous or endogenous compounds such as toxic metabolites from periodontal microorganisms (e.g. indole, amines) contribute to the periodontal inflammatory process. Metabolic activity to foreign substances by metabolizing enzymes determines the individual's susceptibility to toxicity of these compounds.

All these substances are transformed *in vivo* by metabolizing enzymes, both in bioactivation and detoxification. These metabolizing enzymes consist of two main types of enzymes: phase-I oxidative enzymes, which are mainly cytochromes P-450 (CYP), and phase-II conjugating enzymes such as glutathione S-transferases (GSTs), N-

acetyltransferase (NATs). Evidence supports a high concordance between genetic polymorphisms and the metabolic capacities of the metabolizing enzymes (Smith *et al* 1994). It is proposed genetic polymorphisms of the metabolizing enzymes may contribute to an increased risk for periodontitis, especially in subjects exposed to cigarette smoke or other xenobiotics. So far, several studies on the association of gene polymorphisms of the metabolizing enzymes and periodontitis have been reported (Meisel *et al* 2000, Kim *et al* 2004, Kocher *et al* 2002). The purpose of this study is to assess the relationship between the genetic polymorphisms of CYP1A1, GSTM1, GSTT1, NAT2 enzymes and severe chronic periodontitis in smokers and non-smokers of Chinese Han nationality.

Materials and methods

Study population

All subjects were selected from the dental clinic of the Guangdong Provincial Stomatological Hospital. The subject's parents and grandparents were required to be of Han nationality and the patient should have no malocclusion, no history of systemic diseases such as diabetes or cardiovascular diseases. Periodontal status was evaluated clinically by one examiner with intra-examiner calibrations were taken. Probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP) were assessed at six sites per tooth. Based on these periodontal measures, the subjects were divided into two groups:

1. *Severe chronic periodontitis*
   Mean CAL ≥2.5 mm and 1 or more sites in 3 out of 4 quadrants with interproximal CAL measurements of ≥5 mm. No more than 14 missing teeth.
2. *Periodontally healthy or mild gingivitis*
   The data on smoking in this study relied on self-reported questionnaires. Smokers who smoked for ≥10 years with ≥10 cigarettes/day were considered as "smokers", and those who never smoked considered as "non-smokers".

DNA collection

Sterile cotton swabs were used to take buccal swabs from all subjects and left to dry at room temperature overnight. The surface of the cotton swab was removed and transferred to an Eppendorf tube in which 150 ml of 5 µl Chelex-100 solution and 4 ml proteinase K (20 mg/ml) were added. The mixture was incubated at 55°C for 3 hours, then boiled at 100°C for 10 min, centrifuged at 14,000 rpm for 2 min, and supernatant stored at -20°C prior to PCR.

CYP1A1 genotyping

MspI polymorphism

The polymerase chain reaction/restriction fragment length polymorphisms (PCR-RFLP) method was used to detect the mutations at MspI point of CYP1A1 gene.

Primers (50 µM):

P1: 5'-CAG TGA AGA GGT GTA GCC GCT-3'
P2: 5'-TAG GAG TCT TGT CTC ATG CCT-3'

Reaction: Total volume of 38µl, containing template DNA 3.0µl, dNTP (1mM) 6µl, 10×buffer 3.75µl, BSA 3.75µl, $MgCl_2$ solution (25mM) 2.25µl, 0.3µl each primer, Taq DNA polymerase 1.5U. 3 min of initial denaturation at 94°C followed by 32 cycles of 40 sec at 94°C for denaturation, 40 sec at 60°C for primer annealing, and 1 min at 72°C for primer extension, 10 min at 72°C for the last cycle's primer extension. The PCR products were stored at 4°C.

MspI digestion: The PCR products were digested with MspI restriction enzyme: total volume of 10µl, containing PCR products 5µl,

MspI 1.5U, 10×SE buffer 1µl, 37 °C for 16h. Fragments generated were visualized by 7% polyacrylamide gel electrophoresis (PAGE) using silver nitrate staining.

A/G polymorphism at 4889

The allele-specific polymerase chain reaction (AS-PCR) method was used to detect the mutations at 4889 point of CYP1A1 gene.

Primers (50 µM):

P3: 5'-GAA GTG TAT CGG TGA GAC CA-3'
P4: 5'-GAA GTG TAT CGG TGA GAC CG-3'
P5: 5'-GTA GAC AGA GTC TAG GCC TCA-3'

Reaction: Each of primers, P3 and P4, was used for PCR amplification together with primer P5. Total reaction volume was 38µl, containing template DNA 3.0µl, dNTP (1mM) 6µl, 10×buffer 3.75µl, BSA 3.75µl, $MgCl_2$ solution (25mM) 2.25µl, 0.3µl each primer, Taq DNA polymerase 1.5U. 3 min of initial denaturation at 94°C followed by 30 cycles of 40 sec at 94°C for denaturation, 40 sec at 65°C for primer annealing, and 1 min at 72°C for primer extension, 10 min at 72°C for the last cycle's primer extension. The PCR products were stored at 4°C. PCR products were visualized by 7% PAGE using silver nitrate staining.

GSTs genotyping

Multiplex differential PCR (MD-PCR) method was used to analyze the GSTM1 and GSTT1 genotypes by the presence or absence of specific PCR fragments.

Primers for GSTM1 (50 µM):

P6: 5'-GAA CTC CCT GAA AAG CTA AAG C -3'
P7: 5'-GTT GGG CTC AAA TAT ACG GTG G -3'

Primers for GSTT1 (50 µM):

P8: 5'-TTC CTT ACT GGT CCT CAC ATC TC -3'
P9: 5'-TCA CCG GAT CAT GGC CAG CA -3

β-globin primers (50 µM):

P10: 5'-CAA CTT CAT CCA CGT TCA CC -3'
P11: 5'-GAA GAG CCA AGG ACA GGT AC -3'

PCR reaction: Total volume of 38 µl, containing template DNA 3.0µl, dNTP (1mM) 6 µl, 10×buffer 3.75 µl, BSA 3.75 µl, $MgCl_2$ solution (25mM) 2.25 µl, 0.3 µl each primer, Taq DNA polymerase 1.5U. 3 min of initial denaturation at 94°C followed by 32 cycles of 40 sec at 94°C for denaturation, 40 sec at 60°C for primer annealing, and 1 min at 72°C for primer extension, 10 min at 72°C for the last cycle's primer extension. The PCR products were stored at 4°C.

The PCR products were visualized by 7% PAGE using silver nitrate staining.

NAT2 genotyping

Allele-specific polymerase chain reaction (AS-PCR) was used for detecting of the mutations at C481T (m1), G590A (m2) and G857A (m3) of the NAT2 gene.

Primers for m1 point (481T):

P12: 5'-ACA TCC CTC CAG TTA AC-32
P13: 5'-CTC CTG ATT TGG TCC AG-32
P14: 5'-CTC CTG ATT TGG TCC AA-32

Primers for m2 point (590A):

P15: 5'-TTT ACG CTT GAA CCT CG-32
P16: 5'-TTT ACG CTT GAA CCT CA-32
P17: 5'-TGG GTG ATA CAT ACA CA-32

Primers for m3 point (857A):

P18: 5'-CCA AAA CCT GGT GAT GG-32
P19: 5'-CCA AAA CCT GGT GAT GA-32
P920: 5'-CAC TCT GCT TCC CAA GAT-32

PCR reaction: Total reaction volume was 38 µl, containing template DNA 3.0 µl, dNTP (1mM) 6 µl, 10×buffer 3.75 µl, BSA 3.75 µl, $MgCl_2$ solution (25 mM) 2.25 µl, 0.3 µl each primer, Taq DNA polymerase 1.5U. 3 min of initial denaturation at 94°C followed by 30 cycles of 40 sec at 94°C for denaturation, 40 sec at 56°C for primer annealing, and 1 min at 72°C for primer extension, 5 min at 72°C for the last cycle's primer extension. The PCR products were stored at 4°C.

PCR products were analyzed on 7% PAGE using silver nitrate staining

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Subject type	Severe CP (n=112)	Healthy controls (n=78)
Male n (%)	49(43.8)	40(51.3)
Female n (%)	63(56.2)	38(48.7)
Age ($\bar{\chi} \pm s$)	49±6.2	45±8.7
Smoker n (%)	45(40.2)	19(24.4)
Nonsmoker n (%)	67(59.8)	59(75.6)

Table 1. Distribution of subjects according to their age, sex and smoking status

Smoking Status	CYP1A1 MspI genotype	Severe CP	Healthy control	x^2	P-value	OR (95% CI)
nonsmoker	wt/mt, mt/mt	31	39			1.00
nonsmoker	wt/wt	36	20	4.998	0.025	2.265(1.160~4.661)
smoker	wt/mt, mt/mt	20	11	3.518	0.061	2.287(0.955~5.481)
smoker	wt/wt	25	8	8.954	0.003	3.931(1.558~9.918)

Table 2. Interaction of smoking and CYP1A1 MspI wild genotype

Statistical analysis

The frequencies of alleles and genotypes of the genes were calculated. The statistical significance of differences in allele frequencies and genotype frequencies among groups was tested by chi-square test. Odds ratio with 95% confidence intervals were calculated (α=0.05). The SPSS 11.0 statistical program package was used to carry out these analyses.

Results

Baseline characteristics

The number of subjects enrolled in this study was 190. Age, sex, smoking status for the two groups are reported in Table 1.

Association between CYP1A1 genetic polymorphisms and severe CP

CYP1A1 genotypes

MspI point: The wild-type homozygote was characterized by a 340 bp fragment, variant homozygote by 140 and 200 bp fragments, and heterozygote by 140, 200 and 340 bp fragments, respectively (Figure 1).

Ile/Val point: The wild-type homozygote (Ile/Ile genotype) was characterized by a 210 bp fragment on the first lane, variant homozygote (Val/Val genotype) by a 210 bp fragment on the second lane, and heterozygote

(Ile/Val genotype) by a 210 bp fragment on both lanes, respectively (Figure 2).

Interaction of smoking and CYP1A1 MspI wild genotype

The interaction of smoking and CYP1A1 MspI wild genotype was analyzed. Comparing to the subjects who were non-smokers and carried mutant homozygote genotype or heterozygote genotype (calculated odds ratio estimating the relative incidence of periodontitis supposed to be 1), the OR in presence of wild-type homozygote was 2.265 ($P<0.05$) in non-smokers, and the OR increased to 3.931 ($P<0.01$) in presence of both wild-type homozygote and smoking (Table 2).

Interaction of smoking and CYP1A1 Ile/Ile wild genotype

The interaction between smoking and CYP1A1 Ile/Ile wild genotype was analyzed. Comparing to the calculated odds ratio estimating the relative incidence of periodontitis in presence of the variant genotypes (both variant homozygote genotype and heterozygote genotype) and nonsmoking (OR=1), the OR in presence of wild-type homozygote was 1.738 ($p>0.05$), and 1.950 ($p>0.05$) in presence of smoking, and the OR increased to 3.931 ($p<0.01$) in presence of both wild-type homozygote and smoking (Table 3).

Association between GSTM1, GSTT1 genetic polymorphisms and severe chronic periodontitis

GSTM1 and GSTT1 genotypes

GSTM1: The presence or absence of the 215 bp band reflects the GSTM1 positive or GSTM1 null genotype, the presence or absence of the 480 bp band reflects the GSTT1 positive or GSTT1 null genotype, with a constant 268 bp band of the α-globin in all samples as an internal standard control (Figure 3, Figure 4).

Interaction of smoking and GSTM1null genotype

Comparing to the subjects who were nonsmokers and carried GSTM1(+) genotypes (calculated odds ratio estimating the relative incidence of periodontitis supposed to be 1), the subjects carrying GSTM1 null genotype had an OR of 1.057 ($p>0.05$), and the subjects who were smokers had an OR of 1.530 ($p>0.05$), and the subjects who were both smokers and GSTM1 null carriers had and increased OR of 2.8 ($p<0.05$) (Table 4).

Interaction of smoking and GSTT1 null genotype

Compared to the subjects who were nonsmokers and carried GSTT1(+) genotypes (calculated odds ratio estimating the relative incidence of periodontitis supposed to be 1), the subjects carrying GSTT1 null genotype had an OR of 1.462 ($p>0.05$), and the subjects who were smokers had an OR of 2.133 ($p>0.05$), and the subjects who were both smokers and GSTT1 null genotype carriers had an OR increased to 2.880 ($p<0.05$) (Table 5).

Association between NAT2 genetic polymorphisms and severe chronic periodontitis

Genotypes

The fragment obtained from PCR of allele m1 was 217 bp, m2 339 bp, and m3 286 bp, respectively (Figure 5). Rapid acetylators include homozygote (wt/wt) and heterozygote (wt/m1, wt/m2, wt/m3) genotypes. Slow acetylators include variant homozygote genotypes (m2/m2, m3/m3, m1/m2,m1/m3, m2/m3), "homozygote" refers to allele

Smoking status	CYP1A1Ile/Val genotype	Severe CP	Healthy control	x^2	P-value	OR (95%CI)
nonsmoker	Ile/Val, Val/Val	25	30			1.00
nonsmoker	Ile/Ile	42	29	2.336	0.126	1.738(0.854~3.539)
smoker	Ile/Val, Val/Val	13	8	1.645	0.200	1.950(0.697~5.453)
smoker	Ile/Ile	32	11	8.320	0.004	3.931(1.468~8.304)

Table 3. Interaction of smoking and Ile/Ile wild genotype

Smoking status	GSTM1 genotype	Severe CP	Healthy control	x^2	P-value	OR (95%CI)
nonsmoker	+	40	36			1.00
nonsmoker	-	27	23	0.023	0.880	1.057(0.517~2.161)
smoker	+	17	10	0.860	0.354	1.530(0.621~3.769)
smoker	-	28	9	5.514	0.019	2.800(1.166~6.721)

Table 4. Interaction of smoking and GSTM1null genotype

Smoking status	GSTT1 genotype	Severe CP	Healthy control	x^2	P-value	OR (95%CI)
nonsmoker	+	30	32			1.00
nonsmoker	-	37	27	1.124	0.289	1.462(0.724~2.952)
smoker	+	18	9	2.530	0.112	2.133(0.831~5.475)
smoker	-	27	10	5.934	0.017	2.880(1.194~6.944)

Table 5. Interaction of smoking and GSTT1null genotype

combinations rather than to point mutations. Subject with variant homozygote genotype m1/m1 was not found. No subject contained more than two slow acetylator mutations.

Interaction of smoking and NAT2 slow acetylator genotype

The distribution of slow acetylator genotype among the groups of the patients and the controls stratified with smoking were shown in Table 6. There was no significant difference in the distribution of slow acetylator genotypes between non-smoker groups of periodontitis and healthy control ($p>0.05$), but the frequency of slow acetylator genotypes was significantly higher in the smoker periodontitis group than in the smoker healthy control group ($p<0.01$) (Table 6).

Discussion

Association between CYP1A1 genetic polymorphisms and severe chronic periodontitis

The xenobiotic-metabolising enzymes contain two main types of enzymes: the phase-I oxidative enzymes and phase-II conjugating enzymes. The phase-I enzymes, which are mainly cytochrome P-450 enzymes (CYPs), mediate oxidative metabolism. Formation of hydroxylated products from benzopyrene (BP), including aryl hydrocarbon hydroxylase (AHH) activity, is linked with CYP1A1 and several others CYPs. The biological functions of CYP1A1 are considered to be both bioactivation and detoxification (Crofts *et al* 1993).

CYP1A1 enzymes are coded by CYP1A1 gene, whose genetic polymorphisms are associated with the metabolic capacities of CYP1A1 enzymes. To date four kinds of polymorphisms have been found:

1. MspI polymorphisms (Cascorbi *et al* 1996), a T→C transition at position 1194bp downstream of exon 7 in the 3'-flanking region, leading to MspI restriction site
2. Ile/Val polymorphisms (Hirvonen *et al* 1992), an A→G transition at position 4889bp in exon 7, leading to an Ile/Val exchange at codon 462
3. AA polymorphisms (Brockmoller *et al* 1996), a single A-T to G-C transition in the 3' noncoding region approximately 300 bp upstream from the polyadenylation site, which has been found only in African-Americans
4. A C→A transition at position 4887bp in exon 7, leading to a Thr/Asn exchange at codon 461 (Woodson *et al* 1999)

The first and second types of polymorphisms are common in Asians. Studies indicate that the Val/Ile codon difference in the primary structure of the CYP1A1 protein (Val-, Ile-type) was in linkage disequilibrium with the MspI RFLP (Sivaraman *et al* 1994, Taioli *et al* 1995).

Most investigations concerning the relationship between CYP1A1 genetic polymorphisms and susceptibility to disease focus on the association with cancer. Numerous studies have shown an association between CYP1A1 MspI variant homozygote or Val/Val genotypes and the susceptibility to lung cancer, breast cancer, colon cancer, esophageal cancer, etc (Woodson *et al* 1999, Basham *et al* 2001, Garte 1998). However the results are discrepant, sometimes adverse (Hamada *et al* 1995). Some studies suggest that the CYP1A1 MspI variant homozygote genotypes and Val/Val genotype are closely related with high AHH inducibility and high non-induced AHH activity, which correlated with high carcinogen-activating.

The association of genetic polymorphisms of CYP1A1 with the susceptibility of periodontitis has not been determined. The results of this study showed that the wild-type homozygote was highly distributed in the non-smoker periodontitis group with OR value of

Smoking status	Slow acetylator	Severe CP	Healthy control	x^2	P-value	OR (95%CI)
nonsmoker	-	44	46	2.324	0.127	1.000
nonsmoker	+	23	13	2.324	0.127	1.850 (0.835~4.099)
smoker	-	17	11	1.196	0.274	1.616(0.681~3.832)
smoker	+	28	8	8.763	0.003	3.659 (1.506~8.891)

Table 6. Interaction of smoking and slow acetylator genotype

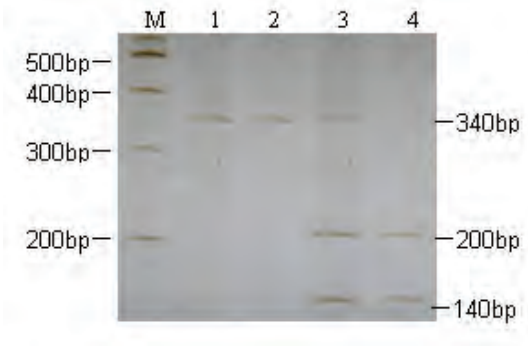

Figure1. Genotypes of CYP1A1 MspI
M:100bp ladder; Lane 1:340bp of PCR products; Lane 2:wt/wt homozygote;
Lane 3:wt/mt heterozygote; Lane 4:mt/mt homozygote

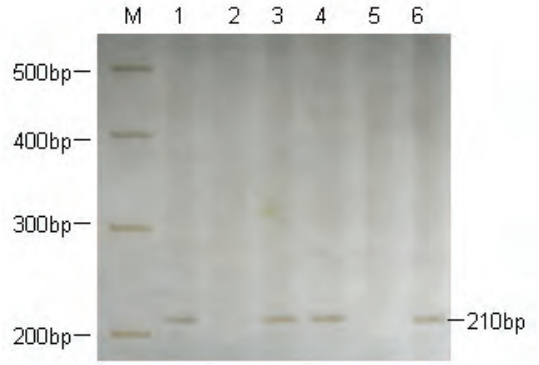

Figure 2. Genotypes of CYP1A1 Ile/Val
M:100bp ladder; Lane 1,2: Ile/Ile homozygote; Lane 3, 4: Ile/Val heterozygote; Lane 5,6: Val/Val homozygote

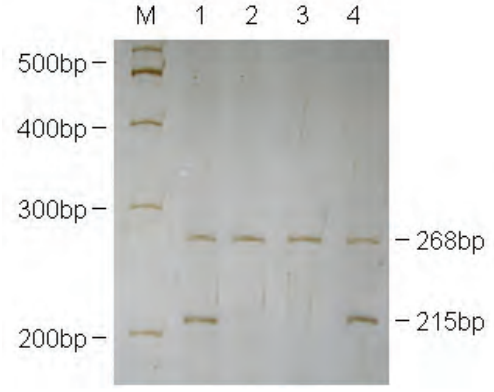

Figure 3. PCR result of GSTM1 genotypes
M: 100bp molecular weight marker; Lane 1,4: GSTM1 positive genotypes; Lane 2,3: GSTM1 null genotypes

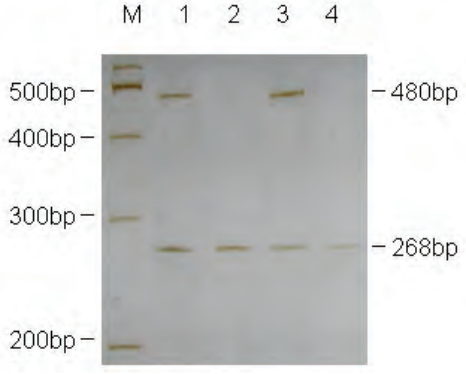

Figure 4. PCR result of GSTT1 genotypes
M: 100bp molecular weight marker; Lane 1,3: GSTT1 positive genotypes; Lane 2,4: GSTT1 null genotypes

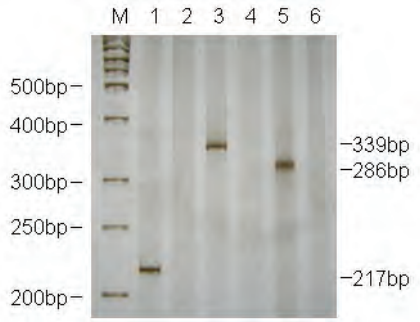

Figure 5. PCR results of NAT2 genotypes
M: 100bp molecular weight marker; Lanes 1 & 2: homozygote of m1; Lanes 3 & 4: homozygote of m2; Lanes 5 & 6: homozygote of m3.

2.265 ($P<0.05$), and the OR value increased to 3.931 ($P<0.01$) in presence of both wild-type homozygote and smoking. There was no difference in the distribution of CYP1A1 Ile/Ile wild homozygote genotype between non-smoker groups of periodontitis and healthy control ($p>0.05$), but the CYP1A1 Ile/Ile wild homozygote genotype was highly distributed in the smoker periodontitis group ($p<0.05$). These results suggest an interaction between cigarette smoking and CYP1A1 MspI or Ile/Val wild-type genotype. Smoking and CYP1A1 wild-type genotype together possibly increased the risk further for severe periodontitis.

Any explanation of the association between the CYP1A1 genetic polymorphisms and the susceptibility of periodontitis remains speculative at this time. Being different from the carcinogen-activating by CYP1A1 in carcinogenesis, a detoxification by CYP1A1 may exist in the xenobiotic substances metabolizing, which plays a protective role in periodontitis onset. CYP1A1 wild genotypes are related to a relative lower metabolizing activity.

Association between GSTM1 and GSTT1 genetic polymorphisms and severe chronic periodontitis

Glutathione-S-transferase isoenzymes (GSTs) belong to phase-II conjugating enzymes. GSTs are multifunctional proteins that catalyze many reactions between glutathione (GSH) and lipophilic compounds with electrophilic centers, including those which are cytotoxic and genotoxic, for instance the polycyclic aromatic hydrocarbons (PAHs). When the reaction of GSH with an electrophile forms a stable covalent bond, the resultant adduct, referred to as a GSH conjugate, is usually no longer toxic and is easier to be excreted.

The GST supergene family is composed of four multigene families: α(GSTA), μ(GSTM), π(GSTP), θ(GSTT) and σ(GSTS) (Habdous *et al* 2004). GSTM1 null homozygote genotype associates with an absent expression of GSTM1 enzymes, which lead to an absence of GSTM1 catalysis to some certain xenobiotic substances. GSTT1 is the isoenzyme of GSTM1. GSTT1 null homozygote genotype has a similar physiological meaning with GSTM1 null genotype (Malats *et al* 2000).

The contribution of GSTM1 and GSTT1 null genotypes to human susceptibility to cancer risk is not consistent. Some studies have reported an association between GSTM1 null genotype and lung cancer (Pinarbasi *et al* 2003), with an increased BPDE-DNA adducts levels in leukocytes. Other investigations suggested an relationship between GSTM1 null genotype and an increased risk of hepatocarcinoma, bladder carcinoma, and other cancers (Lee *et al* 2002, Lear *et al* 2000). It is thought that the deficiency of detoxification to carcinogens caused by null genotypes leads to a high susceptibility to cancer. Other studies have found an association between GSTT1 positive genotype and hepatocarcinoma, which may be due to the carcinogen-activation of GSTT1 (McGlynn 1995).

The association of genetic polymorphisms

of GSTM1, GATT1 and the susceptibility of periodontitis is not clear. The results of this study showed that there was no significant association between GSTM1null genotype or GSTT1null genotype and severe chronic periodontitis in non-smokers ($p>0.05$). But in smokers, GSTM1null genotype or GSTT1null genotype was associated significantly with severe CP ($P<0.05$).

Association between NAT2 genetic polymorphisms and severe chronic periodontitis

Seven different NAT2 mutations were found in human genes; five of which led to amino acid changes: G→A at position 191 (Arg to Glu), detected particularly in people of Afican origin; T→C at position 341 (Ile to Thr); G→A at position 590 (Arg to Gln); A→G at position 803 (Lys to Arg); and G→A at position 857 (Gly to Glu). The remaining two C→T point mutations at position 282 and 481 exert no influence on the amino acid sequence (Vatsis *et al* 1991).

Mutation 481T rarely occurred without 341C and 803 G and mutation 590A was found with mutation 282T 94% of the time. Mutations 481T (m1), 590A (m2), and 857A (m3) are thus the important mutations for detecting polymorphisms of NAT2 gene that determined the phenotypically slow or rapid metabolizers. A number of studies have demonstrated an increased cancer risk in slow acetylators with documented occupational exposure to arylamines (Hou *et al* 2000, Firozi *et al* 2002).

The results showed that there was no significant difference for the distribution of slow acetylator genotypes between non-smoker groups of periodontitis and healthy control ($p>0.05$), but the frequency of slow acetylator genotypes was significantly higher in the smoker periodontitis group than in the smoker healthy control group ($p<0.01$). The results indicated an interaction between slow acetylator genotypes and smoking for the susceptibility to periodontal diseases.

Smoking is an environment risk factor for periodontitis. Xenobiotic substances derived from tobacco could introduce immunosuppression or overstimulation of the immune/inflammatory responses to periodontal bacterial factors (Page and Beck 1997). Possibly, the impaired metabolism of smoking-derived xenobiotic substances may have an immune impact on susceptibility of periodontitis.

