
RESEARCH ADVANCES AND CLINICAL PRACTICE IN PERIODONTICS: BRIDGING THE GAP

Hosted by

Asian Pacific Society of Periodontology

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Edited by
P. Mark Bartold,
I. Ishikawa,
D. Arunachalam

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Table of Contents

Acknowledgments	8
Invited Participants	9
Chapter 1 Exposition of the Conference Theme <i>N. Vergel de Dios (Philippines)</i>	12
Chapter 2 Directions in Periodontal Research in the Asian Pacific Region <i>I. Ishikawa (Japan)</i>	15
Chapter 3 Future Potential Directions in Periodontal Research in the Asian Region <i>T. Taiyeb Ali (Malaysia)</i>	20
Chapter 4 Research Advances in Periodontal Etiopathology <i>L.J. Jin (Hong Kong)</i>	26
Chapter 5 Research Advances in Periodontal Diagnosis <i>J. Zhang (China)</i>	39
Chapter 6 Research Advances in Establishing Contemporary Clinical Protocols in Periodontics <i>D. Arunachalam (India)</i>	49
Chapter 7 Risk Factors for Periodontal Infection Among the Rural Population in Battambang Province, Cambodia <i>P. Sophearoth (Cambodia)</i>	54
Chapter 8 Clinical Applications of Current Research in Periodontal Pharmacotherapeutics <i>N. Laosrisin (Thailand)</i>	60
Chapter 9 Clinical Applications of Current Research in Periodontal Wound Healing & Regeneration <i>N. Surathu (India)</i>	83
Chapter 10 Contemporary Clinical Techniques for Enhanced Periodontal Prognosis <i>K.M. Chung (Singapore)</i>	90

Chapter 11	Contemporary Clinical Directions in Regenerative Periodontics <i>R. Santos-Morales (Philippines)</i>	95
Chapter 12	Future Potential Clinical Directions in Clinical Periodontics <i>P.M. Bartold (Australia)</i>	103
Poster Abstracts		112
Poster 1	Preterm Low Birth Weight and Dental Intervention - Is There Any Link? <i>S. Bala*, S.C. Narula, R.K. Sharma</i>	113
Poster 2	Periodontal Regeneration Based on Multi-layered Cell Sheet Engineering <i>M. Gomez Flores*, M. Hasegawa, M. Yamto, T. Okano, I. Ishikawa</i>	113
Poster 3	Serological Analysis of the Implication of Periodontitis in Vascular Diseases <i>Y. Chen*, M. Umeda, Y. Huang, Y. Takeuchi, D. Wang, Y. Inoue, T. Iwai, I. Ishikawa</i>	114
Poster 4	Synergetic Approach for Optimal Esthetics <i>A. Uppoor*, D. Naik</i>	114
Poster 5	Black Triangles - The Unwanted Outcome of Periodontal Surgery: Methods of Prevention and Correction <i>K.J. Nisha*, K. Nandakumar</i>	115
Poster 6	Periodontal Manifestation in Florid Osseous Dysphasia - A Rare Case Report <i>B. Joseph*, K. Nandakumar</i>	115
Poster 7	Efficacy of Chithra Calcium Phosphate Cement Graft in the Management of Periodontal Osseous Defects <i>J.B. Rajesh</i>	116
Poster 8	A Study to Evaluate Tooth Mobility in Menstruating and Non-Menstruating Women <i>P. Mishra*, P.P. Marawar, G. Byakod</i>	116
Poster 9	Diabetes Mellitus and the Periodontium - Case Report <i>R.B. Pushpalatha</i>	117

Poster 10	Familial Aggregation of Aggressive Periodontitis - A Case Report of Siblings Affected <i>S. Janitha</i>	117
Poster 11	Matrix Disrupter - A Creative Plaque Disrupting Device <i>M. Parvez*, S. Hedge, K.S. Rajesh</i>	118
Poster 12	Evaluation of the Efficacy of Taurine as an Antioxidant in the Management of Patients with Chronic Periodontitis <i>S. Lakshmi</i>	118
Poster 13	A Clinical Evaluation of the Use of Demineralized Bone Matrix and Bioactive Collagen Membranes in Peri Implant Bone Regeneration <i>V. Chadha*, BVS Gupta Rajan</i>	119
Poster 14	Tissue Engineering - Revolutionizing Periodontics Towards Greater Horizons <i>N.K. Sowmya, T.S. Srinivasas</i>	119
Poster 15	Buccal and Coronal Bone Augmentation Using Forced Eruption and Buccal Root Torque - A Case Report <i>V.P. Sharshini</i>	120
Poster 16	Ridge Augmentation with Dense Hydroxyapatite Resorbable Suture (Permaridge) Matrix C <i>S.R. Krishna*, P. Talreja</i>	120
Poster 17	Psychological Stress and Oral Health <i>R. Jain, R Vidhyasagar*</i>	121
Poster 18	Effect of Aspirin Intake on Periodontal Status <i>S. Swamy*, F. Tarannum</i>	121
Poster 19	Effect of 0.2% Tempered Chlorhexidine as an Antiplaque Agent <i>S. Ebenezar, M.D. Jalaluddin, S.K. Nair</i>	122
Poster 20	Investigation of Periopathic Bacterial Transmission From Periodontal Pockets to Peri-Implant Sulci <i>H. Ogawa*, T. Ohshima, F. Kawasaki, K. Gomi, N. Maeda, T. Arai</i>	122
Poster 21	Effects of Fractionated Enamel Proteins on Human Periodontal Ligament Cells Differentiation <i>T. Nagano*, S. Oida, S. Suzuki, A. Kakegawa, K. Gomi, T. Arai, M. Fukae</i>	123

Poster 22	The Antimicrobial Effect of Porcine Amelogenins <i>M. Ebisawa*, T. Ohshima, T. Nagano, K. Gomi, M. Fukae, N. Maeda, T. Arai</i>	123
Poster 23	Comparative Evaluation of CO₂ Laser and Radiosurgery as Techniques in Treatment of Gingival Hyperpigmentation - A Clinical, Histological and Subjective Patient Evaluated Study Followed Over Six Months <i>R.P. Thakur</i>	124
Poster 24	Possible Association Between Preterm Low Birth Weight and Early Periodontitis - A Case Control <i>V.S. Eligar</i>	124
Poster 25	Finite Element Method in Periodontics - Looking Beyond the Obvious <i>M. Taneja*, I. Gupta*, K.L. Vandana</i>	125
Poster 26	Attachment Loss in a Case of Pendred Syndrome - Invidental or Constitutive <i>D. Sharma</i>	125
Poster 27	Primary Tuberculous Gingival Enlargement - A Rare Entity <i>B.V. Karthikeyan</i>	126
Poster 28	Pachyonychia Congenita with Unusual Dental Findings - A Rare Case Report <i>C. Nagaraja</i>	126
Poster 29	Plasma Cell Granuloma of Gingiva - A Case Report <i>P. Devi</i>	127
Poster 30	Role of Platelet-Rich Plasma in Periodontal Regeneration - Fact or Fiction? <i>S. Pai</i>	127
Poster 31	A Comparative Study Between Bioresorbable and Non-Resorbable GTR Membrane in Three Walled Defects <i>P.K. Singh</i>	128
Poster 32	Clinical Efficacy of a Novel Collagen Membrane of Fish Origin in the Management of Periodontal Intrabony Defects <i>S. Anila*, K. Nandakumar</i>	128

Poster 33	Varied Gingival Neoplastic Presentations of the Dreadful AIDS - Case Series	129
	<i>D. Deepa*, J. Kumar</i>	
Poster 34	Local Drug Delivery System Using 8% Tetracycline NCL (Periodontal Plus AB) Evaluated by DNA Probe (DMDX) for the Treatment of Chronic Periodontitis	129
	<i>B. Fernandes</i>	
Poster 35	Guided Tissue Regeneration Using Collagen Membrane - A Practical Approach in the Management of Gingival Recession	130
	<i>A. Madhukant*, S. Hedge, K.S. Rajesh</i>	
Poster 36	Effect of Periodontal Treatment of Monocyte Functions in Periodontitis Patients	130
	<i>D. Hormdee*, T. Nagasawa, G. Koshy, M. Kiji, R. Yashiro, H. Nitta, I. Ishikawa</i>	
Poster 37	Management of an Isolated and Advanced Gingival Recession in a Maxillary Premolar - A Case Report	131
	<i>A. Salman</i>	
Poster 38	Stem Cell Properties of Human Periodontal Ligament Cells	131
	<i>K. Nagatomo*, M. Komaki, K. Noguchi, S. Oda, I. Ishikawa</i>	
Poster 39	Production of BMP-2 by Human Periodontal Fibroblasts Stimulated with TGF-β	132
	<i>R. Yashiro*, T. Nagasawa, M. Kiji, D. Harmdee, G. Koshy, H. Nitta, I. Ishikawa</i>	
Poster 40	Chronic Periodontitis with Gingival Hyperplasia	132
	<i>R. Ma. A. Ferriols</i>	
Poster 41	Multiple Epiphyseal Dysplasia/Bilateral Genu Valgum with Localized Aggressive Periodontitis - A Case Report	133
	<i>G. Sivaram*, K. Bharadwaj</i>	
Poster 42	Assessment of Patient Recapitulation of Post Surgical Instructions	133
	<i>S. Kakarala*, M.G.S. Prasad</i>	

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The 6th International meeting of the Asian Pacific Society of Periodontology was held in Chennai, India on 4 November 2005, immediately preceding the 30th annual conference of the Indian Society of Periodontology. Over 350 delegates attended the APSP meeting making it the largest meeting held to date and indicating the growing interest in this group in the region.

At the Inauguration Ceremony the chief guest Dr CV Birmanandham, Vice Chancellor of the Tamil Nadu Dr MGR Medical University in Chennai opened the meeting. Of note was that Professor Birmanandham as a cardiologist expressed his clear interest in the developing relations between periodontics and cardiology. Following this the President of the APSP Dr Nanette Vergel de Dios declared the meeting open. The one-day program was very full, with 12 presentations from speakers from 9 countries in the region. In addition 43 posters had been selected for presentation from over 200 submissions.

A number of important issues were raised and discussed from these presentations. Major areas of discussion between speakers and delegates included periodontal diagnosis, etiopathology and molecular pathogenesis, antimicrobial therapy (including the use of natural products), periodontal regeneration, implants and epidemiology. There was at times some very good vigorous debate from the audience on these topics.

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 Procter & Gamble and the Sunstar Corporation is very gratefully acknowledged. Without this financial support the APSP meetings would not be possible. I also acknowledge the special support provided by my co-editors Professor Isao Ishikawa and Dr Dhandapani Arunachalam. As always we thank each of the presenters for providing their manuscripts for publication. Finally, this publication would not have been possible if it had not been for the excellent production editing provided by Ms Catherine Offler.

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Chapter 1

Exposition of the Conference Theme

N. Vergel de Dios

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Introduction

On behalf of the Executive Committee of the Asian Pacific Society of Periodontology, I welcome you all to Chennai, India. Time has gone so quickly and it has been two years since we last met in my home country, on the island of Mactan, Cebu, Philippines. Much has transpired around us since then. We saw how natural calamities like the tsunami in December 2004 that swept through our region and more recently the earthquake last October 9th that struck India, Pakistan and Afghanistan, had rendered us helpless and devastated. Likewise, the threat of terrorism not only in our region, but also in the United Kingdom and the United States of America continues to hound us. The second bomb attack in Bali, Indonesia once again shattered our peaceful existence. Internal political conflicts and unrest grips my country; other domestic problems may have beset your own individual countries as well. But life indeed goes on, and since we all share the common goal of continually uplifting the science of periodontology in our region, here we are gathered once again to try and understand how we can apply to clinical practice what we are able to observe in the laboratory.

Evidence-based clinical practice

The 21st century ushered in a shift towards evidence-based clinical practice. Developments in treatment procedures and techniques and the knowledge information explosion including changes in social and cultural patterns have all placed greater demands on clinical decision making (Worthington & Needleman 2005). Clinical decision making involves using the best available evidence to support the treatment options chosen by the clinician with the concurrence of, or in consultation with the patient (Needleman *et al* 2005). Needleman *et al* (2005), further contend that evidence-based dentistry, as an approach to patient care, needs to be substantiated by research data. It cannot however substitute for a well-developed clinical competence or skill (Needleman *et al* 2005).

Periodontal research

We have amongst us in the group, some of the best periodontal researchers in the region. Today, we will examine research directions and advances in periodontal aetiology, diagnosis, and clinical protocol as appropriate in the Asian Pacific region. Our speakers will also discuss the clinical applications of current research in the following areas: disease recognition and

prognosis, periodontal pharmacotherapeutics, and periodontal wound healing and regeneration.

Results of systematic reviews or research synthesis on different areas of periodontal research show that several variabilities and heterogeneities among studies exist and that there is a lack of data on long-term clinical outcomes and patient-centered outcomes (Trombelli 2005).

Osseointegrated oral implants

We have seen how osseointegrated oral implants dominated periodontal research in the early eighties up to the nineties and extensively changed the demeanor of clinical practice in recent times, particularly in the more developed countries of the world. In the Philippines, and elsewhere in the Asian region where oral implant placement procedures can be costly, where the average man earns US\$6 per day, and where no government subsidy could be expected for medical much less dental treatment, clinical practice and research has remained focused on disease control. It has even led to redirecting our efforts and attention back to oral health promotion and prevention of caries and periodontal diseases. Most of our clinical efforts are aimed at tooth retention rather than at extraction of periodontally involved teeth. To replace or substitute these extracted teeth with oral implants may never be considered as a treatment option because tooth substitutes and even fabrication of dentures and prosthodontic appliances to replace missing teeth are not at all affordable to the average dental patient.

Regenerative procedures

The disparity in our socio-economic status bespeaks of the way our specialty is practiced in the region. Researchers in the developed

countries like Australia which ranked number three of the Top 10 in the 2005 Human Development Index of the United Nations and Japan at number eleven, the only Asian country in the Top 20 of the same report (Contreras 2005), focus on reconstructive and regenerative procedures. Regenerative procedures like Guided Tissue Regeneration (GTR) can have additional benefit in terms of clinical attachment gain, probing depths reduction and bone defect fill. I address this question to all of you; will a 0.02 mm or a 2.6 and even up to a 2.9 mm attachment gain really matter to someone whose main concern is to provide for his children's education and which means him foregoing a dental visit to save for his children's tuition and school fees for the next semester?

Reconstructive procedures

I salute the men and women who have devoted time and great effort to discover methods to achieve reconstruction of osseous defects. The development of bone grafts and other biomaterials in the attempt to regenerate lost periodontal tissues is truly noble. The use of grafts and other biomaterials have shown greater bone fill as well as greater clinical attachment gains (Renvert *et al* 1985). However, Trombelli (2005) stated that the addition of a bone substitute to a periodontal membrane does not produce any further benefit in the management of intraosseous defects using GTR as culled from two available systematic reviews. While the shift in research focus of developed member countries had changed over the years to tooth substitutes, i.e. oral implants, among the less developed member countries of the APSP, research continues to be devoted towards tooth preservation and finding clinical procedures by which to control disease and preventing disease recurrence. The importance of supportive

clinical care to decrease the incidence of tooth loss cannot be overlooked.

Enamel matrix proteins

Studies on the effect of Enamel Matrix Proteins (EMP) as an agent for regenerating periodontal attachment apparatus have shown promising results in in-vitro studies (Rincon *et al* 2003, Yoneda *et al* 2003). Again, information on long-term clinical outcomes to confirm the effect of EMP on the stability of periodontal support and tooth survival needs to be ascertained (Trombelli *et al* 2005). To us in the less developed countries, we can only sit back and watch all these developments happen because our governments do not possess the financial capability to support any of our research. We envy you and wish that we too can contribute in all these developments.

The role of the Asian Pacific Society of Periodontology

At the end of today's presentations I hope each of us will be able to address the reason for our being here today. Where do we go from here? How do we help each other out with developing our specialty not only in our individual countries but in our region as a whole? To each of you a fruitful meeting ahead!

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Chapter 2

Directions in Periodontal Research in the Asian Pacific Region

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Introduction

Periodontal research has made great progress in the last two decades. Aside from traditional studies on the etiology, pathogenesis, clinical prevention and treatment of periodontal diseases, new fields, such as the association between periodontal and systemic diseases and the regeneration of periodontal tissues, have been investigated thoroughly. Asian Pacific countries have contributed more and more to these developments in recent years. To investigate periodontal research trends of Asian Pacific countries, the original papers published in three major periodontal journals between 2000 and 2004 were analyzed.

Country of origin analysis

The three major periodontal journals studied were *Journal of Periodontology* (JP), *Journal of Clinical Periodontology* (JCP), and *Journal of Periodontal Research* (JPR). During the period from 2000 to 2004, a total of 2008 original papers were published in these three journals. 879 articles were published in JP, 817 in JCP, and 312 in JPR. We determined which location these papers were from according to the first author's affiliation of each paper. Europe and North America were the most common locations providing

original papers on periodontal research. These two regions published a total of 552 (Europe: 258, North America: 294) original papers in JP, 710 articles (Europe: 571, North America: 139) in JCP, and 129 (Europe: 70, North America: 59) papers in JPR. These papers contributed 62.8%, 86.9%, and 41.3% of the original papers published in JP, JCP and JPR, respectively.

In the last five years from 2000 to 2004, 478 original papers from Asian Pacific countries were published in the three periodontal journals, which accounted for 23.8% of all the original papers. The number of articles published in JP, JCP and JPR was 239 (27.2%), 77 (9.4%), and 162 (51.9%), respectively. Asian countries published most of these articles. The number of papers from Asian and Oceanic regions in JP, JCP and JPR was 228 and 11, 64 and 13, as well as 148 and 14, respectively. More than half of these papers were from Japan. Japanese researchers published 247 articles, accounting for 51.7% of the papers from Asian Pacific countries from 2000 to 2004. Other Asian Pacific countries also published their study results in the three journals during this period. In JP, there were 112 (46.9%) papers from Japan, 42 (17.6%) from Israel, 25 (10.5%) from Korea, 23 (9.6%) from Taiwan, 9 (3.8%) from Australia, 7 (2.9%) from Thailand, 4 (1.7%) from Hong Kong, and 3 (1.3%) from China. In JCP, there were 31

Region	Europe	North America	Asia	South America	Oceania	Africa	Total
2000	47	67	53	12	4	0	183
2001	62	61	41	10	4	0	178
2002	47	50	39	15	3	0	154
2003	46	55	53	20	0	0	174
2004	56	61	42	31	0	0	190
Total	258 (29.4%)	294 (33.5%)	228 (25.9%)	88 (10.0%)	11 (1.3%)	0	879

Table 1. Number of articles from different regions in *Journal of Periodontology* (2000-2004)

Region	Europe	North America	Asia	South America	Oceania	Africa	Total
2000	90	25	14	2	2	2	135
2001	115	29	17	0	4	1	166
2002	131	33	12	5	2	1	184
2003	106	31	9	9	4	0	159
2004	129	21	12	9	1	1	173
Total	571 (69.9%)	139 (17.0%)	64 (7.8%)	25 (3.1%)	13 (1.6%)	5 (0.6%)	817

Table 2. Number of articles from different regions in *Journal of Clinical Periodontology* (2000-2004)

Region	Europe	North America	Asia	South America	Oceania	Africa	Total
2000	12	9	17	2	4	0	44
2001	9	10	27	3	4	0	53
2002	15	16	30	2	2	0	65
2003	21	14	43	7	4	0	89
2004	13	10	31	7	0	0	61
Total	70 (22.4%)	59 (18.9%)	148 (47.4%)	21 (6.7%)	14 (4.5%)	0	312

Table 3. Number of articles from different regions in *Journal of Periodontal Research* (2000-2004)

(40.3%) articles from Japan, 11 (14.3%) from Australia, 7 (9.1%) from Israel, 5 (6.5%) from Taiwan and Jordan, 3 (3.9%) from Hong Kong and Saudi Arabia, and 2 (2.6%) each from Korea, Thailand, China, New Zealand, and Sri Lanka. In JPR, there were 104 (64.2%) papers from Japan, 14 (8.6%) from Australia and Taiwan, 7 (4.3%) from Korea, 6 (3.7%) from Thailand, and 5 (3.1%) from Israel. Israel had a total of 54 articles published in the three journals, including 42 in JP, 7 in JCP and 5 in JPR. Taiwan totalled 42 articles published in the three journals, including 23 in JP, 5 in JCP and 14 in JPR. Both Australia and Korea published a total of 34 papers including 9 and 25 articles in JP, 11 and 2 articles in JCP, as well as 14 and 7 articles in JPR. From other Asian Pacific countries, the total number of articles published in the three journals is 15 from Thailand (JP: 7, JCP: 2, and JPR: 6), 10 from Hong Kong (JP: 4, JCP: 3, and JPR: 3) and 8 from China (JP: 3, JCP: 2, and JPR: 3) and 7 from Jordan (JP: 2, JCP: 5, and JPR: 0).

Impact factors

In the last 5 years, the average impact factors (IF) of JP, JCP and JPR were 1.614 ± 0.358 , 1.606 ± 0.115 , and 1.515 ± 0.289 , respectively. The impact factor of JCP was quite stable, listed as 1.426, 1.641, 1.736, 1.582, and 1.644 from 2000 to 2004 respectively. JP had an impact factor of 1.215 in 2000, and the highest IF of 1.935 in 2001. After that, the impact factor of JP showed a falling tendency as the IF was 1.854 in 2002, 1.499 in 2003, and 1.569 in 2004. On the contrary, the impact factor of JPR has increased steadily during the same period, from 0.946 in 2000 to 1.613 in 2001, 1.776 in 2002, 1.407 in 2003 and 1.831 in 2004. In 2004, the impact factor of JPR was ranked eighth among the journals of dentistry, oral surgery and medicine and was the highest (1.831) among the periodontal journals except *Periodontology 2000* which is a review journal.

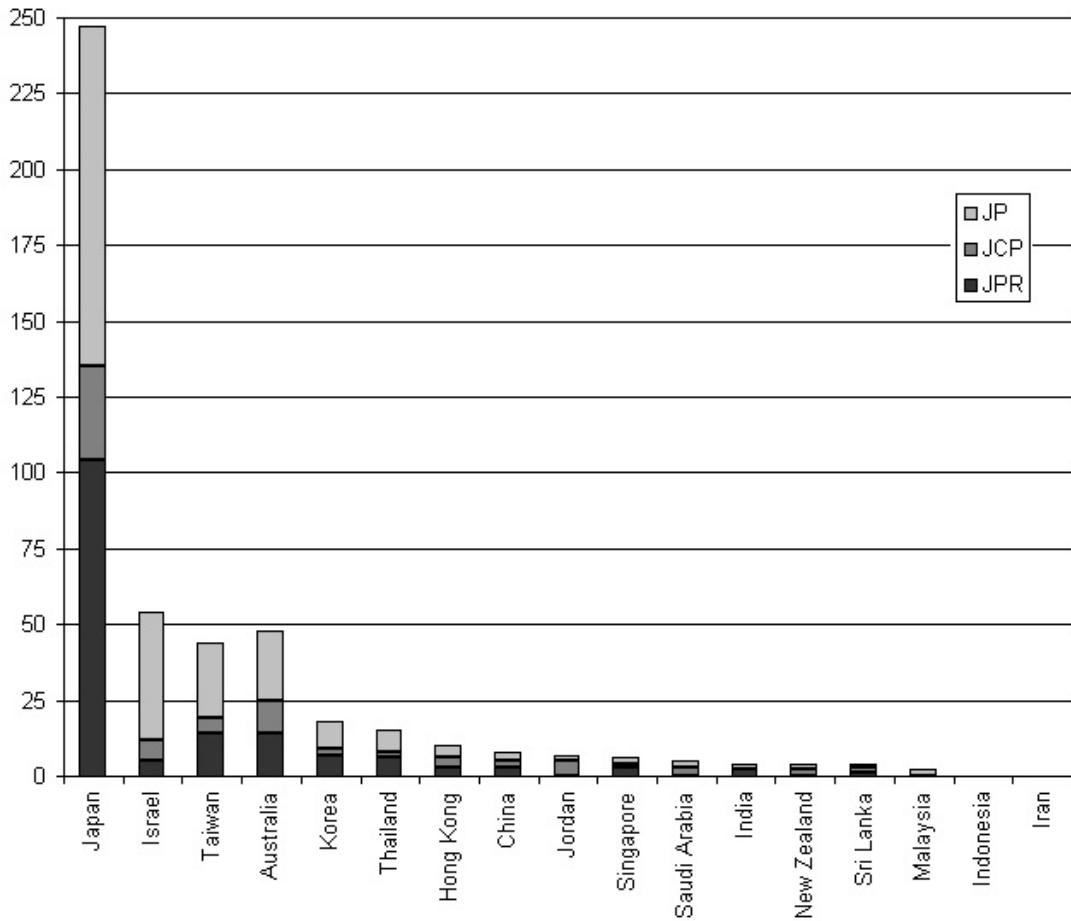


Figure 1. Number of articles published in Journal of Periodontology, Journal of Clinical Periodontology and Journal of Periodontal Research from Asian Pacific Countries (2000-2004)

Keyword analysis

We analyzed the keywords used in the 231 original papers from Asian and Pacific countries, except Japan, from which 247 articles were published in the last five years. The results showed that these papers employed wide variations of keywords and covered a broad range of research fields, including epidemiology, microbiology, immunology, biochemical research, animal studies, and periodontal surgery research.

Conclusions

In comparison to the previous 20 years, the research level and range of the Asian Pacific region has improved considerably. Asian Pacific countries are capable of performing quality periodontal research. Both the quality and quantity of their studies are comparable to those from Europe and USA. The Asian Pacific region has become an important part of the periodontal research community. Since its establishment in Brisbane in 1995, the Asian Pacific Society of Periodontology (APSP) has

played an important role in the mutual understanding and progress on international exchange of scientific and social activities in periodontology. Since its formation, APSP conferences have been held in Seoul, Korea (1997), Bangkok, Thailand (1999), Kuala Lumpur, Malaysia (2001) and Cebu, Philippines (2003). Now an APSP congress is being held in India for the first time with many participants. I hope this conference will have fruitful results and improve the understanding and communication of periodontal researchers from various countries and areas, which will be significant for future progress. In 2007, the next APSP meeting will be held in Beijing, China. I hope there will be more researchers to join us and share their research results and new ideas. The APSP will continue to contribute to the future activities of periodontal research not only in Asian Pacific regions, but also globally.

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Chapter 3

Future Potential Directions in Periodontal Research in the Asian Region

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Introduction

Periodontal research has been remarkably prolific in the last 30–40 years. Although early to moderate periodontitis affects about 50–60% of most populations, severe periodontitis affects 10–15% of the population, which can be a large number of individuals in any given population.

Periodontal diseases are caused by several microbial agents. The oral cavity is inhabited by approximately 500–700 bacterial species (Kazor *et al* 2003) and more than half of these cannot be cultivated. Only 20–30% of these species are considered to be pathogenic (Kornman *et al* 1997a). Microbiological research has identified some of the key pathogens that are implicated in periodontal diseases, the main ones being *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tanarella forsythensis* and *Treponema denticola*. However a susceptible host is necessary for the development of advanced disease even though bacteria are the primary aetiological factor.

Oral microbiota

Conventional culture-dependent techniques

produce limited analysis of the microbiota, resulting in many bacteria remaining uncharacterized. With the advent of molecular techniques based on isolation of DNA, polymerase chain reaction (PCR) amplification of the ribosomal RNA (rRNA) gene and sequence analysis of the cloned 16S RNA gene inserts, discovery of uncultivable organisms has become possible.

Quantification of periopathogens in subgingival plaque is required for understanding the etiologic role of these organisms. Conventional PCR/endpoint PCR technique detects the plateau phase of the reaction but is not suitable for quantification of the pathogens.

Real-time PCR allows monitoring of the exponential phase, facilitating the rapid detection and quantification of bacteria in plaque samples. It is anticipated that real-time PCR will become an indispensable method to aid diagnosis and elucidate the etiologic factors of periodontal diseases, aid prognostic judgement and evaluate treatment outcomes. However the real-time PCR technique is not particularly suitable to examining large numbers of samples for large numbers of different species.

Molecular identification techniques in new probe-target formats (e.g. the checkerboard DNA-DNA hybridization) allows enumeration of larger number of species in an enormous number of samples as first described by

Complex	Species
Red complex	<i>Porphyromonas gingivalis</i> <i>Tannerella forsythensis</i> <i>Treponema denticola</i>
Orange complex	<i>Campylobacter gracilis</i> <i>Campylobacter rectus</i> <i>Campylobacter showae</i> <i>Eubacterium nodatum</i> <i>Fusobacterium nucleatum</i> ssp. <i>nucleatum</i> <i>Fusobacterium nucleatum</i> ssp. <i>polymorphum</i> <i>Fusobacterium nucleatum</i> ssp. <i>vincentii</i> <i>Fusobacterium periodonticum</i> <i>Peptostreptococcus micros</i> <i>Prevotella intermedia</i> <i>Prevotella nigrescens</i> <i>Streptococcus constellatus</i>
Green complex	<i>Actinobacillus actinomycetemcomitans</i> serotype a <i>Campylobacter concisus</i> <i>Capnocytophaga gingivalis</i> <i>Capnocytophaga ochracea</i> <i>Capnocytophaga sputigena</i> <i>Eikenella corrodens</i>
Yellow complex	<i>Streptococcus gordonii</i> <i>Streptococcus intermedius</i> <i>Streptococcus mitis</i> <i>Streptococcus oralis</i> <i>Streptococcus sanguis</i>
Purple complex	<i>Actinomyces odontolyticus</i> <i>Veillonella parvula</i>
Other species	<i>Actinobacillus actinomycetemcomitans</i> serotype b <i>Actinomyces naeslundii</i> genospecies 2 (<i>A. viscosus</i>) <i>Selenomonas noxia</i>

Table 1. Microbial complexes in subgingival plaque

Socransky *et al* (1994). Socransky *et al* (1998) used 40 species-specific DNA-DNA hybridization probes and detected that subgingival plaque contains organisms in 5 major complexes using cluster analysis (Table 1). At present, a Human Oral Microbe Identification Microarray (HOMIM) slide system is under development in order to identify almost all of the 600-700 species in the oral cavity (Boches *et al* 2004).

Host response

Even though bacteria are the primary aetiological factor, a susceptible host is necessary for the development of advanced disease. The resulting immuno-inflammatory response in the periodontal tissues leads to their destruction. The nature of the host response is influenced by genetic risk factors as well as environmental and acquired risk factors, e.g. smoking (Figure 1). The host response is basically protective in nature, however hypo- and hyper-responsiveness of the host response influenced by genetic factors cannot contain the microbial challenge and results in enhanced tissue destruction.

In the 1980s and 1990s, research focussed on mediators of the periodontal inflammatory response to the presence of plaque microorganisms, which included prostanoids (prostaglandin E₂) and cytokines (interleukins and tumour necrosis factors) as well as matrix metalloproteinases (host derived enzymes) and oxygen radicals liberated in the tissues.

The majority of tissue damage occurs as collateral damage resulting from the activation of the host responses triggered by the presence of plaque bacteria.

The IL-1 gene polymorphism has also been investigated for its role in periodontitis (Kornman *et al* 1997b). Patients with one particular polymorphism produce more IL-1 than normal (hyper-responders) and hence are more at risk for periodontal breakdown.

Genetics is a current area of intense research in periodontology in an attempt to identify the genetic traits that place patients at risk for disease so that they can be identified and monitored and preventive steps taken before advanced disease occurs.

Root instrumentation

Having noted that the etiology of periodontal diseases is mainly the dental biofilm, an essential component of treatment is the disruption and elimination of this bacterial complex or regulation of the bacterial ecology. This has largely been achieved by non-surgical mechanical therapy (scaling and root planing) either manually or machine-driven in the initial treatment phase. Root surface instrumentation is the cornerstone of periodontal treatment based on its effectiveness and measurable clinical endpoints which include clinical attachment level, probing pocket depth, bleeding on probing and alterations in the composition of the subgingival microflora. It is imperative to disrupt the subgingival biofilm mechanically and reduce the bacterial bioburden.

Anti-infective treatments - pharmacological agents

Mechanical debridement of root surfaces is difficult, time-consuming and sometimes ineffective due to tooth or root anatomic restrictions and deep pocket probing depth. These setbacks have motivated the introduction and investigation of locally delivered adjunctive pharmacological agents and drug delivery systems, in recent years involving sustained release vehicles.

Systemically administered antibiotics may also be used and are necessary as adjuncts, particularly in the control of advanced chronic and aggressive periodontitis cases.

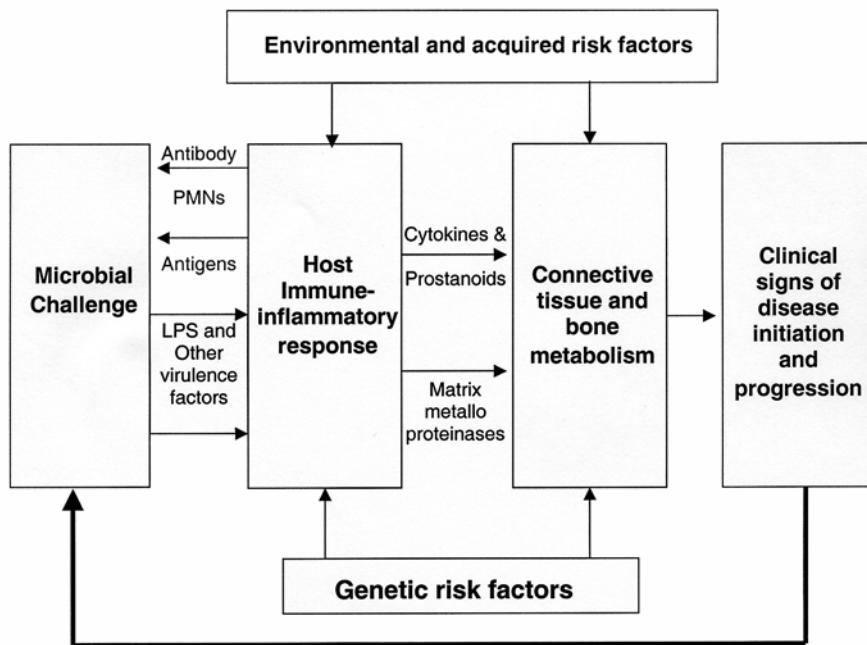


Figure 1. Factors influencing the host response (Adapted from Kornman *et al* 1997).

In the Asian region, the guidelines suggested by the American Dental Association (Imrey *et al* 1994) for determining efficacy of products to supplement scaling and root planing in professional, non-surgical treatment of adult periodontitis could be followed.

Recommendations have been made in the broad areas of:

- Basic study design (parallel-arm or split mouth) with concurrent positive controls
- Clear objectives
- Subject and periodontal site selection
- Clinical conduct
- Choice of outcome variables (CAL/bone support as primary outcomes; probing pocket depth, bleeding on probing, gingival crevicular fluid constituents, microbial assays as secondary outcomes)
- Statistical management and analysis of the data
- Inclusion and exclusion criteria

- Protocols with adequate monitoring against possible systemic and local safety hazards

However the guidelines suggested are viewed as a basis for an evolving document rather than a static standard. Hence certain aspects of the study protocol could be adapted.

Various herbal or traditional concoctions used in the Asian Pacific region should be investigated for their efficacy. Studies should be randomised and blinded (preferably double-blinding) with a trial period of 6 months (clinical attachment gains can take 6 months to stabilize).

Surgical treatment

Periodontal surgery was the most commonly published research area for randomised control trials in periodontitis during 1980-2000. This was the era of novel technologies for regenerative surgery using

enamel matrix proteins and guided tissue regeneration, in combination with various tissue engineered membranes and bone replacement materials. This was also the era of the introduction and investigation of periodontal plastic surgical procedures for the correction of mucogingival problems.

Periodontal research

The frequency of clinical trials in periodontal surgical procedures was followed by studies investigating different approaches for the prevention and treatment of gingivitis and plaque (non-antibiotics) as well as antibiotic regimes.

An important area where randomised control trials in periodontitis was scarce was the use of various diagnostic techniques. Hence future research should give priority to epidemiology, etiology, host responses and clinical periodontal interventions, areas where evidence from randomised control trials are still scarce and high quality trials are of importance. It is easier said than done, especially in the Asian communities, where resources are limited and fair support from industries and health organisations is urgently needed and ought to be sought.

In clinical periodontology, novel treatment procedures, be they non-surgical or surgical, are frequently introduced, however there is a lack of evidence-based reports on the efficacy of these strategies and conflicting reports are occasionally made. In previous systematic review studies (Sjogren and Halling 2000, Sjogren and Halling 2002) it was found that randomised control trials represented just a fraction of all publications in dental research, i.e. only 1 out of 200 dental research publications in the 1980s. This number increased in the 1990s, as a result of debate raised by evidence-based medicine organisations. Hence it is suggested that randomised control trials elucidate and verify

these trends, not only in periodontal treatment but periodontal research as a whole.

The United States of America had the largest number of randomised control trials in periodontitis during 1988-2000 (39%, n = 227), followed by United Kingdom (11%, n = 66). In the Asian Region, Japan had 8, Australia 5 and Thailand 4 (Sjogren and Halling 2002). Although the number of periodontal research publications in clinical trials and randomised control trials in periodontitis increased in the last decade worldwide, the Asian region still lags behind. There could be a number of reasons for this:

- 1 Insufficient funds to conduct research
- 2 Lack of expertise in periodontal research
- 3 Lack of collaboration between the clinicians and basic science researchers
- 4 Lack of multicenter studies, where transfer of knowledge could occur and larger manpower could be available with a greater pool of suitable patients to meet the objectives of the study.

Conclusion

Periodontology is still in a phase of discovery and rapid development. Amidst the many queries and uncertainties regarding etiology and pathogenesis, changes in the management of the disease and new measurement outcomes are developing simultaneously in the light of technological advances available for periodontal research. The needs of the population at large are benefited by new knowledge in preventing and curbing disease. The ultimate aim is to seek the best management option at the individual patient level, and at the macro level to improve public dental health.

The Asian Pacific Society of Periodontology should appoint and form the collaborating centers necessary to initiate and promote these research activities which constitute an important step towards future research in this

region. However, research in the Asian region should be geared to multicenter studies with the collaborating centers sharing centralized study management.

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Chapter 4

Research Advances in Periodontal Etiopathology

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Introduction

Periodontal diseases are amongst the most common inflammatory diseases of humans. They are characterized by bacteria-induced inflammatory destruction of tooth-supporting tissues. Gingivitis is clinically characterized by gingival redness, edema, bleeding, change in color, loss of tissue adaptation to tooth surface, and increased flow of gingival crevicular fluid (GCF) (American Academy of Periodontology, AAP 1999). It may remain contained to the marginal gingival tissues or it may develop into periodontitis with nonreversible destruction of periodontal attachment and alveolar bone, which eventually results in tooth loss. In general, gingivitis precedes periodontitis, but not all gingivitis develops into periodontitis (Lindhe *et al* 1973, Listgarten *et al* 1985, Löe *et al* 1986). A number of epidemiological studies have shown that while periodontal diseases are prevalent in the population, advanced loss of tooth-supporting tissues only affects limited amounts (usually less 20%) of the population in both developed and developing countries, despite dental plaque being a common finding in a majority of the population (Löe *et al* 1986, Holmgren *et al* 1994, Söder *et al* 1994, Baelum *et al* 1996, Papapanou 1996). Studies on the nature of progression of periodontitis have

revealed that the overall pattern of progression is episodic and infrequent with active and quiescent phases, rather than the previously assumed linear fashion. For the majority of periodontitis subjects, most of the diseased sites are relatively stable at any given time, and only a small proportion exhibit active disease progression (Lindhe *et al* 1983, Socransky *et al* 1984). It is conceivable therefore that active periodontitis could occur more frequently and affect multiple sites in susceptible individuals.

Existing evidence in periodontal pathogenesis has demonstrated that periodontal diseases are initiated and perpetuated by a group of predominantly gram-negative and anaerobic bacteria that colonize the subgingival environment. It has become apparent that although bacteria are essential, they are insufficient by themselves for periodontitis to occur and for the disease outcome. Instead, the severity of periodontal diseases is dependent upon a dynamic equilibrium of interactions between microbial challenge and host immuno-inflammatory response. These events are significantly influenced by genetic, environmental or acquired disease modifiers (Page *et al* 1997). This notion is fundamentally important to further understanding the concepts of the etiology, pathogenesis, prevention, diagnosis and treatment of periodontal diseases.

Over the past two decades, substantial new

findings have been obtained regarding the etiopathogenesis of periodontal diseases. The emergence of novel findings and concepts can be well reflected in the recognition of dental plaque as a biofilm; identification and characterization of periodontopathogens including novel microbes and bacterial virulence factors in subgingival biofilm; appreciation of the importance of host-microbe symbiosis and interactions in periodontal health and diseases; molecular mechanisms of periodontal destruction; and the impacts of various risk factors on bacteria-host interactions. This article briefly reviews the current findings and novel concepts regarding the etiology and pathogenesis of periodontal diseases.

Dental bacterial plaque as a biofilm

It has been proven that dental plaque exists in the form of a microbial biofilm (Marsh and Bradshaw 1995), which is defined as “matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces” (Costerton *et al* 1995). With respect to the molecular organization, physicochemical properties and growth characteristics, biofilms are considered etiological communities that evolved to permit survival of the community as a whole and to allow species growth, enhanced virulence and evasion of host defense mechanisms. The structure of a biofilm is characterized by varying areas of high and low bacterial biomass interlaced with aqueous channels of different size (Costerton *et al* 1995, Costerton and Lewandowski 1997) which provide nutrients for the bacterial colony. GCF is the main nutritional component found in the ecosystem accounting for the predominance of asaccharolytic species (Darveau *et al* 1997). Some of the crucial properties of biofilm bacteria which have been identified and characterized include cell to cell communication through quorum sensing or so called cell density-mediated gene expression, gene

transfer, regulation of gene expression, and antimicrobial resistance (Tatakis and Kumar 2005). Of these, the property of antimicrobial resistance allows bacterial cells in the biofilm to develop and enhance resistance to host antimicrobial mechanisms like phagocytosis and antimicrobial agents, which should be taken into account in clinical practice. In this regard, periodontitis is one of the most unusual opportunistic infections where an oral biofilm forms on non-shedding tooth surfaces which could render the host defenses and antimicrobial therapy ineffective (Socransky and Haffajee 1997).

Periodontopathogens and bacterial virulence factors

The primary role of bacteria in the etiology of most forms of inflammatory periodontal diseases has been well established (AAP 1996). Over 500 different species have been identified in subgingival biofilm, but of these only approximately 20-30 species are considered to be of pathogenic significance in periodontal diseases. It is believed that only a limited number of these species play a major pathogenic role in the disease (Dahlén 1993). The transition from gingival health to gingivitis and then periodontitis is associated with an increased total number of subgingival gram-negative anaerobic bacterial species (Van Winkelhoff 1998). Specific microorganisms associated with periodontal health, gingivitis and various forms of periodontitis have been extensively reviewed recently (Tatakis and Kumar 2005) and it seems that different periodontal diseases have somewhat unique profiles of associated bacteria. However, the characteristics of microbiological progression from periodontal health to gingivitis, and eventually to periodontitis are vast and complicated (Moore *et al* 1982). Seminal studies on cluster analysis of subgingival plaque biofilm by Socransky and Haffajee and their

colleagues (1998) have shown that certain specific species frequently occur together in groups or complexes with a color-coded identity. These represent bacterial consortia that are associated with the microbiological transformation from 'beneficial biofilms' in periodontal health, characterized by predominantly gram-positive, aerobic and non-motile microflora, to 'pathogenic biofilm' in periodontitis, characterized by a gram-negative, anaerobic and motile microflora. For details, the readers are referred to Socransky *et al* (1998), Holt and Ebersole (2005) and Socransky and Haffajee (2005). The 'red complex' comprises the three major periodontopathogens, i.e. *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* (previously *Bacreroides forsythus* or *Tannerella forsythensis*). It has been suggested that the 'red complex' presents as a portion of the climax community in the subgingival biofilms which are strongly associated with progressing periodontitis (Socransky *et al* 1998, Holt and Ebersole 2005).

Most of the periodontopathogens concerned exhibit particular virulence factors (Darveau *et al* 1997, Ishikawa *et al* 1997). In the past decade, the study of potential virulence factors produced by periodontal pathogens is a very active area of research. Most of the studies that have investigated virulence factors of known or presumed periodontal pathogens have examined factors produced by *P. gingivalis* (Haffajee and Socransky 2005). The readers should refer to Holt and Ebersole (2005) and Tatakis and Kumar (2005) for more detailed reviews of virulence factors of periodontopathogens.

In general, the expression of virulence is related to various factors including the nature of the microbes, infection patterns, characteristics of the environmental niche and the host responses to the microbial complexes in the biofilm. The common specific virulence

factors associated with periodontopathogens consist of lipopolysaccharide (LPS), heat shock proteins, extracellular proteolytic enzymes, fimbriae, outer membrane proteins, leukotoxin, flagellum, and capsule. Of these, LPS has been intensively studied. It is produced by most gram-negative bacteria, such as *P. gingivalis*, *T. denticola* and *T. forsythia*, and it is a primary inducer of chronic inflammatory diseases and septic shock. LPS is also the best-characterized pathogen-associated molecular pattern (PAMP) able to trigger host inflammatory response by inducing the release of inflammatory mediators and cytokines, such as interleukin (IL)-1 α , IL-1 β , tumor necrosis factor- α (TNF- α), IL-8, and prostaglandin E₂, through CD14 and toll-like receptor-mediated activation of neutrophils, macrophages and fibroblasts. The other effects on host cells deal with induction of nitric oxide secretion, activation of osteoclasts and stimulation of T-helper cell proliferation. It has been shown that unlike other bacteria, *P. gingivalis* LPS does not initiate an immediate innate host inflammatory response and in fact suppresses the innate inflammatory response to bacteria by inhibiting E-selectin expression, which may represent a new virulence factor for this organism (Darveau *et al* 1995, Darveau *et al* 1997, Reife *et al* 1995). Heat shock protein 60 (hsp60) has been increasingly recognized as a crucial molecule in infectious and autoimmune diseases. It enabled stimulatory activity similar to LPS derived from the bacteria. A recent study has shown that it was abundantly expressed in periodontitis lesions and it has therefore been postulated that periodontopathogens stimulate the cells in the periodontium to up-regulate the expression of hsp60, which in turn may stimulate macrophage and possibly other cells to produce pro-inflammatory cytokines, which could contribute to the chronicity and tissue destruction of periodontal disease (Ueki *et al* 2002). It has been shown that the hsp from *Actinobacillus*

actinomycetemcomitans could activate osteoclasts and stimulate epithelial proliferation (Paju *et al* 2000). Traditionally, potential virulence factors produced by pathogens were studied using bacterial cells that were grown *in vitro*. It has recently been realized that a number of pathogens express virulence genes only when they are in their human or animal host (Haffajee and Socransky 2005). An unique and ingenious approach to detecting and distinguishing such virulence factors as well as examples of its use in analyzing virulence factors for periodontal disease has recently been described by Handfield and coworkers (2005).