Since individuals may be exposed to a complex of xenobiotics, periodontal susceptibility is likely to be determined by a number of metabolizing enzymes, rather than a single enzyme. The individual's susceptibility to periodontitis is associated with multiple genes; some are major effect genes and others are minor effect genes. Further investigations are required to assess the combination effect on the risk factors of periodontitis.

The results from this preliminary study seem promising. To determine the association between the metabolizing enzyme gene polymorphisms and severe chronic periodontitis in people of Chinese Han nationality, further investigations on a large scale population should been undertaken.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 7

Prevalence of Systemic Diseases Among Periodontal Patients at the State University Clinic and Two Private Periodontal Practices in the Philippines

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Private Periodontal Practice, Manila, Philippines

Introduction

Recent developments in dental research have highlighted the association between oral inflammatory diseases and certain systemic diseases notably diabetes mellitus, and cardiovascular diseases (Beck and Offenbacher 2001). Periodontal disease, long recognized as a chronic inflammatory condition, has been found to influence the behavior of certain systemic diseases, which also have inflammatory pathways (Mealey and Rose 2005). The cascade of immune reactions triggered by inflammation of the periodontium has been found to have similarities with the immunologic events that occur in systemic conditions such as cardiovascular diseases, respiratory infections and diabetes. In addition to this, certain gram-negative bacteria associated with periodontal disease have been found to influence the pathogenesis of certain systemic diseases. (Dave *et al* 2004).

As researchers further studied the processes by which the human immune system responds to injury and/or invasion of an infectious agent, links among several disease conditions have been recognized. Thus, research geared towards establishing the correlations of these diseases has recently gained popularity.

Seymour *et al* (2006) stated that there are four possible mechanisms to explain the relationship between chronic infections such as periodontal disease and atherosclerosis. These are common susceptibility, inflammation, direct infection of the blood vessels and cross-reactivity or molecular mimicry between bacterial and self-antigens. Earlier than this, Lowe (2001) presented an overview of the potential relationships between infections, including periodontal disease, and atherosclerosis. It was believed that periodontal disease activates pro-inflammatory cytokines, which may contribute to the formation of plaque on arterial walls. Dave *et al* (2004) further expounded on the commonality between the pathogenesis of periodontal disease and cardiovascular diseases. The similarities cited include the release of pro-inflammatory cytokines and expression of macromolecules in both conditions, as well as the possible effects of certain microorganisms. According to the authors, "chronic inflammatory infectious diseases, such as periodontal diseases, have come under close scrutiny for their potential to contribute to both systemic inflammation and bacterial seeding of atherosclerotic plaques".

The role of bacteria has also been investigated. The periodontal pathogen, *Porphyromonas gingivalis* has also been

detected in atheroma plaques and has been found to invade human coronary endothelium and influence atherosclerosis development (Mujakawa *et al* 2004). Bacteremia arising from dental plaque infections has also been suspected to contribute to thromboembolic events (Herzberg and Myer 1998, Spahr *et al* 2006).

Madianos *et al* (2006), in a review article on the association between periodontal disease and coronary heart disease, reported that significant association of these two diseases were apparent in 50% of the cohort, 75% of case controls and 50% of cross-sectional studies analyzed.

Diabetes mellitus is another systemic disease believed to be significantly associated with periodontal disease. Iacopino and Cutler (2000) explained how gram-negative bacterial lipopolysaccharide (LPS), which is present in periodontitis, is capable of producing an exaggerated inflammatory response in a diabetic patient. In addition, Mealey and Rose (2005) and Graves *et al* (2004) cited mechanisms by which the prevalence and severity of periodontitis is increased in a diabetic patient. Firstly, neutrophil defects seen in diabetic patients diminish bacterial resistance. Secondly, patients with poorly controlled diabetes produce advanced glycation end-products, which foster an up-regulation of macrophages, thereby causing a significant increase in pro-inflammatory cytokines. Lastly, the decreased healing capacity seen in diabetic patients is attributed to an increase in both collagenase and tumor necrosis factor-alpha (TNF-α) levels. These are also known to be produced in periodontal disease processes. Taylor *et al* (1996), in a study of the Gila River Indian Community residents, observed that a higher proportion of patients with severe periodontitis had poorer glycaemic control thus implying the possibility of a co-relation between diabetes and periodontal disease. Furthermore, treatment of periodontal infections has been found to improve glycemic control among diabetic patients. (Lamster and Lalla 2001).

In different countries, studies are now being conducted to look into the strength of this association. In the Philippines, the prevalence rates of angina, stroke, claudication (known atherosclerotic diseases) and hypertension were 12.5%, 1.9%, 4.2% and 17.4 % respectively. The prevalence rate for diabetes was 4.6% (Dans *et al* 2003) and it is currently the ninth leading cause of death in the Philippines. It was therefore the aim of this study to determine the percentage of Filipino periodontal patients seeking treatment who may also have associated medical conditions like diabetes mellitus and cardiovascular disorders. This should provide baseline data on the prevalence of cardiovascular disease and diabetes among Filipino periodontal patients in Metro Manila, Philippines.

Materials and methods

All charts of periodontal patients admitted to the Oral Medicine Section of the University of the Philippines College of Dentistry (UPCD) Clinic from June 2000 to April 2007 were screened. Charts of patients in two private periodontal practices (PPP) in Metro Manila seen from January 1997 up to June 2007 were likewise screened. The Oral Medicine Clinic of the UPCD Clinic caters to the periodontal treatment needs of patients admitted to the University Clinic. Undergraduate dentistry clinicians under the supervision of a Clinical Supervisor administer periodontal treatment. The treatment procedures consist mainly of scaling and root planing (SRP) and simple treatment procedures that do not require a specialist's intervention. The majority of patients belong to the lower socio-economic strata of the sample population and could not afford the

higher cost of periodontal treatment in private periodontal practices. On the other hand, patients in the two private periodontal practices (PPP1 and PPP2) are mostly professionals and office workers belonging to the upper socio-economic bracket of the sample population in Metro Manila.

Inclusion and assessment criteria

The subjects/patients had to be at least 18 years old to be included in the study. The medical questionnaire must have been completed by the patient and validated by the student clinician in the case of the UPCD clinic or by the patient attending the private periodontal clinics. Sufficient dental records and adequate dental radiographs (panoramic, periapical and bitewing) had to be available to confirm the presence of periodontal bone loss. No attempt was made to assess the severity of the periodontal disease. Table 1 shows the information gathered from the periodontal patients' records. Information retrieved from the patient's records was then tabulated according to age groups and gender, male (M) or female (F).

- Age
- Gender
- Cardiovascular Diseases
  - Rheumatic heart Disease
  - Mitral Valve Prolapse
  - Sub-Acute Bacterial Endocarditis
  - Hypertension
- Diabetes Mellitus

Table 1. Assessment information gathered from patient records

Results and discussion

This study looked at the prevalence of systemic illnesses, specifically diabetes mellitus (DM) and cardiovascular diseases such as hypertension (HT), rheumatic heart disease (RHD), sub-acute bacterial endocarditis (SBE) and mitral valve prolapse (MVP) amongst periodontal patients seen at the UPCD Clinic and in two private periodontal practices (PPP1 and PPP2). The UPCD Clinic had a total of 324 periodontal patients seen from June 2000 to April 2007; 156 males and 168 females (Table 3). Only 6% of total patients exhibited a systemic illness at the same time (Table 2, Figure 4). 4.62% had cardiovascular disease (Figure 1, Figure 3). The lower prevalence compared with other studies (Georgiou *et al* 2004, Peacock and Carson 1995) may be attributed to certain cultural practices and patient characteristics, which may be inherent to the Philippine setting. The patients that utilise the dental services offered by the undergraduate program of the UPCD belong to the C and D socio-economic strata of our subject population, consisting mainly of minimum wage earners (US$200/month) and the unemployed. They do not seek medical consultation unless they experience symptoms or become too ill to be able to report for work or perform daily activities. Another factor that may contribute to non–detection of associated medical problems is the fact that there is minimal state subsidy for health services and thus ordinary citizens cannot afford regular medical check-ups. On the other hand, it may truly be the case that very few in this particular group of patients have associated systemic disease. The diet of this particular socio-economic group consists mainly of rice and vegetables and occasionally fish, which are healthier food options. Meat and other food products that are more consumed in other parts of the world are more expensive in the Philippines and are thus not affordable by this socio-economic group. Under-reporting or even non-disclosure of medical conditions may also occur because of fear that this may jeopardize immediate access to government-subsidized dental services. Prior medical

Age	RHD		SBE		MVP		HT		DM		Total
	M	F	M	F	M	F	M	F	M	F	
20-30	-	-	-	-	-	-	-	-	-	-	-
31-40	-	1	-	-	-	-	1	1	-	2	5
41-50	-	1	-	-	-	-	3	-	-	-	4
51-60	-	-	-	-	-	-	2	3	2	1	8
61-70>	-	-	-	-	-	-	2	1	1	1	5
Total	0	2	0	0	0	0	8	5	3	4	22

Table 2. Frequency distribution of cardiovascular diseases and diabetes mellitus among periodontal patients at the UPCD Oral Medicine Clinic (June 2000 - April 2007)

Age	UPCD		PP1		PP2	
	M	F	M	F	M	F
20-30	36	39	186	197	129	170
31-40	50	54	157	197	167	230
41-50	38	43	127	167	238	252
51-60	15	25	131	116	178	235
61-70>	17	9	114	128	201	258
Total	156	168	715	805	913	1145

Table 3. Gender distribution of periodontal patients in all groups sampled

Age	RHD		SBE		MVP		HT		DM		Total
	M	F	M	F	M	F	M	F	M	F	
20 –30	2	0	0	0	0	0	2	2	0	0	6
31 - 40	1	0	0	0	1	2	3	5	1	3	16
41 – 50	2	2	0	0	1	1	22	16	3	4	51
51 – 60	4	4	0	0	0	1	27	34	8	5	83
61 – 70 >	6	8	0	0	1	2	42	45	7	10	121
Total	15	14	0	0	3	6	96	102	19	22	277

Table 4. Frequency distribution of periodontal patients with associated systemic illness in a private periodontal practice (PPP1) in Metro Manila, Philippines (Jan 97-Jun 07)

Age	RHD		SBE		MVP		HT		DM		Total
	M	F	M	F	M	F	M	F	M	F	
20 –30	0	3	0	0	0	1	2	4	0	1	11
31 - 40	2	4	0	0	2	2	11	7	3	1	32
41 – 50	6	9	0	0	2	6	54	35	8	9	129
51 – 60	12	4	0	0	0	0	47	54	10	15	142
61 – 70 >	13	14	2	0	0	1	68	80	21	19	218
Total	33	34	2	0	4	10	182	180	42	45	532

Table 5. Frequency distribution of periodontal patients with associated systemic illness in a private periodontal practice (PPP2) in Metro Manila, Philippines (Jan 97-Jun 07)

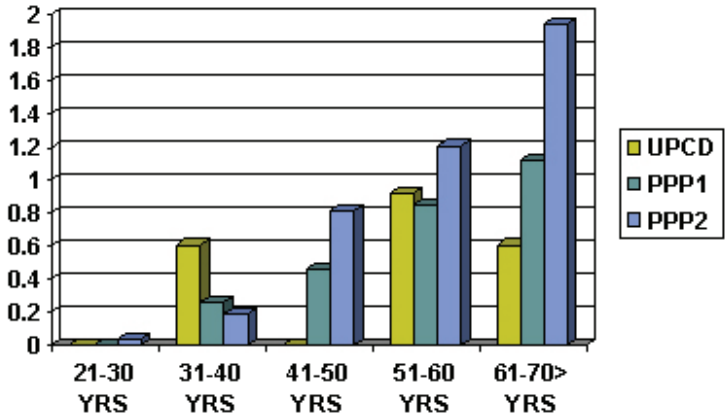

Figure 1. Percentage distribution of periodontal patients with cardiovascular diseases in three sample groups (Manila, Philippines)

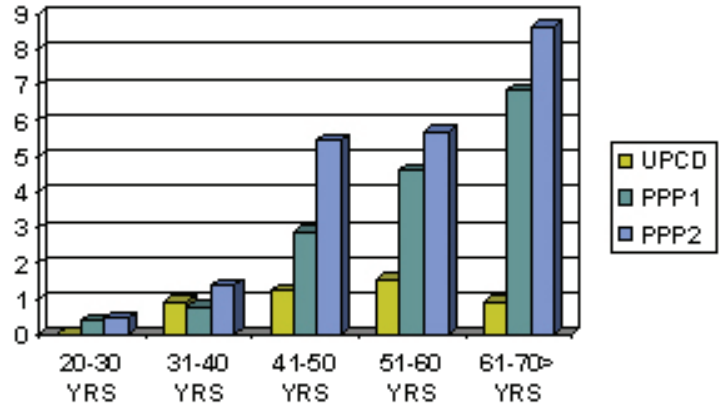

Figure 2. Percentage distribution of periodontal patients with diabetes in three sample groups (Manila, Philippines)

clearance would be required to access certain dental procedures, which as previously mentioned is difficult to obtain.

Data from the two private periodontal practices (PPP1 and PPP2) reflected slightly higher prevalence rates. PPP1 consisted of 715 Males and 805 Females (Table 3). About 88% of this sample did not have associated systemic illnesses. Only 18.22% of periodontal patients reported diabetes mellitus or any form of cardiovascular disease (Table 4, Figure 4). Hypertension was the most common systemic illness seen among periodontal patients in this private periodontal practice. 96 out of the 715 males and 102 out of the 805 females suffered from hypertension. Diabetes mellitus was the next most prevalent medical problem seen in this sample.

In PPP2, 532 out of 2,058 or 25.85% of periodontal patients seen from January 1997 to June 2007 reported that they have diabetes mellitus or a form of cardiovascular disease (Table 5, Figure 4). Prevalence rate for cardiovascular disease was 21.62% while rate for diabetes mellitus was only 4.22% (Figure 2, Figure 3). These rates were still lower than that seen in other studies (Dumitrescu *et al* 2006, Georgiou *et al* 2004, Peacock and

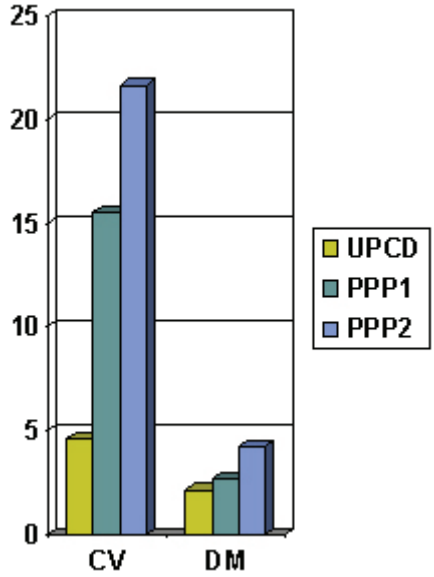

Figure 3. Percentage distribution of periodontal patients with cardiovascular disease and diabetes in three sample groups (Manila, Philippines)

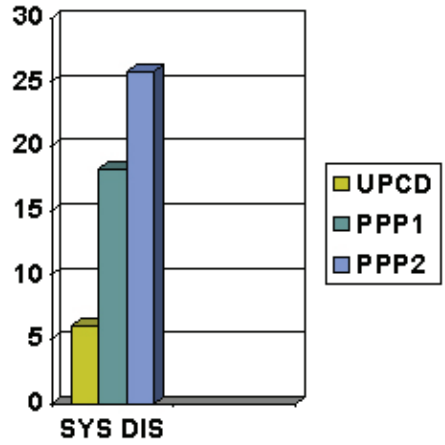

Figure 4. Percentage distribution of periodontal patients with systemic disease in three sample groups (Manila, Philippines)

Carson 1995), but almost similar to that observed by Ozturk *et al* (2006) in a retrospective recording review, where 773 out of 2,737 (28%) subjects with periodontal disease in an urban Turkish population were reported to be suffering from a systemic disease as well. Peacock and Carson (1995) found, upon examination of self-administered health questionnaires, that 52.5% of periodontal patients reported that they have an associated medical condition. The data from the study of Georgiou *et al* (2004) conducted in Brisbane, Australia showed that 33% of periodontal patients reported that they have a systemic illness as well. Cardiovascular disorders were the most frequently reported associated medical condition in both studies. This finding is consistent with our own findings where hypertension was found to be the most frequently reported systemic disorder among periodontal patients in all three samples examined.

Dumitrescu *et al* (2006) looked at the occurrence of self-reported systemic diseases among periodontal patients at a specialist clinic and found that 81.96% of them had reported to have at least one systemic disorder. The authors would like to cite the same reasons stated previously for the observed difference in findings. Filipinos, even those belonging to the A and B socio-economic strata consume a diet consisting of fish, vegetables and meat but serving portions are usually smaller. Like the rest of the population, health is not a priority so there may be more cases that are not recognized or detected. Dental visits are often irregular and aesthetics are often of more concern than health.

Conclusion

The frequency of systemic disease occurring among periodontal patients seems to be much lower compared to that reported in other studies. Only a small percentage of periodontal patients had some form of

systemic disease at the same time. Cultural practices may be cited as factors for the non-detection or under-reporting of these systemic illnesses.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 8

Relationships Between Periodontal Diseases and Systemic Health in New Zealand: An Overview of Research at Otago University

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Introduction

Emerging evidence suggests that periodontitis may affect systemic diseases that significantly increase mortality rates (Georgiou *et al* 2004, Reddy 2007). Systemic conditions that may have a relationship to this intra-oral disease include atherosclerosis, cerebrovascular and cardiovascular disease, obesity, diabetes and the occurrence of preterm birth and/or low birth weight infants (Michalowicz and Durand 2007, Paquette *et al* 2007, Ritchie 2007.) These conditions are found at much higher levels within some New Zealand (NZ) ethnic groups than within the general NZ population.

Ethnicity and systemic disease in New Zealand

New Zealand is a multi-ethnic south-Pacific nation of 4.1 million people, of which Maori, Pacific Island and Asian peoples respectively comprise 15%, 7% and 9.3%. The remaining 68.7% consist predominantly of settlers of European origin (known in NZ by the Maori term "Pakeha"). Mortality rates for Maori are 2.4 fold higher for males and 2.7 fold higher for females than matching European New Zealanders. For NZ Pacific Islanders (PI) the rates are elevated 2.37 (male) to 2.74 (female) times. Heart disease accounts for >40% of the difference in mortality rates between New Zealanders of different ethnicity (Blakely *et al* 2007). However, although this group forms the most rapidly growing section of the population, relatively little is known about the relative mortality rates of NZ Asians.

A major risk factor common to many systemic conditions, as well as periodontitis, is cigarette smoking (Johnson and Guthmiller 2007). The 1996 New Zealand census found significantly higher rates for Maori, with Maori women (43.3%) twice the rates recorded for Europeans (Figure 1). PI males (34.1%) were 1.5 times more likely to smoke than PI females (Borman *et al* 1999). Although NZ Asians smoked less than the general populations, males (18.7%) were four times more likely to smoke than for Asian females (Borman *et al* 1999). By 2002, smoking rates had decreased for Europeans but increased to 49% overall for Maori with peak rates of 55% for young Maori females aged 25-29 years. The Maori Smokefree Coalition, part of a government-sponsored campaign to reverse this trend has gone so far as to accuse the tobacco industry of "Maori murder".

Other conditions that are also disproportionately prevalent in Maori and

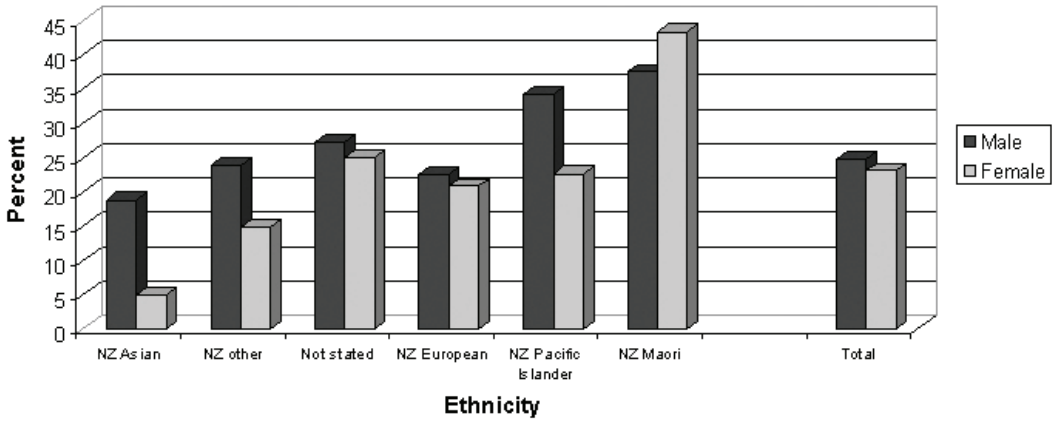

Figure 1. Age adjusted smoking rates from NZ 1996 Census (adapted from Borman *et al* 1999)

Pacific Islanders include atherosclerosis (Bell *et al* 1996, Sarfati and Scott 2000), hypertension (Gentles *et al* 2006) and stroke (Bonita *et al* 1997, Carter *et al* 2006). Stroke incidence and occurrence rates are significantly higher among Maori, PI, and NZ Asian groups than Europeans. The mean age of incidence is a decade earlier for PI and Asians and 60.7 years for Maori compared with 75.6 years for Europeans. The rate of stroke has decreased during the past decade for European females, but has remained static for Maori and increased for PI (Dyall *et al* 2006). Maori are 1.9 times and PI 2.5 times as likely to be obese as Europeans (Russell *et al* 1999) and Maori are twice as likely to be obese smokers (Tobias *et al* 2007). A lack of analysis of the prevalence of obesity in NZ Asians has been identified (Duncan *et al* 2004*).* Diabetes prevalence is 2.8 to 3.4 times higher in non-European New Zealanders; rates of 10.1% for Pacific Islanders, 8% for Maori and 8.4% for NZ Asians are markedly higher than the prevalence of diabetes amongst NZ Europeans at 2.9% (Joshy and Simmons, 2006).

The combination of these systemic conditions is known as "metabolic syndrome" (also known as "insulin resistance syndrome"). This has been characterized as the presence of three or more of the following cardiovascular risk factors; large waist circumference, high triglyceride concentration, low HDL cholesterol concentration, raised blood pressure, or high fasting glucose concentration. Metabolic syndrome is a significant predictor for both cardiovascular disease and type 2 diabetes. Patients who fit these clinical criteria have a doubled risk of stroke or heart attack and a six-fold increased risk of diabetes. The prevalence of metabolic syndrome has been shown to be significantly higher amongst New Zealand Maori and PI compared with other ethnicities (Gentles *et al* 2007). When compared with worldwide figures, 16% of New Zealanders can be classified as having metabolic syndrome (Figure 2). This figure lies between that given for the French (8.5%) and Americans (24%); however Maori (35.5%) and NZ PI (39.0%) have a markedly higher prevalence, approaching that of Native Americans (50.2%) (Cameron *et al* 2004, Simmons and Thompson 2004, Gentles *et al* 2007).

The incidence of pre-term birth and infants with low birth weight shows similar ethnic variation in New Zealand (Craig *et al* 2004a). Rates of pre-term birth are highest for Maori and this has remained fairly consistent at

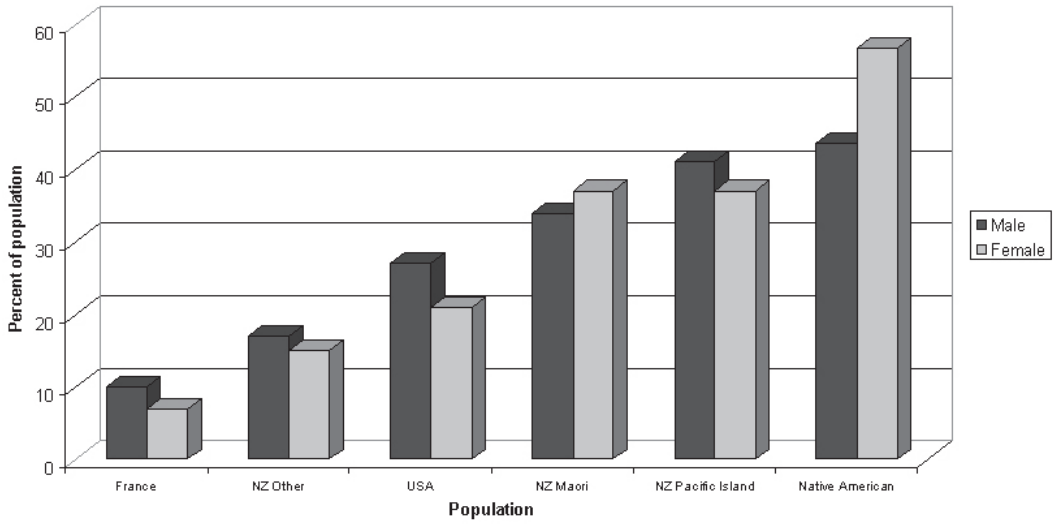

Figure 2. Prevalence of metabolic syndrome - Adult Treatment Panel III definition (adapted from Cameron et al 2003, Simmons and Thompson 2004, Gentles et al 2007)

around 6.5-6.8% during the period 1980 to 2001. Rates for Pacific Islanders have risen slightly 4.4-4.9% over this period, whilst European rates have shown a statistically significant rise from 4.0-5.8% over the same period. These rates appear to be statistically unrelated to differences in socio-economic ranking or the relative proportion of teenage pregnancies amongst the different ethnicities, however higher uptake of preterm inductions and preterm Caesarean deliveries by European women may account for some of these differences (Craig *et al* 2004b); this has been reported as accounting for differences between white and black women in an American study (Demissie *et al* 2001). Small-for-gestational age rates decreased for all ethnic groups during the two decades studied, particularly for Maori, but are still significantly higher amongst Maori and Pacific Islanders.

Ethnicity and periodontitis in New Zealand

Differences between ethnic groups in the prevalence and incidence of dental disease exist but have been relatively poorly documented. The last national dental survey was two decades ago and showed higher rates of periodontitis and edentulism amongst Maori and PI (Hunter *et al* 1992). A longitudinal birth cohort study of 1037 children born in Dunedin, New Zealand in 1972-73 has shown marked differences in the prevalence of periodontitis and tooth loss (Thomson *et al* 2000a, Thomson *et al* 2000b, Thomson *et al* 2006, Broadbent *et al* 2006) but has focused on socio-economic and behavioural factors as explanatory variables, since over 90% of the sample give their ethnicity as New Zealand European. Detailed information on other ethnic groups including NZ Asians is lacking, however a new national dental survey for NZ is currently in the planning stages.