Novel microbes in subgingival biofilm

It is estimated that at least 10^{14} normal or commensal microbes reside on the surfaces of skin, teeth, dentures, dental restorations, prosthetic implants, as well as the mucosal epithelia lining of oral cavity, respiratory, gastrointestinal, and urinary tracts (Cohen and Slavkin 2000). The oral cavity contains approximately six billion microbes representing 500-700 species (Socransky and Haffajee 1994, Wilson *et al* 1997, Paster *et al* 2001). Up to 300 oral bacterial species can be cultured from oral plaque samples, yet it is estimated that another 300 species are uncultivated. Under certain conditions these commensal organisms could become opportunistic pathogens contributing to local and/or systemic infections. Recent evidence has increasingly shown that oral opportunistic pathogens and their resultant oral infections, for instance periodontitis, have been implicated in a number of systemic diseases or disorders, e.g. endocarditis, coronary heart disease, cerebral infarction (or stroke), diabetes, pre-term birth, and aspiration pneumonia (Williams and Offenbacher 2000, Jin *et al* 2003). Our recent studies showed that microbes normally inhabiting non-oral niches could be found from

dental plaque specimens of systemically compromised subjects (Leung *et al* 1998, Leung *et al* 2003). Knowing this, the human oral microbial ecosystem would therefore be highly relevant for the diagnosis and treatment of oral opportunistic infections and related systemic diseases (Paster *et al* 2001).

Environmental surveys based on acquisition of phylogenetically useful microbial sequences, such as that of the 16S rRNA gene, have revealed a great deal of previously unsuspected bacterial and archaeal diversity. In most instances, cultivated members represent <1% of the total extant population (Kroes *et al* 1999). In recent years, the human subgingival crevice has been the focus for intensive studies, as cultivation methods woefully under-represent the true extent of microbial diversity. The current best approach for exploring unidentified species is based on isolating DNA from the target environment, PCR amplifying the rRNA gene, cloning the amplicons into *E. coli*, and sequencing the cloned 16S rRNA gene inserts (Paster *et al* 2001). Recently, a number of novel oral phylotypes (e.g. uncultivated division of TM7) have been identified from periodontitis specimens by molecular phylogenetic methods, such as methanogenic *Archaea*, *Desulfobulbus* sp oral clone R004, *Deferribacteres* sp oral clones BH017 and D084, and *Bacteroides* sp oral clone AU126 (Kroes *et al* 1999, Dewhirst *et al* 2000, Kulik *et al* 2001, Paster *et al* 2001, Kumar *et al* 2003, Lepp *et al* 2004).

Moreover, viruses have also been considered potential members of the oral microbial ecosystem. Human cytomegalovirus, herpes simplex virus and Epstein-Barr virus have been detected in subgingival plaque by nested- or real-time-PCR assay and they were associated with the severity of periodontal disease (Slots *et al* 2003, Kubar *et al* 2005). In general, herpes virus infection can increase the incidence of bacterial and fungal infections, aggravate the severity of concurrent microbial

infections, and accelerate the *tempo* of infectious disease progression (Boeckh and Nichols 2003). Viruses and bacterial pathogens may also act synergistically in oral infectious diseases and further study of these is warranted.

Host-microbe symbiosis and interactions in periodontal health and diseases

The indigenous microbes of humans consist of a number of microbial communities, each with a composition characteristic of a particular body site. It has been estimated that there are ten times more bacteria colonizing a human than the number of human cells in the body, i.e. 10^4 versus 10^3 (Wilson 2005). In the past decade, great progress has been made in further understanding of human-microbe symbiosis (Wilson 2005). In the past, investigations of the bacterial-host interaction focused upon pathogenesis. Currently, it is believed that representative interactions are not pathological but symbiotic. In fact, both humans and bacteria benefit from peaceful coexistence and microbial colonization plays an obligatory role in human health (Tuomanen 2005).

In the context of periodontology, it has recently been proposed that bacteria and their products are a necessary and beneficial component of a healthy periodontium, with the evidence that clinically healthy periodontal tissue contains a highly orchestrated gradient of select inflammatory mediators. These play a key role in the defense of periodontal tissues and the overall health of the individual and these mediators are made in response to a highly specific microbial consortium residing on the tooth surface (Roberts and Darveau 2002). It is conceivable that balanced and appropriate interactions of beneficial microbes and periodontal tissues contribute to a healthy and functional periodontium. Two conceptual advances in the field of immunology have

provided a framework to understand this issue, i.e. the human immune system has evolved to recognize threats (Matzinger 1998) and the theory of pattern recognition originally proposed by Janeway (1992) to explain how the innate host defense system evolved a mechanism to recognize microbes immediately and mount a protective response. The latter proposes that the innate defense system recognizes common conserved structures of different microbes, such as LPS, rather than the specific microbes; and a group of designated pattern recognition receptors, such as lipopolysaccharide binding protein (LBP), CD14, and the Toll-like receptor family (TLR), continually monitor the state of host microbial colonization and elicit appropriate host responses depending upon the microbial structures detected. Pattern recognition receptors therefore provide a link facilitating a molecular dialogue between the commensal bacteria and the appropriate host response (Roberts and Darveau 2002).

It has become increasingly clear that the innate immune system has a much more important and fundamental role in host defense (Medzhitov 2000). Innate host responses are initiated by a variety of microbial PAMPs like LPS and subsequently modulated by LBP, CD14, TLR superfamily (e.g. IL-1RI, TLRs-2 and 4) and MD-2 that recognize various PAMPs and subsequently initiate the transduction of transmembrane signaling cascades through mediation of adaptive proteins, such as MyD88, MyD88-adaptor-like (Mal), IRAK family, TRAF-6 and downstream signaling, leading to the activation of nuclear factor- κ B (NF- κ B) and eventually induction of cytokine gene expression (Aderem and Ulevitch 2000). It has been appreciated that LBP, sCD14 and BPI play important roles in facilitation of neutralizing and clearance of LPS by cells through formation of LPS-LBP-sCD14 complex or BPI-LPS aggregates without leading to cellular activation (Tapping and Tobias 1997, Weiss 2003).

Pattern recognition molecules and antimicrobial peptides in periodontal health and disease – Our recent work

Our recent *in vivo* and *in vitro* studies have focused upon the identification and characterization of common pattern recognition molecules that could be crucially important for periodontal health. We firstly detected sCD14 in GCF and found its levels were significantly higher than those in serum, indicating that this pattern recognition receptor could be produced locally in response to microbial challenge. Furthermore, higher levels of sCD14 in GCF were associated with fewer and shallower periodontal pockets (Jin and Darveau 2001). This study shows that sCD14 may serve a protective role in local host response to bacterial challenge. A descriptions of the expression profile of mCD14 in gingival tissues was undertaken. mCD14 protein and mRNA were commonly detected in healthy or diseased gingival tissues. The mCD14-positive cells were mainly confined to the gingival epithelium-connective tissue interface. Expression levels in periodontally healthy subjects were significantly higher than in the patients. Within the patients, clinically healthy tissues showed greater levels of mCD14 than periodontal pocket tissues and granulation tissues. These findings suggest an important role of mCD14 in the mediation of effective immuno-inflammatory responses to bacterial challenge (Jin *et al* 2004). LBP is an acute-phase reactant, predominantly derived from the liver. It may serve to both neutralize LPS and enhance its biological activities of cellular activation. We recently discovered that LBP protein and mRNA can be locally expressed in gingival epithelia and its expression was mainly confined to the cytoplasm of granular and keratinized layers of gingival epithelium, spreading from the oral sulcular epithelium to oral epithelium with the expression density decreasing gradually from coronal to apical

portion (Ren *et al* 2004). Furthermore, it was also observed that LBP mRNA was more frequently expressed in healthy tissues than in diseased tissues and the expression levels of LBP protein were higher in periodontally healthy tissues than in diseased tissues. It could be speculated that local expression of LBP in gingival tissues might contribute to periodontal homeostasis. Moreover, our recent *in vitro* study revealed that recombinant human LBP (rhLBP) could significantly down-regulate the expression of both mRNAs and peptides of IL-6 in the presence or absence of *E. coli* LPS, and suppress the up-regulated expression of TLR-2 and -4 by *E. coli* LPS (Ren *et al* 2005a). Further studies are warranted to clarify the molecular mechanisms of LBP in regulation of cytokine expression by host cells and to elaborate the relevant clinical implications. Our most recent study further explored the potential interrelationship of *in vivo* expression of LBP and mCD14 in human gingival tissues as well as the co-expression of TLR-2 and -4 in association with periodontal health and disease (Ren *et al* 2005b). A positive correlation was found between LBP and mCD14 peptides in both detection expression and expression levels of these relevant molecules. In diseased tissues, TLR-2 was detected in both pocket epithelia and macrophage-like cells in connective tissues; while TLR-4 was predominantly detected in connective tissues. However in healthy tissues, only a weak expression of TLR-2 was found in gingival epithelia and no TLR-4 expression was detected. In periodontal pocket tissues, mCD14 was co-detected on CD68-labelled macrophages in the underlying connective tissues of pocket epithelium as well as on CD1a-labelled dendritic cells in the pocket epithelium and connective tissues interface. No similar expression profile was detected in healthy tissues from patients and those from periodontally healthy control subjects. These novel findings on an altered cellular expression profile of mCD14 and TLR-2 and -4 in

periodontal pocket tissues imply that these pattern recognition receptors may play a crucial role in periodontal pathogenesis.

It is now well recognized that gingival epithelia serve not merely as physical barriers to microbial challenges, but rather as reservoirs of antimicrobial peptides which enable them to survive under normal as well as harsh environmental conditions. Of the various attributes contributing to innate immunity, a group of well-evolved and conserved antimicrobial peptides, human α - and β -defensins, which are detectable in gingival epithelia, are now considered to be of major importance in innate host defense (Dale 2002). The expression pattern of α - and β -defensins has been described that α -defensins are usually located in the junctional epithelium produced by neutrophils, while β -defensins are distributed in sulcular and oral epithelia, suggesting defensins serve different roles in various regions of the periodontium (Dale *et al* 2001). We recently further described the expression patterns of human β -defensins (hBD) 1-3 in both periodontal health and disease (Lu *et al* 2004, Lu *et al* 2005). The expression of both hBDs-1 and -2 peptides was mainly confined to the granular and spinous layers of gingival epithelium, while hBD-3 peptides were mainly detected in the basal layer in health and the expression extended from the basal layer to the spinous layers in diseased condition. hBD-3 peptide was expressed not only in gingival keratinocytes but also in Langerhans cells and Merkel cells. Furthermore, periodontally healthy tissues expressed higher levels of hBD-2 peptides than clinically healthy tissues from periodontitis patients. These data suggest that appropriate expression of hBDs-1 and -2 may contribute to maintenance of periodontal homeostasis, while hBD-3 peptides may contribute to the maintenance of periodontal homeostasis, possibly through its antimicrobial effect and promotion of adaptive immune responses.

Taken together, our data suggest that an appropriate expression and regulation of host pattern recognition receptors (LBP, sCD14, mCD14, TRL-2 and -4) and local antimicrobial constituents (hBDs) are crucial for maintenance of periodontal homeostasis, despite the relevant regulation mechanisms and intracellular signaling pathways are not fully understood.

Molecular mechanisms in periodontal destruction

Basic principles of infectious diseases indicate that disease expression is a combination of host, microbial agents and environment factors. In the light of the new paradigm of periodontal pathogenesis, it is believed that bacterial flora is necessary but not sufficient for expression of periodontal diseases and there are many other host response factors and environmental/genetic factors which dramatically modify the disease outcome (Page *et al* 1997). The current paradigm of periodontal pathogenesis has shifted and this places renewed emphasis on the host response factors. The complex interplay between the bacterial challenge and innate and acquired host response factors determines the disease outcomes, i.e. the conversion of junctional epithelium to pocket epithelium, formation of periodontal pocket, destruction of periodontal attachment, and alveolar bone loss. It is known that bacterial biofilm can directly cause periodontal injury and that bacteria elicit the most periodontal destruction through indirect mechanisms such as initiation and up-regulation of host destructive inflammation, especially in a periodontally susceptible individual (Page *et al* 1997). With an ongoing microbial challenge, the periodontium is continuously exposed to virulent bacterial components which could alter local cell functions and phenotypes. The host defense cells which are significantly involved are

neutrophils, macrophages, lymphocytes and plasma cells. Meanwhile, complex interactions exist between the defense cells and structural/resident cells including epithelial cells, fibroblasts, osteoblasts and osteoclasts, as well as structural components including various collagens and non-collagenous proteins, through up-regulated cytokines and inflammatory mediators like PGE₂. The total impact of the above alterations is to shift the host response from one in which the host could contain the bacterial challenge to one in which the infection is no longer under control while destructive inflammation is predominant (Kornman *et al* 1997a). The dynamic events of periodontal pathogenesis are determined primarily by the signaling and regulating molecules (e.g. cytokines and prostaglandins) that direct cellular functions. It has been proposed that active periodontitis is characterized by high levels of IL-1 β , TNF- α , INF- α , PGE₂ and MMPs, and low levels of IL-10, TGF- β , IL-1ra and TIMPs that suppress the immuno-inflammatory response and maintain homeostasis (Page *et al* 1997). It is realized that under these pathological conditions, defense cells and structural cells such as fibroblasts are activated in an uncontrolled manner and they produce large amounts of MMPs and pro-inflammatory cytokines and mediators, whilst decreasing the production of TIMPs, resulting in tissue destruction. Subsequently, pro-inflammatory cytokines and PGE₂ mediate hyperactivity of osteoclasts whilst suppressing the osteoblast activity, leading to the resorption of alveolar bone.

Genetic defects and host susceptibility

As mentioned above, although bacteria are essential, they are insufficient for disease to occur nor directly responsible to the severity of the disease, while a susceptible host is necessary for determination of the severity of the disease. It is believed that the disease

severity is rather dependent upon a dynamic equilibrium of bacteria-host interactions which are significantly influenced by various genetic and environmental factors (Page *et al* 1997, Kinane *et al* 2005). In this regard, periodontal disease is obviously a multi-factorial complex disease. It is well evident that severe forms of periodontal disease affect a minority of the subjects (Löe *et al* 1986). A range of risk factors which have been studied include subject determinants, social and behavioral factors, systemic factors, genetic factors, tooth factors and microbial risk factors (Nunn 2003). Emerging evidence also shows that periodontal infection *per se* may be a potential risk factor for systemic diseases like cardiovascular disease (Jin *et al* 2003).

The first convincing demonstration that not everybody is at equal risk to periodontal destruction from periodontitis, despite inadequate oral hygiene and hence exposure to microbial dental plaque, came from an Asian study. Löe and co-workers identified three subgroups within the studied subjects on the basis of periodontal destruction, which were labelled "no progression", "moderate progression" and "rapid progression" subgroups (Löe *et al* 1986). It is now appreciated that the existence and combinations of various factors may significantly account for an individual's risk for periodontal destruction. The current risk factors include subject characteristics; social and behavioral factors, such as tobacco smoking, socio-economic status, nutrition and psychological factors; systemic factors, such as diabetes mellitus, drugs, HIV; genetic factors considering genotype polymorphisms; tooth-level factors, such as anomalies and poor restorations and the microbial composition of dental plaque featuring the holy triumvirate of *A. actinomycetemcomitans*, *T. forsythensis* and *P. gingivalis* (Nunn 2003); and other emerging risk factors, such as obesity (Saito *et al* 2001). Of these, tobacco smoking is believed to be

the most important environmental risk factor for periodontitis (Kinane and Chestnutt 2000).

In recent years, genetic factors as a crucial determining risk for periodontitis have received considerable attention among periodontal researchers. For details, the readers are referred to an excellent recent review by Kinane *et al* (2005). Genetic defects may significantly predispose an individual to severe periodontitis. The current genetic factors include defects of phagocytosis resulting in a hypo-response to the bacterial challenge, or enhanced production of cytokines, prostaglandins, and MMPs, in response to bacterial challenge. There is clearly a fine balance in the nature of the inflammatory response to the presence of plaque, and both under-activity (hypo-responsiveness) and over-activity (hyper-responsiveness) of components of the host response can result in increased susceptibility to disease and destruction of periodontal tissues (Preshaw *et al* 2004). It has been shown that variations in genotypes of inflammatory cytokines and mediators may be independent risk factors for severe periodontitis, such as IL-1 (Kornman *et al* 1997b, Kornman and di Giovine 1998) and other cytokine polymorphisms (e.g. IL-6, IL-10 and TNF), receptor polymorphisms (e.g. R γ RIIa, VDR-TaqI) and other host response polymorphisms (e.g. HLA-DQ β , MMP-1-2G). We recently found that a single nucleotide polymorphism in the MMP-1 promoter region of -1607 bp may be associated with generalized aggressive periodontitis in a Chinese population (Cao *et al* 2005).

Of the gene polymorphisms above, the IL-1 genotype has been intensively investigated. Some studies show that the subjects with the IL-1 composite genotypes of allele 2 of both IL-1 α (+4845) and IL-1 β (+3954) produce more IL-1 than the controls and are at a relatively high risk for developing severe periodontitis than those individuals without the target genotype. Other studies in different

populations have questioned the general usefulness of this test in clinical practice in various ethnic populations. It has been reported that the prevalence of the IL-1 composite genotype was lower in Chinese (2.3%), suggesting the usefulness of IL-1 composite genotype for determining susceptibility in Chinese subjects is dubious (Armitage *et al* 2000). The current concerns for genetic testing in periodontal research seem to focus on assessment of multiple gene polymorphisms, such as IL-1 α , TNF- α , IL-6 (D'Aiuto *et al* 2004), Comprehensive 125 Genes 310 SNPs (Suzuki *et al* 2004), and TNF- β (*Nco*I bi), ACE (I/D), Endothelin-1 (*Taq*I) (Holla *et al* 2001).

Clinical implications and perspectives

Our updated knowledge and concepts of periodontal etiopathogenesis enhance the main targets of conventional periodontal treatment for effective disruption of bacterial biofilm and control of plaque retention factors, through mechanical instrumentation by non-surgical approaches with periodontal surgery as necessary. It has been shown that mechanical instrumentation is by far the most effective approach to control plaque biofilm and it will continue to be the cornerstone of periodontal therapy. Research advances in the pathogenesis of periodontitis and appreciation of the crucial role of the host response have significantly contributed to the newer treatment strategies, i.e. emphasizing the identification of high risk individuals through risk assessment and controlling risk factors in clinical management of periodontitis, and development of adjunctive host modulatory therapies to modulate destructive components of the host response for better periodontal treatment outcomes. The new research advances in etiology and pathogenesis of periodontal disease hold promise for development of novel strategies for preventing and controlling periodontal disease in humans.

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Chapter 5

Research Advances in Periodontal Diagnosis

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Introduction

As our understanding of the etiology, pathogenesis and natural history of periodontal diseases improves, many interesting developments are occurring in periodontal diagnosis.

Periodontal destruction is due not only to the direct effects of pathogenic bacteria, but also to secondary destruction caused by the host response (Genco 1992). Bacteria are necessary, but not sufficient to cause disease, a susceptible host is also required (Loe *et al* 1986). A comprehensive diagnosis should include assessment of systemic risk factors and genetic susceptibility of the diseases, in order to predict risk for further destruction and determine the type and frequency of treatment. Furthermore, periodontal disease does not progress at a constant rate (Socransky *et al* 1984). In actuality it is episodic, with periods of exacerbation and remission. It is important to assess current disease activity, since the type of treatment may vary depending on whether the patient is in an "active" or "quiescent" phase of disease.

As described above, the intent of periodontal diagnosis is not only to provide information about whether the patient is suffering from periodontal disease and which type of periodontal disease is present, but also to

identify current disease activity, predict the risk for future disease progression, guide treatment planning, evaluate treatment outcomes and monitor disease progression during maintenance phase.

Traditional diagnostic procedures, including clinical and radiographic assessments, are still the foundation of periodontal diagnosis, but they have certain limitations. These methods provide only retrospective information about past disease, cannot diagnose disease activity and many are not precise enough to detect small amounts periodontal damage. Hence, many variables may affect the results and as a result the reproducibility of measurements is relatively low. As a consequence of these limitations, it is difficult to monitor disease progression by comparing a series of non-standardized clinical or radiographic measurements.

Therefore, in the past decade much effort has been devoted to improving conventional techniques and developing new diagnostic approaches. This paper reviews important developments in this area and offers a description of traditional and novel diagnostic approaches, including clinical and radiographic techniques, evaluation of microbial challenge, monitoring host biochemical response, genetic susceptibility tests and risk assessment.

Clinical diagnostic procedures

Traditional clinical evaluation is still the foundation of periodontal diagnosis. Clinicians will typically observe the periodontal tissues for plaque and calculus accumulation and gingival redness, measure probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP). Recently, newly developed electronic devices, including controlled-force electronic probes, Periotest and Periotemp have been introduced to facilitate clinical diagnosis.

The accuracy of periodontal probing can be affected by a number of factors such as the inflammatory status of the tissue (Armitage *et al* 1977), the angle of the probe (Van der Weijden *et al* 1994) and, most importantly, the probing force (Mombelli *et al* 1992). It has been widely recognized that measurements taken with conventional manual probes are subject to a variety of errors. Manual probing cannot reliably measure PD or CAL changes less than 2-3 mm (Hassell *et al* 1973).

Since probing force is the major variable that affects measurement accuracy, in the past decade a variety of controlled-force electronic probes have been invented to minimize measurement errors due to this factor. One of these devices is the Florida Probe. This computer-linked device incorporates constant probing force, automated electronic measurement and direct data entry with computer software. The Florida Probe has been proven to be superior to conventional manual probe as it can reliably measure as small as 1 mm of attachment change (Yang *et al* 1992). This device has not been widely used in clinical practice, partly due to the cost and complexity of the device.

Other electronic devices

The Periotest is a form of automated

electronic instrument to measure tooth mobility. Periotest delivers a standardized force to a tooth and the time required for the tooth to rebound to its original position is measured on a scale from 0 to 50. This instrument is now commercially available and may be useful for documenting the change of tooth mobility over time.

Subgingival temperature measurement has been suggested as a diagnostic tool for the quantitative assessment of periodontal inflammation. An electronic instrument called Periotemp has been developed for this purpose. It is the shape of a periodontal probe, with a thermocouple device at the end of the handpiece. It allows for collection of temperature, as well as PD and BOP, with one pass of the instrument. Subgingival temperature is measured to a precision of 0.1°C and is then referenced to the sublingual temperature. Sites with higher temperatures had more than twice the risk for future attachment loss than those with lower temperatures (Haffajee *et al* 1992). Further studies are needed to demonstrate the accuracy of this device and its usefulness in clinical diagnosis.

Radiographic examination

Periapical and bitewing radiographs are invaluable diagnostic aids. They are essential for determining the extent and severity of bone destruction around teeth. However, conventional radiographic assessment of bone levels has four major sources of error:

- 1 Variation in projection geometry.
- 2 Variations in contrast and density due to differences in film processing, kilovoltage and exposure time.
- 3 Masking of osseous changes by other anatomic structures.
- 4 The unaided eye is only able to detect radiographic changes when approximately 30%-50% of bone mineral has been lost.

These factors make it difficult to detect small changes in bone levels using a series of conventional non-standardized radiographs (Hirschmann *et al* 1994).

In the past decade “digital subtraction radiography” has become more widely used in dentistry. It is a computer-assisted image-processing technique used with standardized radiographs. Standardized radiographic films are exposed at certain intervals (every 6–12 months) and digitized. Computer software “subtracts” the initial from the follow-up image, clearly displaying the difference between the two. Bone loss is visualized as a dark area and bone gain as a light area. Thus, the subtraction radiography technique allows the comparison of sequential images over time and helps to identify subtle changes in alveolar bone that would otherwise be missed by unaided eye (Jeffcoat *et al* 1996).

The development of local CT, which distinguishes itself by using a small-field high-resolution detector to generate a limited high-resolution 3D volume, makes it possible to show a patient 3D reconstruction of alveolar bone. This technology is still new, but is very promising for the imaging of alveolar bone.

Microbiological tests

Microbiological tests are not indicated for most adult periodontitis patients, but they may help to more precisely define the cause of disease and guide therapy for specific patients, such as patients with aggressive or refractory periodontitis.

There are a number of advantages in using bacterial markers for periodontal diagnosis. For example, for those specific patients as mentioned above, microbiological testing may provide clinically useful information including the identification of putative periodontal pathogens or unusual superinfecting microorganisms and antibiotic sensitivity, which allows dentists to choose the appropriate

antibiotic chemotherapy. In some cases the bacterial markers appear to be predictive of disease activity. Overall the chairside tests are simple to use and the results are available in a short time. The visual results produced can be shown to the patients and serve as a motivational tool to enhance patient compliance.

Despite these features there are some problems associated with microbiological tests that may limit their diagnostic value. Multiple bacteria are simultaneously involved in the initiation and progression of periodontitis. No single pathogen can be proved to be the cause of disease. Therefore because the levels of putative pathogens necessary to cause periodontal destruction are host-dependent and quite variable, it is difficult to choose certain levels of particular bacterial species to assay as a marker for actively progressing sites of disease. Since it is not possible to sample all the sites in the mouth, dentists must choose ‘appropriate’ sites to sample. The fact that most tests target specific organisms is also a problem. All microbiological tests will only detect the specific bacterial species which are searched for. They are unable to identify ‘unexpected’ or ‘unusual’ species. Finally, the tests are expensive to use on a regular basis.

Because of these limitations, it must be emphasized again that microbiological tests should be reserved for patients with unusual forms of periodontal disease such as aggressive periodontitis or refractory periodontitis. Since these forms usually have a poor response to conventional therapy, microbiological tests may provide useful information to guide treatment plan.

Types of microbiological tests

There are several methods for assessing subgingival plaque samples, including darkfield and phase-contrast microscopy, culture and sensitivity, DNA probes, restriction

endonuclease analysis, polymerase chain reaction (PCR), immunoassay and bacterial enzyme-based assay.

Darkfield and phase-contrast microscopy

This method has been used to detect motile rods and spirochetes (Greenstein *et al* 1985). It has been proven to be of no value in predicting progressing sites in appropriately treated and well-maintained patients.

Culture and sensitivity

Until now, bacterial assays remain the only method that can determine whether bacteria are sensitive or resistant to specific antibiotics. This information can provide important guidelines for antibiotic use. The main drawbacks of this method include:

- 1 Samples must be sent to the laboratory within one or two days to maintain bacteria viability.
- 2 Not all bacteria can be readily cultured.
- 3 The proportional recovery of cultivable species is unlikely to match their proportions in periodontal pocket.
- 4 Only a limited number of laboratories are equipped to grow anaerobic bacteria.
- 5 It takes several days for results to be available.

DNA probes

A DNA probe is composed of a marker molecule and a specific single-stranded nucleic acid sequence that is complementary to the characteristic DNA sequence in specific bacteria (Conrads 2002). Thus, the labeled single-stranded DNA probe can bind to (or hybridize with) the complementary DNA sequence and identify the target bacteria in plaque sample. Bacteria counts are determined by the amount of binding. This method is very

sensitive and can detect as few as 100 bacteria. It is relatively rapid, providing results within 24 hours and it allows concomitant analysis of large numbers of samples with respect to a multitude of bacterial species. Fastidious species not readily grown on culture media can also be identified. Therefore, DNA probes have become a valuable research tool. They are particularly useful in epidemiological studies of microecology, but their usefulness in clinical practice has not been established. This is because probes have only been constructed for a limited number of putative pathogens and they provide no information about the antibiotic sensitivity of bacteria.

PCR

This technology is the most sensitive test currently available for detecting bacteria. A modification of the original PCR technology, “real-time” PCR, permits not only detect of specific microorganisms in plaque, but also its quantification (Conrads 2002). PCR assay in combination with synthesized 16SrRNA probes enable the detection of virtually any microorganisms in dental plaque samples.

Immunoassays to detect bacterial antigens

Each bacterial species has a specific surface antigen that is unique for that microorganism. In immunological assays, a specific antibody labeled with a fluorescent or a colorimetric reaction system is used to bind to and identify the target bacterial antigen in a plaque sample. This technology is very sensitive and at the same time can be very specific if controls are used to check for non-specific reactions (Snyder *et al* 1996). The limitation of the method is that it can only detect species for which a suitable antibody is available.

Bacterial enzyme-based assay

A group of three subgingival pathogens (*Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola*) produce trypsin-like enzymes that can degrade a synthetic peptide BANA (Loesche *et al* 1990). Thus, the capacity to hydrolyze this substrate has been incorporated into a chairside diagnostic test called 'Perioscan'. This test employs Evans black dye that produces a permanent blue-black color when reacted with BANA hydrolysis product. The result is read by eye as positive, weak positive, or negative. Positive enzymatic activity indicates the presence of at least one of the three bacteria. This enzyme assay provides a rapid and inexpensive way of screening samples of these bacteria. The main drawback of this test is a lack of quantitative data and the inability to determine which of the three bacteria are responsible for the enzyme production.

Biochemical assays of gingival crevicular fluid

Gingival crevicular fluid (GCF) is a form of inflammatory exudate which can be analyzed to assess local inflammatory process. Biochemical assays of GCF have been extensively studied in an attempt to search for a sensitive biochemical marker to predict disease progression before it can be detected by traditional methods. A very large number of potential markers have been investigated. Some of these have proven to have considerable potential as valuable indicators for disease activity and several commercial chair side kits based on some of these biochemical markers have been developed. However, it is unknown whether they have any diagnostic value in treated and well-maintained patients. It is also unclear whether the results of such tests are applicable to individual sites or if they are best applied to the patient in terms of predictive

value. More longitudinal studies are still needed to confirm the predictive ability of these markers.

In general, these potential markers can be classified into three groups: inflammatory mediator and products, host-derived enzymes and tissue-breakdown products.

Inflammatory mediators and products

Prostaglandin E₂ (PGE₂)

This mediator plays an essential role in bone resorption. There is already convincing evidence that correlates PGE₂ levels in GCF to the severity of periodontal disease (Offenbacher *et al* 1986). Sites with periodontitis have significantly increased levels of PGE₂ compared to healthy sites. Level of PGE₂ are markedly elevated during disease progression and appropriate treatment can significantly lower the PGE₂ levels in GCF. This makes PGE₂ a very promising candidate marker for disease progression.

Cytokines

Cytokines are potent local mediators of inflammation. Cytokines that have been investigated as potential diagnostic markers for disease activity include IL-1 α , IL-1 β , TNF α , IL-6 and IL-8 (Ebersole *et al* 1993), which are associated with bone resorption and neutrophil chemotaxis.

Antibacterial antibodies

An important aspect of the host response to periodontal infection is the development of specific antibodies to periodontal pathogens. Studies have shown that there are strong correlations between serum and GCF levels of certain antibodies. However, the clinical usefulness of antibody assays is limited because extensive variation exists on a site and patient

basis with regards to local and systemic antibody production.

Host-derived enzymes

The inflammation process is associated with accumulation of inflammatory cells and release of various enzymes from these cells. These enzymes can degrade periodontal connective tissue components. Therefore, host-derived enzymes may be good candidate markers for disease progression. Many kinds of enzymes such as neutrophil elastase (Armitage *et al* 1994), neutral proteinases (Eley *et al* 1992), β -glucuronidase (Chung *et al* 1997), MMPs (Ingman *et al* 1996), cathepsins (Chen *et al* 1998), lactate dehydrogenase (Atici *et al* 1998), myeloperoxidase (Yamalik *et al* 2000), tryptase (Eley *et al* 1992), alkaline phosphatase (Binder *et al* 1987), arylsulfatase (Lamster *et al* 1988) and aspartate aminotransferase (Atici *et al* 1998) have been investigated for their association with periodontal inflammation and as markers of disease activity.

Neutrophil elastase

Neutrophil elastase (NE) is a serine proteinase stored in neutrophil. Studies indicated that GCF samples from sites with periodontitis have significantly higher total NE activity than GCF from healthy or gingivitis sites. Treatment has shown to result in significant decrease in GCF NE level. A commercial chair side test kit has been developed to detect GCF NE. This qualitative test uses filter paper strips impregnated with a synthetic elastase-sensitive oligopeptide substrate. When exposed to elastase, a fluorescent marker is released by the enzyme-substrate reaction that can be detected by ultraviolet light. The intensity of fluorescence is read visually.

Neutral proteinase

Neutral proteinase is a more general measure of the enzymes that degrade the structural components of gingiva. A chairside qualitative test for neutral proteinase in GCF has been marketed. This test system uses a filter paper strip to collect GCF and then place it on a reaction card impregnated with dye-labeled bovine collagen. As the collagen is lysed, a blue dye appears on the strip. Although the test can detect the presence or absence of the enzyme, the diagnostic value of such information is unclear since the enzyme present at most inflamed sites.

β -glucuronidase

This lysosomal enzyme is found in neutrophils. Elevated GCF β -glucuronidase levels have been reported to have some predictive value in identifying patients who are at higher risk for disease progression. It is unknown if there would be any diagnostic value in a well-maintained population.

Matrix metalloproteinases

Matrix metalloproteinases are a group of Zn⁺⁺ and Ca⁺⁺-dependent proteolytic enzymes. They are involved in physiological degradation and remodeling of extracellular matrix components. In pathological conditions such as periodontitis, the amount and activity of MMPs will increase markedly, leading to breakdown of connective tissue and periodontal destruction. Recent research has highlighted the importance of MMP-8. It is detected in an active form in the GCF of periodontitis patients, whereas it is mainly in a latent form in gingivitis. It has been reported that more than 80% of periodontal disease activity could be explained by MMP-8, MMP-1 and MMP-2.

Cathepsins

These are a group of acidic lysosomal enzymes. Cathepsins B, H and L are cysteine proteinases that play an important role in intracellular protein degradation. Cathepsin G is a serine proteinase derived from neutrophils. It is secreted with elastase and proteolytically activates latent collagenase. In patients with periodontitis, highly significant correlations have been noted between GCF levels of cathepsins and disease severity. Significant decreases in cathepsins levels have been noted after treatment. It has been suggested that the cathepsins may have some use for monitoring the response to treatment.

Aspartate aminotransferase

Aspartate aminotransferase (AST) is an enzyme normally found in the cytoplasma of cells. Detection of AST in GCF indicates cell death which is believed to be associated with disease progression. Several cross-sectional and longitudinal studies have proved the association between AST and loss of periodontal attachment. A chairside test kit for AST in GCF is currently available.

Tissue-breakdown products

Tissue-breakdown products such as glycosaminoglycans (Embery *et al* 1982), hydroxyproline (Akalin *et al* 1993) and bone specific proteins (Bowers *et al* 1989) have been studied as possible marker for progressive destruction.

Glycosaminoglycans

Glycosaminoglycans (GAGs) are polysaccharide components of proteoglycans, which are widely distributed in connective tissue. Among GAGs, C-4-S has received special attention, since it represents 93.8% of

GAG content of alveolar bone. The appearance of C-4-S in GCF has been suggested as a promising diagnostic marker for bone resorption.

Hydroxyproline

This is a prominent amino acid of collagen and its appearance in GCF has been investigated as a marker for connective tissue destruction. Results indicate that collagen degradation is a prominent feature of both gingivitis and periodontitis, so GCF hydroxyproline level cannot distinguish between sites with gingivitis or periodontitis.

Bone specific proteins

Bone contains a number of proteins within its matrix which are characteristic of mineralized tissue. At sites of periodontitis these bone specific proteins may pass into GCF. Therefore, they have been considered as possible markers of bone resorption and hence periodontal disease activity. These proteins include osteonectin, osteocalcin and telopeptides of type I collagen.

Osteonectin is a noncollagenous protein of bone which is thought to play an important role in the initial phase of mineralization. The amount of GCF osteonectin has been shown to increase in line with the site probing depth, therefore it may be a possible marker for disease severity.

Osteocalcin is a 5.4KD calcium-binding protein and is the most abundant non-collagenous protein of bone. It has been reported that GCF osteocalcin increases remarkably during the development of periodontitis and is significantly correlated with clinical parameters.

Telopeptides of type I collagen (ICTP) is a 12-20KD fragment of bone type I collagen. Elevated ICTP has been shown to coincide with bone resorption rate. In several studies, significantly higher ICTP concentrations in GCF

were found in periodontitis patients. A positive correlation was found between the total amount of GCF ICTP per site and clinical parameters like PD and radiological bone loss.

Genetic tests

Many preliminary studies have shown that genetic factors strongly influence susceptibility and severity of periodontitis. Periodontitis is a chronic immuno-inflammatory disease. Susceptibility and severity of periodontitis may be influenced by the intensity of host immune and inflammatory response to bacterial LPS. Therefore, genes regulating the production of various immune and inflammatory mediators are potential candidate genes that may influence host susceptibility and severity of periodontitis. These candidate genes include IL-1 gene family (IL-1A, IL-1B and IL-1RN), TNF gene family (TNF and TNF B), COX II gene, Fc γ RII A and Fc γ RIII B genes. Polymorphisms or mutations in transcription-regulating areas of these genes may lead to inter-individual differences in the production of these proinflammatory cytokines and antibodies. It is proposed that individuals carrying certain genotypes will tend to produce significantly more IL-1 α , TNF β , PGE $_2$, less IgG $_2$ or Fc-gamma receptor with lower adherendity to IgG, when challenged by bacterial LPS. These individuals may experience a more vigorous inflammatory reaction or a less effective immune response and be inclined to more extensive tissue destruction.

So far a number of studies have supported this hypothesis. Komman *et al* (1997) reported that a specific IL-1 genotype (namely IL-1A+4845 allele II H/IL-1B+3954 allele II composite genotype) was associated with severe periodontitis. The odds ratio of having this genotype in severe versus mild disease in non-smokers was 6.8. The genotype positive individuals are at higher risk of developing severe periodontitis than genotype negative

individuals (odds ratio 18.9 for age 40-60 years). Recently, a commercial genetic test kit has been introduced to test patient susceptibility for severe chronic periodontitis. It may be concluded that genetic testing has a great potential for future use in disease prevention, treatment planning, making maintenance schedules and preventing over-treatment or under-treatment. A clearer understanding of the genetic heterogeneity of periodontitis may also lead to important revisions to currently used classification system.

Risk assessment

Risk assessment is a way to identify the potential risk factors for periodontitis so that they may be avoided, reduced or managed. During the past decade many risk factors have been identified. Confirmed risk factors for periodontitis in adults include genetic influences (Rao *et al* 1979), smoking (Haber *et al* 1993), specific pathogens (Haffejee *et al* 1997) and diabetes (Soskolne *et al* 2001). Infrequent dental attendance and poor compliance (Becker *et al* 1984), depression (Aleksejeniene *et al* 2002) and female osteoporosis (Von Wowern *et al* 1994) can be considered to be risk indicators of periodontitis.

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Chapter 6

Research Advances in Establishing Contemporary Clinical Protocols in Periodontics

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Introduction

The objective of this paper is to briefly review the established clinical protocols in the management of destructive periodontal disease, through an analysis of the current concepts in its etiopathology. Taking into account the emergence of newer etiological factors in destructive periodontitis, an attempt will then be made to present a new protocol in the management of periodontal disease, with special reference to the Asian Pacific region utilizing a regionally customized approach.

Current protocols

Current established protocols in the management of destructive periodontal disease, though effective, have many deficiencies (Figure 1).

In seminal studies by Hirschfeld and Wasserman (1978) and McFall (1982) it was observed that some patients lose teeth from periodontal disease despite regular maintenance appointments or display almost no benefits from regular maintenance appointments.

In 1997, Hafajee described the ongoing loss of probing attachment in over 30% of adult patients who had received 3 hours or more of initial scaling and root planing and then

supportive periodontal therapy every 3 months throughout a 9 month study period.

In 2001, Rosling *et al*, described the persistence of tooth loss in 64% of his study population susceptible to periodontal disease, despite maintenance therapy every 3-4 months.

Several etiological factors have been implicated in the development of periodontal diseases with the principal focus on virulent infectious agents, host inflammatory elements and environmental factors. In order to make rational decisions in the management and maintenance care of periodontal patients, it is becoming increasingly evident that a thorough knowledge of the relevant microflora in the etiopathology is imperative.

The current understanding of the microflora of healthy gingiva points towards *Streptococcus viridans* and *Actinomyces* as the dominant species. Chronic gingivitis is dominated by a picture of increase in the populations of gram positive facultative and gram negative anaerobic flora. The composition is typical of *Spirochaetes*, motile rods, *Fusobacterium*, *Prevotella* and *Treponema*. Chronic periodontitis has a quantitatively higher common plaque, with a composition dominated by *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Campylobacter rectus* and *Treponema spp.*

Aggressive periodontitis is, however,

characterized by specific bacterial infections, gram negative anaerobic dominance and frequently by spirochaetal infections. The composition is typically dominated by *Actinobacillus actinomycetamcomitans*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythia* and *Treponema denticola*.

Recent additions to this list of putative pathogens are human cytomegalovirus and the Epstein-Barr virus where the double hit concept of etiopathogenesis is proposed by a combination of herpes viral and bacterial causes (Contreas and Slots 2000).

With the increasing evidence of specific microbial factors in the etiopathogenesis of aggressive periodontitis, current treatment options rely heavily on chemomechanical approaches, the use of systemic antibiotics and local drug delivery systems (American Academy of Periodontology 1996).

However single drug therapies do not appear to afford an ideal protocol (Sakellari 2000). Drug combination therapies offer better value in treatment (Slots and Ting 2000), with recommended combinations of *Amoxycillin* 375 mg - 1 tds for 8 days, *Metronidazole* 250 mg - 1 tds for 8 days. For specific *A. actinomycetamcomitans* infections the recommended combination is *Ciprofloxacin* 500 mg - 1 tds for 8 days together with *Metronidazole* 500 mg – 1 tds for 8 days.

However, systemic antibiotic therapy is not without the risk of adverse reactions, high costs, lack of effectiveness in chronic periodontitis, emergence of resistant bacteria and adverse reactions that may be age related. There are concerns regarding the inappropriate use of antibiotics and the emergence of resistance bacteria and the subsequent effects on commensal ecology. Further microbial analysis may be warranted in the use of these agents.

Current evidence on the role of local antibiotic therapy is still not conclusive as to their effectiveness and efficiency to support

their use in a standard protocol for the management of aggressive periodontal conditions. To paraphrase "...*clinical outcomes of several studies do not point to a significant utility of topical antibiotics in periodontal treatment, at least not when employed in conjunction with thorough mechanical debridement*" (Slots 2000).

The Asian continent has particular local variables such as low per capita income and a poor dentist to population ratio that further compounds the difficulty of patient management. Treatment options in Asia are heavily influenced by the lack of informed choice, patient's value system, financial resources which results in a large percent of untreated disease.

Any treatment protocol that has the potential to work in the Asian environment must strike a balance between the benefits, risks and financial costs of disease prevention and treatment. This specific need of the Asian population has directed chemotherapeutic options towards the use of antimicrobials more than antibiotic therapy, with optimal chemotherapeutic options being the use of 0.12% to 0.2% chlorhexidine gluconate as a mouth rinse, twice daily for 2 weeks.

Recently the description of an antibacterial protocol consisting of subgingival irrigation of freshly prepared dilute sodium hypochlorite (0.1-0.5%) irrigation and the subgingival 10% povidone iodine irrigation applied repeatedly with a disposable endodontic syringe for a contact time of 5 minutes by Slots (2000) may find a more relevant application specifically for the Asian population

Amongst the antibacterial agents, chlorhexidine gluconate (0.12 - 0.2%) has little bactericidal activity against enteric gram negative rods, stains tooth colored restorations and has been documented to be toxic to gingival fibroblasts.

Chlorine (sodium hypochlorite 0.1 - 0.5%) however is an ideal antimicrobial agent with a

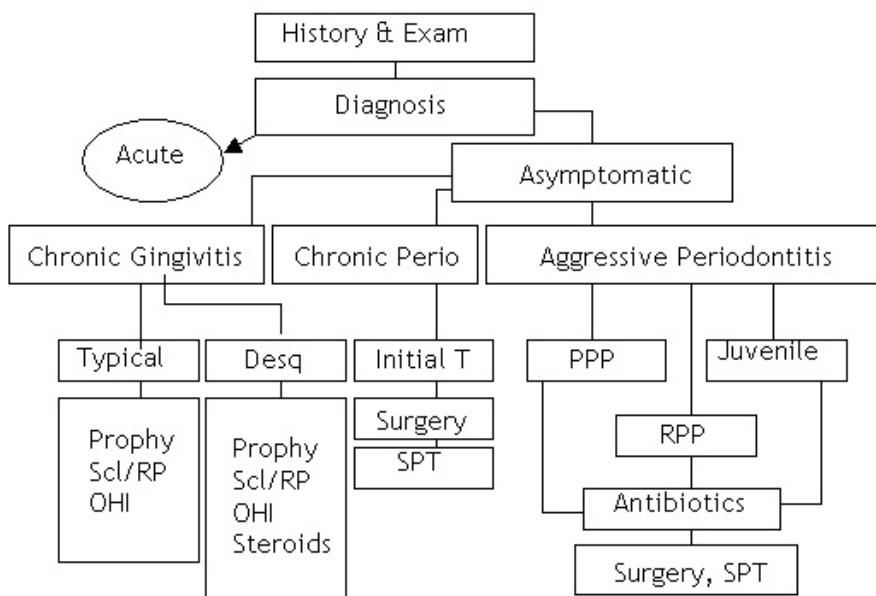


Figure 1. Established clinical protocols (Adapted from Hall, 1997)

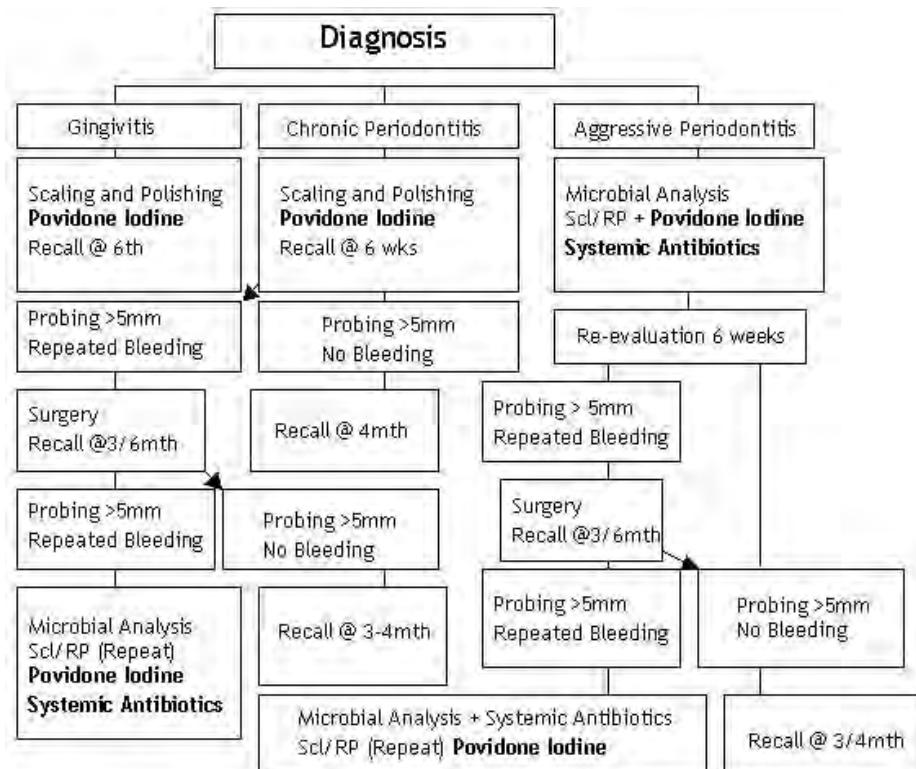


Figure 2. Standardized treatment protocols for periodontal disease

broad antimicrobial activity, rapid bactericidal action, relative non-toxicity, no color, no staining, ease of access, very low cost and no known contraindications

Fluoride rinses and gels, although not as effective as chlorhexidine or chlorine, have anticaries properties and hence are strongly recommended.

The most efficacious protocol amongst antibacterial agents however, relies on subgingival 10% povidone iodine irrigation applied repeatedly with a disposable endodontic syringe for a contact time of 5 minutes. Amongst the reasons for proposing 10% povidone iodine are that it is a potent antimicrobial agent, acts on bacteria, yeasts, protozoa and the herpes virus, reaches pathogens in deep periodontal sites, is inexpensive, safe, widely available and there is no known microbial resistance to this agent. The only contraindications to this protocol are for individuals with iodine hypersensitivity, thyroid disease, pregnancy and nursing mothers

The standardized treatment protocols recommended for various manifestations of periodontal disease are as follows in Figure 2.