Periodontitis and systemic conditions at Otago: pre-term birth

A number of projects have been or are being carried out at Otago University investigating links between systemic

conditions and periodontitis. The first NZ study into the relationship between periodontitis and pre-term birth has been completed and submitted for publication (English 2005). In this retrospective clinical case-control study, 30 mothers with preterm birth/low birth weight (PTLBW) infants ≤35 weeks gestation and <2500g birth weight were compared with 30 age- and ethnicity-matched control mothers. Demographic details were collected and two randomly selected diagonally opposite quadrants had clinical attachment level and pocket depths recorded within one month of delivery. Mesial and distal crestal bone height was measured in posterior bitewing radiographs. Although hampered by a small sample size, the results suggested that PTLBW mothers had more periodontitis ($p < 0.001$) with an adjusted odds ratio of 9.2:1. This small pilot study concluded that, after adjusting for other known risk factors, the clinical presence of maternal periodontitis appears to be associated with increased risk of a premature low birth-weight birth in a NZ population. Further work is required to determine whether there is a relationship between periodontitis and pre-term birth in NZ Maori and Pacific Island groups, which may account for some of the known differences in birth outcomes for these ethnic groups.

Animal model for investigating periodontitis and pre-term low birthweight

The domestic sheep model for neonatal research is well established in NZ and Australia (Bloomfield *et al* 2003). Research in Western Australia has demonstrated that intra-amniotic injection of *P. gingivalis* and *A. actinomycetemcomitans* LPS into pregnant sheep results in high rates of fetal lethality compared with *E. coli* LPS, which may account for a proportion of unexplained stillbirth and other pregnancy complications (Newnham *et al* 2005).

It is known that this animal species is vulnerable to naturally-occurring periodontitis (Ismael *et al.* 1989). Clinical, microbiological and serological parameters of this animal model for periodontitis research have been established at Otago (Frisken *et al* 1989, Duncan *et al* 2003). An investigation comparing periodontally-healthy sheep (HS) and periodontally-diseased sheep (PDS) found significantly higher serum IgG titers to *P. gingivalis*, *T. forsythensus*, *P. intermedia* and *F. nucleatum s*trains and to fimbriae and cysteine proteases ($p = 0.05$ to 0.001) as well as significant associations between tooth loss and serum IgG titers to *P. gingivalis* ($p > 0.01$) in PDS. The researchers concluded that periodontitis-susceptible and non-susceptible sheep can be identified for periodontal research. Future research opportunities now exist to combine the sheep obstetric and periodontitis models, in order to establish whether periodontal pathogens such as *P. gingivalis* cross the placental barrier and initiate premature labour.

Current research at Otago: gene analysis

Current research into periodontitis and systemic conditions takes advantage of the newly-established Otago Centre for Gene Research. Three doctoral projects have commenced examining (i) the effect of smoking upon periodontal ligament fibroblasts and gingival fibroblasts using an *in vitro* wound model; (ii) the pattern of gene expression in white blood cells from patients with gingivitis and periodontitis; and (iii) T-cell immune responses to *P. gingivalis*, in subjects with both atherosclerosis and moderate-to-severe periodontitis. Results from these studies are not expected for another 18 months.

Future research at Otago

These are a number of promising lines of inquiry within New Zealand populations that may be pursued further at Otago. The pilot study into pre-term birth now requires a larger scale study that includes Maori, Pacific Island and New Zealand Asian patients. Further investigation of the mechanisms that link periodontitis and pre-term birth may be examined using the sheep animal models. Potential also exists to examine links with other well-documented systemic conditions that affect New Zealand populations, leveraging off the experience we are developing with genetic analysis. For example, does gene array analysis reveal differences between Maori populations with and without diabetes, and with and without periodontitis?

Conclusions

Investigations into systemic conditions and periodontal diseases are now a focus for periodontal research at Otago University. Current and future studies will employ emerging technologies to analyse New Zealand's unique human populations. Examination of underlying disease mechanisms may include established animal models.

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Chapter 9

Bisphosphonate Associated Osteonecrosis of the Jaws: Implications for Periodontics and Implantology

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Introduction

In an era with a focus on the field of periodontal medicine and an increasing involvement with medically compromised patients, it is not unusual to encounter pharmacological interactions that have a bearing on the progress, prognosis and treatment outcomes of periodontal diseases. In some patients the focus may not be on periodontal disease being the cause in a true cause and effect relationship, but these interactions nevertheless have place in any scientific discussion on periodontal medicine. Of very recent interest is bisphosphonate associated osteonecrosis (BON) of the jaws and the possible implications of this condition in periodontal and implant treatment (Marx 2003, Migliorati 2003). The most common dental co-morbidity in patients with this condition is periodontitis (Marx *et al* 2005) and a close co-relationship has been established with surgical bone manipulation as well (Migliorati *et al* 2005). No true cause and effect relationship have yet been traced (American Dental Association 2006) and no data from clinical trials are currently available (Migliorati 2005). Additionally there is no diagnostic methodology in place to identify individuals who are at risk (American Dental Association 2006).

What is bisphosphonate associated osteonecrosis?

BON usually presents as a painful soft tissue swelling, which is often extensive and sometimes infected. It is typically characterised by loosening of teeth, drainage and exposed bone (Migliorati *et al* 2005). Most often, the patient is asymptomatic until bone becomes clinically visible (American Dental Association 2006). There may also be other presenting signs such as numbness, heaviness and dysesthesia.

What are bisphosphonates?

Bisphosphonates are analogs of inorganic pyrophosphates. They are commonly used to treat bone loss in conditions such as Pagets disease and osteoporosis. These drugs inhibit osteoclastic differentiation and induce apoptosis with consequent imbalance in the bone remodelling process at the basic multicellular unit level (Sato *et al* 1991, Nagashima *et al* 2005, Xing and Boyce 2005). As a result, these drugs have an antiangiogenic effect (Fournier *et al* 2002, Wood *et al* 2002) and promote an increase in trabecular thickness and bone mass (Sato *et al* 1991). There is also a cumulative suppression of bone turnover that results in an impairment of the

biomechanical and reparative properties of bone (Ensrud *et al* 2004, Odvina *et al* 2005). Animal studies have also demonstrated an inhibition of microdamage repair (Mashiba *et al* 2000, Mashiba *et al* 2001, Li *et al* 2001).

What is the problem?

The primary problem lies in the fact that many patients are unaware of whether they are receiving bisphosphonate therapy. Many patients for whom therapy is of serious consequence are receiving intravenous (IV) bisphosphonates and not oral bisphosphonates, therefore the importance of becoming acquainted with conditions that receive IV bisphosphonate therapy cannot be overemphasised. Many IV bisphosphonates have unusual half lives that extend into several years and many patients may not disclose a history of past therapy (American Dental Association 2006). This is not unusual as patients generally only disclose medication that they consume regularly and IV therapy is usually only provided in a hospital environment. Such patients typify the potential BON patient suffering from a high degree of bone damage. In comparison, in the case of oral therapy, less than 1% of the dosage is absorbed by the gastrointestinal tract, as opposed to over 50% of the drug becoming bioavailable for bone matrix incorporation in the case of IV therapy (Berenson 1997, Ezra and Golomb 2000).

Most often, sustained oral therapy is provided in osteoporosis. Given the high number of patients who suffer from this condition and the even higher numbers who are at risk, the incidence of bisphosphonate therapy is naturally large and growing. What is even more alarming is the fact that high numbers who are at risk use bisphosphonates prophylactically (Santini *et al* 2004). The potency of effect on bone metabolism is also dependent on the specific type of bisphosphonate being administered

Compound	Preclinic Atiresorptive Relative Potency	Route of Administration
Short Alkyl or Halide Side Chain		
Elidronte (Didronel)	1	Oral/Intravenous
Cyclic Chloro Side Chain		
Tiludronate (Skelide)	10	Oral
Aminterminal Group		
Pamidronate (Aredia)	100	Intravenous
Alendronate (Fosamax)	100-1000	Oral
Cyclic Nitrogen-Containing Side Chain		
Risedronate (Actonel)	1000-10,000	Oral
Ibandronate (Boniva)	1000-10,000	Oral
Zoledronic acid (Zometa)	≥10,000	Intravenous

Figure 1. Antiresorptive potency of bisphosphonates observed in human clinical trials (Adapted from Watts 1998)

(Migliorati *et al* 2005). A wide variety of prescription bisphosphonates are available for oral therapy and their antiresorptive potency varies widely. Some bisphosphonates have a high antiresorptive potency even in oral administrative applications (Table 1). Potency is varied by using various R2 side chains in the molecular composition of the drug (Migliorati *et al* 2005). While most bisphosphonates employ a phosphorus-calcium-phosphorus chain that acts like a bone hook, incorporating an OH group R1 chain enhances binding of the drug to hydroxyapatite and consequent bone matrix incorporability of the drug in question (Migliorati *et al* 2005). Further variation in the R2 chain can determine clinical potency (Migliorati *et al* 2005). Figure 1 demonstrates a typical bisphosphonate molecular model that may be varied for clinical potency. The pathobiological model is also consequently variable depending on the drug in question and a typical model is presented in Figure 2.

Implications for regular periodontal care

Prophylactic measures in the case of all IV bisphosphonate therapy patients and those prescribed high potency oral bisphosphonates is of paramount importance. These patients must maintain a high level of oral hygiene and must undergo regular oral prophylaxis. Tori and other forms of bony exostoses must be eliminated as they are viewed as being at increased risk of developing BON (American Dental Association 2006). In any form of dental therapy, surgical manipulation of bone and extractions must be avoided (Bagan *et al* 2006). It is not unusual for a patient to present with spontaneous 'osteochemonecrosis' even in the absence of any exacerbative procedure (Ruggiero *et al* 2004).

In cases where therapy is being considered, it must be understood that while the median duration between therapy and the onset of BON is usually around 25 months, it is often common to see a much reduced duration between dental therapy and onset (Markiewicz *et al* 2005). In a patient receiving IV bisphosphonate therapy or high potency oral bisphosphonate therapy, the common strategy is to observe the patient while treatment is performed in only one sextant in a carefully controlled environment under antibiotic therapy (American Dental Association 2006). If healing is uneventful, then multisextant therapy usually follows. It has been observed that periodontal disease which involves medullary bone seems more likely to precipitate BON (American Dental Association 2006). Primary tissue closure may also be of substantial importance and given the reduced tissue vascularity as a result of the anti angiogenic effect of bisphosphonates, bone grafting and guided tissue regeneration may have limited consideration in such cases (Fournier *et al* 2002). Implant placement may also depend on the extent of treatment. The bottom line seems to be to implement conservative treatment as much as possible and to protect the healing wound site. Vinyl appliances may be used to cover potential BON sites after therapy (Migliorati *et al* 2005). Ironically, some studies have actually shown that the drugs may be beneficial in producing enhanced healing subsequent to surgical periodontal therapy (Lane *et al* 2005).

Further research directions

With regard to basic science research, there exists a substantial paucity of animal studies simply because of the lack of availability of a suitable animal model. Most studies are conducted on animal models that use ovariectomized postmenopausal rodents and simulate osteoporosis by glucocorticoid-induced and senescence-related osteopenia (McHugh *et al* 2003). The cost and duration

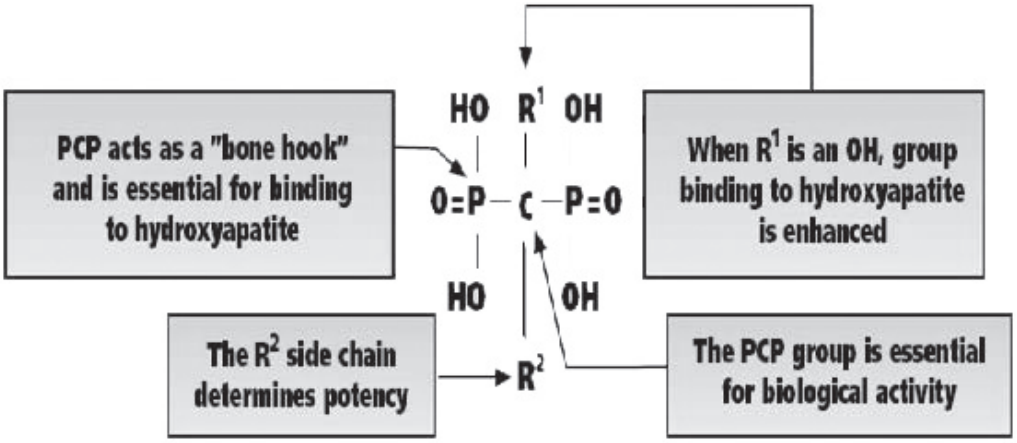

Figure 1. Chemical structure of bisphosphonates demonstrating that the manipulation of the basic structure will change the biological activity and the potency of the drug (Adapted from Migliorati 2005)

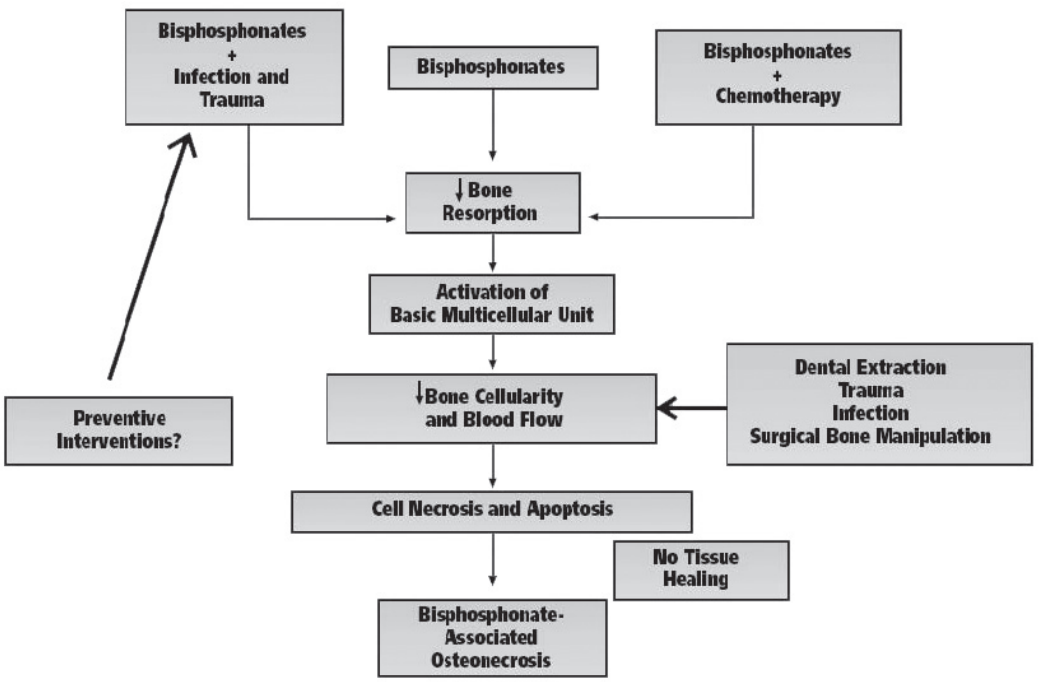

Figure 2. Pathobiological model for the development of bisphosphonate-associated osteonecrosis (Adapted from Migliorati 2005)

of developing such an animal model makes it difficult to evolve and the veracity of such a model in duplicating osteoporosis is also questionable. The model is by no means a representation of the true balance of bone metabolism with a balance of formation and resorption (McHugh *et al* 2003). A model worthy of consideration is one similar to the original animal models that were used in early animal experiments with bisphosphonates to establish safety for regulatory approvals (Schenk *et al* 1986, Muhlbauer *et al* 1991). Unlike conventional animal models, these models use a very young 21 day growing (weanling) rat model. Computerised tomography may be employed to advantage in this model to measure bone density with 0.1 mm slices to decrease voxel (3D pixel) size (McHugh *et al* 2003). This technique called Peripheral Quantitative Computerised Tomography scanning cannot replace the information obtained from histostaining, immunostaining or histomorphometric analysis but allows for rapid and non-invasive evaluation of potential therapeutic anti-resorptive compounds in a shorter amount of time as opposed to conventional animal models. Our own unpublished studies have clearly shown that employment of this model may be to advantage in demonstrating significant dose-related changes in BMD following bisphosphonate therapy. Significant decreases in growth (weights) and increases in cortical bone area were also observed in our study. The model is also being used to evaluate bone matrix changes around specially designed inserted implants in an effort to establish peri-implant healing mechanisms that will further understanding of the effect of inserting implants into bisphosphonate incorporated bone matrices.

Future directions in basic science research must focus on molecular mechanisms that lead to BON. An enhanced understanding of the role of bisphosphonates in the alteration of bone remodelling is needed in order to enable a better pharmacogenetic understanding that will help us to identify at risk patients. In terms of clinical research, clinical trials may not have the power or duration to provide us with usable data. A greater emphasis on understanding treatment outcomes for periodontics and implantology in patients undergoing bisphosphonate therapy is required. Discontinuation of bisphosphonate therapy and its effect on bone healing also needs to be better comprehended. A collaborative BON register that would help to consolidate various case reports worldwide would also provide vital data to enhance current understanding.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 10

Current Evidence of Periodontal Medicine in Thailand

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Introduction

Periodontal medicine is a new and exciting branch in the practice of periodontology. This term encompasses the associations between periodontal infection and other health concerns such as cardiovascular problems, pulmonary disease, diabetes, osteoporosis, smoking and increased risk of delivering a pre-term low birth weight baby. The term periodontal medicine also refers to the treatment of periodontal disease using medication adjunct to non-surgical means. Another aspect of periodontal disease addressed by periodontal medicine is the genetic predisposition of some individuals to advanced tissue destruction.

The consistent evidence presented by lead researchers for an association between periodontal disease and other systemic diseases includes:

- People with severe periodontal disease may be twice as likely to have the type of stroke caused by blocked arteries as those with good oral health.
- People with severe periodontitis may be more than six times as likely to have poor glycemic control as those without periodontal disease.
- Decreased bone mineral density and osteoporosis appear to be related to tooth loss. Hormone replacement therapy seems to be effective in saving teeth.
- Smoking is a major risk factor for periodontal disease. A smoker's risk of developing periodontal disease is increased anywhere from 2-10%, depending on the amount that person smokes.
- 18% of all pre-term, low-birth-weight births may be attributable to periodontal disease.
- Genetics can amplify or reduce the severity of periodontal disease. High production levels of interleukin-1 (IL-1), which indicate that people may be more susceptible to severe periodontal disease, run in families. A genetic test can now be administered to discover if a person has this genetic marker, and this knowledge could provide further information about a patient's prognosis.

In Thailand, limited numbers of epidemiological and pathological studies in the field of periodontal medicine have been carried out. Most of these studies have investigated the relationship between periodontal disease and diabetes. There are also some studies investigating the association between periodontal status and smoking, which the Thai government has now endorsed. The Ministry of Public Health of Thailand has launched a nationwide campaign to encourage

Age group	Male (%)	Female (%)
15-29	2.0	1.6
30-44	5.2	5.1
45-59	11.4	12.6
60-69	13.8	18.9
70-79	12.3	15.7
> 80	10.1	9.2
Total	**6.4**	**7.3**

Table 1. Prevalence of diabetes (by fasting glucose) in Thailand as of 2004

all dentists to counsel their patients during periodontal treatment to quit smoking.

This paper will describe the studies related to periodontal medicine in Thailand. The smoking cessation campaign in Thailand will also be discussed.

Evidence-based research concerning diabetes and periodontal disease in Thailand

The International Diabetes Federation (IDF) and the World Health Organization (WHO) have reported that there are an estimated 7 million new cases of diabetes diagnosed each year. The increasing incidence of diabetes is more significant in developing countries. Approximately 4 million people die from diabetes every year. The prevalence of diabetes in the Thai population is shown in Table 1. Table 2 lists the mortality rate of the top ten diseases in the Thai population.

Recent estimates show that around 246 million people around the world have been diagnosed with diabetes and without adequate prevention and control this number will reach 380 million in the next 20 years (Zimmet *et al* 2003). This chronic disease is amongst the most prevalent, expensive and preventable of all health problems. Type 2 diabetes is the more common type, and can be managed by exercise and diet modification. Complex and multifactorial metabolic changes in type 2 diabetes often lead to functional impairment and organ damage. The cardiovascular system, in particular, is at 2-4 times higher risk in diabetics than in healthy people. The WHO has estimated direct and indirect expenditures due to diabetes to be about 15% of overall health expenditures. In Thailand, the Ministry of Public Health has reported that about 2.4 million (9.6%) of people over 35 years old have been diagnosed with diabetes with most of them being type 2 diabetics.

The association between diabetes and periodontal diseases has been extensively studied. Several reports have shown that diabetes is associated with an increased prevalence, extent and severity of gingivitis and periodontitis. In 1999 and 2006, the American Academy of Periodontology reported that patients with diabetes might have a strong risk factor for periodontal disease due to a compromised immune system.

A variety of polymorphonuclear leukocyte defects have been described in diabetics such

Rank	Male Disease category	%	Female Disease category	%
1	HIV/AIDS	17	HIV/AIDS	9
2	Traffic accident	9	Stroke	7
3	Stroke	5	Diabetes	7
4	Liver cancer	4	Depression	4
5	Diabetes	3	Liver cancer	3
6	Ischaemic heart disease	3	Osteoarthritis	3
7	COPD (Emphysema)	3	Traffic accident	3
8	Homicide and violence	3	Anemia	3
9	Suicides	3	Ischaemic heart disease	3
10	Drug dependence/Harmful use	2	Cataracts	2

Table 2. Mortality rate by gender in the Thai population as of 2002
Data from: http://www.m-soiety.go.th/mso/doc/no_43/c4.pdf

as defects in chemotaxis, adhesion and phagocytosis. These defective functions lead to an impaired bacterial destruction process in periodontal pockets, resulting in progressing periodontal disease. The monocytes/ macrophages of diabetics may be hyperresponsive to bacterial antigens, leading to a significantly increased production of pro-inflammatory cytokines and mediators. Salvi *et al* (1997) described a higher TNF-α response of monocyte to *P. gingivalis* in patients with diabetes. In addition, the level of proinflammatory cytokines and mediators in the gingival crevicular fluid (GCF) of patients with diabetes are influenced by blood sugar levels. High levels of blood sugar inhibit collagen synthesis and reduce osteoblastosis resulting in impairment of osseous healing and bone turnover.

Ficara *et al* (1975) reported that elevated blood glucose in the GCF of poorly controlled diabetics might favor the growth of certain pathogenic microorganisms in periodontal pockets. Microvascular changes which may impair regeneration, thereby affecting the periodontal tissues, have been found in patient with diabetes.

Periodontal disease can affect the pathogenesis of diabetes and influence diabetic complications. The literature implies that periodontal disease can adversely affect the metabolic control of diabetics and the control of blood sugar levels. A 2 year longitudinal study showed that patients with diabetes and periodontal disease had 6 times higher blood sugar levels than patients with diabetes only (Taylor *et al* 1996, Taylor *et al* 1998). The severity of periodontal disease was also found to be associated with glycemic control (Grossi *et al* 1998). Another study by

Saito *et al* (2004) revealed an association between the severity of periodontal disease and the development of glucose intolerance in non-diabetics.

In Thailand, a cross-sectional survey has been done in elderly employees of the electricity generating authority of Thailand in order to identify the risk indicators of periodontal disease (Torrungruang *et al* 2005a). The study group consisted of 2005 elderly Thai adults, aged 50 to 73 years old. They received detailed medical and periodontal examinations including measurement of plaque scores, probing depth and clinical attachment levels (CAL). These individuals were categorized into mild (CAL <2.5 mm), moderate (CAL <2.5 to 3.9 mm), or severe (CAL $\geq$ 4.0 mm) periodontitis. The degree of association between the severity of periodontitis and various independent variables was investigated using multinomial logistic regression analysis. The results suggested that age, gender, education, oral hygiene status, smoking and diabetes were significantly associated with periodontal disease severity in this study group.

Periodontitis and cardiovascular disease may share common risk factors such as smoking, hyperglycemia, dyslipidemia, behavioral factor, aging and being of the male gender. Further studies of the same group of subjects were performed to investigate the association between periodontal disease and hyperglycemia, hyperlipidemia and to determine the negative effect of periodontal disease on plasma glucose and lipid levels (Kungsadalpipob *et al* 2007). The results showed the periodontitis group had higher fasting blood glucose (FBS) levels than the non-periodontitis group and the percentage of poor glycemic was highest in the severe periodontitis. The periodontitis group exhibited significantly higher mean FBS and triglycerides (TG) and lower mean high density lipoprotein (HDL) levels ($p<0.01$), but no significant differences in mean cholesterol and low density lipoprotein (LDL) levels when compare to the non-periodontitis group. A significant association between periodontitis, hyperglycemia and dyslipidemia was found. This indicated that hyperglycemia and dyslipidemia may be associated with periodontitis and that periodontal infection may impair metabolic control in older adults. However, these data do not allow us to determine whether periodontal disease causes an increase in hyperlipidemia and hyperglycemia or if periodontal disease shares hyperlipidemia and hyperglycemia as common risk factors or these coincidentally occurred.

Treatment of periodontal disease and the resulting decrease of oral inflammation may have a positive effect on the diabetes condition. It was shown that effective treatment of periodontal infection and reduction of periodontal inflammation, such as scaling and root planning combined with systemic doxycycline, was associated with a reduction in level of glycated hemoglobin (Grossi *et al* 1997). The study demonstrated that the doxycycline-treated group showed an improvement in periodontal status and a nearly 10% reduction of mean HbA1c from the pretreatment (Grossi *et al* 1997). However, clinical studies evaluating the benefit of periodontal treatment on glycemic control in diabetic patients were limited (Tables 3 and 4).