Treatment protocols

Plaque-induced gingivitis

- Oral hygiene instruction and motivation
- Scaling and polishing
- Disinfection of subgingival sites with 10% povidone iodine with a contact time of at least 5 min
- For contraindicated cases: 0.1 - 0.5% NaOCl
- 6 month follow up

Chronic periodontitis (responsive)

- Extensive debridement, Scl + RP
- 10% Povidone-iodine subgingival irrigation

- Re-evaluation in 6 weeks
- Probing depth >5 mm or bleeding: surgery
- Additional re-evaluation in 3 months
- No probing depth >5 mm, no bleeding, effective plaque control
- Recall in 3-4 months

Chronic periodontitis (non-responsive)

If probing depth >5 mm and repeated bleeding persists perform:

- Microbial analysis
- Mechanical debridement
- Subgingival irrigation with 10% povidone iodine
- Antibiotics guided by microbial analysis.
- Additional evaluation in 6 weeks and later at 3 months

Aggressive periodontitis

- Subgingival sampling
- Oral hygiene instruction
 - Scl + RP with subgingival irrigation with 10% povidone iodine performed over several appointments
- Systemic antibiotics based on microbial analysis
 - Amoxycillin 250mg, 1 tds for 8 days
 - Metronidazole 250mg, 1 tds for 8 days
- Re-evaluation in 6 weeks
- If no bleeding or probing depth >5 mm
 - Schedule for recall in 3 months
- If repeated bleeding or probing depth >5 mm
 - Additional microbial analysis
 - Further mechanical debridement
 - Subgingival irrigation with 10% povidone iodine
 - Additional course of systemic antibiotics
 - Frequent follow up

Supportive periodontal therapy for these patients relies on education, and the use of dilute

sodium hypochlorite (0.1 - 0.5%) irrigation via a subgingival irrigation device.

Summary and conclusions

In the light of current evidence that the elimination of contributing factors and the use of subgingival scaling and root planing, as well as surgical procedures, may not always effectively result in the remission of the disease process, several researchers have devised systemic and local antibiotic delivery protocols as well as supplemental local antimicrobial applications to effectively combat the disease. These protocols are particularly exciting for Asian populations, where the high prevalence of the disease is compounded by low per capita income and a poor dentist to population ratio that further increases the difficulty of patient management. Treatment options in Asia are also heavily influenced by the lack of informed choice, patient's value system and financial resources and this often results in untreated disease.

This paper highlights the use of a 10% povidone iodine solution for subgingival application by an endodontic syringe and a thin cannula using a protocol where the active solution can remain in the periodontal pockets for a minimum period of 5 minutes. The paper further highlights the use of a fresh solution of 0.1% sodium hypochlorite solution for home subgingival irrigation. Such a protocol seems to afford a predictably safe, effective and acceptable treatment option to patients especially with regard to its ease of application and reduced financial costs.

Further research could focus on the development of newer drugs, dosage and duration of antimicrobial therapies in the management of periodontal disease by relying on the premise of markedly suppressing or eliminating periodontopathic microflora.

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Chapter 7

Risk Factors for Periodontal Infection Among the Rural Population in Battambang Province, Cambodia

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Introduction

Cambodia is a constitutional monarchy in Southeast Asia with a population of more than 13 million people. Most Cambodians are Theravada Buddhists of Khmer extraction, but the country also has a substantial number of Cham and small hill tribes.

The country shares borders with Thailand to the west, Laos to the north, Vietnam to the east, and the Gulf of Thailand to the south. There are three major political parties in Cambodia: the Cambodian People's Party, FUNCINPEC and the Sam Rainsy Party. Currently, the Cambodian People's Party, led by Prime Minister Hun Sen, is the ruling party. In 2004, after a year of negotiations, a coalition between the Cambodian People's Party and the royalists' FUNCINPEC came to power in the National Assembly. Administratively, Cambodia is divided into 20 provinces and 4 municipalities. It is also divided by District (srok), Communion (khum), Great Districts (khett), and also Islands (koh).

In the Battambang province there are 12 Districts, 89 Communes and 611 Villages. Within these there are 4 referral hospitals and 74 Health Centers. The demographics of this province are shown in Table 1. The general living conditions in both urban and rural regions of Battambang are shown in Table 2.

To date there have been very few studies concerning the periodontal health of Cambodians (Amarasena *et al* 2002). Therefore, this study was initiated in order to determine the periodontal health of residents in one province of Cambodia.

The Battambang Periodontal Health Project

This project investigated the incidence of periodontal infection in a rural area of Cambodia. It was a collaborative effort between the Organization of International Support for Dental Education (OISDE) and Faculty of Odontostomatology, Health Science University (HSUFO) under the support of JICA. The purpose of this project was to investigate periodontal conditions of residents in Battambang province.

Methods

Patient history

A detailed history was taken for each patient and this included obtaining relevant information concerning general demographic and living information, oral hygiene understanding and oral hygiene habits. A medical history also included information

<i>Population</i>	
• Total 793,129	
• Males 388,599	
• Females 404,530	
<i>Population density</i>	
• 68 per km ²	
<i>Urbanization Rate</i>	
• 17.6%	
<i>Population distribution by broad age groups</i>	
• 0 – 14: 44.4%	
• 15 - 64: 52.5%	
• 65 and above: 3.2%	
<i>Literacy rate</i>	
• Both Sexes: 65.0%	
• Males: 72.4%	
• Females: 58.0%	
<i>Employment activity</i>	
• Both Sexes: 50.7%	
• Males: 54.7%	
• Females: 46.9%	
<i>Unemployment rate</i>	
• Both Sexes: 8.0%	
• Males: 6.8%	
• Females: 9.3%	

Table 1. Demographic information of Battambang province

relating to general medical conditions, systemic conditions, and smoking history. Systemic conditions of particular interest included diabetes, respiratory disease, heart disease, hypertension, malaria and dengue fever (Table 3).

Oral/periodontal examination

Detailed oral and periodontal examinations were carried out which included recording missing teeth, oral hygiene, plaque index, calculus index, and an occlusal assessment. Details of these measurements are shown in Table 4.

Facility	Distribution
Toilet facility	19.9%
Firewood	92.4%
Charcoal	4.4%
Kerosene	1.6%
Liquified Petroleum Gas	0.6%
Other	1.0%

Table 2. Distribution of household facilities

Risk factors

Specific risk factors which were assessed included smoking, oral hygiene, systemic conditions and quality of living environment.

Results

In this study a total of 164 individuals were assessed. Of these 66 were male and 98 were female. Most of the subjects studied (85%) lived in rural or remote regions with only 15% living in towns. The majority of subjects suffered from little tooth loss (none or one tooth missing – 77%) and only 9% of the subjects had more than 9 teeth missing (Table 5).

Oral hygiene

Approximately 15% of the study population regularly carried out oral hygiene practices while up to 75% either very rarely or never practiced any form of oral hygiene (Table 5). These practices were reflected in the findings for general oral hygiene where 11% of the subjects had very good levels of oral hygiene and 47% had bad to very bad oral hygiene.

- Diabetes
- Heart disease
- Respiratory disease
- Liver disease
- Kidney disease
- Brain disease
- Hypertension
- Gynaecological disease
- Skin disease

Table 3. Systemic conditions assessed

Calculus levels were found to be moderate to heavy in 66% of the population with only 4% of the study group having no calculus deposits.

Periodontal pockets

The average pocket depth for each patient in the study ranged from 3.0 to 6.5 mm (Figure 1). Only around 8% of the populations had pocket depths averaging 5 mm or more.

Occlusal analyses

Of the subjects studied 57% did not have any occlusal problems while 7% had some prosthetic occlusal problems, 32% had a malocclusion of some form and 8% had an orthodontic problem.

Systemic disease

Participants in this study were assessed for the incidence of one or more systemic conditions as listed in Table 3. Approximately 70% of the study population did not suffer from any systemic conditions whereas 18% had a single condition and 12% had multiple conditions (Figure 2).

Smoking

Only 4% of the study population did not

smoke. The majority of smokers smoked more than 21 cigarettes per day and this represented 80% of the study population (Figure 3).

Discussion

This study investigated the periodontal health of residents within the Battambang province in Cambodia. This has been an important study because there is a paucity of information pertaining to oral disease patterns including periodontal disease in Cambodia. In 2002 a house-to-house survey was conducted to assess the periodontal status of 1948 subjects aged 15-74 years in a rural commune in Cambodia using Community Periodontal Index (CPI) and measuring attachment loss (Amarasena *et al* 2002). The periodontal status of Cambodians increased with age as indicated by both CPI and loss of attachment. Calculus was the most common finding among Cambodians pointing to overall poor oral hygiene levels. Notwithstanding the poor oral hygiene, however, severe periodontitis as denoted by 6 mm or greater periodontal pockets was rare even in the elderly, while edentulousness was not frequently observed until 65 years.

The present study confirmed many of the findings from the Amarasena study. For example although around 90% of the population had poor oral hygiene the incidence of severe and advanced disease was low, with only 8% of the populations demonstrating average pocket depths of 5 mm or more. These findings are in line with most other epidemiologic studies which indicate that advanced periodontal destruction is rare in nearly all societies whether they be industrialized or third world (Albander and Rams 2002).

Indeed, the prevalence of periodontal disease in adults in a variety of communities has been determined. The data from all racial populations shows that the prevalence of periodontal disease around the world ranges

	Grade			
	0	1	2	3
Missing teeth	None	2-4	5-8	>9
Oral hygiene freq	Regular	Sometimes	Occasional	Never
Oral hygiene control	Good	Fair	Bad	Very bad
Plaque index	No plaque	Thin film	Moderate plaque along gingival margin	Heavy accumulation along gingival margin and interdentally
Calculus	None	Mild deposits	Moderate deposits	Heavy deposits
Occlusal problems	None	Prosthetic	Malocclusion	Orthodontic
Living conditions	Town	River district	Village	Outside village or mountain
Smoking	Never	<10/day	<20/day	>21/day
Systemic conditions	None	One	Two	Three

Table 4. Qualitative assessments of study

	Grade			
	0	1	2	3
Missing teeth	46	31	14	9
Oral hygiene freq	18	5	75	5
Oral hygiene control	11	42	34	13
Plaque index	5	30	39	26
Calculus	4	30	38	28
Occlusal problems	53	7	32	8
Living conditions	15	8	26	51
Smoking	4	6	10	80
Systemic conditions	70	18	10	2

Table 5. Percentage distribution of qualitative assessments

between 5 to 20 % (Hugoson and Jordan 1982, Albandar *et al* 1999, Diamanti-Kipioti *et al* 1995, Miyazaki *et al* 1991). This distribution of periodontal disease appears to have remained unchanged for the last twenty years (Hugoson *et al* 1998).

While plaque is considered a necessary component for the development of periodontal disease, its absolute role must be questioned. For example good evidence exists that in developed countries the frequency of tooth brushing is high, and oral hygiene levels are improving (Bartold *et al* 1998). However, the incidence of periodontal disease has remained static over the past 20 years. Interesting, in the present study despite an apparent lack of oral hygiene the incidence of advanced periodontal disease is no different than many other regions.

It is now recognized that plaque may account for only 20% of the risk for developing periodontitis (Page 1998, Grossi *et al* 1999). Indeed it is well accepted now that periodontitis is a multifactorial problem with three factors interplaying before disease becomes manifest: a susceptible host, environmental factors conducive to disease development and altered host responses leading to tissue destruction. In this context the findings of this study show that interestingly 30% of the population had one or more systemic condition.

In conclusion, this study on a small Cambodian provincial population has revealed that periodontal infection is very strongly involved with all age groups in the targeted area. Because oral health services have not been provided in the area almost 60% of the people do not have any knowledge of appropriate

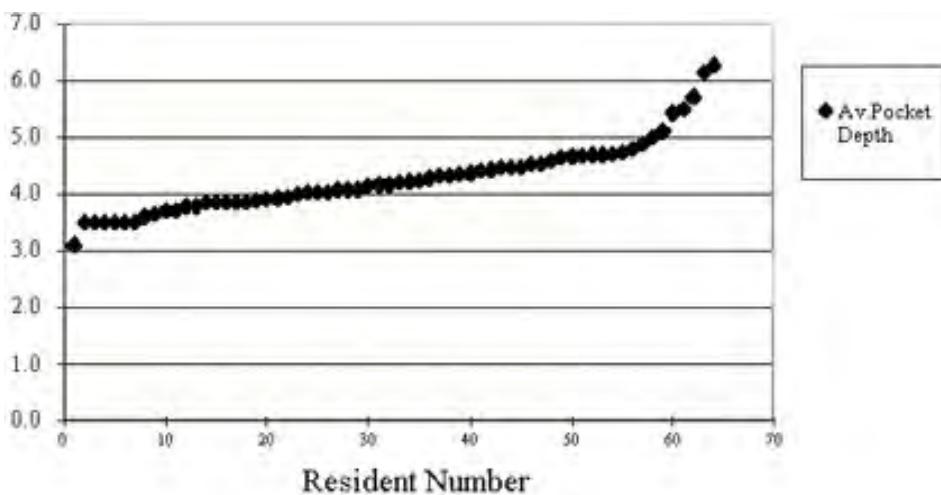


Figure 1. Average pocket depths in study population

plaque control procedure and only 11% of the people had good oral hygiene. Smoking and systemic disease were high in this population and indicates the possibility that these modifying conditions may influence the manifestation of advanced periodontal disease.

Future efforts to deal with periodontal disease in these areas must consider not only oral hygiene but also major modifying factors such as smoking and systemic health.

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Chapter 8

Clinical Applications of Current Research in Periodontal Pharmacotherapeutics

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Introduction

Periodontitis is an infectious disease with a number of specific characteristics including connective tissue degradation and alveolar bone loss. Subgingival periodontopathic microbiota accumulate on the root surface to form an adherent layer of plaque with the characteristics of a biofilm which can interact with host tissues even without direct tissue penetration. Mechanical supragingival plaque control is indispensable to minimize the reemergence of periodontal pathogens and the reestablishment of a biofilm in treated sites.

The microbial etiology of periodontal disease provides the rationale for the use of adjunctive antimicrobial agents in the prevention and treatment of the disease. Although mechanical removal of supra and subgingival calcified and non-calcified plaque deposits has been proven effective to control gingival inflammatory lesions, as well as to halt the progression of periodontal attachment loss, some patients may experience additional benefits from the use of systemic or topical antimicrobial agents. To be effective these antimicrobial agents must be available at a sufficiently high concentration, not only within the periodontal tissues, but also outside, in the environment of the periodontal pocket. Such agents are able to significantly affect supra and subgingival plaque

accumulation and/or suppress or eradicate periodontal pathogenic microflora. Properly selected local antiseptic and systemic antibiotic therapies can provide periodontal treatment that is generally effective, low-risk and affordable (Trombelli and Tatakis 2003). This paper will review current research related to the clinical applications of systemic antibiotics and local drug delivery in the treatment of periodontitis. In addition, different commercial classes of local delivery systems, the use of regional herbs for periodontal pharmacotherapeutics and important factors in designing a strategy for their use will also be discussed.

Systemic antibiotics in the management of periodontitis

Patients with gingivitis or chronic periodontitis usually respond well to mechanical debridement and topical antiseptics and may not obtain clinically significant additional benefit from antibiotic therapy (Slots and Rams 1990). Nonetheless, antibiotics may be prescribed for periodontal patients who do not respond to conventional mechanical therapy and continue to exhibit progressing loss of periodontal attachment despite diligent conventional mechanical periodontal therapy. In addition, antibiotics may be prescribed for patients with acute periodontal infections associated with

systemic manifestations, for patients with aggressive types of periodontitis (Schenkein and Van Dyke 1994), for prophylaxis in medically compromised patients and as an adjunct to surgical and nonsurgical periodontal therapy. Patients with acute or severe periodontal infections (periodontal abscesses, acute necrotizing gingivitis/periodontitis) may also need antibiotic therapy (Johnson and Engel 1986).

Many studies have investigated the use of systemic antibiotics as adjunctive treatments in the management of chronic periodontitis (Preshaw 2004). Evidence exists suggesting that antibiotic use in chronic periodontitis may result in an improvement in clinical attachment level. However, many questions regarding the indications for this therapy remain unanswered (Haffajee *et al* 2003). Few published studies are of adequate quality to be considered and the outcomes are varied. Drawing definitive conclusions is difficult. Therefore, given the lack of reliable published data, systemic antibiotics cannot be indicated as adjuncts in the treatment of chronic periodontitis (Preshaw 2004).

The efficacy of periodontal antibiotic therapy is determined by the antimicrobial spectrum and the pharmacokinetic characteristics of the drug (Pallasch 1996) and by local environmental factors (van Winkelhoff *et al* 1996) including:

- 1 Drug binding to tissues.
- 2 Protection of pathogens through binding, consumption, or degradation of the drug by non-target microorganisms.
- 3 Subgingival plaque biofilm protecting the pathogens.
- 4 Total bacterial load relative to the maximum achievable antibiotic concentration.
- 5 Effectiveness of the host defenses.
- 6 Pathogens in periodontal tissues, root surfaces, and extra-dental sites not affected by the therapy.

Tables 1a-f list investigations that have evaluated the effect of antibiotic therapy in periodontitis patients (The American Academy of Periodontology 2004). Most studies on patients experiencing disease progression suggest that properly selected systemic antibiotics may provide significant additional clinical benefit to conventional mechanical periodontal therapy, particularly in patients with recurrent or refractory periodontitis. Combination drug therapy may be useful in periodontitis that involves a variety of periodontopathic species with differing antimicrobial susceptibilities or to overcome the drug-protective effects of the biofilm.

Systemic antibiotic therapy has certain advantages over topical application of antimicrobial agents. Systemic antibiotics may enable the simple, easy administration of the drug to multiple sites of disease activity. They may also eliminate or reduce pathogens which colonize oral mucosa and other extra-dental sites including the tongue and tonsillar areas (van Winkelhoff *et al* 1988, Muller *et al* 1995, Asikainen and Chen 1999), which may reduce the risk for future translocation of organisms and recolonization of the periodontal pocket, thereby potentially reducing the risk for recurrent disease progression. Disadvantages of systemic antibiotic therapy, as compared to locally applied antimicrobial agents, include an inability for systemic drugs to achieve high gingival crevicular fluid concentration (Goodson 1994), an increased risk of adverse drug reactions (Walker 1996), increased selection of multiple antibiotic resistant microorganisms, and uncertain patient compliance (Loesche *et al* 1993). Table 2 shows common antibiotic therapies in the treatment of periodontitis.

The role of local drug delivery for periodontitis

In order to deliver medications into

Metronidazole

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Loesche et al, 1992	15 test, 18 placebo/ ppa	MET 250 mg tid, 7d	Scaling	Placebo medication	1-1.5	Test showed more reduction in PD, more gain in CAL, reduced level of pathogens and less need for surgery.
Saxen & Asikainen, 1993	27/LAgp	MET 200 mg tid, 10d or TET 250 mg qid, 12 d	Scaling	Scaling	18	Best clinical results with MET. <i>Aa</i> eliminated in 100% of MET group, in 44% of TET group and in 67% of scaling group.
Nieminen et al, 1996	18 test, 15 control/ ppa	MET 250 mg tid, 10 d	Scaling	Modified Widman flap surgery	18	Test showed less BOP and more reduction in PD.
Palmer et al, 1998	31 test, 27 control/ ppa	MET 200 mg tid, 7 d	Ultrasonic Scaling	Ultrasonic Scaling	6	Test and control group showed no statistically significant differences.
Soder et al, 1999	32 test, placebo/ ppa	MET 400 mg tid, 7d	Scaling/ surgery or no surgery	Placebo medication	60	Pd significantly reduced in test but not in placebo group.

Table 1a. Clinical studies of Metronidazole therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; LAgp = localized aggressive periodontitis; MET = metronidazole; TET = tetracycline; PD = probing depth; CAL = clinical attachment level; BOP = bleeding on probing.

Clindamycin

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Gordon et al, 1985 1990	13/ppa	CLIN 150 mg qid, 7d	Scaling	None	12	CLIN and scaling improved clinical variables, reduced motileorganisms and decreased annual rate of sites with disease activity from 1.05 to 0.5%.
Walker and Gordon, 1990	13/ppa	CLIN 150 mg qid, 7d	Scaling	None	24	CLIN and scaling improved clinical variables and reduced annual rate of sites with disease activity from 8.0% to 0.5%. <i>Pg</i> , <i>Pi</i> and <i>Pm</i> were reduced or absent from 12 months.

Table 1b. Clinical studies of Clindamycin therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; LAgp = localized aggressive periodontitis; CLIN = clindamycin; *Pg* = *Porphyromonas gingivalis*; *Pi* = *Prevotella intermedia*

Azithromycin

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Smith et al, 2002	23 test, 21 control/ ppa	AZI 500 mg qd, 3d	Scaling	Placebo medication	5	AZI reduced BOP and PD better than placebo: double-blind study

Table 1c. Clinical studies of Azithromycin therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; AZI = azithromycin; PD = probing depth; BOP = bleeding on probing.

Penicillins/Amoxicillin

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Kunihira et al, 1985	16/LAgP	PEN 250mg qid, 10d every 3 mth for 9 mth	Surgery	Placebo medication	9	No advantage to adjunctive PEN, double-blind study
Haffajee et al, 1995	40/ppa	AMOX/CLA 250 mg tid, 30 d	Surgery	Pacebo medication	10	AMOX/CLA improved PD and CAL compared to placebo or ibuprofen. More decrease in <i>Aa</i> , <i>Pg</i> , <i>Pi</i> and <i>Cr</i> with AMOX/CLA than with placebo.
Winkel et al, 1999	10 test, Control/ppa	AMOX/CLA 500 mg tid, 10d	Surgery	Placebo medication	12	No clinical or microbiological difference between test and control double blind study.

Table 1d. Clinical studies of Penacillin/Amoxicillan therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; LAgp = localized aggressive periodontitis; PEN = penicillin; AMOX = amoxicillin; AMOX/CLA = amoxicillin/clavulanic acid; PD = probing depth; CAL = clinical attachment level; *Aa* = *Actinobacillus actinomycetemcomitans*; *Pg* = *Porphyromonas gingivalis*; *Pi* = *Prevotella intermedia*; *Cr* = *Campylobacter rectus*

Tetracycline/Doxycycline

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Rams and Keyes, 1983	21/ppa	TET 250 mg qid, 14d	None	Placebo medication	11	TET reduced PD, motile organisms and crevicular leukocytes recalcitrant sites: double-blind study.
Novak et al, 1988	4 LAgP	TET 250 mg qid, 42 d	None	None	2-48	TET without scaling reduced PD, CAL and BOP for up to 4 years.
Saxen et al, 1990	7 test, 7 ppa	DOX 100 mg qd, 13 d	Scaling	Placebo medication	20	DOX reduced <i>Aa</i> better than placebo double-blind study.
Haffajee et al, 1995	Control/LAgp 40 ppa	TET 250 mg qid, 30 d	Scaling	Placebo medication	10	TET improved PD and CAL compared to placebo or ibuprofen. More decrease in <i>Aa</i> , <i>Pg</i> , <i>Pi</i> and <i>Cr</i> with TET than with placebo.
Ramberg et al, 2001	35 test, Control/ppa	TET 250 mg qid, 21 d	Scaling	Scaling	12	TET improved CAL but not PD or BOP. TET had no beneficial effect beyond 1 year post-treatment.