In Thailand, a study of effect of periodontal therapy on uncontrolled type 2 diabetes mellitus (DM) subjects was carried out by Promsudthi *et al* (2005). Fifty-two diabetic patients, age 55-80 years (mean age = 61 years), with glycated hemoglobin (HbA1c) 7.5-11.0% (8.98 ± 0.88) and severe periodontitis were included in this study. The treatment group received mechanical periodontal treatment combined with systemic doxycycline, 100 mg day for 14 days. The

Authors	Study design	Duration	HbA1c	
			Baseline (%)	Change (+/- %)
Miller *et al* 1992	Type 1 DM Test (n=9) SCRP No control	2 mo	9.4	-0.4 No stat analysis
Aldridge *et al* 1995	Type 1 DM Test (n=12) SCRP Control (n=10) no Treatment	2 mo	Test 9.8 Control 9.7	Test +0.6 Control -0.2 NS
Smith et al 1996	Type 1 DM Test (n=18) SCRP No control	2 mo	8.2	+0.1 NS
Westfelt et al 1996* *This study did not report mean HbA1c, but reported only the number of subjects in each HbA1c category.	Type 1 DM (n=14) SCRP Type 2 DM (n=6) SCRP Total Test (n=20) no control	2, 5 yr	< 6.0 n=2 6.0-7.9 n=6 8.0-9.9 n=10 > 10 n=2	0-2 yr 0-5 yr n=3 n=3 n=4 n=5 n=11 n=9 n=2 n=2 NS
Grossi et al 1997	Type 2 DM (n=113) SCRP/H_2O+Doxy SCRP/CHX+Doxy SCRP/Iodine+Doxy SCRP/CHX+placebo SCRP/H_2O+placebo Doxy 100 mg/d 2 wk	3, 6 mo	 10.5 10.4 10.3 10.7 9.2	3 mo 6mo -0.9 -0.2 -0.5 -0.1 -0.5 -0.1 -0.1 -0.2 -0.2 -0.1
Christgau et al 1998	Type 1 DM (n=7) Type 2 DM (n=13) Control: No DM (n=20) SCRP all groups	4 mo	 DM 6.5 No DM 4.3	 DM +0.2 No DM +0.4 NS
Stewart et al 2001	Type 2 DM Test (n=36) SCRP Control (n=36) no Treatment	10 mo	Test 9.5 Control 8.5	Test -1.9 Control -0.8
Iwamoto et al 2001	Type 2 DM Test (n=13) SCRP + Local minocycline x4 No control	2 mo	7.96	-0.84
Rodrigeus et al 2003	Type 2 DM Test (n=15) SCRP + Amox/Clav Control (n=15) SCRP	3 mo	Test 9.5 Control 8.8	Test -0.3 Control -1.2
Kiran et al 2005	Type 2 DM Test (n=22) SCRP Control (n=22) no treatment	3 mo	Test 7.31 Control 7.00	Test -0.86 Control +0.31
Jones et al 2007	Type 2 DM Test (n=15) SCRP + Doxy 100 mg/d 2wk + CHX 4 mo Control (n=83) no Treatment	4 mo	Test 9.9 Control 10.2	Test -0.65 Control -0.51 NS

Table 3. The effect of periodontal treatment of hemoglobin A1c levels: International Studies

Authors	Study design	Duration	HbA1c	
			Baseline (%)	Change (+/- %)
Promsudthi et al 2005	Type 2 DM Test (n=27)	3 mo	Test 8.98	Test -0.19
	SCRP +Doxy 100 mg/d 2wk		Control 9.17	Control +0.12
	Control (n=25) no Treatment			NS
Nisapakultorn et al 2007				
	Type 2 DM Test (n=20)	3, 6 mo		3 mo 6 mo
	SCRP +Doxy 200 mg +100 mg/d 10d		Test 9.1	-0.7 -0.5
	No control			

Table 4. The effect of periodontal treatment of hemoglobin A1c levels: Thai Studies

Legend for Table 3 & 4.
(+) increase HbA1c ; (-) decrease HbA1c Number in bold and italic were statistically different from baseline.
Amox/Clav = amoxicillin plus clavuranic acid; CHX = chlorhexidine gluconate mouth rinse; DM = diabetes mellitus; Doxy = doxycycline; n = number of subjects; NS = no statistical diference; SCRP = scaling and root planning

control group received neither periodontal treatment nor systemic doxycycline. The results showed that periodontal treatment significantly improved periodontal status in the treatment group, but the reduction in the level of fasting plasma glucose (FPG) and HbA1c was not significant. In the control group, no significant changes in clinical periodontal parameters, FPG and HbA1c levels were observed, except for significant increase in attachment loss. These indicated that the periodontal condition of older Thais with uncontrolled diabetes is: (a) significantly improved 3 months after mechanical periodontal therapy with adjunctive systemic antimicrobial treatment, and (b) rapidly deteriorates without periodontal treatment.

Recently, another study to investigate the effect of periodontal treatment with doxycycline administration on glycemic control of poorly controlled type 2 DM patients was carried out in a Thai population with a longer observation period (Nisapakultorn *et al* 2007). Twenty diabetic patients were included. All subjects received blood chemistry, periodontal treatment, consisting of scaling and root planning and systemic doxycycline (Table 5). A significant difference in HbA1c was observed only between baseline and 3 months after periodontal treatment (Table 6).

A study to investigate the effect of chemical plaque control agents on the diabetic status was performed (Soongyai 2003). The effect of a triclosan/copolymer/fluoride dentifrice during the maintenance period following non-surgical periodontal therapy was observed in 50 type 2 diabetic patients. Subjects using the dentifrice exhibited improved clinical attachment levels, reduced periodontal pocket depths and maintained low levels of inflammatory mediators (IL-1α and PGE_2) compared to those using the standard fluoride dentifrice over the 3 month maintenance interval. However, both groups showed no significant change in metabolic status. The results suggested that this oral hygiene regimen could significantly improve and maintain periodontal status, but has no effect on the metabolic status of diabetes mellitus.

Smoking and periodontitis: research and cessation program in Thailand

A wealth of data show that smoking is strongly associated with periodontitis. The majority of tooth loss in adults aged 19-40 years is associated with smoking more than 15 cigarettes a day (Holm 1994). A linear dose–response relation between smoking and bone loss has also been shown. In a study of over 1,400 people, Grossi *et al* (1995) found that, compared with a nonsmoker, a light smoker (<10 cigarettes a day) was 2.0 times more likely to have alveolar bone loss. In a heavy smoker (>10 cigarettes a day), the odds ratio was 7.3.

The biologic basis for smoking as a risk factor for periodontal disease is clear. Smoking inhibits neutrophil function in saliva as well as in connective tissues (Bennet and Reade 1982). It suppresses immunoglobulin G2 antibody response and enhances the release of interleukin-1 beta (IL-1β), affecting osteoblast function (Payne *et al* 1996). In addition, it constricts the gingival blood vessels, which in part accounts for the lack of bleeding on probing found in most smokers. Periodontal therapy, both surgical and nonsurgical, is less likely to be effective in smokers and disease is more likely to recur than in non-smokers (Preber and Bergström 1986, Preber and Bergström 1990).

The percentage of smokers in the Thai population is low in comparison to those in other countries. More than 10 million people or one fifth of the Thai population are smokers. The ratio of male and female smokers is about 18:1. More than 74% of smokers have low education and low income and more than 90% of smokers start smoking before 24 years old. It appears that the smoking rate has increased in the last few years, especially in young females where the rate of smoking increased threefold over a five year period (Table 5). There have been a few studies in Thailand investigating the association between periodontal status and smoking.

A cross-sectional survey was performed to determine the effect of cigarette smoking on the severity of periodontal disease among older Thai adults (Torrungruang 2005b). The study population consisted of 1,960 subjects (age 50 to 73 years old) of which 48.7% were non-smokers, 14.4% were current smokers and 36.9% were former smokers. Current smokers had a higher percentage of sites with plaque, deeper mean probing depths and greater mean clinical attachment levels than former smokers and non-smokers. The odds of having moderate and severe periodontitis for current smokers were 1.7 and 4.8 times greater than non-smokers, respectively. Former smokers were 1.8 times more likely than non-smokers to have severe periodontitis. Quitting smoking reduced the odds of having periodontitis. For light smokers (<15 pack-year), the odds for severe periodontitis reverted to the level of non-smokers when they had quit smoking for 10 years or more. For moderate and heavy smokers (15 or more pack-years), the odds of having severe periodontitis did not differ from those of non-smokers when they had quit smoking for 20 years or more. The results indicated a strong association between cigarette smoking and the risk of periodontitis and that quitting smoking appears to be beneficial to periodontal health.

Due to the strong association of smoking with periodontal disease, it would be prudent to advise our patients that they are at a greater risk of developing periodontal disease, less likely to respond well to periodontal therapy and more likely to suffer post-surgical complications. Patients requiring dental surgery might be referred for smoking cessation counseling or treated by more conservative means.

A smoking cessation campaign initiated by the Thai government, has been launched nationwide by the Ministry of Public Health.

	Baseline	1 month	3 months	6 months
Serum fructosamine	302.2 ± 44.3	302.2 ± 40.7	NA	NA
HbA1c (%)	9.1 ±1.0	NA	8.4 ± 1.0	8.6 ± 1.2
Total cholesterol	184.9 ± 36.3	NA	184.8 ± 27.8	191.2 ± 45.4
Triglycerides	156.6 ± 102.8	NA	158.7 ± 76.1	164.9 ± 114.4
HDL	51.5 ± 8.2	NA	52.8 ± 9.5	52.2 ± 15.6
LDL	123.85 ± 33.9	NA	119.6 ± 27.3	123.0 ± 32.9

Table 5. Blood chemistry values at baseline and after periodontal treatment (mean ± SD) (Nisapakultorn *et al* 2007)

	6 mths before treatmt	3 mths before treatmt	Baseline	3 mths after treatmt	6 mths after treatmt
HbA1c (%)	9.1 ± 1.4	9.2 ± 1.5	9.1 ±1.0	8.4 ± 1.0*	8.6 ± 1.2

*Significant different from baseline (P value =0.01

Table 6. HbA1c before and after periodontal treatment (mean ± SD) (Nisapakultorn *et al* 2007)

Age group		Smoking rate (%) Year 1999	Year 2003
Young adult (15-24 Yrs)	Male	24.0	32.1
	Female	0.3	0.9
	Total	12.3	15.2
All ages		20.5	21.6

Table 7. The percentage of smokers in Thai population in the year 1999 and 2003
Data from: http://www.m-soiety.go.th/mso/doc/no_43/c4.pdf

This campaign encourages all dentists to counsel their patients to quit smoking. The dentists' role in helping periodontal patients stop smoking is justified but has not been investigated in Thailand. A study to determine the effectiveness of a brief smoking cessation program integrated into periodontal treatment visits and to assess the potential periodontal effects of smoking cessation was carried out (Kudngaongarm 2002). Seventy-two smokers with periodontitis were recruited in this study (35 test and 37 control subjects). All patients received routine periodontal treatment. In addition, the test group received a smoking cessation program during the periodontal treatment visit. The results showed that a brief smoking cessation program integrated into periodontal treatment can have a marked effect in reducing tobacco consumption. The potential periodontal benefit is also demonstrated in the patients who quit or reduced smoking.

Conclusion

Periodontal medicine has recently received remarkable attention from Thai dental researchers. Several epidemiological studies have been carried out to confirm the association between periodontal diseases and diabetes or smoking. However, this association needs to be further confirmed by longitudinal studies. The study of the effect of periodontal treatment on systemic conditions should also be investigated. In Thailand as well as in other Asian Pacific countries, there have been a limited number of studies investigating the relationship between periodontal diseases and other health conditions such as cardiovascular problems, pulmonary disease, osteoporosis and pre-term birth. Research collaborations should be developed among the Asian Pacific countries in order to better understand the association between periodontal and systemic diseases and allow for the improvement of oral and systemic health of the population.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 11

Relationship of Periodontal Disease to Pre-term Low Birth Weight Infants in a Selected Population in Malaysia: A Pilot Study

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Introduction

According to the World Health Organization, pre-term (PT) birth is defined as labour that begins before the gestational week of 37, and low birth weight (LBW) is delivery of an infant with a birth weight under 2.5kg. Approximately 11% of all pregnancies lead to pre-term low birth weight (PTLBW) (Goldenberg and Rouse 1998) and despite considerable progress in medical care, this rate is increasing in western countries (Ventura *et al* 1999). Each year, more than US$5 billion is spent in the United States for neonatal care, with the majority used for care of PTLBW infants. LBW has a higher incidence rate in Asia (15%) than in other areas of the world (Williams *et al* 2000). According to Health Facts 2007 (Ministry of Health of Malaysia Preliminary Report) 8.6% (32,991) infants were born with LBW in Malaysia in 2006.

PTLBW is a significant cause for mortality and morbidity among infants (McCormick and Wise 1993) and is the major cause of neonatal mortality (McCormick 1985). Although introduction of neonatal intensive care methods during the 1960s and the subsequent development of surfactant therapy in the 1980s resulted in improvements in the survival rates of PTLBW neonates, PTLBW infants are still 40 times more likely to die during the neonatal period (Shapiro *et al* 1980). More than 60% of the mortality that occurs among infants without anatomic or chromosomal congenital defects is attributable to PTLBW (McCormick 1985).

PTLBW infants who survive the neonatal period are at a significant risk of developing serious and lasting health problems, e.g. neurodevelopmental disturbances, health problems (such as asthma, upper and lower respiratory tract infections and ear infections) and congenital anomalies (McGaw 2002). PT delivery is the cause of one-half of all serious long term neurological morbidity (McCormick 1985). The spectrum of neurological defects range from subtle degrees of neuromotor abnormality to cerebral palsy. Rates of cerebral palsy in the subset of infants with very LBW of less than 1.5 kg are close to 20% (Hack *et al* 1983).

Various risk factors have been associated with the delivery of PTLBW infants. Known maternal risk factors include high (>34 years) or low (<17 years) maternal age, height, weight, ethnicity, socio-economic status, alcohol, tobacco and drug abuse, nutritional status and stress level. Low maternal bodyweight, birth interval, previous PTLBW, inadequate pre- and ante-natal care, maternal hypertension, diabetes mellitus, urinary and genital tract infections, cervical incompetence

and multiple pregnancies may also be important (Davenport 2002, Jeffcoat *et al* 2003, Offenbacher *et al* 1996). However, a significant proportion of LBW deliveries are of unknown etiology. Approximately 25% of PTLBW deliveries occur without any of the known risk factors (Romero 1988a) and 58% of the variance of the incidence of PTLBW remains unexplained (Holbrook 1989). Furthermore, despite efforts in understanding and managing controllable risk factors, the incidence of PTLBW deliveries remains high. It may be due to limited understanding of risk factors that contribute to PTLBW delivery (Gibbs *et al* 1992). Thus many groups, including the United States National Academy of Sciences, have suggested continued research into the causes and management of PTLBW (National Center for Health Statistics of United States 1992).

Periodontal disease is the second most frequent oral disease in the world. It consists of a chronic inflammatory process in periodontal tissues, caused mainly by infection with gram-negative bacteria in the accumulated dental plaque on the tooth surface (Mumghamba *et al* 1995). Inflamed periodontal tissues produce significant amounts of inflammatory chemical mediators especially IL-1, IL-6, PGE_2 and TNF-α. Persistent bacterial aggression in periodontal tissues, together with the inflammatory response by the host, may have important consequences in systemic damage, and in pregnant women, gestational complications and fetal effects (Offenbacher *et al* 1996).

Infection is one of the major causes of PTLBW deliveries, responsible for 30% to 50% of all PTLBW cases (McGaw 2002). Several studies have demonstrated an association between infection and PTLBW, e.g. genitourinary tract infections (Offenbacher 1996). Several scientists investigating the molecular basis of PTLBW point to common cellular and biochemical pathways that seem to mediate the pathogenesis of PTLBW, e.g. cytokines such as IL-1, IL-6, PGE_2 and TNF-α (Hillier 1988, Gibbs *et al* 1992, Romero 1993). During normal pregnancy, the intra-amniotic levels of these mediators rise steadily until a critical threshold level is reached to induce labor, cervical dilation and delivery. Thus, infections that contribute to abnormal production of these mediators (e.g. genitourinary tract infections) may result in PTLBW deliveries. However, even in the absence of any clinical or subclinical genitourinary tract infections levels of PGE_2 and TNF-α are elevated and this suggests probable sources of infections are of unknown origin (Romero *et al* 1988b, Romero *et al* 1989).

The theory that remote sites of infection might contribute to PTLBW is supported by a number of studies using the pregnant hamster model (Collins *et al* 1994a, Collins *et al* 1994b, Collins *et al* 1995). Pregnancy outcomes were evaluated in these animals after either the establishment of experimental periodontitis (Collins *et al* 1995), the establishment of a non-disseminating subcutaneous tissue infection with *P. gingivalis*, a common Gram-negative periodontal pathogen (Collins *et al* 1994a) or intravenous injection of LPS from *P. gingivalis* (Collins *et al* 1994b). Fetal weights were significantly lower in the experimental animals and the severity of the fetal effects was directly related to the levels of PGE_2 and TNF-α. Periodontal disease has been suggested to cause PTLBW. A statistically significant correlation between PD and PTLBW delivery was first demonstrated by Offenbacher *et al* in 1996. Mothers with periodontal infection had more than 7 times the risk of delivering a PTLBW infant and 18.2% of the PTLBW deliveries occurring might be attributable to PD (Offenbacher *et al* 1996). It has been supported by several other studies, such as Madianos *et al* (2002) and

Jeffcoat *et al* (2001). A co-relational significance in pre-term birth and periodontitis at 21-24 weeks gestation existed (Jeffcoat *et al* 2001). Women with progressive PD were nearly 5 times more likely to give birth before 37 weeks (Madianos *et al* 2002) than healthy women.

In another study by Offenbacher *et al* (1998), GCF levels of PGE_2 were significantly higher in mothers of PTLBW infants. It was suggested that a dose-response relationship for increased PGE_2 as a marker of current PD activity and decreasing birth weight existed. Four periodontal pathogens (*B. forsythus, P. gingivalis, A. actinomycetemcomitans, T. denticola*), characteristically associated with mature plaque and progressing periodontitis, were detected at significantly higher levels in the mothers of PTLBW infants.

The American Academy of Periodontology recommends that all women who are pregnant or planning pregnancy undergo periodontal examinations so that appropriate preventive or therapeutic services, if indicated, be provided. Jeffcoat *et al* (2001) suggested that care providers consider treating pregnant women who have been diagnosed with periodontal disease with scaling and root planning to reduce their risk of pre-term birth. Providing treatment to pregnant women with periodontal disease may the reduce risk of pre-term birth (Mitchell-Lewis *et al* 2001, Lopez *et al* 2002).

However, a number of recent studies found no association between periodontitis and pregnancy outcomes. Davenport *et al* (1998) did not find periodontitis was more severe among mothers with PTLBW deliveries in a predominantly Bangladeshi population living in East London. Noack *et al* (2005) found no association between periodontitis and pregnancy outcome. Davenport *et al* (2002) reported an adverse association between maternal mean pocket depth and PTLBW. Animal studies to prove a plausible biological hypothesis for a causal link between maternal periodontal infection and PTLBW had no concordant results (Collins *et al* 1994a, Collins *et al* 1994b, Galvao *et al* 2003).

Hence the association between PD and PTLBW varies among the different studied populations. Some studies strongly support this hypothesis, while others refute this association. The association between PD and PTLBW probably exists in the presence of other environment or genetic risk factors (Teng *et al* 2002).

The rationale for the present study was the serious consequences of PTLBW and the lack of any related evidence in female populations of Malaysia. The results may spearhead further investigations for public health promotion and education as well as oral health delivery requirements, ultimately reducing general health budget costs in managing PTLBW babies in Malaysia. The objective of this investigation was to assess the effect of periodontal status on the delivery of preterm low birth weight infants in a selected population in Malaysia.

Materials and methods

Study design and population

Two ante-natal clinics in Selangor (Kuala Lumpur General Hospital and National University of Malaysia Hospital) and three ante-natal clinics in Perak (Gunung Rapat Health Clinic, Jelapang Health Clinic and Waller Courp Health Clinic) provided a large accessible community of pregnant women comprising of a multi-ethnic population, namely Malays, Chinese and Indians.

A prospective cohort study design was chosen as the most appropriate as it can demonstrate a temporal relationship between PD (exposure) and PTLBW infant (outcome). It also allowed direct measurement in an exposed and non-exposed population. The

study group was defined as mothers with PD while the control group was defined as mothers without PD.

Approval was granted for the study from the Ethics Committee (Faculty of Dentistry, University Malaya) prior to the study. Approval to conduct the study was also obtained from the Department of Obstetrics and Gynecology in all the hospitals and health centres involved.

Study population

The participation from pregnant women who attended routine ante-natal care at the prenatal care clinics was requested. Only those between 28-36 gestational weeks were included in this study. Exclusion criteria included systemic conditions such as severe anaemia, diabetes, cardiovascular disorders, hepatic deficiency, high blood pressure, venereal diseases, urinary tract infections, bacterial vaginitis and viral infections. This study was based on convenience and volunteer sampling methods. Potential participants were identified through folder checking of all those available on the selected days for periodontal examination at the prenatal care clinic. A total of 127 mothers met the criteria, 76 of which agreed to volunteer for the study. However, only 73 participants' complete data could be collected. Informed consent was obtained from the subjects.

Maternal data including the results of blood, urine and MGTT tests were obtained from the patient's folder and recorded at this stage.

Questionnaire administration

Before the periodontal examination, an interviewer-administered questionnaire was completed for each participant. The questionnaire was designed to gather personal data, demographic details (e.g. age, gender, race, socio-economic status) and habits including oral hygiene habits, tobacco use, parafunctional habits and dental visit regularity. The general maternal health during and prior to pregnancy, and history of previous PTLBW babies were recorded as well.

Clinical periodontal examination

Clinical periodontal examinations included plaque index, gingival index, papillary bleeding index, probing pocket depth (PPD), probing attachment loss (PAL) and total number of missing teeth. Probing pocket depths and attachment levels were measured with a William's periodontal probe for selected teeth at 4 sites, disto-buccal, mesio-buccal, mid-buccal and mid-palatal. Partial mouth recording (16 teeth) was conducted on central incisors, canines, first premolars and first molars (or alternative teeth) in each quadrant.

The examinations were conducted using a portable dental chair under a portable dental light source at all centres, except at the General Hospital Kuala Lumpur where the examination was conducted using a fixed dental chair and light sources available in a nearby dental clinic. An assistant recorded the dental and periodontal findings on a standard examination form.

All the periodontal examinations were conducted by the first and second authors who are experienced periodontists. A standardized procedure was utilized.

Definition of pregnancy outcomes

PT birth and LBW were the primary pregnancy outcome measures considered. According to the World Health Organisation, PT birth was defined as labour that begins before the gestational week of 37, and LBW as delivery of an infant with a birth weight under 2.5 kg. Estimation of gestational age was based on antenatal records.

To aid the analysis of the data and result discussion, participants were grouped according to primary pregnancy outcomes into:

- PT birth group if they delivered before 37 weeks of gestation;
- LBW group if they delivered a baby with a birth weight under 2.5 kg; and
- PTLBW group if they delivered a baby with a birth weight under 2.5kg before 37 weeks of gestation.

Pregnancy outcome data collection

Collection of pregnancy outcome information was obtained using patients' postnatal records. The data were recorded on standardized data collection forms. In addition to gestational age at delivery and birth weight of the delivered baby, data concerning delivery information and pregnancy complications such as type of delivery, onset of delivery, medication administered, total blood loss during delivery, and other complications such as genitourinary tract infections, were also collected.

Classification of periodontitis

A subject was categorized with PD if she had ≥2 teeth with ≥5 mm probing pocket depth and loss of attachment ≥3 mm. Subjects without these criteria were regarded as periodontally healthy.

Statistical analysis

The results were analyzed using SPSS Edition 12 and the statistical packages used were Chi Square test, Independent Sample T-test and Logistic Regression analysis.

Results

Ethnicity and age profile of study population

Tables 1 and 2 list the ethnicity and age distribution of the study subjects. The final study sample comprised 73 pregnant mothers within the age range of 22-48 years and average age of 29.1 years. Mother's age is a well known risk factor for PTLBW (Williams 2000). By including subjects who are not too young (younger than 18 years old) nor too old (older than 36 years old) it is easier assess the true relationship between PD and PTLBW without interference from this risk factor.

According to Shiono (1986), ethnicity can affect the incidence of PT delivery which may contribute to higher incidence of LBW infants. The final study sample comprised of 42 Malays (57.5%), 19 Chinese (26%) and 12 Indians (16.2%). The ethnic composition for Malays and Chinese in this study is similar to the ethnic composition in the Malaysian population, with the exception for the Indian group, which was higher in the study. However, there are no relevant studies on ethnic differences between the Malay, Chinese and Indian ethnic groups in relation to PTLBW prevalence.

Distribution of disease and healthy subjects

37 (50.7%) pregnant women were diagnosed with PD (minimum 2 teeth with ≥5 mm PPD and ≥3 mm PAL) and 36 (49.3%) without PD. This finding supports previous studies reporting 50% of pregnant women suffer from PD. According to Heaman *et al* (2001) and Goepfert (2004), PD affects as many as 50% of pregnant women in the United States. The present study group had a similar prevalence of PD among the pregnant women and it can be assumed that this study population would be fairly representative of the general population in Malaysia as well

Ethnicity	Frequency	Percentage
Malays	42	57.5
Chinese	19	26.0
Indians	12	16.4
Total	73	100.0

Table 1. Ethnicity of study population

Age	Frequency	Percentage
20-29 years	39	53.4
30-39 years	30	41.1
≥40 years	4	5.5
Total	73	100.0

Table 2. Age of study population

Group	Number of Subjects	Percentage
Periodontally Diseased	37	50.7
Healthy	36	49.3
Total	73	100.0

Table 3. Distribution of diseased and healthy subjects in the study population

Risk Factor	PD	Healthy	p-value	OR	95% CI
Irregular Dental Visits	35 (94.6%)	25 (69.4%)	0.005*	7.70	1.57, 37.82

* significant

Table 4. Effect of irregular dental visits to PD occurrence in the study population using Chi-square test

PD Clinical Parameter	Periodontal Disease Group Mean	SD	Healthy Group Mean	SD	p value
Probing pocket depth	2.54	0.67	1.65	0.45	<0.001**
Probing attachment loss	2.31	1.11	0.23	0.30	<0.001**
Papillary Bleeding Index	0.80	0.54	1.61	0.63	<0.001**
Plaque Index	0.68	0.38	0.43	0.21	0.004*
Gingival Index	0.56	0.55	0.86	0.38	0.027*

* Significant **Highly Significant

Table 5. Comparison of PD Parameters in the disease and healthy groups of the study using Independent Sample T-test

Gestational week	Birth weight >2.5kg	≤2.5kg	Total
>37 weeks	57	2	59
≤37 weeks	11	3	14
Total	68	5	73

Table 6. Gestational week and birth weight cross tabulation

	PD n(%)	Healthy n(%)	p-value	RR	95% CI for RR
PT delivery	4 (10.8%)	10 (27.8%)	0.066	0.39	[0.13, 1.13]
LBW infant	3 (8.1%)	2 (5.6%)	0.66	1.46	[0.26, 8.23]

Table 7. Association between PD status and PT or LBW deliveries using Chi-square test

others globally.

According to Health Facts 2007, 8.6% infants were born with LBW in the year 2006. Only 6.8% infants were born with LBW in our study sample. This may be due to the removal of potentially interfering co-variables. In this study, pregnant mothers with systemic diseases were excluded. Furthermore, the number of subjects smoking or having extreme maternal ages was also minimal.

Association between PD and its risk factors

There are many risk factors associated with PD, such as oral hygiene habits, smoking habits and regularity of dental visits. In this study, irregular dental visits show significant association with PD occurrence compared to other risk factors ($pd \leq 0.005$).

Association between PD parameters and PD prevalence

All the clinical periodontal parameters in this study show a highly significant relation with PD group as compared to the Healthy group. Hence, these parameters can be excellent PD indicators and the results indicate the disease sample was representative.

Association between PD status and PT or LBW deliveries

Among the study subjects, 14 women had PT delivery and 5 had LBW infants. Only 3 women gave birth to PTLBW infants (Table 6). Of those with PD, 4 (10.8%) had PT delivery and 3 (8.1%) had LBW infants (Table 7). PD status was not significantly associated with PT delivery or LBW infant ($p>0.05$).

Association between socio-demographic factors and PT or low birth weight deliveries

Table 8 demonstrates the effect of socio-demographic factors on the delivery of PTLBW infants. None of the socio-demographic factors including ethnicity and age had an impact on the pregnancy outcome. However, looking at the odds ratio there is some evidence that previous tobacco use may increase the risk of PTLBW delivery, although the result is not conclusive due to the small sample size. This finding agrees with previous studies (Shiono *et al* 1986, Kierse 1989) which have shown that cigarette smoking has been related to PT birth and LBW.

Clinical periodontal parameters as PTLBW predictors

Table 9 shows that none of the PD parameter means associated significantly with either of the two groups ($p>0.05$) using the Independent Samples T-test. The data were analyzed further using Logistic Regression Analysis (Table 10). None of the PD parameter means associated significantly with PT or LBW status ($p>0.05$). Further Stepwise Logistic Regression analysis was used to further test these relationships (demographic and clinical) for PT or LBW status (Table 11). Among all the clinical parameters, only Plaque Index mean was shown to be a significant predictor ($p<0.03$).

Discussion

Comparison with other studies

This study addressed the question of whether pregnant women with PT contractions (risk for PT birth) or mothers with PTLBW deliveries have a periodontal status worse than women of the same age with risk-

Variable	PLBW	Normal	OR	95% CI
Presence of previous live births	6	23	0.89	[0.28, 2.78]
Previous tobacco use	2	3	2.57	[0.39, 16.91]
History of urinary tract infection	0	5	0	[0, 0]
Previous LBW infant	2	4	1.89	[0.31, 11.41]
Previous PT delivery	1	4	0.88	[0.09, 8.51]

Table 8. Association between socio-demographic factors and PT or low birth weight deliveries using Chi-square Test

PD Variables	Group	Mean	Std. Error	95% Confidence Interval		p-value
PI	PT / LBW	.58	.04	.49	.67	0.17
	Normal (T&W)	.43	.08	.26	.60	
GI	PT / LBW	.71	.07	.58	.84	0.85
	Normal (T&W)	.74	.13	.49	.99	
PPD	PT / LBW	2.14	.10	1.94	2.33	0.20
	Normal (T&W)	1.87	.18	1.50	2.24	
PAL	PT / LBW	1.36	.18	1.01	1.71	0.10
	Normal (T&W)	.73	.33	.061	1.39	
PBI	PT / LBW	1.19	.10	.99	1.38	0.49
	Normal (T&W)	1.33	.19	.97	1.70	

Table 9. Comparison of PD variables in the PT/LBW and normal delivery subjects using Independent Samples T-test

PD variables	Model Fitting Criteria -2 Log Likelihood of Reduced Model	Likelihood Ratio Tests Chi-Square	df	p value
Intercept	53.15	0.41	1	0.52
PI mean	56.20	3.46	1	0.06
GI mean	53.72	0.99	1	0.32
PPD mean	54.10	1.37	1	0.24
PAL mean	53.13	0.40	1	0.53
PBI mean	53.13	0.40	1	0.53

Table 10. Clinical periodontal parameters as PLBW predictors using Logistic Regression Analysis

	Model Fitting Criteria -2 Log Likelihood of Reduced Model	Likelihood Ratio Tests Chi-Square	df	p value
Intercept	56.53	0.00	1	0.99
PI mean	61.21	4.68	1	0.03*

Table11. Clinical periodontal parameter as PLBW predictor using Stepwise Logistic Regression

free pregnancies who give birth to normal weight healthy infants. PT and LBW infants present important health problems worldwide, as these factors are major causes of neonatal morbidity and mortality. LBW can result from PT birth or intrauterine growth restriction, or both. It is difficult to separate the PT component of LBW. It is estimated that approximately 50% of PT infants weigh less than 2.5 kg, whereas only 2% of full-term infants weigh below that threshold (Nault 1997).

The results of this study are in contrast to the association between PD and a higher risk for PTLBW reported in other studies (Offenbacher *et al* 1996, Offenbacher *et al* 1998, Jeffcoat *et al* 2001, Madianos *et al* 2002, Lopez *et al* 2002). To examine the association between PD and PTLBW, regression models were used. Adjustment for potential confounding variables (maternal age, race, smoking, drug use, bacterial vaginosis, socio-economic status) in these models is of high importance to avoid study bias (Madianos *et al* 2002). Thus the association between periodontitis and PTLBW supported in some studies may be due to inadequate adjustment or unknown confounding factors. Most studies did not control for potential confounding factors.

The definition of PT birth used in our study includes births that followed spontaneous labour or spontaneous rupture of membranes, because there is considerable evidence that the

risk factors for both are similar and the distinction is artificial (Guinn *et al* 1995). Since the determinants of PT birth and intra-uterine growth restriction appear to differ (Kramer 1987), we analyzed the data evaluating the risk factors for LBW and PT delivery together. The only risk factor in the present study that showed significant association with PT and LBW was mean Plaque Index.

PD has been identified as a potential risk factor for PTLBW (Offenbacher *et al* 1996), and it might be one of the factors associated with some of the approximately 50% of PT births that occur in women without established risk factors (Kramer 1987).

The mechanisms by which PD may cause PTLBW or PT birth have still not been determined, but there is evidence that this association has a biologically feasible basis. It has been suggested (Offenbacher *et al* 1996) that the effect of PD on PTLBW could result from stimulation of fetal membranes on prostaglandin synthesis by cytokines produced by inflamed gingival tissues, or through the effect of endotoxins derived from periodontal infection. Endotoxins can stimulate prostaglandin production by amnion macrophages (Romero *et al* 1988a) and decidua *in vitro* (Romero *et al* 1989). In animal models, it has been shown that endotoxins produce fetal growth retardation (Beckman *et al* 1993, Offenbacher *et al* 1998). On the other hand, peripheral monocytes obtained from some patients with PD showed enhanced release of inflammatory mediators such as PGE_2, IL-lβ, and TNF-α, when challenged with bacterial endotoxins (Shapira *et al* 1994, Salvi *et al* 1997). Endotoxins derived from periodontal pathogens in women with PD might induce PT labour through primed monocyte-macrophage activation in peripheral blood and deciduas.

Some obvious differences between the present study as compared to others are the differences between our study populations, study designs and sample size. Their populations included very high percentages of Afro-Americans, up to 58-82% (Offenbacher *et al* 1996, Jeffcoat *et al* 2001, Mitchell-Lewis *et al* 2001). In addition, the subjects in these studies were of low socio-economic status. There are marked racial differences in the prevalence of both PTLBW infants (Hogue *et al* 1995, Kleinman *et al* 1987) and in the prevalence of severe forms of periodontitis (Brown *et al* 1996, Loe and Brown 1991, Melvin *et al* 1991). In our study, the pregnant women who participated were both from the Malay, Chinese, or Indian ethnic groups and mainly of middle socio-economic status (67.3%). The ethnic distribution of the Malay and Chinese groups reflected the general Malaysian population although the percentage of Indian subjects was slightly higher. Study design, whether prospective or retrospective, may influence the results of these studies (Offenbacher *et al* 1996). Noack *et al* (2005) reported that difference in sample size of the study may also influence the results of these studies.

The findings of the present study are consistent with a number of recent studies, e.g. Holbrook *et al* (2001), Klinger *et al* (2001), Sundell *et al* (2002), Davenport *et al* (2002), Moore *et al* (2004). They failed to find an association between PD status and PTLBW deliveries. Indeed, the British case-control study showed a general tendency for decreasing odds ratio of PTLBW with increasing mean probing pocket depth (Sundell *et al* 2002). Teng *et al* (2002) summarized the explanations for the differences between the results of the British study and the previous reports; there may in fact be no association; the differences may reflect differences in the study populations; and PD may be associated with PTLBW but only in the presence of other specific environmental or genetic risk factors. His

comments are probably also relevant to our findings.

The results of our study, where most of the data collection was conducted before the deliveries, show that PD was evaluated as an independent risk factor for PTLBW. However some of these adverse pregnancy outcomes are frequently associated with potentially correctable lifestyles like tobacco abuse, or with infectious diseases, that, like PD, can be eliminated prior to or during pregnancy prior to delivery.

Limitations of the study

Due to logistics restriction and some patients declining participation, the final study sample comprised of only 73 pregnant mothers. A larger sample size would have allowed us to subject the data to more statistical analyses. A larger sample size would also contribute to greater power of the study, which directly influences the representation of the study sample to the study population.

Being a pilot study, the present investigation was conducted in Selangor and Perak only. The assumption was made that the study population in Selangor and Perak represents the total ante-natal population in Malaysia. The sample size is not sufficient to further discuss the relationship of PD in ethnic groups to PTLBW babies in the Malays, Chinese and Indians individually.

Clinical Implications

Although the present study shows no significant association between PD and PTLBW deliveries in Malaysia, prophylactic scaling and polishing of teeth of pregnant women is recommended in order to prevent adverse pregnancy outcomes as indicated by the positive relationship in some studies as well as the fact that pregnant women are prone to develop PD. The present study has revealed that PD affected as many as 50.7% of pregnant women and the possible need for the health delivery system to address the needs of the ante-natal mothers.

Conclusions

In this study population, PD was not shown to be a risk factor for PT delivery or LBW infants. Only the mean Plaque Index showed some association with the outcome of PT deliveries and LBW infants. In spite of the results of this study, it is highly recommended that all antenatal mothers have access to periodontal treatment.

Recommendations for future studies

1. A study be conducted on a larger sample size from various Obstetrics and Gynaecology Clinics in various states.
2. Studies on the direct measurement of specific periodontal pathogens in the foetal environment and the measurement of the resulting inflammatory mediator levels be conducted that would be helpful in better understanding the relevant pathogenesis.
3. Undertake the investigation of microbiological and biochemical factors affecting PTLBW deliveries and other pregnancy outcomes.
4. The measurement of gingival crevicular fluid constituents to be used as a clinical parameter or indicator.
5. Further studies are needed to support oral care delivery to ante-natal mothers in order to reduce PD and ultimately adverse pregnancy outcomes. This relates to public health concerns and could ultimately reduce health budget costs in managing PTLBW babies in this country.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 12

Periodontal Infections, Systemic Inflammation and Cardiovascular Disease: Current Evidence and Perspectives

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Introduction

Periodontal diseases are among the most commonly occurring yet unusual infections in humans, due to the anatomically unique dento-gingival structure and the nature of pathogenic plaque biofilm infection. They are highly prevalent and can affect over 90% of the worldwide population. Periodontitis is characterised by bacteria-induced inflammatory destruction of tooth-supporting structures and alveolar bone (Jin 2006). The impact of periodontal disease on an affected individual is increasingly apparent and becomes more significant with the progression of the disease; from gingival recession with dentine hypersensitivity at relatively early stage, towards tooth mobility, pathological migration, and eventually tooth loss, thereby affecting chewing and speech functions, esthetics, psychological aspects, and quality of life. Currently, periodontitis remains the most common cause of tooth loss in adults (Krebs and Clem 2006).

Over the last 100 years, four key discoveries in the understanding of periodontal disease in humans have been made. These are:

1. Appreciation of the essential role of pathological plaque biofilm in the initiation and progression of periodontal diseases
2. Recognition of the natural history of periodontal diseases and the establishment of periodontal risk concept
3. Recognition of the crucial role of susceptible host in the pathogenesis of periodontitis
4. The emergence of periodontal medicine.

Periodontal diseases have been regarded as the source of focal infection for over 100 years (Miller 1891). Since 1989, emerging scientific evidence shows an association of periodontal diseases with cardiovascular disease (CVD) after adjustment of common confounders, although a causal interrelation remains to be clarified (Pihlstrom *et al* 2005, Demmer and Desvarieux 2006). This article updates the current evidence on the association of periodontal infections with systemic inflammation in the context of their links with CVD and will:

1. Describe the nature and uniqueness of periodontal infections - so called the 'silent' infections
2. Present the current evidence of their association with CVD
3. Elaborate on the potential mechanisms by which periodontal infections may affect the atherogenesis and CVD
4. Update on the contribution of periodontal infections to systemic inflammation and the potential effect of periodontal treatment

on reduction of the risk of CVD. The relevant implications and perspectives will be elaborated.

The uniqueness of periodontal infections

It is well known that bacterial biofilm is a common cause for a number of human diseases, including the two most common oral diseases; caries and periodontal disease (Costerton *et al* 1999). Periodontal diseases are initiated and perpetuated by a group of predominantly gram-negative and anaerobic bacteria found in the dental plaque biofilm (Darveau *et al* 1997, Jin 1999). Periodontal diseases are unusual human infections due to the unique anatomic dento-gingival structure and the nature of pathogenic plaque biofilms. The non-shedding tooth surface is a stable interface for microbial colonisation that allows micro-organisms to continually maintain an immediate proximity to the periodontal tissues. The attached micro-organisms are immersed in an aqueous environment where the bacterial infection is less able to be controlled by the potent mechanisms of host defense and antimicrobial therapy (Socransky and Haffajee 1997). It is therefore conceivable that the pathogenic plaque biofilm is extraordinarily persistent and difficult to control without appropriate professional care. In moderate to severe periodontitis, the highly inflamed pocket epithelia are easily broached, allowing large doses of bacterial toxins and other inflammatory products access to the underlying connective tissues and blood vessels. It is apparent that uncontrolled periodontal infections may therefore serve as a reservoir for spillover of bacteria, bacterial products and inflammatory cytokines/ mediators (Page *et al* 1997, Jin *et al* 2003) into the circulation with resultant various levels of bacteremias, endotoxemias and cytokinemias, subsequently affecting the vascular endothelium and other cells and tissues distant from periodontal tissues (Thoden *et al* 1984, Page 1998).

The crucial role of infection and inflammation in atherogenesis and CVD

CVD is a leading cause of premature death in humans. Current evidence shows that long-lasting infections and chronic low-grade systemic inflammation play a crucial role in the onset and progression of atherosclerosis and CVD (Mattila *et al* 1995, Maseri *et al* 1996, Danesh *et al* 1997, Ross 1999, Libby *et al* 2002). The initiation, growth and complications of atherosclerotic plaque are considered to be an inflammatory response to injury. Virtually every step in atherogenesis is believed to involve cytokines, other bioactive molecules, and cells that are characteristic of inflammation, which consequently leads to the development of acute coronary and cerebrovascular syndromes (Pearson *et al* 2003). Intensive studies have been undertaken for the identification and development of various diagnostic measurements and biomarkers for assessment and monitoring of the levels of systemic inflammation and endothelial dysfunction, such as C-reactive protein (CRP), proinflammatory cytokines (e.g. IL-1, IL-6 and TNF-α), oxidized low-density lipoproteins, tissue plasminogen activator (t-PA), adhesion molecules (e.g. intercellular adhesion molecule-1, selectins), other acute-phase reactants (e.g. erythrocyte sedimentation rate), Willebrand factor concentration, as well as indicators of cellular responses to inflammation (e.g. elevated white blood cell count) (Pearson *et al* 2003, Danesh *et al* 2004, Joshipura *et al* 2004). The main sources of the inflammation cascade include atherogenesis in coronary artery and other arteries, connective tissue diseases and, more notably, chronic, low-grade local infections

and inflammations, such as gingivitis and periodontitis, prostatitis, bronchitis, urinary tract infections, and gastric inflammation (Pearson *et al* 2003).

Association of periodontal diseases with CVD

The association of periodontal diseases with CVD has been documented in a series of studies (Jin *et al* 2003, Beck and Offenbacher 2005, Pihlstrom *et al* 2005, Demmer and Desvarieux 2006). A recent 16-year follow-up study has shown that experience of periodontitis with missing molars in young adults might be associated with certain life-threatening diseases (e.g. circulatory disease) which contribute to premature death (Söder *et al* 2007). Currently, most longitudinal studies have shown an association between periodontal diseases and CVD after adjusting for confounding factors, such as age, sex, diabetes, cholesterol levels, blood pressure, obesity, smoking status, dietary patterns, ethnicity, education background and socioeconomic status, although a causal association remains to be clarified (Demmer and Desvarieux 2006).

Recent novel research has refined the study protocols to focus more directly on the interactions of infectious and inflammatory periodontal disease processes with appropriate outcome measures of atherogenesis and subclinical CVD. It has been shown that periodontal disease might be a significant independent risk factor for development of peripheral vascular disease (Mendez *et al* 1998), increased intima media wall thickness (Beck *et al* 2001) and the development of early atherosclerotic carotid lesions (Söder *et al* 2005). It is proposed that the inflamed and ulcerated pocket epithelium may form an easy port of systemic access to circulation system for oral microorganisms, bacterial products and pro-inflammatory mediators in inflamed periodontal tissues through blood dissemination (Gemmell *et al* 1997, Loos *et al* 2000, Geerts *et al* 2002), contributing significantly to chronic, systemic vascular challenge, and directly resulting in platelet aggregation, adhesion and vasculitis, and the subsequent cholesterol deposition, thromboembolic events and atheroma formation and development of CVD (Page 1998). It is also evident that increased systemic antibodies to periodontopathogens were significantly associated with atherosclerosis, CHD and risk of CVD progression (Pussinen *et al* 2004, Pussinen *et al* 2005, Beck *et al* 2005). Carotid intima-medial wall thickness, a measure of subclinical atherosclerosis, increased with levels of periodontopathogens (Desvarieux *et al* 2005). Periodontal pathogens have also been identified in atheromatous plaques (Chiu 1999, Haraszthy *et al* 2000), and they could actively invade human endothelial cells (Li *et al* 2000) as well as vascular endothelium (Haraszthy *et al* 2000, Iwai *et al* 2005), which could trigger an inflammatory response that may translate into endothelial dysfunction (Lalla *et al* 2003), and induce the formation of foam cells (Curtis *et al* 1993, Kuramitsu *et al* 2001). It has recently been shown that *P. gingivalis* could play a crucial role in the initiation and exacerbation of atherosclerosis (Li *et al* 2002, Lalla *et al* 2003, Giacona *et al* 2004). These data further support the notion of the possible role of periodontal infection in the pathogenesis of CVD (Desvarieux *et al* 2003, Spahr *et al* 2006). Further active studies are highly warranted to investigate concerns of the complexity of interactions among common risk factors, exposures used for assessment of periodontal disease and infections, and the outcome measures of CVD (Beck *et al* 2005). It is therefore essential to target the direct assessment of periodontal infection exposure and the resultant systemic inflammation in relation to appropriate measurement of

subclinical status of CVD and related biomarkers.

Periodontal infections, systemic inflammation and CVD

It is considered that all stages of atherosclerotic plaque may be an inflammatory response to injury (Pearson *et al* 2003, Jin and Wang 2007). CRP has proved to be the strongest and most significant predictor of the risk of future cardiovascular events among several plasma variables (Ridker *et al* 2000, 2004, Pearson *et al* 2003). Therefore, changes of CRP levels in peripheral blood in periodontitis patients may reflect the underlying mechanisms linking periodontitis with atherogenesis and CVD (Loos 2005). Recent studies showed that periodontal infections may significantly contribute to systemic inflammation with the observation on the association of periodontitis with elevated number of peripheral blood leukocytes (Loos 2005), lower number of erythrocytes and lower levels of haemoglobin (Hutter *et al* 2001), and elevated biomarkers of systemic inflammation and endothelial dysfunction such as CRP, IL-6, low-density lipoprotein cholesterol (LDL-C) and t-PA, which may partly explain the documented association of periodontal disease with CVD (Loos 2005, Ioannidou *et al* 2006, Jin and Wang 2007). It has been shown that both CRP (Buhlin *et al* 2003, Craig *et al* 2003, D'Aiuto *et al* 2004, D'Aiuto *et al* 2005a, D'Aiuto *et al* b) and IL-6 (Loos *et al* 2000, Mengel *et al* 2002) were related to the severity and extent of periodontitis. In recent years, CRP and IL-6 have been studied intensively as possible markers of systemic inflammation involved in periodontal infections (Mengel *et al* 2002, Loos 2005, Ioannidou *et al* 2006), as the plasma or serum levels of these markers were consistently higher in periodontitis patients than in healthy controls.

Recent studies showed that control of periodontal infection through non-surgical treatment could reduce serum levels of CRP and IL-6 (Mattila *et al* 2002, Ide *et al* 2003, D'Aiuto *et al* 2005a, D'Aiuto *et al* 2005b, Offenbacher and Beck 2005). Our recent study in Chinese adults with untreated periodontitis also showed that periodontal treatment decreased both the serum levels of CRP and count of inflammatory cells (Tse *et al* 2007). Periodontal treatment could also improve the endothelial function in patients with severe periodontitis (Seinost *et al* 2005, Tonetti *et al* 2007). There is increasing evidence from clinical trials that the improvement of endothelial function may result in lower rates of cardiovascular events (Bonetti *et al* 2003). In addition, it is worthy to note that intensive periodontal therapy could reduce the levels of total and LDL cholesterols (D'Aiuto *et al* 2005a, D'Aiuto *et al* 2005b). Hence, periodontal disease may represent a modifiable risk factor for CVD (Tse *et al* 2007).

Clinical implications and perspectives

Dentistry and medicine have, to a great extent, been somewhat separated during the last 160 years, despite the fact that they have the same patients in common (Cohen 2001). The link between oral infections and general health represents a new and crucial area for professional concerns and further research that has far-reaching scientific and clinical implications. It is apparent that a strong collaboration between dental and medical professionals would contribute to better diagnosis and treatment across specialities (Cohen 2001, Jin *et al* 2003). It has recently been acknowledged that as the interrelationship between the mouth and the rest of the body becomes clearer, dental professionals, medical doctors and patients need to rethink the term 'oral health' and its

potential impact on general health (Guynup 2006). It is essential that healthcare professionals should remain updated on developments in periodontal medicine. Practicing dentists have be more attentive to periodontal diseases and screen for periodontal conditions in their patients on a daily basis, including an appropriate risk assessment of periodontal infection as an infectious burden on systemic health and the need for relevant risk control in dental practice. Meanwhile, patient education should be enhanced by emphasizing the importance of oral health in general well-being. Essentially, further integration of dental and general medicine requires more communication and collaboration between dental and medical practitioners, and greater co-responsibilities and effective team approaches in the clinical management of their shared patients thereby providing more effective and predictable treatment outcomes as well as achieving better oral health and general health.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 13

A Perspective of Periodontal Systemic Relationships in Smokers in the Asian Pacific Region

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Background

The high prevalence of smoking in Indonesia can be expected to have significant implications on the health of the population. This includes oral health conditions such as periodontal disease, as the harmful effect of smoking on periodontal condition has been widely reported (Swenson 1978, Bergstrom and Preber 1994, Linden and Mullaly 1994, Martinez-Canut *et al* 1995, Bergström *et al* 1996, Selvi 1997, Boström *et al* 2001, Lindhe 1995). However, chronic periodontitis, i.e. a sustained inflammation of the periodontal tissue associated with loss of gingival and eventually alveolar bone attachment, can also be present in non-smokers, depending on factors such as maintenance of oral hygiene and inherited susceptibility. To assess the regional impact of smoking, it is therefore necessary to evaluate and compare the periodontal condition of smokers and non-smokers in the population concerned. In Indonesia the impact of smoking has characteristic features due to the the common local habit of smoking clove (kretek) cigarettes which have a high tar content. Due to the inflammatory characteristics of chronic periodontitis, it can be expected that the potential differences between smokers and non-smokers are reflected in the immune reactions of the oral tissues. Usually clinical indicators such as pocket depth are used to evaluate the health status of the periodontium (Lindhe 1995, Rateitschak *et al* 1985). However, pro- and anti-inflammatory cytokines have been suggested occur at levels that could indicate the status of chronic periodontitis (Preiss and Meyle 1994, Lee *et al* 1995, Boström 1998, Yamazaki and Nakajima 2004, Rawlinson *et al* 2003, Giannopoulou *et al* 2003). This approach is adopted here to determine the effect of smoking on the severity of chronic periodontitis in relation to oral hygiene and levels of IL-1β, TNF-α and IL-10 in the gingival crevicular fluid (GCF).

Materials and methods

A total of 102 smokers and 25 non-smokers between 25-64 years of age were selected from consenting male patients in three Indonesian dental hospitals and clinics. The subjects were checked for oral hygiene and periodontal status. Patients with caries, aggressive periodontitis or systemic disease, or who had undertaken antibiotic treatment within the

previous two months were excluded from the study.

The oral hygiene of the subjects was evaluated by using the oral hygiene index (OHI) which is determined as the sum of the modified Ramfjord plaque and calculus indices (modified to include all teeth) on buccal and lingual surfaces (Rateitschak *et al* 1985, Preiss and Meyle 1994). The OHI ranges were divided into three categories, with OHI≤2.0 ranked as fair, 2.0<OHI≤4.0 as moderate, and 4.0<OHI≤6.0 as poor oral hygiene.

The periodontal condition of the subjects was assessed in terms of the papillary bleeding index (PBI, mean score (0 to 4) of inspected tooth surfaces), gingival pocket depth (number of tooth surfaces in classes of P<4 mm, >4-≤6 mm, >6 mm) and gingival attachment loss (AL, number of locations in classes of AL<4 mm, >4-≤6 mm, >6 mm).

Samples of gingival crevicular fluid (GCF) were extracted from 65 randomised subjects with ≥-4 mm pocket depth without bleeding. GCF was collected by injecting 200 microliters of saline into the gingival pocket and withdrawing the saline-GCF mixture until a total of 500 microliters was obtained. ELISA was used to determine the levels of IL-1β, TNF-α and IL-10. Fisher's exact test, t-test and Anova were used for statistical analysis.

Results and discussion

The determined criteria of chronic periodontitis (mild, moderate and severe) based on numbers of pocket depth, numbers of attachment loss and papilla bleeding index are summarised in Table 1.