Table 1e. Clinical studies of Tetracycline/Doxycycline therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; LAgp = localized aggressive periodontitis; TET = tetracycline; PD = probing depth; CAL = clinical attachment level; BOP = bleeding on probing; *Aa* = *Actinobacillus actinomycetemcomitans*; *Pg* = *Porphyromonas gingivalis*; *Pi* = *Prevotella intermedia*; *Cr* = *Campylobacter rectus*

Combination Therapy

Reference	N Patients/ Disease	Antibiotic/ Dose, Time	Concurrent Treatment	Control	Follow-up time (mth)	Outcomes
Van Winkelhoff et al, 1992	22/LAgP, 90/ppa	MET 250 mg + AMOX 375 mg tid, 7 d	Scaling	None	3-9	Improved clinical status. <i>Aa</i> was eliminated in 97% of patients and <i>Pg</i> in 88%.
Berglundh et al, 1998	8 test, 8 control/ppa	MET 250 mg + AMOX 375 mg tid, 14d	With and without scaling	Placebo	12-24	Improved clinical status. <i>Aa</i> and <i>Pg</i> were markedly suppressed or eliminated 2 months post-antibiotic. Combined mechanical and antibiotic therapy was most effective.
Flemming et al, 1998	18 test, 20 control/ ppa	MET 250 mg + AMOX 375 mg tid, 8d	Scaling	Scaling	12	Patients harbouring <i>Aa</i> benefitted clinically and microbiologically from the antibiotic therapy.
Winkel et al, 2001	18 test, 20 control/ ppa	MET 250 mg + AMOX 375 mg tid, 7d	Scaling	Placebo	6	More improvement in PD, CAL and BOP and more suppression of <i>Pg</i> , <i>Tf</i> and <i>Pi</i> in the antibiotic group than in the placebo placebo group.

Table 1f. Clinical studies of combination therapy in patients with severe periodontitis (Adapted from American Academy of Periodontology 2004)

ppa = progressive periodontitis in adults; LAgP = localized aggressive periodontitis; MET = metronidazole; AMOX = amoxicillin; PD = probing depth; CAL = clinical attachment level; BOP = bleeding on probing; *Aa* = *Actinobacillus actinomycetemcomitans*; *Pg* = *Porphyromonas gingivalis*; *Pi* = *Prevotella intermedia*; *Tf* = *Tannerella forsythensis*

Antibiotic	Adult Dosage
Metronidazole	500 mg/tid/8 days
Clindamycin	300 mg/tid/8 days
Doxycycline or minocycline	100-200 mg/qd/21 days
Ciprofoxacin	500 mg/bid/8 days
Azithromycin	500 mg/qd/4-7 days
Metronidazole + amoxicillin	250 mg/tid/8 days of each drug
Metronidazole + ciprofloxacin	500 mg/bid/8 days of each drug

Table 2. Common antibiotic therapies in the treatment of periodontitis (Adapted from American Academy of Periodontology 2000)

Trade name	Medication	Provider
Actisite®	Tetracycline fibres	Alza Corp, Mountain View, USA
Elyzol®	Metronidazole gel	Dumex, Copenhagen, Denmark
Periocline®	Minocycline Ointment	Sunstar, Japan
Dentomycine®	Minocycline Ointment	Lederle, UK
PerioChip®	Chlorhexidine Chip	Perio Products Ltd, Jerusalem, Israel
Atridox®	Doxycycline hyolate in a resorbable polyment	Atrix Labs, Ft Collins, USA
Arestin®	Minocycline monospheres	OraPharma Inc, Warminster, USA

Table 3. List of commercial products with a local delivery system for antibiotics

periodontal pockets to suppress or eradicate the pathogenic microorganism or modulate the inflammatory response, local application of pharmacological agents must fulfill three criteria: the medication must reach the intended site of action, remain at an adequate concentration and last for a sufficient duration of time (Goodson 1989). Once a drug reaches the site of action at an effective concentration, it must remain at the site long enough for the pharmacological effects to occur. The duration of exposure required is dependent upon the mechanism by which the antimicrobial agent inhibits or destroys target bacteria.

Longer therapeutic duration requires the use of a subgingival drug reservoir that can release the medication to counteract its continuous loss due to crevicular fluid flow. A local delivery device consists of a drug reservoir and a limiting element that controls the rate of medicament release. Local delivery devices can be divided into two classes according to the duration of medicament release: sustained release devices and controlled delivery devices. Sustained release formulations are designed to provide drug delivery for less than 24 hours. On the other hand, controlled delivery systems should have a duration of drug release that exceeds 1 day (Langer and Peppas 1981, Langer 1990).

There are a number of delivery systems with regulatory approval or pending approval by the US Food and Drug Administration or by the regulatory bodies of the European Union. To date, six commercial products have been available (Table 3). Elyzol and Periocline are not marketed in the United States at this time.

The clinical efficacy of local drug delivery has been evaluated primarily using several outcome measures: reduced probing depths, increased clinical attachment levels, decreased bleeding on probing and reduced disease progression.

Tetracycline fibers : Actisite®

Tetracycline fibers are a non-resorbable cylindrical drug delivery device made of a biologically inert, plastic copolymer loaded with 25% tetracycline HCl powder (Goodson *et al* 1983). The fiber is applied to completely fill the pocket and is maintained in site with a cyanoacrylate adhesive for 7 to 10 days (Goodson *et al* 1991, Tonetti *et al* 1994). At the end of the therapeutic period, the fiber must be removed.

Following application of tetracycline fibers, a suppression of the subgingival microbiota has been observed (Mombilli *et al* 1996, Goodson *et al* 1991). However, fibers releasing high local concentrations of tetracycline for 10 days were unable to eliminate the bacteria in the periodontal pocket (Goodson *et al* 1991). Use of tetracycline fibers resulted in a significant improvement in all tested outcome measures similar in magnitude to the improvement from scaling and root planing (Table 4a) (The American Academy of Periodontology 2000).

The long term therapeutic effect of these fibers was addressed in 12 month follow up and in a large, controlled, randomized, single-blind, multi-center, split-mouth study (Drisko *et al* 1995). When the efficacy of tetracycline fibers was compared to scaling and root planning alone or combination therapy there were no significant differences in mean effects with regard to probing depth reduction or gain of clinical attachment.

Metronidazole gel : Elyzol®

Metronidazole gel is a bioabsorbable delivery device containing 25% metronidazole benzoate in a matrix consisting of glyceryl mono-oleate and sesame oil (Norling *et al* 1992). The gel is applied subgingivally with a syringe and a blunt cannula. Decay of the drug concentration in crevicular fluid follows an exponential pattern which is compatible with a

sustained drug delivery (Stolze 1992).

The pivotal studies have compared sequential applications of metronidazole gel with a control group consisting of scaling and root planing only (Table 4b) (Ainamo *et al* 1992, Kinane and Radvar 1999, Klinge *et al* 1992, Rudhart *et al* 1998). In general, there were no clinical differences between therapies with regard to probing depth reduction and decrease in prevalence of bleeding on probing (Ainamo *et al* 1992, Kinane and Radvar 1999, Klinge *et al* 1992, Pedrazzoli *et al* 1992).

An additional study compared scaling and root planing to combined therapy (metronidazole plus scaling and root planning) (Stelzel and Flores-de-Jacoby 1996). This study reported a statistically significant, but clinically insignificant, improvement with combined therapy.

Meta-analysis has been used to assess the effectiveness of local delivery of metronidazole alone or as an adjunct to mechanical therapy in patients with chronic periodontitis. The results demonstrated the effectiveness of metronidazole as an adjunct to scaling and root planing in the treatment of chronic adult periodontitis, but clinical significance and dissemination of antibiotics should be taken into account in the evaluation of metronidazole as an alternative to scaling and root planing (Pavia *et al* 2004).

Minocycline ointment: Periocline®

Minocycline ointment is a bioabsorbable sustained delivery system consisting of 2% minocycline HCl in a matrix of hydroxyethyl-cellulose, aminoalkyl-methacrylate, triacetine, and glycerine. Magnesium chloride is added to modify the drug release properties (Satomi *et al* 1987). Minocycline is a bacteriostatic antibiotic. However, no data are available regarding the extent of its subgingival drug reservoir. A controlled, randomized, clinical trial compared the adjunctive effect of minocycline

ointment (4 administrations of drug at weekly intervals) or vehicle control to a single session of scaling and root planning (Table 4c) (van Steenberghe *et al* 1993). The results indicated that the combination of the ointment with scaling and root planning was significantly better than the vehicle control in pockets ≥ 7 mm when probing depths were evaluated at 1 and 3 months. No significant differences were observed in clinical attachment levels or a bleeding index.

Chlorhexidine chip : Periochip®

This product is a bioabsorbable local delivery device comprised of 34% chlorhexidine gluconate in a cross-linked gelatin matrix. Each chip is a 5 mm long, 5 mm wide, 1 mm thick pliable strip loaded with 2.5 mg of chlorhexidine gluconate. This controlled delivery system is gently pushed into the pocket, and chlorhexidine has been detected in gingival fluid in excess of 125 µg/ml for 1 week following a single application (Soskolne *et al* 1998). Two large scale randomized multicenter trials have assessed the efficacy of chlorhexidine chip plus scaling and root planing (Soskolne *et al* 1997, Jeffcoat *et al* 1998). Data analyses indicated that attachment level gains and probing depth reductions were statistically significantly better in the combined treatment group than in the scaling and root planing group (Table 4d).

Doxycycline polymer: Atridox®

A biodegradable formulation containing 10% by weight doxycycline, 33% by weight poly DL lactide and 57% by weight NMP (N-methyl 1,2-pyrrolidone) is available for the treatment of chronic adult periodontitis. An initial study reported that doxycycline hydiate applied in a vehicle was superior to 5% sanguinarine chloride or the vehicle alone for attaining probing depth reduction and gaining clinical attachment (Polson *et al* 1997). There

Tetracycline Fibers: Actisite®

Reference	Populn	Design	Study Type	No. of subjects	Observ period	Regimen	Treatment	Decreases in PD (mm)	Increases in AL (mm)	% Decrease BOP sites
Goodson et al, 1985	AP	Split mouth	CRT	10	12 mth	Single application	Untreated S/RP Fibers	0.43 0.54 0.57	0.23 0.50 0.54	NA
Goodson et al, 1991	AP	Split mouth (single sites treated after prophylaxis)	CRT	107	2 mth	Single application	Untreated S/RP Vehicle control Fibers	0.46 0.57 1.02	0.38 0.40 0.65	-23.4 -26.2 -20.6 -50.5
Heijl et al, 1991	AP	Split mouth	CRT	10	2 mth	2 consecutive applications	Untreated S/RP Fibers	0.02 1.78 1.98	NA 1.78 2.15	+4.0 -81.0 -82.0 -83.0
Newman et al, 1994	Maintenance (non-resp sites)	Split mouth	CRT	113	6 mth	Single application	S/RP Fibers & S/RP	1.08 1.81	1.08 1.56	-50.0 -63.0
Drisko et al, 1995	AP	Split mouth	CRT	122	12 mth	Single application, no further	S/RP 10 day fibers 20 day fibers	-1.17 -1.0 -1.3	-1.2 -1.2 -1.3	~60.0 ~60.0 ~60.0

					therapy	10 day f&S/RP	~1.5	~1.5	~60.0
Tonetti et al, 1998	Bleeding furcations in maintenance patients	Parallel group design	CRT	20	3 mth	Single application	S/RP Fibers & S/RP	0.8 1.6	0.7 0.9
Kinane and AP Radvar, 1999	Parallel group design	CRT	79	6 mth	Single application	S/RP Fibers & S/RP	0.71 1.38	0.54 0.69	38.0 43.8

Table 4a. Results of studies investigating treatment of periodontitis with Tetracycline fibers (Adapted from American Academy of Periodontology 2000)

Metronidazole Gel: Elyzol®

Reference	Populn	Design	Study Type	No. of subjects	Observ period	Regimen	Treatment	Decreases in PD (mm)	Increases in AL (mm)	% Decrease BOP sites
Klinge et al, 1992	AP	Split mouth	CRT SB	61	3 mth	Various applications	2xMET25% over two weeks	1.2	NA	43.0
							2xMET125% 1xS/RP	1.0	NA	49.0
							4xMET25%	1.2	NA	40.0
							1xS/RP	1.3	NA	41.0
Ainamo et al, 1992	AP	Split mouth	CRT SB	206	6 mth	Two applications	2xMET25%	1.3	NA	32.0
							1xR/RP			
							2xMET25%	1.3	NA	~50.0+
							1xS/RP	1.5	NA	~50.0
Steizel and Flores-de-Jacoby, 1996	Recall	Split mouth	CRT	30	6 mth	Two applications	1xR/RP	1.5	NS	39.0
							2xMET25%	1.3	NA	~50.0
							1xS/RP	1.5	NA	
Kinane and Radvar, 1999	AP	Parallel group	CRT SB	79	6 mth	Single application	S/RP MET25% & RP	0.71	0.54	38.0
								0.93	0.54	33.2
Rudhart et al, 1998	Recall	Split mouth	CRT SV	46	175 days	Two applications	2xS/RP 2xMET25%	1.6	0.50	NA
								1.6	0.70	NA

Table 4b. Results of studies investigating treatment of periodontitis with Metronidazole gel (Adapted from American Academy of Periodontology 2000)

Minocycline Ointment: Periocline®

Reference	Populn	Design	Study Type	No. of subjects	Observ period	Regimen	Treatment	Decreases in PD (mm)	Increases in AL (mm)	% Decrease BOP sites
Van Steenberghe et al, 1993	ChP	Parallel group	CRT DB	103	3 mth	Four applications once every two weeks	4xMIN2% & RP 1.4	1.7	0.8	-1.2
Timmerman et al, 1996	ChP	Parallel Group	CRT DB	20	18 mth	Seven applications over 12 mths	7xMIN2% & RP 1	0.5	0.5	-1.2
Kinane and Radvar, 1999	AP	Parallel group	CRT SB	79	6 mth	Single application	1xMIN2% & RP 0.95	0.3	0.3	-1.1

Table 4c. Results of studies investigating treatment of periodontitis with Minocycline Ointment (Adapted from American Academy of Periodontology 2000)

Chlorhexidine Chip: Periochip®

Reference	Populn	Design	Study Type	No. of subjects	Observ period	Regimen	Treatment	Decreases in PD (mm)	Increases in AL (mm)	% Decrease BOP sites
Soskine et al, 1997	ChAP	Split mouth	CRT DB	118	6 mth	Single application	S/RP S/RP & chip	0.70 1.16	0.47 0.31	
Jeffcoat et al, 1998	ChAP	Parallel group	CRT DB	447	9 mth	Single application	S/RP S/RP & chip	0.65 0.95	0.58 0.75	
							S/RP - placebo	0.69	0.55	

Table 4d. Results of studies investigating treatment of periodontitis with Chlorhexidine chip (Adapted from American Academy of Periodontology 2000)

Local herbal product	Vehicle	Level of research
Streblus asper		Gel development Microbiological test Clinical safety Clinical trial
Andrographis paniculata gel	Glyceryl monooleate (GMO) + triglycerides	Gel formulated Microbiological test Clinical trial Comparison to commercial gel (Elyxol, Minocycline)
Cymbopogon citrates		Microbiological test

Table 5. List of medicinal plants that have been investigated for local usage in Thailand as adjunctive periodontal treatment

are no data available regarding the ability of doxycycline polymer to enhance periodontal health when used in conjunction with root planing.

Minocycline microspheres: Arrestin®

Minocycline microspheres are a subgingival sustained release product containing minocycline hydrochloride incorporated into a bioresorbable polymer, Poly(glycolide-co-DL-lactide) or PGLA. Each unit-dose cartridge delivers minocycline hydrochloride equivalent to 1 mg of minocycline free base.

Large scale randomized multicenter trials have assessed the efficacy of minocycline microspheres plus scaling and root planing in patients with generalized moderate to advanced adult periodontitis. Subjects treated with scaling and root planing plus minocycline microspheres were found to have statistically significantly reduced probing pocket depth compared with those treated with scaling and root planing alone or scaling and root planing plus vehicle at 9 months after initial treatment (Williams *et al*

2001). Other studies have indicated that treatment with scaling and root planing plus minocycline microspheres is consistently more effective than scaling and root planing alone in providing clinically relevant site-based responses in patients with chronic periodontitis (Paquette *et al* 2004) and smokers (Paquette *et al* 2003).

The development of medicinal plant extracts for local delivery usage

Recently, medicinal plants as new sources of antimicrobial agents have already attracted considerable research interest. A wide variety of plant extracts have been reported to have antimicrobial effects and anti-inflammatory properties (Atindehou *et al* 2002, Kweifio-Oka 1991, Mahasneh 2002, Matsuda *et al* 1997, Taweechaisupapong *et al* 2000, Taweechaisupapong 2002). In Thailand, several research groups have investigated the potential of local herbal products as an adjunctive periodontal treatment (Taweechaisupapong *et al* 2000,

Taweechaisupapong 2002, Amornchat *et al* 1991, Rassameemasamaung *et al* 1998, Atsawasawan *et al* 1998, Kuphasuk *et al* 1998a, Kuphasuk *et al* 1998b, Sookkhee *et al* 2004, Sirirut and Rojanapanthu 2005). These plants include *Streblus asper* (Taweechaisupapong *et al* 2000, Taweechaisupapong *et al* 2002), *Andrographis paniculata* (Amornchat *et al* 1991, Rassameemasamaung *et al* 1998, Atsawasawan *et al* 1998, Kuphasuk *et al* 1998a, Kuphasuk *et al* 1998b), and *Cymbopogon citratus* (Sookkhee *et al* 2004) (Table 5). Although studies on the effect of these medicinal plants in the treatment of periodontal disease are still in early stages, there is already evidence demonstrating the beneficial effects (Figures 1-3) (Sirirat 2005). Further studies are warranted.

Case 1

A 39 year old Thai male had deep probing depth and bleeding on probing on anterior teeth and upper molars. Periodontitis was more severe on teeth 11, 12 and 31 with pus exudation, 2 mm gingival recession and second degree tooth mobility.

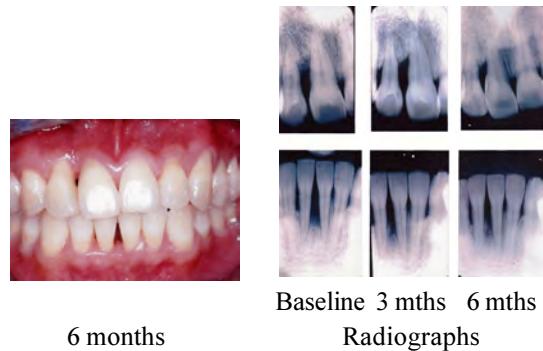


Figure 1. 39 year old male with chronic periodontitis - response to treatment

Case 2

A 41 year old Thai male was referred for generalized chronic periodontitis treatment. The clinical photographs of lower anterior teeth at baseline examination show gingivae with severe inflammation and edema on both labial and lingual aspects. 8 mm of probing pocket depth on the mesial of 31 and 41, bleeding on probing and first degree tooth mobility were noted.



Baseline 3 months 6 months

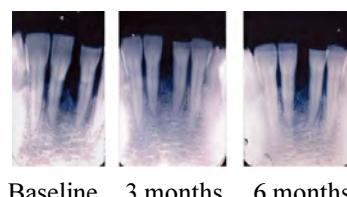


Figure 2. 41 year old male with generalised chronic periodontitis - response to treatment

Case 3

A 54 year old Thai female with severe destruction of alveolar bone on the distal aspect of tooth 41 was referred for periodontitis

treatment. Clinical examination exhibited deep pockets (10 mm), no gingival recession and second degree tooth mobility. Vitality testing on 41 was positive.



Figure 2. 54 year old female with severe destruction of alveolar bone - response to treatment

Conclusion

Two systematic reviews have evaluated literature-based evidence in an effort to determine the efficacy of currently available anti-infective agents, with and without concurrent scaling and root planing, in controlling chronic periodontitis. One meta-analysis completed on 19 studies that included scaling and root planing and local sustained-release agents compared with scaling and root planing alone indicated significant adjunctive probing depth reduction or clinical attachment gain for minocycline gel, microencapsulated minocycline, chlorhexidine chip and doxycycline gel during scaling and root planing compared to scaling and root planing alone (Hanes and Purvis 2003). Use of antimicrobial irrigants or anti-infective sustained-release systems as an adjunct to scaling and root planing does not result in significant patient-centered adverse events. While another review

indicated the most positive results occurred for tetracycline, minocycline, metronidazole, and chlorhexidine (Bonito *et al* 2005). Adjunctive local therapy generally reduced probing depth levels. Differences between treatment and scaling and root planing only groups in the baseline-to-follow-up period typically favored treatment groups but usually only modestly (e.g. from about 0.1 mm to nearly 0.5 mm probing depth reduction) even when the differences were statistically significant. The effects on clinical attachment gains were smaller and statistical significance less common. Whether such improvements, even if statistically significant, are clinically meaningful remains a question.

Current data suggest that local delivery of antimicrobials into a periodontal pocket can improve periodontal health. However, these drug delivery systems do not provide a superior result when compared to scaling and root planning. Therefore, the benefits of utilizing local delivery systems as a monotherapy are questionable. In conjunction with scaling and root planning, the adjunctive use of local drug delivery may enhance the results in sites that do not respond to conventional therapy. A few localized persistent lesions, in otherwise well controlled patients, may offer the greatest potential for success with this treatment modality.

Therefore, as with systemic antimicrobial therapy, local antimicrobial therapy should not be used routinely in situations when efficacious results can be accomplished with scaling and root planning.

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Chapter 9

Clinical Applications of Current Research in Periodontal Wound Healing & Regeneration

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Introduction

Clinical concepts in periodontal regeneration are constantly undergoing evaluation and evolution, however, the quest to repair periodontal tissues destroyed by inflammation to their original anatomic and functional form is not new. The literature documents the use of several cellular and non-cellular graft materials from natural and synthetic sources in an attempt to regenerate periodontal tissue (AAP Consensus report). Much of the earlier clinical work focused on the regeneration of bone, but eventually evidence of the participation of several tissue types in the healing periodontal wound provided an enhanced understanding of the underlying mechanisms (Aukhil 2000). This led to the guided cell repopulation theory that formed the basis for clinical techniques such as guided tissue regeneration (Melcher 1976, Murphy and Gunsolley 2003). With the advent of oral implantology, attempts were made to clinically extrapolate some of these techniques to the regeneration of bone around implants with an osseous deficit. Recent research has however dramatically affected our previous understanding of wound healing and this has led to the development of tissue engineering techniques that seek to harness biological principles of wound healing to enhance the

results of periodontal regeneration (AAP Position Paper 1996). The use of various growth factor isolates, or autologous plasma concentrates, are examples of such efforts. Nevertheless, questions remain about current techniques and the continual quest to make periodontal regeneration economical and predictable. Distinctions must also be made between the kind of regeneration that is sought around teeth and implants as the composition of the tissues in either case is different. While certain principles may be biologically applicable in both situations, there is nevertheless a clinical difference.

Mechanisms of wound healing

Our current understanding of the mechanisms that underlie ideal periodontal or peri-implant wound healing can be best described by the Venn diagram in Figure 1. At least four principle factors typically play a key role, with the availability of a cellular source probably being the most important factor. The periodontal wound is characterized by various tissue types, that are in turn represented by their cellular precursors or by undifferentiated cells that could potentially differentiate into precursor cell lines (Wang and MacNeil 1998). The availability of the right cells in the healing wound is therefore primary to regenerative

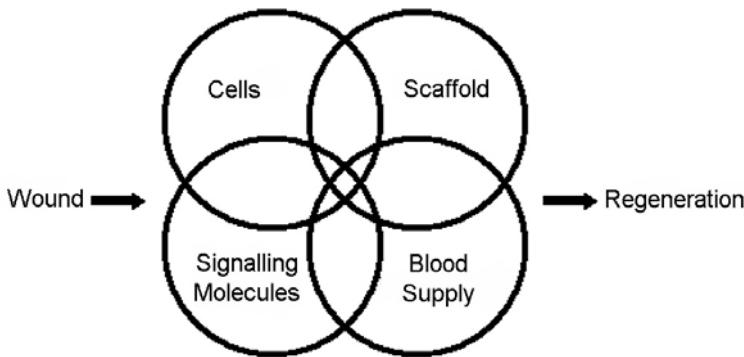


Figure 1. Venn diagram of the scheme of regeneration

success. The differentiation sequence is in turn wrought by a second and equally important factor; signalling molecules (AAP Position Paper 1996). These may represent a wide variety of biochemical constituents of the healing wound that work concomitantly to regulate the complex physiology of the regenerative process (Ripamonti and Reddi 1997). The ability of this system to bring about complete regeneration of the periodontal wound is sometimes limited by the extent of tissue deficit, and the need for a third factor in the form of a scaffold or matrix in such situations seems inevitable. A scaffold provides a mechanical matrix that offers physical support to regenerating tissue (Spector 1994). Scaffold chemistry could also potentially contribute to enhanced wound healing. A final fourth factor that plays a key role is vasculature, which provides angiogenic elements to the regenerating wound and provides a transport mechanism for participating biochemical constituents (Polson and Proye 1983, Wikesjö *et al* 1992). The presence or absence of periodontopathic bacteria also affects wound healing, often detrimentally (Selvig *et al* 1992). Undeniably, the elimination of infection is therefore key to achieving ideal regenerative results, even in the presence of all contributory factors.

Graft materials

The use of various graft materials as a part of regenerative periodontal therapy has become commonplace. These materials vary widely from autologous bone to human cadaver sourced allografts to animal sourced xenografts. The use of synthetic alloplasts is also very common (Reynolds *et al* 2003).

The first documented use of an autograft in periodontal therapy was by Hegedus in 1923 and their use in regenerative therapy in both periodontics and implantology is still apparently mandated.

The limited availability of autografts initially led to much interest in allografts and xenografts and their use was based largely on the fact that they were naturally derived and chemically identical. Cadaver sourced allografts have also been subjected to biochemical processes such as decalcification and freeze drying in order to enhance their osteoinductive potential, ostensibly by the exposure of bone morphogenic proteins (Urist 1965, AAP Position Paper, Pearson *et al* 1981). Several years of use have however brought into question the potential of these materials to consistently induce cellular differentiation of osteoprecursor cell lines. Variations in biologic activity as a result of differing processing

protocols have also been demonstrated and renewed standards for potency evaluation, a defined age/systemic status for cadaver donors and the development of assays for inductive capacity have all been suggested (Schwartz *et al* 1998, Schwartz *et al* 1996). Xenografts on the other hand are of questionable consistency in replicating the physical structure of human bone in order to act as a suitable scaffold, as most of these materials are classified as 'osteoconductive', in apparent recognition of their inability to actually induce new bone formation. The age and the systemic status of the source animal, as well as the actual osseous location from which the graft is harvested, are factors that affect the consistency of the physical structure of a xenograft. Allografts and xenografts have also frequently presented concerns about cross-infections and immunogenicity by virtue of a natural source (Sogal and Tofe 1999). Therefore, as a gold standard for grafts, cellular autografts seem to hold the most potential for use.

Signaling molecules

Several attempts have also been made to isolate various biochemical constituents of the wound healing process, with a view to providing them in the immediate environment and thereby presumably accelerate the rate of healing (Giannobile and Somerman 2003). Comprising largely of proteins that are derived from various cells (principally platelets and macrophages), these constituents function concomitantly and often upregulate individual function of respective constituents that are present in the environment (Sculean *et al* 2002, AAP Position Paper 1996). Their isolation and use in clinical therapy may therefore have limited application. Nevertheless, studies suggest that these attempts may be a step in the right direction with the documented clinical success of use of autologous plasma concentrates that provide higher

concentrations of platelets and related growth factors (Camargo *et al* 2002). The ability to incorporate these techniques into a chairside procedure and the potential for use of autologous concentrates with graft materials is also appealing from the standpoint of ease of use. It must be emphasized however, that these molecules have a role to play only in the presence of cellular graft materials.

Initial studies with recombinant human proteins and matrix proteins are also encouraging, but there is very limited evidence from human clinical trials with these materials (Kinoshita *et al* 1997, King *et al* 1998). Similarly, the use of a new putative collagen binding peptide utilizing a combination of a bovine hydroxyapatite matrix and a synthetic clone of the 15 amino acid sequence of Type I Collagen, also has limited evidence (Yukna *et al* 1998, Qian and Bhatnagar 1996, Bhatnagar *et al* 1999).

Scaffold matrix and blood supply

The use of non-cellular grafts materials is primarily an attempt to provide a physical scaffold matrix for the regenerating wound. The physical importance of such a matrix comes into play in situations where the volume of osseous deficit extends beyond the regenerative capacity of surrounding or augmented tissue (Meffert *et al* 1985). In such situations the topography of the osseous defect, number of peripheral osseous walls and the proximity of normal bone, all play a role. The physical and chemical nature of the scaffold is nevertheless contributory and it is important to ascertain that this does not become a deterrent to the regenerative process (Le Geros 1990). The physical nature of the scaffold must provide for ideal inter- and intra-particular porosity to allow angiogenesis and osteoconduction of the regenerating fibroangiomatous elements of the wound (Kenney *et al* 1996). The nature of such

porosity should be interconnected and continuous in order to allow proper tissue ingrowth (Carranza *et al* 1987). In addition, the porosity of the scaffold has important implications for the rate of resorption of the graft material, thus allowing its replacement by natural bone (Surathu 1994). Given the same chemistry, a graft particle that is dense would resorb far more slowly than one that is porous, a phenomenon that is explained by increased vascular and cellular access to multiple surfaces of a graft particle that is porous.

The resorptive ability of a graft particle is in itself however dependent on the chemistry of the material that constitutes it. Depending upon the crystallinity, molecular structure and chemical constitution, alloplasts can vary between totally resorbable to totally non resorbable. In an ideal scaffold configuration, the material must eventually undergo complete replacement by natural bone. In implantology, the rate of such resorption and replacement may also be of some clinical consequence to the treatment plan. Alloplasts have been constituted by various calcium phosphate ceramics, bioactive glasses and the like and these materials have varying rates of resorption. It may be that biphasic calcium phosphate ceramics constituted in the right chemical ratio may hold promise for ideal resorption dynamics in the future (Daculsi *et al* 1989).

The use of a scaffold also mandates the use of guided tissue or bone regeneration techniques as there is ample evidence to suggest that failure to do so results in improper/unproportional constitution of the wound by various tissue types (Nyman *et al* 1982). Guided tissue regeneration materials in use today are largely resorbable in nature, by virtue of the convenience that such a technique provides in terms of elimination of a secondary surgery for extrication of the material (Teparatz *et al* 1998). Resorbable materials are largely constituted either by synthetic polymers

(Caffesse 1997) or animal collagen (Black *et al* 1994, Blumenthal 1993), of which the latter seems preferable due to the fact that polymers tend to metabolize into end products that are acidic in nature (Mattson *et al* 1999). Current collagen materials also maintain their integrity for sustained clinical periods and offer the advantage of an ideal tensile strength that allows tacking or suturing to immobilize the material, since mobility may be detrimental to healing (Egelberg 1987). Collagen has also been demonstrated to possess a hemostatic function that may facilitate early clot formation and wound stabilization (Steinberg *et al* 1986), in addition to a chemotactic function for fibroblasts that may aid cell migration and to promote primary wound closure (Poslethwaite *et al* 1978).

Vascular supply to the healing wound is undoubtedly also important and emphasis continues to be placed on the formation of a stable blood clot with ideal fibrin linkages that enhance connective tissue matrix formation. Disruption of the fibrin linkages has been shown to promote long junctional epithelial healing as well (Polson and Proye 1983, Wiksjö *et al* 1992).

Periodontopathic pathogens

Favorable clinical results have most often been observed in healthy patients demonstrating good plaque control and compliance with recommended oral hygiene measures. Studies have also noted an inverse relationship between plaque contamination of retrieved membranes and clinical attachment gain. Colonization of membranes with black pigmented species and the presence of bacteria in samples treated with regenerative procedures has been shown to correlate with diminished healing response (Selvig *et al* 1992, Nowzari and Slots 1994).

Conclusion

The quest for techniques that make periodontal regeneration predictable continues. An amalgamation of all that we currently know seems to suggest that autologous grafts and signaling molecules will play a large role in the future. The use of alloplastic materials with ideal porosity and resorbability seems supportive at best, in combination with resorbable guided regeneration devices. The emphasis on prevention and maintenance however seems central to all attempts to regenerate tissue that is lost to disease. In the near future, we will possibly encounter enhanced clinical techniques that will employ advancements in material science and our improved understanding of wound healing biology, for greater success.

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Chapter 10

Contemporary Clinical Techniques for Enhanced Periodontal Prognosis

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Introduction

Recent advances in periodontics have been largely a result of research. This has translated into a better understanding of the etiopathogenesis of periodontal disease, the biological capability of periodontal regeneration and the ability to undertake tooth replacement using dental implants. This paper aims to provide a brief overview along three fronts, namely diagnostics and risk analysis, guided tissue regeneration and dental implants, which have the potential to provide clinical techniques for enhanced periodontal prognosis of our patients with periodontal disease.

Diagnosis of periodontal disease

As research has revealed the various mechanisms and parameters involved in the etiopathogenesis of periodontal disease, we can now consider how such knowledge can be utilized to improve diagnosing our patients. Traditional methods of relying primarily on radiographs, probing depths, clinical attachment levels, bleeding and plaque index to diagnose periodontal disease have been shown to be rather inadequate and therefore in need of updating. In a recent review, Persson (2005) posed a fundamental question concerning which of the relevant parameters should be

considered before a diagnosis is made. The paper indicated that traditional pocket depth, bleeding index and radiographs produced the primary data sets that are utilized in our diagnosis; despite knowing such data sets have serious weaknesses in aiding diagnosis and lack predictive value on tooth prognosis. The review compared the limitations of site-based vs subject-based diagnosis of periodontal disease. A number of issues were highlighted:

- 1 Standard site-based clinical parameters (such as bleeding upon probing, plaque scores, tooth mobility, probing depths, clinical attachment loss) collected do not take into account any subject-based information and therefore considered 'poorly' used in the diagnostic process.
- 2 Such site-based clinical measurements have poor intrinsic diagnostic and prognostic value.
- 3 Dental radiographs serve only as a cumulative record of bone destruction caused by periodontitis.
- 4 Other site-specific laboratory based information (i.e. analysis of specific bacteria, markers of inflammation, antibody titres recovered from gingival crevicular fluid (GCF)) can be used to provide diagnostic evidence of infection and/or host response and this should be routinely used by dentists.

Parameters	Relative value of individual parameters					
	0	2	4	6	8	10
Infection (Microbiological assay)						
Host immunity (Antibody assay)						
Serum inflammatory markers						
System diseases (Diabetes)						
Clinical factors (Pocket depths, bleeding on probing, radiographs)						
Genetics (Genetic factors)						
Behavioural factors (Tobacco use)						

Table 1. Model of periodontal disease diagnosis (Persson 2005)

- 5 Traditional clinical data (ie. bleeding upon probing, pocket depth, radiographs) need to be re-examined so that they can be translated into providing subject-based information.
- 6 Subject-based data (ie. serum assays of antibody titres, microbiological assays) can provide differential diagnosis of periodontitis and therefore should be incorporated into the diagnostic process.
- 7 Any laboratory based methods must be cost effective and have standardized protocols so as to provide useful diagnostic information.

In conclusion, Persson (2005) proposed a

potential composite model that considered both site and subject based diagnostic information (Table 1).

Such a model would be helpful not only in the diagnosis of periodontal disease, but would also provide useful information in analyzing the relative risk of future periodontal disease activity at the site and subject level. It will be a challenge for the various research centres around the world to conduct collaborated research and to produce meaningful data that will help develop this model and revolutionize the diagnosis of periodontal disease and thereby help the clinician determine individual tooth prognosis.



Figure 1. Tooth #21 presenting with persistent sinus

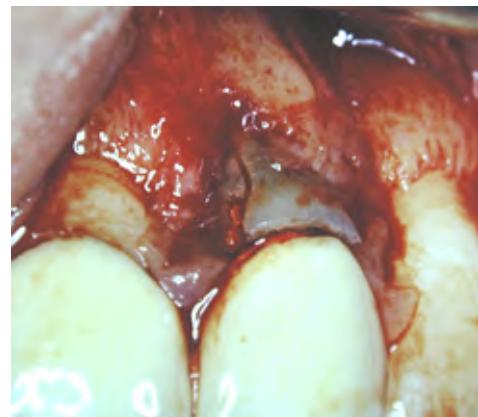


Figure 2. #21 Open flap surgery reveals vertical root fracture

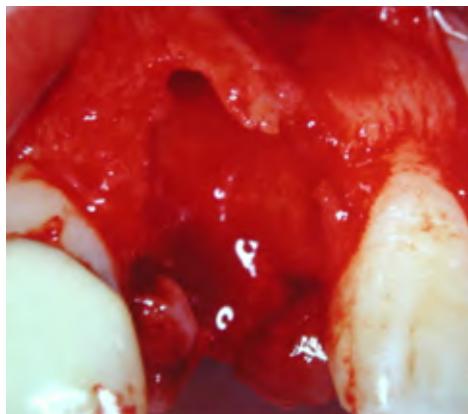


Figure 3. Extraction of #21 reveals a 5 mm loss of vertical buccal bone plate with sizable periapical granuloma



Figure 4. #21 Socket grafted with Bio-Oss and covered with Bio-Guide collagen membrane then closed and allowed to heal



Figure 5. Stage 1 dental implant surgery (Note new buccal bone plate)



Figure 6. Final prosthesis in place

Guided periodontal regeneration

Another major front in periodontics is regenerative therapy. Early work (Gould 1977, Gould 1980, McCulloch and Melcher 1983, Melcher 1970) into periodontal wound healing provided the biologic basis and information for subsequent research in clinical techniques to achieve periodontal regeneration. Guided periodontal regeneration (GTR) originated following a report by Nyman and co-workers (1982). This technique can be described as a surgical procedure whereby a barrier or material is placed at a site to assist, enhance or allow selective repopulation by preferred cells that will heal with the result of reconstitution of tissue that resembles the original tissue architecture. Thus true periodontal regeneration will result in formation of new cementum, alveolar bone and connective tissue attachment/periodontal ligament.

GTR procedures have impacted on the treatment and management of various periodontal conditions. Today, a wide variety of membranes and grafting materials are available for use. In addition, from the principle of GTR, the technique of guided bone regeneration (GBR) was developed. This procedure has enabled clinicians to help patients recover from the loss of bone volume in different defects to facilitate restorative treatments. The importance of GBR procedures was further underlined in a recent study by Donos *et al* (2005) that concluded onlay autografts with GBR provided superior long-term volume stability of the graft when compared with grafted sites without GBR.

A systematic review by Murphy & Gunsolley (2003) concluded that GTR is consistently more effective than open flap debridement in the gain of clinical attachment and probing depth reduction in the treatment of infrabony and furcation defects. Furthermore a recent position paper by the Academy of Periodontology (2005), concluded

that GTR has value as a regenerative procedure, particularly in 3-walled infrabony and gingival recession defects. This procedure can thus be recommended for use in clinical practice (eg. for the treatment of infrabony, furcation and recession defects). However, the report also cautions the clinician of the limitations of regenerative procedures, in that although periodontal regeneration is a possible objective of several periodontal therapeutic modalities, outcomes of such modalities are not always predictable. Perhaps, research may in time identify other factors (local and host factors involved in the healing response/potential) that possibly influence the outcomes of regenerative procedures and thereby improve the predictability. Future research will continue to look into the development of bioactive membranes or 3D cell matrices serving as scaffolds for cultured periodontal stem cells in regenerative procedures. This, together with the ability to modify healing response/potential, will have the potential to provide consistent and predictable treatment outcomes in periodontal regeneration.

Dental implants

Since the principle of osseointegration was first described by Branemark and co-workers (1969) dental implants have provided another invaluable modality of tooth replacement therapy to offer to our patients. The spectrum of research into various aspects involving dental implants has helped us to better understand the processes involved in osseointegration. This has subsequently impacted on both the design of dental implants and the clinical techniques involved. This range, from smooth machined surface titanium implants involving surgery in the strict environment of the operating theatre, to the current variety of micro-textured surfaces and implantation in a chair-side setting, dental implants as a modality of tooth replacement, has helped periodontists offer a long-term

predictable alternative to attempts to maintain the teeth affected by advanced periodontal disease.

Together with GBR and sinus elevation techniques (Boyne 1980), even compromised sites (see Figures 1-6) can be restored with dental implants. More recent techniques involving immediate implant placement following tooth extraction, as well as single stage implant procedures can be done provided the clinician adheres to careful and appropriate case selection for such procedures. Dental implants, as a modality of treatment have changed the way tooth replacement therapy is viewed by both the clinician and our patients. With the advent of more and cheaper dental implant systems, the costs for our patients will continue to drop thereby making it more affordable for more.

Conclusion

The future of periodontics appears bright. However, we face a number of exciting challenges ahead. Among them, include the development of a better diagnostic and prognostic model to guide us towards accurate diagnosis and treatment for our patients, more predictable techniques and materials for periodontal regeneration and dental implants.

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Chapter 11

Contemporary Clinical Directions in Regenerative Periodontics

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Introduction

Regeneration has been the focus of periodontal treatment over the past decade. Regeneration has been defined as the reproduction or reconstitution of a lost or injured part (Glossary of terms, AAP, 2003). Periodontal regeneration is the regeneration of the tooth's supporting structures including alveolar bone, periodontal ligament and cementum. (Glossary of terms, AAP, 2003) Researchers have been focusing on different ways to accomplish this using the following agents and procedures:

- 1 Growth and amelogenin-like factors.
- 2 Biomodification of root surfaces.
- 3 Bone substitutes from varying sources, autogenous, demineralized freeze dried bone allograft, freeze dried bone allograft, bovine-derived xenografts, alloplasts, etc.
- 4 Guided tissue regeneration for intrabony and furcation defects.
- 5 Guided tissue regeneration for root coverage.

Growth and amelogenin-like factors

A great deal of attention has been given to growth and amelogenin-like factors for periodontal regeneration. Growth factors are

natural biological mediators which have the ability to control vital events such as DNA synthesis, chemotaxis, cell differentiation and matrix synthesis, which are all involved in tissue repair (Anusaksathien *et al* 2002). The 2003 World Workshop lists the following as examples of growth factors used experimentally to treat periodontal disease:

- Platelet-derived growth factor (PDGF)
- Transforming growth factor- β (TGF- β)
- Basic fibroblast growth factor (FGF-2)
- Insulin-like growth factor (IGF-1)
- Bone morphogenic proteins (BMPs)
- Vascular endothelial growth factor (VEGF)
- Parathyroid hormone (PTH)

It is important to note that these growth factors are still under study and various stages of development and are not approved for human use at this time.

Enamel matrix derivative

Enamel matrix derivative (EMD) is derived from a developing porcine tooth germ. EMD is made up of 90% amelogenins, with the remaining 10% primarily proline-rich non-amelogenins, tuftelin, tuft protein, serum, ameloblastin, amelin, and salivary proteins (Brookes *et al* 1995). Current studies reveal that these proteins have been extraordinarily

well-conserved throughout evolution (Hammarstrom 1997) and maintain high homogeneity with human enamel proteins (Gestrelius *et al* 1997). Numerous clinical studies have reported improvement of periodontal parameters with the application of EMD to root surfaces in intrabony defects (Froum *et al* 2001, Heijl *et al* 1997, Zetterstrom *et al* 1997). In comparison with GTR and EMD, clinical trials have found comparable results with the use in intrabony defects (Pontoriero *et al* 1999, Sculean *et al* 1999) without one being superior to the other. EMD has also been found to be safe and has not shown any antibody response or other local or inflammatory occurrences (Zetterstrom *et al* 1997). Histologically, EMD has proven to show true periodontal regeneration consisting of cementum, periodontal ligament and alveolar bone aside from remarkably improving soft tissue measurements (Heiji 1997, Mellonig 1999, Yukna and Mellonig 2000). Current evidence supports the use of EMD in periodontal osseous defects to increase clinical attachment level and reduce probing depth, although long term benefits have yet to be established with further long term clinical trials (Giannobile and Somerman 2003)

Biomodification of root surfaces

Root surfaces act as a wound surface where periodontal regeneration can take place, thus it should provide an area suitable for cell attachment and fiber growth if regeneration is to be achieved. Many studies in the periodontal literature target citric acid, EDTA and tetracycline HCl as the media by which this is attained. Periodontal disease causes changes on the root surfaces of teeth, namely, collagen fiber loss (Selvig 1969), root surface contamination by bacteria and endotoxin (Adriens *et al* 1988, Aleo *et al* 1974), changes in the mineral density and composition of the

surface (Selvig *et al* 1977, Selvig *et al* 1962). Therefore, researchers seek to alter these changes caused by the disease on the root surface that can encourage regeneration. The mechanism by which these chemicals operate on the root surfaces is not well understood, but it has been hypothesized "that demineralizing agents act by exposing collagen fibers within the root matrix thereby facilitating attachment by other fibers in the periodontium and/or by decontaminating the root surface via elimination of endotoxin and bacteria, and/or by removal of the root debris allowing for the unobstructed attachment of regenerative cells to the root surface" (Mariotti 2003).

Although many studies have shown some connective tissue attachment to cementum after application of these demineralizing agents (Cole *et al* 1980) the results are not universal (Stahl *et al* 1983). No clinical significance with its utilization has been shown to prove its claim. Further clinical studies have been suggested to fully establish its supposed objective (Mariotti 2003).

Bone replacement grafts

Bone replacement grafts (BRG) have been used as a therapeutic measure to treat periodontal osseous defects. A wide variety of BRGs are available and have been categorized into 4 main types:

- 1 Autogenous.
- 2 Allografts.
- 3 Hetero-Xenografts.
- 4 Alloplastic materials.

Autogenous grafts

Autogenous grafts are usually obtained from the maxillary tuberosity, healing extraction sites, edentulous spaces of the jaw, mandibular retromolar area or osseous coagulum from bone while osteoplasty or osseous resection are being

performed at surgical sites (Mann 1964, Ellegard *et al* 1971, Hiatt 1973). Studies have shown 1.2 mm probing bone gain in areas grafted with intraoral autogenous bone compared to 0.8 mm in non grafted controls (Renvert *et al* 1985). Superior fill compared to open flap debridement has been successfully shown with use of bone blend or osseous coagulum (Froum *et al* 1975). Autogenous grafts have been the material of choice provided it is available and patients are informed of the advantages and disadvantages.

Allografts

The most extensively studied allograft is demineralized freeze dried bone allograft (DFDBA). Results from animal studies have indicated that demineralization of a cortical bone allograft improves its osteogenic potential by exposing bone inductive proteins referred to as bone morphogenic proteins (BMP) which induces host cells to differentiate into osteoblasts (Urist *et al* 1970, Harakas 1984, Mellonig *et al* 1981). A controlled study has shown a mean bone fill of 2.6 mm (65% bone defect fill) in grafted sites treated with DFDBA versus 1.3 mm (30% bone defect fill) in non grafted sites (Mellonig 1984). Histologically, when DFBA is placed into intrabony defects a new attachment apparatus forms, including new bone, cementum, and periodontal ligament while open flap debridement has shown only periodontal repair characterized by formation of a long junctional epithelium (Bowers *et al* 1989, Bowers *et al* 1991).

Freeze dried bone allograft (FDBA) has been studied less but also has shown some possible clinical efficacy in osseous defects (Barnett *et al* 1989, Mellonig 1991). More controlled studies of the clinical benefits are needed to show its efficacy in achieving good consistent results. Attention has been given to this graft for the potential to act as a scaffold-

base carrier for biologically active molecules because of its clinical characteristic as a good space maintainer (Rosen *et al* 2002). In fact DFDBA and FDBA have been used as a carrier for biologically active molecules and further studies are recommended to determine its potential as scaffold-based carriers for growth and amelogenin like factors (Reynolds *et al* 2003).