The observed OHI levels and severity of periodontitis in smokers and non-smokers are summarised in Tables 2 and 3 respectively. Clearly, smokers were more significantly associated with poor oral hygiene and severe periodontitis than non-smokers (Fisher exact test, $p < 0.05$). The number of smokers in the two first classes of oral hygiene up to OHI=4.0 were roughly equal (36-38%), and about one quarter were classified as poor oral hygiene (OHI>4.0). In contrast, most non-smokers (72%) showed OHI≤2.0 and none were classified into the class of poor oral hygiene. Mild periodontitis was observed in 35% of smokers but in 76% of non-smokers, whereas moderate periodontitis appeared in 20% of smokers and in 4% in non-smokers, and severe periodontitis was observed in 44% of smokers and in 20% of non-smokers. These differences were significant, but there were too few subjects (2 in total) with no periodontitis to show a statistically significant difference.

The measured levels of cytokines in the saline-GCF mixture for smokers and non-smokers are shown in Table 4 and Figure 1A. The mean levels of pro-inflammatory cytokines IL-1β and TNF-α are significantly higher in smokers than in non-smokers, and the corresponding levels of the anti-inflammatory cytokine IL-10 significantly reduced ($p < 0.05$, Fisher's exact test). This demonstrated that smoking influenced the immune response of periodontal tissue, so that chemotaxis and fagositosis of neutrophils are disturbed. Futhermore, IgG antibodies in smokers were decreased compared to non-smokers, consequently protection against infection in smokers was decreased (Novak and Novak 2006).

The mean difference in the status between smokers and non-smokers is further enhanced in terms of the cytokine ratios IL-1β:IL-10 and TNF-α:IL-10 (Figure 1B). This figure shows that these ratios in smokers were higher than non-smokers.

As shown in Table 5 and in Figure 2A, poor oral hygiene as indicated by the oral hygiene index (OHI) was associated with significantly increased levels of IL-1β and TNF-α, and reduced levels of IL-10 in all subjects ($p < 0.05$). Figure 2B shows the levels of IL-1β,

Chronic Periodontitis	Pocket	AL	PBI
Mild	P<4 1-80	AL<4 1-80	0
	P46 1-8	AL46 1-11	
	P>6 0	AL>6 0	
Moderate	P<4 1-80	AL<4 1-80	0 - 0,27
	P46 >8	AL46 >11	
	P>6 0	AL>6 1-6	
Severe	P<4 >80	AL<4 >80	>0.27
	P46 >8	AL46 >11	
	P>6 >1	AL>6 >6	

Table 1. The criteria of chronic periodontitis based on numbers of pocket depth, attachment loss and papilla bleeding index (P = pocket depth, AL = attachment loss)

	Oral hygiene index			
Group	**0 – 2.0**	**2.01-4.0**	**4.01-6.0**	**Total**
Smokers	37 (36.3%)	39 (38.2%)	26 (25.5%)	102 (100%)
Non-smokers	18 (72.0%)	7 (28.0%)	0 (0.0 %)	25 (100%)
Total	55	46	26	127

Table 2. Oral hygiene index levels in smokers and non-smokers

	Severity of periodontitis				
Group	**None**	**Mild**	**Moderate**	**Severe**	**Total**
Smokers	2 (2.0%)	36 (35.3%)	20 (19.6%)	44 (43.1%)	102 (100%)
Non-smokers	0 (0.0%)	19 (76.0%)	1 (4.0%)	5 (20.0%)	25 (100%)
Total	2	55	21	49	127

Table 3. The severity of periodontitis in smokers and non-smokers

Cytokine		Non-smokers	Smokers	p
IL-1β	Mean	2.78	6.32	0.0121
	SD	0.38	2.63	
	Median	2.86	5.14	
TNF-α	Mean	4.06	8.21	0.0007
	SD	1.64	2.13	
	Median	3.28	8.12	
IL-10	Mean	12.76	7.08	0.0025
	SD	1.97	3.34	
	Median	13.74	5.45	

Table 4. Observed levels GCF (pg/μl) of IL-1β, TNF-α and IL-10 in the GCF of smokers and nonsmokers (N = 65)

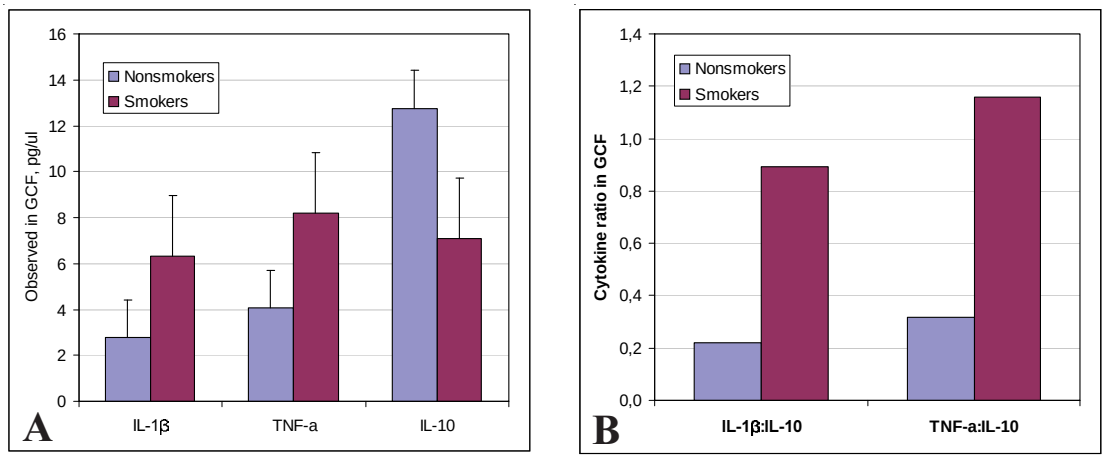

Figure 1. (A) Observed levels of IL-1β, TNF-α and IL-10 (mean and SD) and (B) Mean ratios of IL-1β:IL-10 and TNF-α:IL-10 in the GCF of smokers and non-smokers.

Cytokine		Oral hygiene index 0-2.0	2.01-4.0	4.01-6.0	p
IL-1β	Mean	3.35	4.93	8.78	0.000
	SD	0.75	1.48	1.75	
	Median	3.12	4.86	8.88	
TNF-α	Mean	5.29	7.52	9.89	0.000
	SD	1.53	1.37	1.57	
	Median	5.04	7.09	10.04	
IL-10	Mean	11.45	7.73	4.91	0.000
	SD	2.27	2.89	2.30	
	Median	11.82	8.99	4.23	

Table 5. Observed levels GCF (pg/ml) of IL-1β, TNF-α and IL-10 in the GCF of subjects according to their oral hygiene index (N=65)

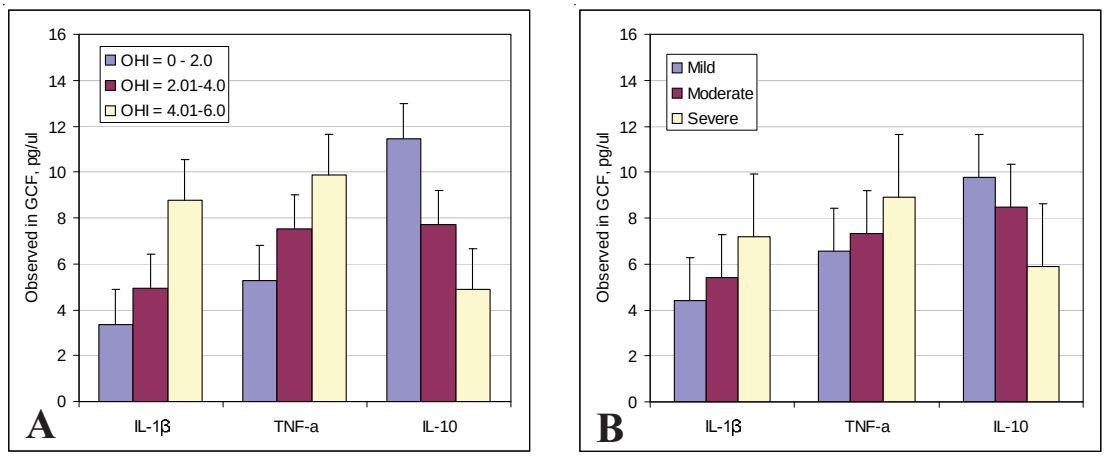

Figure 2. (A) Levels of IL-1β, TNF-α and IL-10 (mean and SD) in GCF according to the oral hygiene index and (B) according to severity of periodontitis (N = 65)

Cytokine		Severity of periodontitis Mild	Moderate	Severe	p
IL-1β	Mean	4.41	5.42	7.17	0.0007
	SD	1.88	1.84	2.75	
	Median	3.62	5.07	7.79	
TNF-α	Mean	6.54	7.35	8.90	0.0005
	SD	2.11	1.68	2.11	
	Median	6.61	7.78	9.39	
IL-10	Mean	9.77	8.49	5.79	0.0002
	SD	3.05	2.95	3.13	
	Median	10.49	9.17	4.29	

Table 5. Observed levels of IL-1β, TNF-α and IL-10 in the GCF of subjects according to the severity of periodontitis (N=65)

TNF-α and IL-10 (mean and SD) in GCF according to the severity of periodontitis.

Levels of IL-1β, TNF-α and IL-10 in severe periodontitis were significantly different from those in moderate or mild periodontitis ($p<0.01$) (Table 6).

Conclusion

The severity of chronic periodontitis, the oral hygiene index and the levels of IL-1β and TNF-α in the GCF were significantly higher and the levels of IL-10 in the GCF were significantly lower in smokers than non-smokers.

The severity of chronic periodontitis as mild, moderate or severe can be based on the number of sites with varying extent of pocket depth, loss of epithelial attachment, PBI and the levels of IL-1β, TNF-α and IL-10 in the GCF.

The methods used in this study for obtaining GCF are recommended for use in further studies.

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Chapter 14

Dentistry in Cambodia

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Background

There is only one dental school in Cambodia, which is part of the University of Health Sciences and the Ministry of Health. The Faculty of Dentistry was founded in 1953 along with the Faculties of Medicine and Pharmacy. The first dental training programme was for auxiliary dentists and followed the French system. In 1972, a Doctor of Dentistry course was begun; however, these dental students were unable to complete their course due to political upheaval caused by the reign of the Khmer Rouge from 1975 to 1979. Many of the dental students and auxiliary dentists were killed or died of hardship during that period; only 34 survived.

By 1979, the school building had been stripped of almost all clinical and laboratory equipment, materials, books, and educational materials. Between 1982 and 1987, the building was used as a dormitory for the medical and pharmacy students.

Between 1981 and 1984, the thirty-four surviving auxiliary dentists underwent additional training with Vietnamese professors and were upgraded to the level of Doctor of Dentistry (DD). Training of new auxiliary dentists (OSD) began in 1984. In 1987 a new DD course began. Admission of students into the auxiliary dentist course was stopped in 1993. Since 1995 OSDs have had the chance to continue their studies and upgrade to DDs.

Commencing in 1990 the Faculty received assistance from a Non Governmental Organisation (NGO) called World Concern. The dental school building was renovated, basic equipment and materials purchased, some teaching provided and English was introduced into the curriculum. World Concern has now has been replaced by an NGO called International Cooperation for Cambodia (ICC). In 1996, the school's clinic was renovated and supplied with new dental chairs/units by the Association of Technical Cooperation Germany. Several other groups have also helped to develop the dental school including New Asian World (Japan), Aide Odontologique Internationale (France), Organization of International Support for Dental Education, and Latter Day Saints Charities (LDSC). Individual volunteer dentists and some overseas university groups have also provided support.

Structure and facilities

Administratively, the school comprises three main sections - Administration, Technical (Clinical), and Research and Prevention. The Dean is assisted by two Vice-Deans who are in charge of the Administration

and Clinical Sections.

Clinically, the Faculty is divided into nine departments: Paedodontics, Oral Surgery, Operative Dentistry (Restorative/ Endodontics), Periodontics, Prosthodontics, Orthodontics, Radiology, Oral Pathology, and Community Dentistry.

The dental clinics are located on the ground floor of the dental school where students practice under the supervision of clinical tutors. The clinic has been set up primarily for teaching dental students and to serve the poor from all over the country.

The Research and Prevention Office at the Dental School was only recently established. This office is supported by Aide Odontologique Internationale, which is also helping to improve cross-infection control in the clinics. The Paedodontic Clinic has been assisted by New Asian World from Japan, a group of Australian and New Zealand dentists, and LDSC. Moreover, the school was donated a Dental Bus by the Aichi Dental Association in Japan in 1991. The bus has been providing free dental treatment for poor children in and around Phnom Penh, however the equipment is now mostly in disrepair.

A small dental library is located in the second floor of the school building. Almost all the textbooks and dental journals in the dental library are in English. However, most are out-dated. Therefore, new dental textbooks and journals are urgently needed. The English Language Laboratory is attached to the library and both are managed by ICC.

The Cambodian Dental Association's Office is located at the second floor of the school. Most of the CDA Board Members are from the Faculty. The CDA has striven to improve the quality of dental education by contacting allied dental professional organisations around the world to help provide continuing dental education for dentists. Those organisations include the World Dental Federation, the Academy of Dentistry International and the Asia-Pacific Dental Federation.

Staff

There are currently 39 staff at the Faculty, including 24 lecturers/clinical supervisors (all DD's), 7 auxiliary dentists (OSD's) and 8 dental assistants (who are actually general nurses). Two of the dental assistants have undergone dental nurse training at the Regional Nurses Training Centre in Kampong Cham. Most of the lecturers are general dentists with no overseas postgraduate qualifications. One member of staff has completed a post-graduate Diploma in Oral Surgery in New Zealand, and one a Master of Public Health in Thailand. Two of the staff have undergone Health Personnel Education training in Cambodia and several others have gone overseas for short-term training and study visits.

Since 1990, several expatriate dentists have worked at the dental school as advisors, teachers and clinical tutors. There is a pressing need for volunteer clinical tutors at present.

ICC has supplied one expatriate to assist the Dean with administration and management.

Student numbers

The total number of students in the Faculty is 194. The dental class size is between 20 and 25 students. This included some OSD's who are now upgrading their degrees to become DD's. From 2003 the number of students has been increased, with the addition of 20 to 30 fee-paying students in each new class. Given the present facilities and staffing it will be difficult to provide quality education for such a big group, however the additional income is expected to provide the Faculty with much needed income.

Student selection

Students wishing to enter the Faculty of Dentistry are firstly assessed on the basis of their high school examination results. Since 2003, a grading system (A, B, C, D, E, F) has been used to grade the students in each subject. The top 20 applicants who have received grade A to C passes in Biology, Chemistry, Mathematics, and General Culture are offered entrance to the Faculty without sitting any further exams. However, only 12 students were offered a scholarship in the school year 2002-03. An additional 38 students who could afford to pay US$ 850 per year were selected, based on their examination scores.

Course length

The course length is presently 7 years in total. During the first three years of the course students study the basic sciences. The next two years emphasise basic dental sciences and pre-clinical subjects. Clinical dentistry is only introduced during the final two years. The curriculum has been revised several times in the last decade, usually following revisions to the Medicine and Pharmacy courses. The latest changes have reintroduced a five-year course for a Certificate in Dentistry. These five-year-trained dentists will not be DD's. To complete the degree of Doctor of Dentistry, students will need to study for 2 more years and present a thesis or dissertation. These changes are in line with the Faculties of Medicine and Pharmacy and have been influenced by the French system. To run these two courses simultaneously will be difficult, graduates in the Certificate programme will have had little clinical experience, and will therefore have to complete thefull seven years in order to practice dentistry safely. Job descriptions have yet to be written for the two types of dental graduate.

Undergraduate course curriculum

To be in line with medical and pharmacy curriculae, the curriculum for dental undergraduate has been divided into two categories. The first category is a five year course for a Certificate of Dental Science. The second one is the seven year courses for the degree of Doctor of Ondontostomatology. The proposed new curriculum for a 5 and 7 year courses is as follows:

Course contents (Plan of study)

First year: General Anatomy, Physiology, Biophysics, Biochemistry, Embryology, Histology, Human Disease, Surgical Pathology, Dental Anatomy, Biomaterials, Occlusion, and Laboratory Practice. Additionally, French and English are also taught (but without examination).

Second year: Physiology, Bacteriology, General Anatomy and Pathology, Surgical Pathology, Human Disease, Head and Neck Anatomy, Fixed Prosthodontics, Removable Prosthodontics, Dental Pathology, Operative Dentistry, Biomaterials, Laboratory Practice, French and English.

Third year: General Pharmacology, Fixed Prosthodontics, Removable Prosthodontics, Occlusiology, Radiology, Immunology, Orthodontics, Endodontics, Dental Pathology, Operative Dentistry, Oral Biology, Periodontology, Laboratory Practice, French, and English.

Fourth year: Removable Prosthodontics, Paedodontics, Orthodontics, Complete Dentures, Oral Pathology, Basics Dental Surgery, Endodontics, Oral Biology, Dental Pharmacology, Lab Practices, Clinical Practice, French, and English.

Fifth year: Paedodontics, Periodontology, Minor Oral Surgery, Endodontics, Dental Pharmacology, General Anaesthesia, Code of Dental Practice, Fixed Prosthodontics, Community Dentistry, Oral Pathology, Oral Medicine, Clinical Practice and English.

Sixth year: Periodontology, Paedodontics, Code of Dental Practice, Oral Surgery, General Anaesthesia, Endodontics, Community Dentistry, Clinical Practice and English.

Seventh year: Internship, Clinical Practice, and English.

Since some students will finish their courses in five years in the future, the curriculum will need to take this into account and job descriptions for those dentists will need to be defined. Expatriate consultants to assist in revising the curriculum have been suggested so as to ensure that the Cambodian course and graduates are similar to others in the South East Asian region.

Languages

Most subjects at the Faculty are taught in Cambodian, with mainly French medical and dental terminology. English (with Khmer translation) has been taught at the dental school since 1990, by both expatriate and Cambodian teachers. All of the students learn basic French and English, although English is continued for longer and is spoken by more by students than French. The teaching programme of both General and Dental English has been supported by ICC. Most General English teachers are from overseas while Dental English teachers are local Cambodian dentists.

Lecturers

- Full time lecturers
- Part-time lecturers
- Visiting lecturers: Dr Callum Durward (New Zealand), Dr Pederson (USA), Dr Jon Hammond (UK), Prof Takashi Miyata (Japan)
- Local clinical tutors
- Overseas clinical tutors: Dr Joceline Loane (Australia), Dr Chern Chern (Singapore), Dr Yew On (Singapore)

Academic calendar

Postgraduate courses

Since 2000, two postgraduate diploma courses have started. These part-time courses are conducted by groups of visiting overseas specialists. So far, courses have commenced in Paediatric Dentistry and Dental Public Health. Diplomas in Periodontics and Orthodontics are due to start at the end of 2003. The Faculty would like to introduce courses in other clinical areas (including oral surgery, endodontics, prosthodontics, restorative dentistry and so on) if overseas volunteer teachers and support can be found. The primary purpose of these courses is to upgrade the staff at the Faculty so that they can teach more effectively. It is very difficult to find opportunities for Cambodian dentists to study overseas.

Needs

There are significant problems at the Faculty related to the knowledge and skills of the teaching staff, teaching methodologies and resources, management, infection control, clinical supervision, dental equipment maintenance and repair, instruments and materials, the curriculum, the assessment system, pre-clinical and laboratory training, opportunities for postgraduate study and access to up-to-date dental textbooks and journals.

Visiting dentists

Volunteer visiting dentists are needed to work in the student dental clinics as clinical tutors, as well as alongside the Cambodian lecturers in the classroom. Both short and long-term volunteers are being sought. An organization that could help coordinate a visiting dentist programme would be valuable.

Library resources

The Faculty needs donations of up-to-date dental textbooks and journals. The library only has current subscriptions to two French dental journals. Students have no textbooks.

Equipment and materials

There are shortages of all types of dental equipment and materials (both clinical and laboratory). Some items are purchased locally using income from the clinics, however the Faculty relies heavily on donations from overseas. Since the school is striving to improve infection control, dental equipment and materials for this purpose are also needed. There are no preclinical simulator heads on which students can practice at present. The Faculty would also benefit from having its own data projector. Much of the equipment at the Faculty is in disrepair. A visiting dental repair technician could probably restore much of this equipment to working order, while at the same time teaching local staff about maintenance and repair requirements. One key piece of equipment in short supply at the Faculty is the dental handpiece. These cannot be repaired in Cambodia and poor maintenance and sterilization procedures are of great concern.

Postgraduate diploma programmes

Two postgraduate diploma programmes are already underway in Cambodia in paediatric dentistry and dental public health. Two more (in periodontics and orthodontics) are due to start within the next 6 months. One of the main purposes of these courses is to upgrade the knowledge and skills of the dental school staff. The courses require an overseas dentist (or group) to assist their Cambodian counterpart to write a curriculum for the course and coordinate visits by volunteer dentists. The courses need to have both theoretical and practical/clinical components, and should last 2-3 years (part-time). Dentists to help conduct courses in oral surgery, restorative dentistry, prosthodontics and endodontics are presently being sought.

Postgraduate study opportunities

The Faculty is seeking opportunities to send staff overseas to undertake postgraduate courses. Shorter study tours are also of value. Most dentists at the dental school can now communicate in English.

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Chapter 15

Mucogingival Issues Today

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Introduction

The management of oral soft tissues has undergone significant changes in recent times. Previously, mucogingival issues focussed on the width of attached gingiva and whether its presence is essential to gingival health. However, many studies have provided evidence that the presence of attached gingiva is not needed to maintain oral health (Miyasato *et al* 1977). Although, it may not be necessary for health, nonetheless, it is important from an aesthetic perspective. Currents trends are towards a restorative driven approach in the treatment and management of dental conditions. Additionally, patients enlightened by the media expect exacting and demanding aesthetic outcomes.

Dentists need to work in conjunction with dental technicians to ensure that the fabricated crown has the right shape, size, specific staining patterns and the appropriate colour in terms of hue, chroma, and value. However, it does not end there, since a beautiful crown would need to be 'supported' by suitable gingivae. A healthy and aesthetic gingival tissue profile is important. Therefore, the soft tissue that 'frames' the crown of our teeth needs to be of an appropriate profile, contour, shape and colour. This aesthetic approach is the current practice and has spawned a variety of surgical techniques. Various definitions and descriptions have been used for such techniques including mucogingival surgery, aesthetic periodontal surgery and periodontal plastic surgery.

This article provides a brief overview of some of the clinical parameters associated with mucogingival issues today as well as illustrating some clinical examples of management using arguably one of the most popular techniques today, the subepithelial connective tissue graft.

Clinical parameters in mucogingival issues

Clinical factors associated with mucogingival issues can be broadly classified into tooth associated factors, soft tissue factors and others. They are seldom the sole factor that would adversely impact mucogingival condition but are often associated with other factors such as inflammatory mechanisms resulting in the loss of gingival tissue.

Tooth factors

Tooth position

The position of the tooth with respect to the dento-alveolar bone at the bone crest level. A tooth that is in a maligned position, either

buccally or lingually, is likely to display poor proportion, contour or some deficit of gingival tissue (Weinberg 1960).

Root position

Similarly, any portion of the root that is positioned outside the dento-alveolar housing will result in either root fenestration or dehiscence. This, in conjunction with other factors, may predispose the tooth to recession of gingival tissue (Getral and Mathews 1976).

Anatomical factors

Certain anatomical features of the tooth like cervical enamel projection, enamel pearls or a cementicle may, with other co-factors, lead to loss of gingival tissue.

Periodontal disease

Being the leading cause of tooth loss in the middle age population, the pathogenic process results in loss of attachment and can potentially cause loss of gingival tissue, resulting in defects.

Soft tissue factors

Frenal attachments

The position and extent of frenal attachments can potentially predispose the tooth to cause loss of gingival tissue.

Gingival biotype

A thin gingival tissue profile is less likely to resist processes and/or mechanisms that lead to loss of gingival tissue.

Others

Tooth brushing

Over brushing or inappropriate use of hard or electric toothbrushes will result in chronic trauma to gingival tissue, leading to recession.

Orthodontic treatment

Orthodontic forces applied in a manner that will move a tooth outside the limits of the dento-alveolar bone housing will leave the tooth at increased risk of loss of gingival tissue.

Other iatrogenic factors

Restorations with poor margins or those which cause trauma to the gingiva may induce or promote chronic irritation which results in inflammation and subsequently tissue destruction.

Management of mucogingival problems therefore must begin with identifying the causative factors that contribute to the condition. To provide a lasting solution the clinician must address all of the relevant factors effectively and then decide on the appropriate treatment. There are also a number of different ways to classify gingival recession (Sullivan and Atkins 1968, Miller 1985).

Available techniques

The free gingival graft has previously been the most common procedure utilized to treat and manage clinical conditions that present with lack of attached gingival. Dentists have several techniques from which to choose from. These techniques can be broadly classified into 4 groups:

1. Free grafts: Traditional free full-thickness gingival grafts, sub-epithelial connective tissue grafts.
2. Pedicle gingival grafts: Lateral sliding, rotational, double papilla, coronal repositioned, semilunar flap.
3. Combination procedures involving both free & pedicle techniques.
4. Guided tissue regeneration: Use of membranes both non-resorbable and resorbable (collagen), cadaver epithelial membranes (Alloderm).

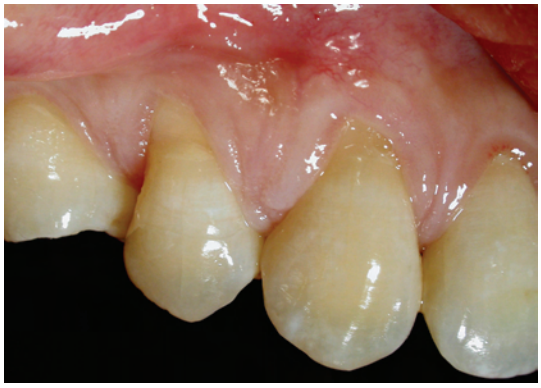

Figure 1. Pre-operative photo of Case 1. Gingival recession on teeth 13 and 14.

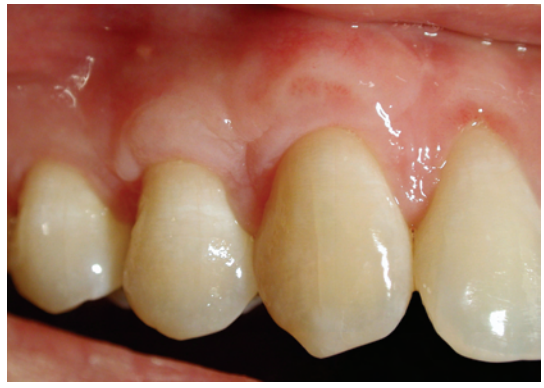

Figure 2. Post-operative photo of Case 1. Teeth 13 and 14 after 1 month healing.

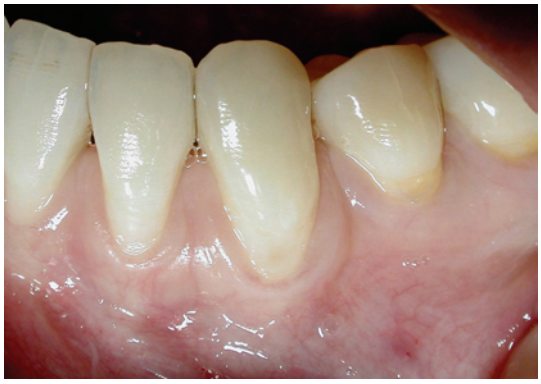

Figure 3. Pre-operative photo of Case 2. Recession on tooth 33.

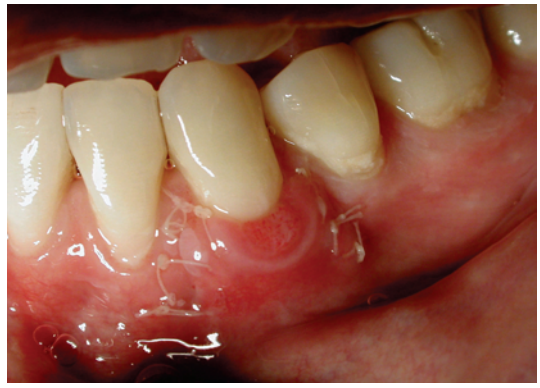

Figure 4. Post-operative photo of Case 2. Tooth 33 after 1 week healing.

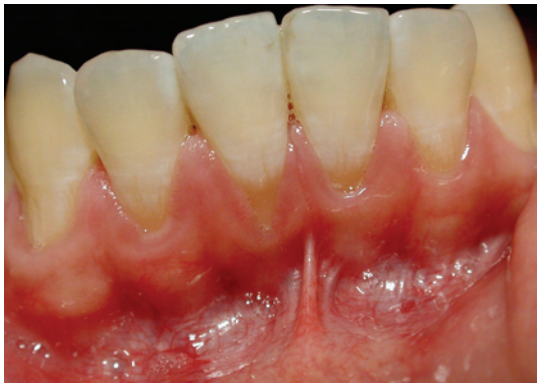

Figure 1. Pre-operative photo of Case 3. Recession on teeth 31 and 41.

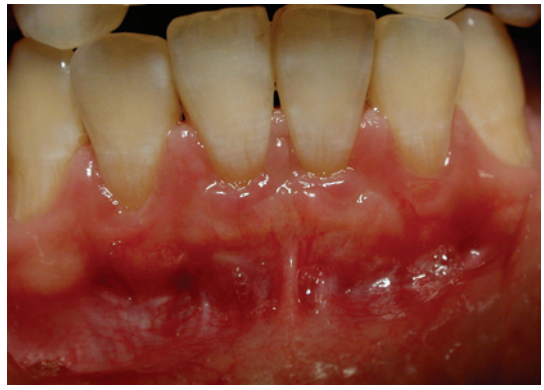

Figure 2. Post-operative photo of Case 4. Teeth 31 and 41 after 1 month healing.

Clinical cases

This article showcases clinical examples where a sub-epithelial connective tissue graft was utilized. This technique, first described by Langer and Langer (1985), provides clinicians with a procedure with very predictable and aesthetic treatment outcomes.

Case 1

The patient presented with buccal gingival recession on the upper right canine and first premolar. The patient was experiencing extreme hypersensitivity that a variety of toothpastes failed to relieve (Figure 1). Sub-epithelial connective tissue grafts were indicated and the results revealed complete root coverage, resolution of hypersensitivity, as well as a very pleasing aesthetic outcome (Figure 2).

Case 2

The patient presented with 2 mm on the lower left canine with severe hypersensitivity (Figure 3). A sub-epithelial connective tissue graft was indicated and provided very satisfactory results. The 1 week post operative review revealed complete root coverage with the establishment of a wide and thicken band of keratinized gingival tissue (Figure 4).

Case 3

This patient presented with recession around the lower central incisors and a prominent high frenum (Figure 5). This patient's main concern was the poor aesthetics caused by the recession. A combination of frenectomy with a sub-epithelial connective tissue graft and coronally advanced flap was indicated. The procedure was successfully carried out and the patient was happy with the outcome (Figure 6).

Conclusion

This mini-article revisits mucogingival issues that periodontists frequently face. Today, an enlightened patient pool challenges us with their expectations. However, armed with a variety of clinical techniques, our clinicians can confidently offer treatment outcomes that will deliver predictable and aesthetically pleasing results to our patients.

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A Perspective of Periodontal Systemic Relationships for the Asian Pacific Region
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Chapter 16

Smoking and Genetics: Disease Modifiers in Periodontal and Systemic Diseases

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Introduction

Current literature indicates that several systemic diseases are linked with periodontal disease including diabetes, cardiovascular disease, atheroma, pre-term low birth weight and adverse pregnancy outcomes. Periodontal disease and these systemic diseases share multiple common modifiers and risk factors including inflammation /infection from various sources, shared modifiable risk factors such as smoking, and non-changing risk factors such as specific gene polymorphisms. Hence the association between these diseases may be through a combination of direct mechanisms and/or through shared risk factors.

Inflammation/infection from various sources

Firstly, we must consider the pathogenesis of atherosclerosis in our attempts to understand potential common risk factors and etiological agents. Older, but still pertinent, concepts in the etiopathology of atheroma include lipid infiltration of the intima and thrombogenic theories. Modern concepts, which now co-exist with the older concepts, include the 'reaction to injury/inflammation theory' which are responses to insult of various sorts including mechanical, infection, hypercholestrolemia, immune, toxins and viruses. Atheroma is clearly not well understood from an etiopathological perspective in that we have a clearer picture of the time course and the pathology but not of the etiology and what the pivotal steps are that determine atheromatous formation.

Loos *et al* (2005) reviewed the contribution periodontal disease might make to atheroma formation. Loos *et al* hypothesized that daily episodes of bacteremia originating from periodontal lesions are the cause of changed systemic markers in periodontitis. This may explain the association with cardiovascular disease. Bacteremias emanating from the periodontal tissues are widely reported in the literature particularly in patients with periodontitis and can occur through simple tooth brushing as well as through clinical intervention such as scaling (Kinane *et al* 2005). Episodes of bacteremia originating from any part of the body may contribute to the inflammatory burden and a predisposition to systemic disease.

Several papers support this theory. Briggs *et al* (2006) reported on associations between poor periodontal status and heart disease. Spahr *et al* (2006) suggested that the association between periodontitis and coronary heart disease is particularly affected

by the periodontal microbial pathogen burden. Furthermore, Grau *et al* (2006) discussed the influence of infection (including periodontitis) on stroke risk. Stroke and periodontitis are conceivably the most connected oral and systemic diseases with an odds ratio for periodontal disease patients developing stroke of more than twice that of non-periodontitis subjects.

Traditional risk factors in atheroma include hemostatic factors such as fibrinogen, white blood cell counts and specific elevated lipids. Kweider *et al* (1993), examined fibrinogen and white blood cell counts in periodontitis patients and healthy controls. They discovered similar elevations in periodontitis patients to those noted in heart disease sufferers. Other studies have reported that plasma lipids and blood glucose levels were elevated similar to heart disease patients in subjects with destructive periodontal disease (Losche *et al* 2004). They concluded that hyperlipidemia and hyperglycemia which are major risk factors for cardiovascular disease are also present in periodontitis.

The question arises as to how periodontitis may affect blood vessels. Three theories have relevance here; bacteraemias emanating from the gingival crevice, bacterial products entering the blood and lymphatics, or activated inflammatory or immune cells from the periodontitis lesion may circulate in the blood and adversely affect the endothelia at distant sites. Secondary responses from the blood borne consequences of periodontal disease could be the acute phase proteins, cytokines and adipokines produced by the bacteraemias and host response factors issuing chronically over time into the blood stream from periodontitis lesions. These factors may also damage vessels.

Histopathological analyses of periodontal lesions clearly show microbial plaque in intimate contact with host cells. The gingival crevice is the site of considerable host pathogen interaction which will involve a variety of toxins and protease being released by the bacteria as well as host proteases, antibodies, complement and antimicrobial peptides to name but a few of the molecules that with bacteria may enter the body through the lymphatics or bloodstream. They travel to distant sites to create de-novo inflammation. Given the excessive bacteraemias and inflammatory molecules present, in response to plaque perturbation of the crevice in gingivitis and periodontitis, it is clear that reducing the plaque and/or reducing the inflammation could benefit the host by diminishing systemic events or inflammatory burden from the inflamed periodontium. Thus the 'tongue-in-cheek' statement 'floss or die' uttered by Dr Raul Garcia in 1997 gains some credence as does the more all-encompassing 'brush, floss, rinse or die' (reflecting intensive oral home care regimens taught and practiced in the US).

The inflammatory burden concept

The term 'floss or die' was coined to draw attention to the associations between periodontal and systemic diseases. This has been changed to 'brush, floss, rinse or die' by several US commentators to stress the importance of dental care to overall health. It is at this stage better to state that periodontal disease is a local condition with potential systemic consequences much like other inflammatory conditions such as in diabetes, the adipose tissue in obese subjects and the atheromatous lesions of those susceptible to heart and cerebrovascular disease. All of these conditions are considered, along with many other chronic conditions, to contribute to the overall inflammatory burden of the body. Reduction in any of them, including periodontal disease, (Tonetti *et al* 2007) is considered important in reducing risk of other serious systemic conditions. The

inflammatory burden concept is depicted in Figure 1. A major contributor to the inflammatory burden is smoking and this will be discussed next.

Smoking

Numerous investigations of the relationship between smoking and the periodontal diseases have been performed over the past 15 years. Both cross-sectional and longitudinal studies provide strong epidemiological evidence that smoking confers a considerably increased risk of periodontal disease. Numerous studies of the potential mechanisms whereby smoking tobacco may predispose to periodontal disease have been conducted. It appears that smoking may affect the vasculature, the humoral immune system, the cellular immune and inflammatory systems. A 10 year longitudinal radiographic study of alveolar bone loss showed that smoking was a significant predictor of future bone loss (Bolin *et al* 1986), and in a 5 year study of attachment loss, smokers were found to be at an increased risk of attachment loss (Beck *et al* 1997). In a further 1 year longitudinal study, smokers exhibited both greater attachment loss and bone loss when compared with their non-smoking counterparts (Machtei *et al* 1997). Smokers were also shown to be at significantly greater risk of further attachment loss when compared to non-smokers. Thus both cross-sectional and longitudinal studies indicate a strong relationship between smoking and increased risk of periodontal breakdown.

A study examining the relationship between smoking and attachment loss demonstrated a dose-dependent response (Grossi *et al* 1994). The odds for more severe attachment loss in smokers compared to non-smokers ranged from 2.05 (light smokers) to 4.75 (heavy smokers). These results support the findings of a later study which reported that probing depth was significantly correlated with smoking pack years (Baker and Tondreau 1985). Furthermore, years of exposure to tobacco products have been shown to be a statistically significant risk factor for

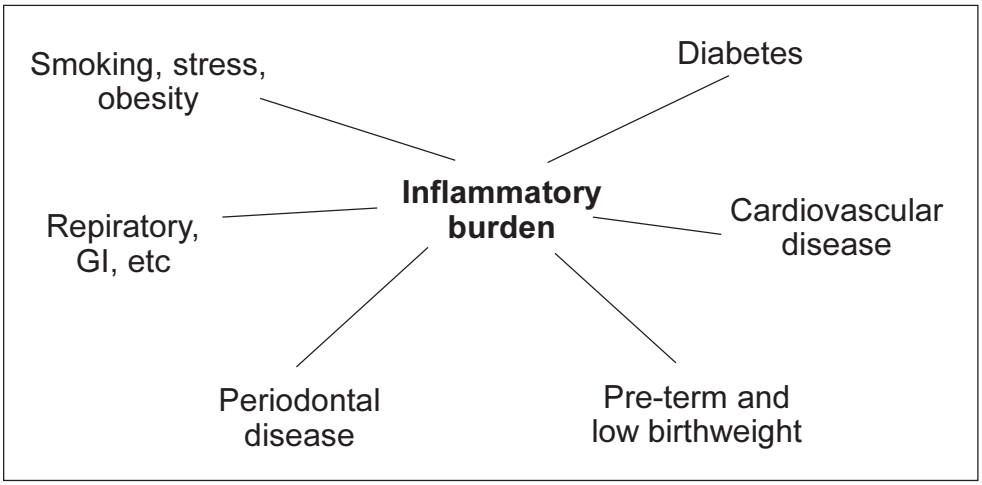

Figure 1. Contributors to the systemic inflammatory burden. This figure indicates how periodontal disease mayh be linked with other diseases through the inflammatory burden and like other local chronic inflammatory conditions itself contributes to this overall body burden. Note the two-way association with diabetes and cardiovascult disease (itself considered an inflammatory lesion similar to the adipose tissue producing adipokines in obese patients)

periodontal disease in 1,156 community dwelling New England elders, regardless of other social and behavioral factors (Jette *et al* 1993). Thus factors such as quantity of cigarettes smoked and duration of the habit may need to be considered in assessing risk of periodontitis due to smoking.

Nicotine and the periodontium

Nicotine may cause a vasoconstriction in the peripheral blood vessels and may reduce the clinical signs of gingivitis (Clarke *et al* 1981). Evidence for this reduction in clinical disease expression comes from various sources including Bergstrom (1990), who compared the compliance of smokers and non-smokers with an oral hygiene intervention program. The plaque index decreased in both groups and despite the similarity in plaque index, gingival bleeding was significantly lower in smokers than in non-smokers. These results suggest that, in smokers, the clinical expression of gingivitis (i.e. chronic inflammation) in response to plaque is suppressed.

A study with similar findings was conducted by Danielsen *et al* (1990) who set up an experimental gingivitis study on smokers and non-smokers. Similar amounts of plaque accumulated in the 2 groups during the period of oral hygiene absence, but clinically, smokers exhibited less gingival inflammation than non-smokers. A study by Holmes (1990) compared crevicular fluid flow in non-smokers with clinically healthy gingiva to the crevicular fluid flow of smokers in the areas physically exposed to smoke (maxillary lingual) and in areas not physically exposed to smoke (maxillary buccal). Smokers had significantly less crevicular fluid flow than non-smokers. Interestingly the exposed lingual areas of smokers showed no significant difference from the less exposed buccal areas. This suggests that the effect of nicotine may not be local or, if it is local, may be modified by the saliva and its affects dispersed. The authors suggest that the effect of tobacco smoke on clinically healthy gingiva may be through vasoconstriction rather than direct physical irritation.

Kinane and Radvar (1997) investigated the response of smokers and non-smokers to instrumentation, with and without subgingival antimicrobials. They reported that gingival crevicular fluid (GCF) volumes were significantly lower among smokers than non-smokers. In this study, it was noted that after therapy the decrease in the GCF volume of smokers was less than that of non-smokers regardless of treatment. However, the actual mean GCF volumes still remained lower in smokers than non-smokers. These findings are consistent with a diminished peripheral blood flow leading to a diminished GCF flow.

Epidemiology

One of the largest studies of risk factors for periodontal disease, was undertaken in Erie County, New York State, USA. Involving 1,361 subjects aged 25-74 years, this study showed that those who smoked were at greater risk of experiencing severe bone loss than those who did not smoke, with odds ratios ranging from 3.25 to 7.28 for light and heavy smokers, respectively (Grossi *et al* 1995). In a Swedish investigation of 155 patients with periodontal disease, a significantly higher percentage were found to be smokers than in the population at large and the risk ratio was reported as 2.5 (Bergstrom 1989).

A study of 540 Swedish adults 20-70 years of age has revealed that the three variables, smoking, greater age and higher mean plaque levels were potential risk factors for severe periodontitis (Norderyd and Hugoson 1998). After controlling for confounding factors such as age, sex, plaque and calculus, in a study of 615 American adults, the odds of having a

mean probing depth of at least 3.5 mm in one randomly selected posterior sextant was reported as five times greater for smokers than non-smokers (Stoltenberg *et al* 1993).

A case-control study of the relationship between life-events and periodontitis has shown smoking to be statistically associated with periodontal disease, after controlling for oral health behavior and socio-demographic variables (Croucher *et al* 1997).

Although the greatest number of investigations of the relationship between smoking and periodontal disease are cross-sectional, a few longitudinal investigations exist. In a 10 year longitudinal radiographic study of alveolar bone loss which began in 1970, it was shown that in those subjects who had at least 20 teeth at the start of the study smoking was a significant predictor of future bone loss (Bolin 1986). Moreover, in a five year study of attachment loss in 800 community dwelling adults, smokers were found to be at an increased risk for attachment loss (Beck *et al* 1997).

In a further 12 month longitudinal study, in which a wide range of clinical, microbiological and immunological indicators were correlated with disease progression, smokers exhibited both greater attachment loss and bone loss when compared with their non-smoking counterparts. Smokers were shown to be at significantly greater risk for further attachment loss when compared to non-smokers, the odds ratio being quoted as 5.4 (Machtei *et al* 1997).

Dose-response

Grossi *et al* (1994) examined the relationship between smoking and attachment loss and demonstrated a dose-dependant response. The odds for more severe attachment loss in smokers, compared to non-smokers, ranged from 2.05 for light smokers to 4.75 in heavy smokers. These findings support those of Alpagot and colleagues who reported that probing depth was significantly correlated with 'pack years' (i.e. packs of cigarettes smoked per day multiplied by the number of years the subject has smoked for) (Alpagot *et al* 1996). Furthermore, years of exposure to tobacco products have been shown to be a statistically significant risk factor for periodontal disease in 1,156 community dwelling New England elders, regardless of other social and behavioral factors (Jette *et al* 1993).

A Spanish survey involving 889 patients, reported that gingival recession, pocket depth and probing attachment level were significantly related to smoking status and that attachment levels were proportionate to the amount of cigarettes smoked. Smoking one cigarette per day, up to ten, and up to 20, increased probing attachment level by 0.5%, 5% and 10% respectively. However, only in the latter group did loss of attachment differ significantly from that of non-smokers. This led the authors to conclude that tobacco usage increases disease severity, and that this effect is clinically evident above a certain level of usage (Martinez-Canut *et al* 1995).

This suggestion of greater periodontal destruction above a certain level of smoking was suggested in a previous investigation of alveolar bone loss. Expressed as a percentage of tooth root length in 723 dentate adults, alveolar bone height was shown to be significantly lower in individuals smoking more than 5 g of tobacco per day compared with those smoking between 1 and 5 grams of tobacco per day (Wouters *et al* 1993). Norderyd and Hugoson (1998) examined 547 Swedish adults and found moderate to heavy smoking (greater than or equal to 10 cigarettes per day) was associated with severe periodontitis but that light smoking (less than 10 cigarettes per day) was not.

The strong association found between smoking and advanced periodontitis is

consistent with the hypothesis that smoking has cumulative detrimental effects on periodontal health (Horning *et al* 1992). So, there is good evidence that the more a patient smokes, the greater the degree of periodontal disease will be experienced. Furthermore, there is a suggestion that this may worsen above a certain threshold.

In one of the few studies which assessed cotinine, the severity of periodontal destruction measured either as clinical attachment level or crestal bone height, was shown to be positively correlated with serum cotinine levels (Gonzalez *et al* 1996). Cotinine is the principle metabolite of nicotine and provides a valuable quantitative measure of smoking status. Patients' cotinine levels have recently been shown to correlate directly with outcomes of progressive periodontal breakdown (Machtei *et al* 1997).

Cessation

Further evidence of the role of smoking in periodontal disease, comes from studies of patients who stop smoking. If smoking is associated with increased risk for periodontal disease, a reduction or elimination of tobacco use should reduce this risk and should be beneficial to the patient.

In a study comparing the prevalence of cigarette smoking amongst patients attending specialist periodontal and general dental practices, a dose response was observed. After controlling for age and sex, the odds ratio for those who currently smoked compared to those who never smoked was 3.3, whilst former smokers compared to people who had never smoked tobacco had a ratio of 2.1 with regards to the presence of moderate or advanced periodontitis (Haber and Kent 1992). The prevalence and severity of periodontitis has been shown to be less in former smokers compared to current smokers, leading Haber to conclude that stopping smoking is beneficial (Haber, 1994). In a ten year radiographic follow-up of alveolar bone loss, it was reported that progression of bone loss was significantly retarded in those who had ceased smoking during the study, compared with those who continued unabated (Bolin *et al* 1993).

Prospective observations of tooth loss in 248 women and 977 men, with a mean follow-up time of six years, indicated that individuals who continued to smoke cigarettes had in the order of 2.4 to 3.5 fold risk of tooth loss compared with non-smokers. The rates of tooth loss in men were significantly reduced after they quit smoking cigarettes, but remained higher than those in non-smokers. The authors concluded that stopping smoking significantly benefits an individual's likelihood of tooth retention, but it may take decades for the individual to return to the rate of tooth loss observed in non-smokers (Krall *et al* 1997).

Smoking summary

Cross-sectional and longitudinal studies indicating a strong relationship between smoking and increased risk for periodontal breakdown has been observed. Research performed in the last decade has proven beyond doubt that smokers have more periodontal problems than non-smokers. The evidence that smoking is a risk factor for periodontal disease is strengthened by the consistency of findings in different studies and in different populations, such as North America, the Nordic countries and the United Kingdom. The strong epidemiological evidence that smoking confers an increased risk of periodontal disease is further supported by evidence emanating from studies looking at patients who stopped smoking. The prevalence of periodontal disease is less in former smokers than in those who continue to smoke. This indicates that refraining from

smoking affords the individual greater periodontal health.

Genetic modifiers

Individuals respond to their environment differently and this response is influenced by their genetic profile which can produce variations in tissue structure (innate immunity), antibody responses (adaptive immunity) or inflammatory mediators (non-specific inflammation). Genetic factors which have been considered to influence the host response and may be relevant to periodontal disease could be considered in two main categories:

1. Those obvious genetic factors which result in overt systemic diseases such as Papillon Lefèvre syndrome (PLS) and leucocyte adhesion deficiency (LAD) and in which subsequently periodontal manifestations appear; or
2. Those more covert genetic factors which otherwise do not perceptibly affect the general health of the subject but predispose to periodontitis none-the-less.

In the first group the genetic defect results in a significant inability of the individual to protect against disease and is often related to defects in basic proteins, enzymes (cathepsin G in PLS for example) or receptors (adhesion receptor defects in LAD), i.e. deficiencies in innate or non-specific immunity. In contrast the second group of more covert genetic defects would likely result in reductions in number or function of inflammatory or immune related molecules and may alone not be sufficient to cause the disease but they may contribute with other genes and environmental elements (e.g. microbial plaque or smoking) to worsen the disease or increase the risk of developing the disease. It is likely that in the first category the disease developing from the major gene defect will have periodontal destruction as one of the many clinical features, but the type of periodontal destruction and its aetiopathogenesis may differ from that of typical periodontal disease. In the second scenario, the genetic defect may be a combination of several genetic variations, some with more influence than others, but not one with a major influence such that it would cause disease on its own. Clearly these genetic predispositions will be difficult to identify and to quantitate their individual attributable risk for the disease in question.

Proving the association between single nucleotide polymorphisms and disease

Recommendations emanating from population polymorphism association studies are invariably overstated in the periodontal and medical literature and are not based on a proper interpretation (conservative or ambitious) of the findings. If researchers are lucky and find an association between a polymorphism and periodontal disease their immediate priority should be to determine if the polymorphism actually does anything i.e. to address whether the polymorphism creates a silent mutation (synonymous) or not (non-neutral). A non-synonymous alteration could mean that the polymorphism results in the production of a protein with enhanced activity as has been claimed for the IL-1 polymorphisms (Pociot *et al* 1993) which Kornman and colleagues (1997) have associated with periodontal disease. A further approach might be to consider what other genes the polymorphism is close to on the chromosome and whether these may plausibly have a role in the aetiology of periodontal disease.

Recently the concept of looking for candidate genes and single nucleotide polymorphisms is in question. This approach has not been proven useful in diseases such as diabetes where whole genome scanning has

delivered gene variations with strong predictability for the disease. Performing whole genome scans appears to be the most fruitful way forward in attempts to link genes and diseases. In future the single nucleotide polymorphism-association study approach may fall into disrepute.

Other important considerations should be to check for population biases, case/control selection, possible confounders (and correction for these), and whether there probability values are strong enough to discount false-positive associations which may creep in if allowance has not been made for multiple comparisons. The typical next stage is to roll out the investigation to consider the association in larger and diverse populations which would be independently assessed and the results reported in the literature (whether they confirm or deny the positive association first claimed). Associations of polymorphisms with periodontal disease studies are at best hypotheses generating exercises and clinicians should be clear about the extreme limitations of these approaches when trying to develop robust associations which can be used as clinically relevant risk evaluators for patients. In the vast majority of cases, associations will not relate to the function of the gene, but to prove a functional relationship will require considerable multidisciplinary effort in population, pathogenesis and molecular genetics research.

Many investigators have suggested a role for IL-1 in the initiation and progression of periodontitis and have quoted *in vitro* and *in vivo* studies showing that IL-1 activates the degradation of the extracellular matrix and bone of the periodontal tissues, and elevated tissue or gingival fluid levels of IL-1β have been associated with periodontitis. Kornman *et al* (1997) quotes abstracts of *in vitro* studies from Pociot *et al* (1993) and others which claim that the 1 polymorphism associated with severe periodontitis in their study is also known to correlate with a 2- to 4-fold increase in IL-1β production. A problem with this line of reasoning is that IL-1 is a proinflammatory cytokine intimately involved in all inflammatory reactions as well as immune and reparative or healing responses. In addition, IL-1 is one of many proinflammatory cytokines (IL-6, TNF-α etc) which have overlapping activities and thus some redundancy is exists in the system. Furthermore, IL-1 has many controlling mechanisms which include inhibition of transcription, release, receptor antagonists etc and thus is highly regulated such that any polymorphism coding for increased production of this molecule could readily be controlled by the elaborate positive and negative feedback loops associated with its regulation.

The claimed association of severe periodontitis with smoking and the IL-1 genotype (in smokers the composite IL-1 genotype did not influence susceptibility) poses further problems. Does smoking and the overproduction of IL-1 work along the same pathogenic pathway and thus do the combination of both factors not give a summation of effect but make the other redundant? Or is the overall effect of smoking is so dominant that the composite genotype has little or no effect? These explanations, while feasible, require much more knowledge of both disease causing mechanisms associated with smoking and this genotype, but more so for the genotype given that the association with periodontitis is not as established as the literature on smoking (Kinane and Chestnutt 2000).

Socransky *et al* (2000) investigated the association between the composite genotype and carriage of periodontal species. They found the mean counts of specific species were higher in general in IL-1 genotype positive over negative subjects. The species detected

at higher levels were those frequently associated with measures of periodontal inflammation. A further study aimed at studying the composite genotype and inflammation was performed by Lang *et al* (2000). Genotype-negative subjects had significantly lower percentage BOP (p=0.0097) and it was concluded that the increased BOP prevalence and incidence observed in IL-1 genotype-positive subjects indicates that some individuals have a genetically determined hyper-inflammatory response that is expressed in the clinical response of the periodontal tissues.

Engebretson *et al* (1999) also found that in shallow sites of patients who were positive for the composite genotype of Kornman *et al* (1997) elevated levels of IL-1β were found in their gingival crevicular fluid. Smoking was not considered in the study and no statistically significant differences were noted for deeper pockets. Interestingly in the 22 chronic periodontitis patients examined only seven were positive for the susceptible genotype.

Summary of the findings on the IL-1 composite genotype in periodontitis

It appears that this IL-1 composite genotype has equivocal ability in detecting susceptibility to periodontitis and may at best be limited in its utility to only specific populations. It would appear from the mixed reports on this composite genotype that it is:

1. Unlikely to be relevant in aggressive periodontitis
2. It is at best in linkage disequilibrium with the gene contributing susceptibility to chronic periodontitis
3. Appears to confer risk independent of that attributable to smoking
4. The polymorphism is at best one of several involved in the genetic risk to chronic periodontitis which is likely to be a disease in which multiple genes may confer risk
5. The polymorphism is only a useful marker in defined populations and is relatively absent in some (Armitage *et al* 2000) and too prevalent (Walker *et al* 2000) in others to be a genetic marker with utility
6. Demonstration of the functional significance of this gene polymorphism has yet to be confirmed
7. Clinical utilisation of these composite polymorphisms for risk assessment and prognostic determination is currently premature.

Genetic screening for periodontitis risk

The current practical clinical utility of genetic knowledge in periodontics is limited. However, performing clinical periodontal assessments of siblings of AgP probands is one of the most useful actions we can perform in order to ensure the early diagnosis of this disease. By these means we may detect susceptible patients early and instigate therapy which may prevent the more significant disease aspects occurring. In the pursuit of better genetic diagnostic tests for chronic and aggressive periodontitis we must plan our research using plausible biological arguments and carefully avoid bias and misinterpretation of genetic associations with disease.

Summary

The three systemic conditions currently linked with periodontal disease and supported by extensive literature are diabetes, atheroma and pre-term low birthweight or adverse pregnancy outcomes.

Periodontal disease and these systemic diseases shared multiple common modifiers and risk factors and these include:

1. Inflammation/infection from various sources
2. Shared modifiable risk factors such as

smoking
3. Non-changing risk factors such as specific gene polymorphisms.

This review has attempted to argue that the association between these diseases may be through a combination of direct mechanisms and/or through shared risk factors essentially immutable risk factors such as genetics and changeable risk factors or environmental factors like smoking. In time we shall conceivably develop complex risk assessment models for multifactorial diseases such as atheroma and periodontal disease. These models should allow us, by proper weighting and through thorough assessments of current clinical status and previous environmental exposures, to accurately predict disease risk and thus invoke preventive measures early.

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Poster Presentations

The following is a record of the posters presented at the
7th Meeting of the Asian Pacific Society of Periodontology

Human Periodontal Ligament Cell Sheet Labeled with Nanogel-quantum Dot Hybrid Particles Transplanted in Athymic Rat Model

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Background: Prior research has used the cell sheet technique in an animal model for periodontal regeneration, obtaining a new cementum formation and the attachment of new collagen fibers to this newly formed cementum. The cell sheet could serve as a resource of the cells necessary for regeneration. However the fate of transplanted cells has not been fully elucidated. It is an important issue in this kind of transplantation study to elucidate the destiny of the periodontal cell sheet after transplantation. To monitor the interaction of the transplanted cell sheet, we intend to use nanogel-quantum dot (QDs) which contains an inorganic fluorescent nanocrystals that provide an useful alternative for studies that require long-term and multicolor imaging of cellular and molecular interactions. The aim of this study is to elucidate the fate of transplanted human periodontal cell sheet (HPDL) labeled with nanogel-quantum dot particles.

Methods: Nanogels of cholesterol-bearing pullulan modified with amino-groups (CHPNH) were used as a carrier to introduce quantum dots (QDs) into HPDL cell sheets after culturing in temperature-responsive dish for 21 days with osteodifferentiation medium containing ascorbic acid, dexametasone and β-Glycerophosphate. The HPDL cell sheets were harvested then transplanted into buccal mandibular defects of athymic rats and sacrificed at 24 hrs, 1 week and 2 weeks post-transplantation. Mandibles were excised and cryosections were prepared and observed using confocal laser scanning fluorescence microscopy.

Results: QDs, effectively labeled HPDL cell sheets through the observation period. At 2 weeks the fluorescence was diminished and only present at the apical side of the defect. In vitro study showed that the HPDL cells were labeled over 3 weeks. These results indicate that nanogel-quantum dots have potential as a research tool in the studies of intracellular delivery system.

Conclusions: Nanogel-quantum dots were found to be useful as a carrier to introduce QDs into HPDL cell sheets under culture and after transplantation into athymic rat model. Therefore our results indicate that nanogel-quantum dot might be useful for further characterization of HPDL cell sheets and could serve to elucidate the fate of transplanted cells.

**Recipient of Best Poster Presentation Award - First Place*

Gingipain-Active Extracellular Protein Preparations from *Porphyromonas gingivalis* ATCC 33277 Induce Apoptosis of Bovine Artery Endothelial Cell

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Objectives: Accumulating evidence shows that periodontitis may play a role in the process of cardiovascular diseases. Apoptosis has been recognized as a central component in the pathogenesis of atherosclerosis. The periodontal pathogen *Porphyromonas gingivalis* has been shown in atherosclerosis plaques, and may challenge endothelial cells. This study was to explore whether *P. gingivalis* is associated with endothelial cell apoptosis.

Methods: Extracellular protein was extracted from the culture supernate of *P. gingivalis* ATCC 33277, and the protein preparations were shown to have Rgp and Kgp activities. After exposure of bovine artery endothelial cells (BAEC) to the gingipain active protein preparations, apoptosis of BAEC was examined using Hoechst 33342 staining, DNA fragment assay and immunoblotting for cleaved caspase-3. To test the role of gingipain in this process, the extracellular protein was pretreated with *N*a-p-tosyl-L-lysine chloromethyl ketone hydrochloride (TLCK), an inhibitor of the protease activity. Then BAEC was exposed to protein preparations with or without pretreated by TLCK, respectively. Apoptosis of BAEC in two groups was tested by Annexin-v/PI staining.

Results: After treatment by gingipain active protein preparations, BAEC exhibited a rapid loss of cell adhesion properties. Apoptosis was detected by Hoechst33342 staining, DNA fragment assay and immunoblotting respectively. The loss of BAEC adhesion was significantly delayed and the apoptosis of BAEC was partly inhibited by TLCK.

Conclusions: Gingipain active protein preparations from *P. gingivalis* culture supernate induced apoptosis of bovine artery endothelial cells. It is suggested that *P. gingivalis* may contribute to the pathogenesis of cardiovascular diseases.

**Recipient of Best Poster Presentation Award - Second Place*

Study of Human Osteoblast Attachment to Guided Tissue Regeneration Membranes Which were Coated Either with Platelet-Rich Plasma or Platelet-Poor Plasma

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Objectives: To determine whether human alveolar bone osteoblast attachment to commercial available guided tissue regeneration (GTR) membranes can be enhanced by coating with freshly prepared human platelet-rich plasma (PRP) and platelet poor plasma (PPP).

Methods: Human osteoblasts established from tissue explants were used at 4th passage in culture. Human whole blood from healthy subjects was collected and centrifuged twice to produce the PRP fraction and PPP fraction. Double-sided adhesive tape was used to fix 3 mm discs of each membrane and cover-slides to the bottom of a 24-well tissue culture plate. GoreTex-ePTFE™, GoreTex-Resolut™ and Inion-GTR™ membranes were studied. Cover-slides were positive control. Membranes or cover-slides were exposed to PRP, PPP or PBS respectively for 2 hours. Membranes and cover-slides were seeded with osteoblasts ($5x10^4$ cells/ml) and allowed to attach for 24 hours. Wells were then rinsed with PBS, fixed at room temperature for 2 hours using 10% buffered formalin and stained with hematoxylin. The number of attached cells per mm^2 was counted using a light microscope with graticule. Scanning electron microscopy (SEM) was used to observe the ultrastructure of osteoblast attachment to the membranes.

Results: PRP and PPP treated membranes significantly enhanced osteoblast attachment compared to PBS treated membranes ($P<0.05$); Osteoblast attachment in the PRP-treated membranes is more than in the PPP-treated membranes ($P<0.05$); Cover-slides showed more osteoblast attachment than the three membranes ($P<0.05$); Comparing three membranes, GoreTex-Resolut™ and Inion-GTR™ membranes showed higher cell attachment than GoreTex-ePTFE™ membranes ($P<0.05$). SEM showed that osteoblast attachment to the membranes treated by PRP were spindle and well stretched and there were platelets, fibrins in a interlaced mesh on the membranes appearing to grow in a multilayer style. The osteoblasts attached to the membranes treated by PPP or PBS were round and partially attached.

Conclusion: PRP and PPP can improve attachment of osteoblasts in the three membranes. PRP altered and enhanced the method of attachment of osteoblasts to the membranes.

**Recipient of Best Poster Presentation Award - Third Place*

Posters Presented at the 7th Meeting of the Asian Pacific Society of Periodontology

Attachment Characteristics of Junctional Epithelium Cells in Cell-Tooth Slice Co-Culture Model

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Production and Bioactivity of Recombinant Enamel Proteins

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Effect of LPS on HMGB-1 Expression in Human Periodontal Ligament Fibroblasts

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Expression of PAR-2 in Human Periodontal Ligament Cells Activated by Periodontopathic Bacteria

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Lymphocyte Sub-Population in Periodontal Disease

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Effect of PGE_2 on VEGF Production in Human Periodontal Ligament Cells

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Comparision of Overexpression of CBFA1, BMP2 in Dental Follicle Cells

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The Relationship between IL-1β, IL-6 in Saliva, CRP in Serum and Nifedipine-Induced Gingival Overgrowth

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Explore the Method of Detecting Several Substances in a Single GCF Sample

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The Effect of Initial Periodontal Therapy on C-Reactive Protein Levels in Serum

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A Longitudinal Study of Volatile Fatty Acids in Gingival Crevicular Fluid of Patients with Periodontitis Before and After Nonsurgical Therapy

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Hypoxia Regulates Osteogenic Differentiation of Human Periodontal Ligament Stem Cells (PDLSCS)

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Differentiation of Mesenchymal Stem Cells from Human Periodontal Ligament

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Construction of 3D Complex of Porous β-Tricalcium Phosphate/Collagen Scaffolds and Dog Periodontal Ligament Cells

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The Effect of Different Platelet-Rich Plasma Concentrations on Human Periodontal Ligament Cells *in vitro*

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Expression and Regulation of Vitamin D Receptor, RANKL and Osteoprotegerin MRNA in Human Periodontal Ligament Cells with Different VDR Taq Genotypes

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Construction of a Eukaryotic Expression Vector Containing the Enhanced Green Fluorescence Protein and the Recombinant Human Bone Morphogenetic Protein-2

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ChemR23 Over-Expression Modulates Leukocyte Recruitment *in vivo*

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Effect of Photodynamic Therapy on Cytokines in GCF of Chronic Periodontitis

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Study on the Effect of Estrogen upon the Expression of OPG in the Alveolar Bone of Rat with Experimental Periodontitis

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Anterior Ridge Augmentation by Jawbone Expansion without Primary Closure

Tsai J, Huang D, Tuan L

Combination of Simvastatin and TGF-β1 Receptor Inhibitor Promotes Differentiation of Osteoblastic MC3T3-E1 Cells

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A Retrospective Case Study on 67 Consecutively Placed Short (7 mm and 5 mm) Sintered Porous Surface Implants: 8-24 Months Post-Loading Results

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Long-Term Results of Tetracycline-Loaded Collagen Membrane in Guided Tissue Regeneration: A 72-Month Case Report

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A Study of the Distance from Height of Contour to Cementoenamal Juction of Extracted Tooth

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Periodontal Conditions in Adult Chinese

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Periodontal Disease Status Among Smokers

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A Systematic Approach for the Management of Post Extraction Bony Defects in the Esthetic Zone

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Periodontal Risk Assessment: An Approach Towards Better Management of Medically Compromised Periodontal Patients

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Crown and Root Surface Area of the First Molar

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Bacterial Diagnosis Using Real-Time PCR Assay for Japanese Aggressive and Severe Chronic Periodontitis and Subsequent Treatment: Full Mouth Disinfection versus Conventional SRP

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Survival Analysis of Implants Placed in the Sinus Floor Elevated Maxilla

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Study on IGG Antibody Titers to PG in Serum in Patients with Aggressive Periodontitis

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Prevalence of Putative Periodontal Microorganisms in Subgingival Plaque of Patients with Aggressive Periodontitis

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Peripheral Blood Cell Analysis for Patients with Aggressive Periodontitis

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Serum IGG Antibody Titer to Different Serotypes of *Actinobacillus Actinomycetemcomitans* in Patients with Aggressive Periodontitis

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Plasma Calprotectin, Polymorphism of S100A8 and Aggressive Periodontitis

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Effect of Non-Surgical Periodontal Treatment on Prevalence of Putative Periodontal Microorganisms of AgP Patients

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A Familial Analysis of Clinical and the Genetic Polymorphisms of Interleukin-1 Gene Cluster Polymorphisms of Aggressive Periodontitis in Chinese Population

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Measurement and Estimation of the Single Root Surface Area

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Effect of Sulcular Volatile Sulphur Compounds Level on Initial Periodontal Therapy

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The Impact of Yishenqinghuo Recipe on Alveolar Bone Reconstruction in Rats with Experimental Periodontitis

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Reevaluation of the Curative Effects and of "Bushenguchiwan" Recipe in Periodontitis

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The Severity of Gingival Enlargement Induced by Cyclosporin–A and Nifedipine - An Experimental Study in Rabbit

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Triclosan for Gingival Health: A Systematic Review
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Clinical Efficacy of a Herbal Dentifrice in the Management of Gingivitis and Plaque
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Clinical and Microbiological Effects of Subgingival Application of Gel Containing Streblus Asper Leaf Extract in the Treatment of Chronic Periodontitis
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Clinical Safety of Subgingival Application of Gel Containing Streblus Asper Leaf Extract in the Treatment of Chronic Periodontitis Patients
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Research on the Relationship between Gene Polymorphisms of IL-1 Family and Chronic Periodontitis
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Differential Expression Patterns of Wnt Signaling Genes in Human Periodontal Tissues
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FcγRIII Gene Polymorphism and its Association with Periodontal Disease in a Population in Tamilnadu, India
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Correlation between Single Nucleotide Polymorphisms in Vitamin D Receptor Gene and Risk of Periodontitis in a Chinese Population
Liu KN, Zhang L, Meng HX, Tang XL, Xu L, Chen ZB, Shi D, Feng XH

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Functional Polymorphisms in the Promoter Region of MMP-2, MMP-9 and TIMP-2 Gene and Generalized Aggressive Periodontitis in a Chinese Population

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Actinobacillus Actinomycetemcomitans Infection and Acute Coronary Syndrome

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Anti-Sulfur Producing Oral Bacterial Activity of the Root Extracts of Chrysopogon Zizanioides (L) Roberty

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The Effect of *Actinobacillus Actinomycetemcomitans* Lipopolysaccharide on Rat Periodontal Tissues

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Porphyromonas Gingivalis Vesicle Induced Cellular Inflammatory Responses of Gingival Epithelial Cells *in vitro*

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Cloning of the RGPACD Gene of Porphyromonas Gingivalis and its Expression in *E.Coli*

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Screening of Virulence Genes of *Porphyromonas Gingivalis* W83 in Chronic Periodontitis
Lichen L, Liu J, Zhang D, Zhaojian, Yaping P
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***Porphyromonas Gingivalis* Infection Accelerate Intimal Thickening in Iliac Artery of Balloon-injured Rabbit Model**
Zhang MZ, Liang JP, Li CR, Jiang YT, Jiang W, Sun Y, Shu R
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Nasal Immunization with Pires-Fima:IL15 Provide Protection against *Porphyromonas Gingivalis* Challenge in Rats
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Lippopolysacchride of *Porphyromona Gingivalis* Shows Weaker Effect on Prostagladin E_2-Biosynthetic Pathway than that of *Escherichia Coli* in THP-1 Cells
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Lipopolysaccharide (LPS) of *Porphyromonas Gingivalis* Induce IL-1β, TNF-α and IL-6 Production by THP-1 Cells in a Way Different from that of *Escherichia Coli* LPS
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The Initial Research on Sieving PG Outer Membrane Proteins as the Candidate Antigens of Periodontitis Vaccine Using Shotgun Proteomics Method
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Possible Association between Mother's Periodontal Status and Preterm Low Birth Weight
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Non-Surgical Periodontal Therapy and Serum Lipid Levels in an Asian Population of Diabetics

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Study on the Association of Periodontal Status with Three Types of Angiographically Defined Coronary Heart Disease

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Evaluation of Effectiveness of Community Periodontal Care Intervention in Type 2 Diabetic Patients with Chronic Periodontitis

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Comparison of Periodontal Parameters between Populations with Systemic Healthy and Post-Acute Cerebral Infarction

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Relationship between Chronic Periodontitis and Post-Acute Myocardial Infarction

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Identification of Epithelial Cell Rests of Malassez in Regenerating Periodontal Tissues Following Enamel Matrix Derivative Application Using Light and Electron Microscopy

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An Experimental Study on the Effect of Astragalus Polysaccharides on Chitosan/Polylactic Acid Scaffolds Repairing Alveolar Bone Defects in Dogs

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Athymic Rat Model: A Pilot Study

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In Situ Tissue Engineering of Periodontal Tissues by Seeding with Cryopreserved and Uncryopreserved Bmscs

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Application of Human Periodontal Ligament Cells for the Tissue Engineering: A Study in Immunodeficiency Mice

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Enhancement of Periodontal Tissue Regeneration by Transplantation of Bone Marrow Stromal Cells

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Literature Analysis in Prominent Periodontal Journals between 2004 and 2005

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Using Delphi Technique in a Syllabus for Periodontics

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Expressions of BMP-2 and bFGF in Mouse Tooth Development

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Mineralized Characteristics of Human Periodontal Cell Sheet in a Temperature-Responsive Dish

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Biological Effects of Enamel Matrix Proteins on Rhesus BMSCs

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Effects of EMPS on Proliferation and Mineralization of Human BMSCs

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Effects of Human Bone Morphogenetic Protein-7 Gene on Bone Marrow Stromal Cell *in vitro*

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The Expressions of Cbfa1 and Proteins Related to Bone Morphogenesis in Human Tooth Germ Development

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Expression of Core Binding Factor A1 in the Mineral Stage Tooth Germ of Mice

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Expression of Epidermal Growth Factor and its Receptor in Soft Tissue Passway of Tooth Germ

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Characteristics of Epithelial Cell Rests of Malassez During Tooth Emergence and Occlusal Function

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Preliminary Study on Herpes Simplex Virus Type 1 Infection of Human Oral Epithelial Cells *in vitro*

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A Clinical and Histological Evaluation of Regenerative Techniques Employed in Alveolar Socket Preservation Prior to Implant Placement

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Establishment of Experimental Rat Model of Alveolar Bone Resorption

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The Influence of HTGF-β1 Gene Transfection on Gingival Fibroblasts' Osteogenesis

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The Biological Character of Gingival Fibroblast Transfected with the TGF-Â1 Gene after Being Stimulated by LPS

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Effects of Advanced Glycation End Products on the Human Gingival Fibroblast Proliferation and Type Collagen Synthesis

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Study on Expression of TLR4 on Human Gingival Fibroblasts Cells

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Effects of Amorphouys Calcium Phosphate/Hyaluronic Acid Composite on Cell Adhesion and Proliferation *in vitro*

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Transient Expression of Constructed Eukaryotic Vectors Carrying Encoding Gene of Soluble Human Interleukin-1 Receptor (I) and Tumor Necrosis Factor-Alpha Receptor (P55) in Human Gingival Fibroblast Cells

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The Transmembrane Transport of Tetracyclines by Human Gingival Fibroblasts

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Analysis the Minocycline Content Uptaked by Mature Rat Alveolar Osteoblasts *in vitro*

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The Expression of Scleraxis in Human Periodontal Ligament Cells of Different Passages *in vitro*

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The Effect of Basic Fibroblast Growth Factor on the Gene Expression of Decorin by Periodontal Ligament Cells in Culture

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Biological Effects of Phenytoin on Cultured Human Periodontal Ligament Cells *in vitro*

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Toll-Like Receptor 4 Signaling Plays a Role in Triggering Periodontal Infection

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Signal Pathway Analysis of Periodontal Ligament Cells after Treatment with Enamel Matrix Proteins

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Lentivirus-Mediated Human Amelogenin Gene Delivery in Human Periodontal Ligament Cells

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Experimental Model of Orthodontic Tooth Movement in DM Rats and the Expression of Col-I, MMP-1 and TIMP-1 in the PDL

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Photodynamic Effects of ZnPC-PL on Beagle Dog Marrow Stromal Cells, Human Periodontal Ligament Cells and *Porphyromonas Gingivalis in vitro*

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Effect of MMP-1 Promoter Genotype on the MMP-1 Production in Human Periodontal Ligament Cells

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Human Periodontal Ligament Fibroblasts in Dex-GMA/Gelatin Scaffolds Containing Microspheres Loaded with either BMP-2, or IGF-1, or a Mixture of Each Type of Microspheres

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Synthesis of Periodontal Ligament Fibroblasts/Platelet-Rich Plasma Gel and its Osteogenic Activity Expression

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Biological Transport of Tetracycline Hydrochloride by Human Periodontal Ligament Fibroblasts

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Expression of Toll-Like Receptor 2 and 4 on Human Periodontal Ligament Fibroblasts and their Regulation by Porphyromonas Gingivalis Lipopolysaccharide

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Effect of Estrogen and Raloxifene on the Proliferation and Mineralized Nodules Production of Human Periodontal Ligament Fibroblasts

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Research into the Function of Tea Polyphenol Enhancing the Attachment and Proliferation of HPDLFS, and Treatment Effect on Bandicoot Experimental Periodontitis Model

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Immunolocalization of Extracellular Matrix Metalloproteinase Inducer in Healthy and Inflamed Human Gingiva

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Ultrasound-Mediated Microbubble Destruction Enhances Bone Morphogenetic Protein-2 Gene Expression in Mouse Skeletal Muscle

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A Study on the Effect of Platelet-Rich Plasma, Activated by Different Concentrations of Thrombin, on the Repair of Cranial Defects

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Experimental Study on the Gene Expression of OPG of Alveolar Bone of Diabetes Mellitus Rats

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Local Expression of Thymic Stromal Lymphopoietin in Human Gingival Tissues

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The Influence of Initial Periodontal Therapy on Cytokines and C- Reactive Protein in Patients with Chronic Periodontitis and Coexistent Coronary Heart Disease

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Changes of Substance P in Gingival Crevicular Fluid in Response to Periodontal Treatment

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Measurement and Significance of Interleukin-18 in Blood Serum from Periodontitis

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Curative Effects and Experience of Treating Furcation Involvement with BAM Artificial Bone

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The Application of Micro-Infrared Spectrometry Technique in Periodontitis Diagnosis

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Application of a Modified Connected Tissues Graft to Mucogingival Surgery of Anterior Region

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Prevalence of *Actinobacillus Actinomycetemcomitans* in Chinese Chronic Periodontitis Patients and Periodontally Healthy Adults

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Bio-Oss and Bio-Gide Treatment Periodontal Bone Pocket Damage Clinical Curative Effect Observation

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The Clinical Effect of Crown Lenghthening and its Contributing Factors

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A Novel Way to Repair Periodontal Defects by Using Functionalized Chitosan Thermosensitive Hydrogel

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The Comparison of Periodontal Disease Patients' Oral Heath Behavior between Prior Treatment and Post-Treatment

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Research into the Curative Effect of Bibaike Jiaonang as an Adjunctive Treatment in Severe Periodontitis

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Association of Vitamin D Receptor Gene Polymorphism with Aggressive Periodontitis in Persons of Han Nationality

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The Effect of Nifedipine on the Expression of Type I Collagen in Gingival Fibroblasts

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Clinical Primary Study of Traditional Chinese Medicine on Chronic Periodontitis

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Influence of Baicalin on Alveolar Bone in Rat Experimental Periodontitis Assessed by Micro-Computed Tomography (Micro-CT)

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The Adjunctive Effect of Doxycycline on Patients with Chronic Periodontitis

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Effect of Phenytion on Proliferation Index and Apoptosis Index of Gingival Keratinocytes in Rat

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Recent Observations of Minocycline Hydrochloride Ointment in the Adjunctive Treatment of Periodontitis

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Correlated Research Between the Estrogen Receptor Gene Xbai-Pvu Polymorphism and Aggressive Periodontitis

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The Study of Correlation between Levels of Fibrinogen, the β455g/A Fibrinogen Gene Polymorphism, and Chronic Periodontitis

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Screen Outer Membrane Protein Antigen of Pg for Periodontitis Vaccine by SELDI-TOF MS

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Association between *Porphyromonas Gingivalis* and the Clinical Parameters Following Scaling and Root Planning Assessed by Taqman Real-Time PCR

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Interleukin-8 Regulation of Human Gingival Fibroblasts by *Porphyromonas Gingivalis* with Different FIMA Genotypes

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Comparision of the Effect of Inactivation of Periodontal Pathogens *in vitro* with Two Kinds of Photosensitizers

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The Association between Interleukin-1 Receptor Antagonist-Genotype and Chronic Periodontitis of Diabetes Mellitus

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Detection of the Presence of Luxs Gene in Strains of *Porphyromonas Gingivalis*

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Phosphorylcholine Expression in *Actinobacillus Actinomycetemcomitans* does not Increase its Susceptibility to CRP-Mediated Complement Killing

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