Xenografts

The most common xenograft is a natural bone mineral of bovine origin. This is a highly purified osteoconductive mineral structure that is made from natural bone in a multistage purification process under strict safety standards. Bovine porous bone mineral closely mimics human cancellous bone as compared to other allografts or synthetic hydroxyapatite materials when assessing parameters such as inner surface area, porosity, crystalline size and calcium-to-phosphorous ratio (Valdre *et al* 1995). A few studies have documented the ability of this xenograft to enhance bone formation in situations such as those around implants (Berglundh 1997), critical sized osseous defects (Schmitt *et al* 1997) and sinus elevations (Valentini *et al* 1997). Although there are some histologic studies demonstrating new attachment formation after its use (Camelo *et al* 1998, Camelo *et al* 2001), further investigation should be done to investigate the predictability of their outcome. Randomized clinical trials that confer clinical outcome data and uncontrolled human histologic studies are still to be performed in order to verify its ability to produce true periodontal regeneration (Reynolds *et al* 2003). Furthermore, limited but well-substantiated evidence also shows that xenogenic bone mineral matrix and bovine collagen/mineralized bovine bone matrix exhibits the ability to produce regeneration in intrabony defects (Camelo *et al* 1998, Nevins

et al 2003). New studies have shown that bovine bone in combination with EMD has promising results, however the predictability of such outcome still needs to be investigated (Zuchelli 2003).

Alloplasts

Alloplasts are various inorganic synthetic graft materials. Two groups of alloplasts are available (Garrett 1996):

- 1 Absorbable: Plaster of paris, calcium carbonate, absorbable ceramics such as tricalcium phosphate and absorbable hydroxyapatite.
- 2 Non-absorbable: Dense hydroxyapatite, bioglass, calcium coated polymer consisting of polymethacrylate and hydroxyethylmethacrylate.

Reviews of these materials have generally shown that their use leads to considerable improvements in probing depth and clinical attachment levels, increase in bone level, reduce crestal bone as compared to open flap debridement procedures in intrabony defects (Reynolds *et al* 2003). Histologically they act almost exclusively as biologic fillers inducing little bone fill and very limited, if any, periodontal regeneration (Yukna 1993, Yukna 1994.) The Glossary of Terms by the American Academy of Periodontology (2001) defines bone fill as the “clinical restoration of bone tissue in a treated periodontal defect”. This term does not address the presence or absence of periodontal regeneration or new connective tissue attachment. Thus evidence shows that alloplastic grafts produce periodontal repair rather than regeneration (Reynolds *et al* 2003).

Guided tissue regeneration for intrabony and furcation defects

For many years GTR has been shown to regenerate periodontal tissues lost as a result of periodontal disease. The procedure allows

progenitor cells residing in the periodontal ligament to form a new connective tissue attachment. When other cell populations are blocked from the healing area, then the cells in the periodontal ligament (PDL) can repopulate the root surface and thereby produce regeneration of cementum, PDL and bone. A landmark study (Gottlow *et al* 1984) strongly suggested that when the epithelial and gingival connective tissue cells are excluded from the healing area with the use of a physical barrier membrane, the periodontal ligament cells are allowed to repopulate the previously diseased root surface and thus produce regeneration.

Since its discovery, many physical barriers (absorbable and non-resorbable) have been used in different configurations to fit the periodontal defects in need of regeneration. The most common non resorbable membrane is from GORE-TEX and made of expanded polytetrafluoroethylene (e-PTFE) designed for periodontal regeneration. Because this type of membrane does not resorb, even after healing, it requires a second procedure to remove it. Thus, researchers have developed resorbable membranes in order to eliminate the need for second stage surgery. The most common of these bioresorbable membranes are polylactic acid, polyglycolic acid and collagen membranes. Similar satisfactory results can be expected with bioabsorbable materials as with non-bioabsorbable materials (Hugoson *et al* 1995, Cortellini *et al* 1996).

Based on the systemic review in 2003 by Murphy and Gunsolley, the following conclusions regarding GTR can be made:

- 1 GTR procedures demonstrated a greater gain in clinical attachment level (CAL) and reduced probing depths as compared to open flap debridement when used for intrabony defects. Barrier types (resorbable versus non-resorbable membrane types) did not differ significantly when meta analyses among the studies were carried out.

- 2 GTR procedures demonstrated gains in vertical probing attachment level (VPAL) and horizontal probing attachment level (HOPA) with reductions in vertical probing depth as compared to open flap debridement when used for furcation defects. Barrier types seemed to affect the heterogeneity of the data as VPAL was only enhanced with the use of e-PTFE and polymeric barriers.
- 3 For furcation defects, there seems to be better regenerative outcome when augmentation material was used with barrier membranes than without. For intrabony defects, however, there was no advantage in the use of augmentation materials with barrier membranes.
- 4 Recession of the gingival margin increased when physical barriers were used in intrabony defects. Whether it was a resorbable or a non resorbable membrane that was used in intrabony defects, no difference between the two were noticed when compared.
- 5 In furcation defects, the use of coronally positioned flaps in an effort to fully cover the barrier membranes was associated with better clinical outcomes.
- 6 In the treatment of intrabony defects, intensive post operative care can lead to shallower probing depths.

Guided tissue regeneration for root coverage procedures

Indications for root coverage procedures are for esthetics, hypersensitivity, prevention of further recession defects and correction of anatomic deficiencies that may affect tissue health such as frenum pull. In the past, different procedures to perform root coverage involved autogenous soft tissue grafting and/or repositioning of flaps to cover denuded root surfaces and gain attached gingiva. Recently,

allogenic tissue grafts have been introduced in order to eliminate the use of the palate as a second surgical site. Moreover, GTR procedures with barrier membranes and GTR procedures using EMD have been introduced to cover recession defects.

When comparing connective tissue (CT) grafts with GTR or allogenic grafts, studies have shown that CT grafts provided a greater gain in root coverage than the GTR procedures (Jepsen *et al* 1998, Zuchelli *et al* 1998). For gains in width of keratinized tissue, studies have shown that CT grafts had greater gain in keratinized tissue than the GTR or allogenic graft procedures (Novaes *et al* 2001, Wang *et al* 2001, Aichelmann-Reidy *et al* 2001, Borghetti *et al* 1999). Therefore, it has been shown through meta analysis from the systemic review (Oates *et al* 2003) that autogenous connective tissue grafts offer significantly more advantages than GTR with bioabsorbable barriers in terms of root coverage and width of keratinized tissue. Moreover, with use of allogenic dermal tissue grafts, limited studies support their advantages over CT grafts. A more recent study reported on short term and long term comparison of root coverage with an acellular dermal matrix and a subepithelial connective tissue graft. Long term results (four years) showed that subepithelial grafts remained more stable with time than those treated with acellular dermal matrix. There were also smaller probing depth reductions and less increase in keratinized tissue with the acellular dermal matrix than the subepithelial connective tissue graft (Harris 2004).

Future investigations

Future investigations are still needed to investigate the long term stability of the different ways to regenerate lost periodontium due to periodontal disease. It is of major interest for all clinicians to have predictable and successful results each time a technique is

performed. It is therefore imperative for future investigations to give specific indications as far as choice of material for a particular defect, location of defect in combination with patient factors (oral hygiene habits, smoking, susceptibility, etc.) are concerned so that clinicians have a better guide to the ultimate goal of regeneration.

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Chapter 12

Future Directions in Clinical Periodontics

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Introduction

Periodontology is a specialist discipline of dentistry, whose objective is the scientific study and clinical application of the pathology and pathophysiology of the periodontium. It encompasses a broad range of scientific and clinical areas including the social sciences, behavioral science, microbiology, immunology, cell biology, molecular biology, nanotechnology and surgical sciences. There is no single field of dentistry which is not touched by the basics of periodontology. As depicted in the illustration below periodontology can be considered the foundation of dentistry or perhaps even the hub of dentistry (Figure 1).

Today periodontal research is arguably the most active and progressive field of research in dentistry. Four of the top fifteen dental journals are devoted to periodontics and periodontal research receives the largest portion of dental research funding in the USA. With the recent development of the subdiscipline of periodontal medicine, or the study of periodontal and systemic diseases, the medical relevance of periodontology to the general well being of the population cannot be underestimated.

Over the past 20 years there have been some exceptional advances made in the field of periodontology. This paper discusses what, in the authors opinion, have been some of the most

significant advances which have impacted on the clinical practice of periodontics.

Recognition that there are many different forms of periodontitis

The manifestation of periodontal disease in various forms has been recognized for many decades. However, by the late 1980's it had become apparent that there was a need for a new classification of the periodontal diseases. Accordingly, in 1989 the American Academy of Periodontology held an International Workshop for a Classification of Periodontal Diseases and Conditions (Armitage 1999). Through general consensus at the meeting, a new classification was devised and is shown in Table 1.

This classification provides a degree of uniformity in nomenclature of the diseases. Accordingly it provides a framework in which to scientifically study the etiology, pathogenesis and treatment of periodontal diseases. In addition this classification provides clinicians with a rationale for the treatment and prevention of care for their patients.

Recognition that subgingival plaque is a biofilm

Dental plaque can be defined as the diverse



Figure 1. Periodontics is the hub of dentistry

microbial community embedded within a matrix of host and bacterial polymers growing on teeth as a biofilm. The oral microflora is highly diverse with around 600 distinct microorganisms present including viruses, fungi, protozoa but mostly bacteria. The oral microflora appears to develop naturally and, in general, benefits the host. The largest number of microbes are found on tooth surfaces and especially in stagnant sites. In health the microflora is essentially of an indigenous nature and largely non-pathogenic. With the development of gingival inflammation there is an overall increase in the amount of plaque and the gingivitis response represents a non-specific response to a non-specific build up of dental plaque. With periodontitis there is a clear change in the plaque composition, with the emergence of a unique subgingival flora which may even vary with the type of periodontitis. In recent years the groups (complexes) of bacteria associated with periodontal health and disease have been classified according to their pathogenicity and ecological role. These are often named according to various color classifications (Socransky and Haffajee 2002).

I. Gingivitis

Plaque associated
Non-bacterial gingivitis
Not previously defined

II. Periodontitis

Aggressive periodontitis
Chronic periodontitis
Necrotizing periodontitis
Systemic disease-associated periodontitis
Periodontal abscess
Periodontitis associated with pulpal disease
Not previously defined

III. Developmental or acquired deformities and conditions

Tooth related issues
Mucogingival issues
Occlusal conditions

Table 1. 1989 Classification of periodontal diseases

The clinical significance of dental plaque is that it must be removed, or at least controlled, in order to prevent the development of substantial disease. According to the nature of the plaque and associated disease, various chemical adjuncts to mechanical removal can be used. For example if the problem is simple accumulation of supragingival plaque then relatively non specific agents such as chlorhexidine, triclosan or cetylpyridinium may be used to control its accumulation. Such agents are, however, of limited use for the management of subgingival plaque. Accordingly control and management of subgingival plaque has been aided through the rational use of antibiotics delivered either systemically or locally.

Interestingly, despite clear sensitivity of single periodontal pathogens to a variety of antibiotics, such agents are often of little efficacy in a clinical setting (Gilbert *et al* 1997). With the recognition that subgingival plaque is a biofilm the reasons for lack of efficacy of antibiotics became apparent. A biofilm can be considered as a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Biofilms are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. As such, biofilms can be particularly resistant to antibiotics (Socransky and Haffajee 2002). Hence a mainstay of clinical practice must be to dislodge and disrupt the subgingival biofilm. In doing so the delicate subgingival ecology is destroyed and this is usually sufficient to reduce the pathological nature of the plaque and allow the host tissues to recover. Thus mechanical therapy remains the mainstay of clinical practice. Whether disruption of the biofilm through mechanical means renders the subgingival plaque more susceptible to antibiotics remains equivocal.

Recognition that bacteria are necessary but not sufficient for periodontitis to develop

Despite the dramatic increase in use of oral hygiene aids, efforts by the dental profession in oral hygiene instruction and the associated general improvement in oral hygiene in the community, the incidence of severe periodontitis remains largely unaffected (Bartold *et al* 1998). Improved oral hygiene practices, devices and products have been shown to have a positive effect as manifested by a reduction in plaque scores and in the prevalence of gingivitis, but they have little effect on periodontitis.

Indeed, our clinics are full of patients who appear to have minor plaque deposits yet manifest with very significant periodontal destruction. Others may have significant deposits of plaque yet very little overt disease. These situations represent the paradox of the periodontal diseases (Figure 2) and highlight the need for careful clinical evaluation and a resistance to categorize patients merely on the level of their plaque levels or oral hygiene.

Hence while oral hygiene seems to be improving in the community, severe periodontitis remains a significant problem. This highlights that there is more to the development of periodontitis than mere plaque accumulation. It is now well recognized that dental plaque accounts for around 20% of the risk for development of severe periodontitis (Page 1998, Grossi *et al* 1999). Therefore, while plaque is considered necessary for the development of periodontitis it is not sufficient alone to cause the disease. There are clearly many other factors at play which lead to the development of disease. Thus, to focus solely on plaque control as a means of managing periodontitis is bound to lead to inappropriate and unsuccessful treatment.

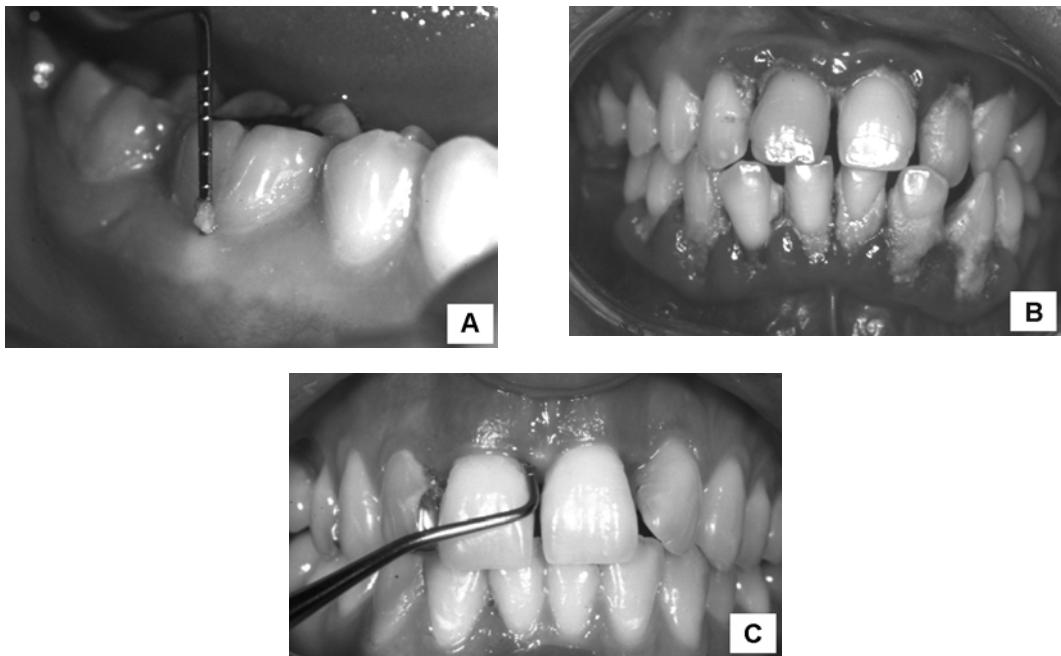


Figure 2. The paradox of the periodontal diseases

- A. Lots of plaque but little disease
- B. Lots of plaque and lots of disease
- C. Minimal plaque and advanced disease

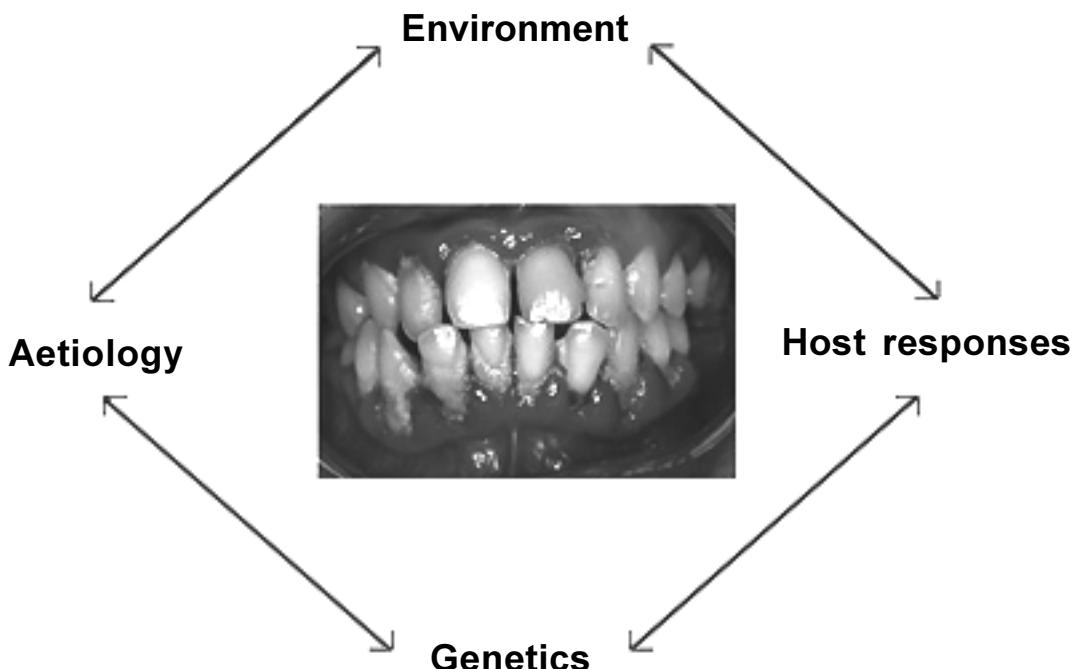


Figure 3. Periodontitis is a multifactory (ecogenetic) disease

Recognition that periodontitis is a multifactorial disease

In light of the above, and with the recognition that plaque accounts for around 20% of the risk for developing periodontitis, it has become apparent that periodontitis is a multifactorial disease incorporating bacterial infection, host responses, environmental modification and genetic susceptibility (Figure 3).

On the basis of this it is now proposed that the periodontal disease are referred to as “Eco-genetic diseases”.

Such concepts now dictate that the ultimate goal of periodontal therapy must take these factors into account. Accordingly, the standard of care would dictate that management of the periodontal diseases must be to control the infection, remove any predisposing factors and manage any modifying factors.

Understanding the molecular pathogenesis of periodontitis

Over the past 20 years there has been an enormous effort to investigate and understand the molecular pathogenesis of the periodontal diseases. Early concepts of periodontitis constituted a continuum paradigm in which plaque accumulation led to gingivitis, which if left untreated and plaque allowed to accumulate, periodontitis would result and tooth loss would be inevitable. Over time this paradigm was shown to be significantly flawed. It was far too simple and did not reflect the clinical condition of periodontitis. Through many epidemiological, natural history, pathology, microbiological and immunological studies concepts have changed and the importance of the host response in containing and controlling the disease has become increasingly apparent. However even this has not been enough to explain the widespread clinical variance of the disease. With time it became apparent that both

environmental factors and genetic factors also contributed significantly to the etiopathology of the various periodontal diseases. In 1997 Page and Kornman elegantly summarized the findings to date in what has become a classic diagram which accurately and succinctly summarizes our current understand of the molecular pathogenesis of periodontitis (Figure 4).

While of obvious academic interest, the understanding of molecular pathways in periodontitis also has significant clinical ramifications. For example, although periodontitis is initiated by the subgingival plaque, the mediators of connective tissue breakdown during this inflammatory disease are primarily generated by the host's response to the micro-organisms. This offers numerous opportunities to explore means of pharmacological anti-inflammatory control of these mediators and development of adjunct medications (Figure 5).

Development of the subdiscipline of periodontal medicine

The relationship between periodontal diseases and the manifestation of various systemic conditions has been reported for decades. Conversely, the influence of systemic diseases on the periodontium has also been long recognized and reported. However, it has not been until relatively recently that an evidence based approach has been used to study the intricate relationships between periodontitis and systemic health. No longer can the periodontal diseases be considered a local inflammatory response to infections limited solely to the periodontal tissues. The results from well controlled studies now strongly support the notion that the periodontal diseases can have widespread systemic effects. These findings have led to the development of the subdiscipline of periodontal medicine (Rose *et al* 2000).

While it is easy to see how systemic factors

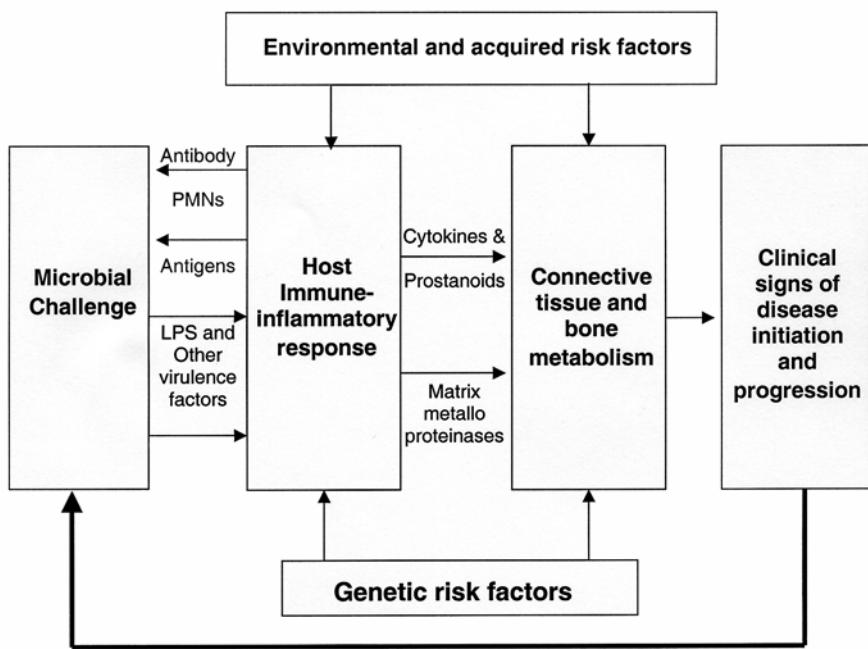


Figure 4. Molecular pathogenesis of periodontitis (Adapted from Kornman *et al* 1997).

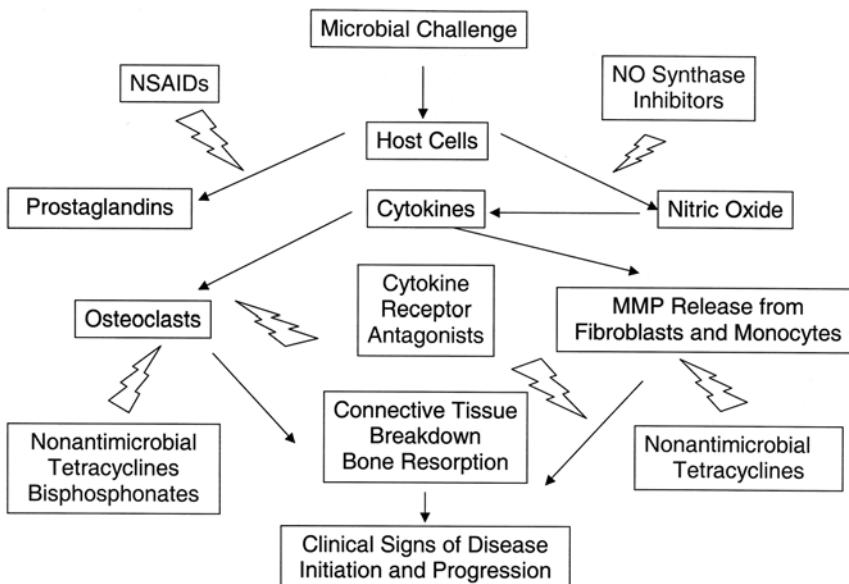


Figure 5. Periodontal inflammation and potential sites for pharmacologic control

can modify the various forms of periodontitis through their effect on normal host defence mechanisms, the converse can, at time seem tortuous. Nonetheless evidence is accruing rapidly that periodontal infections and inflammatory responses can impact significantly on many systemic conditions. A current list of conditions for which various associations with periodontitis can be made is shown in Table 2.

Association	Causality
Rheumatoid Arthritis	Diabetes
Osteoporosis	Preterm Low Birth Weight
Obesity	Cardiovascular Disease Pulmonary Disease

Table 2. Potential systemic diseases associated with periodontitis

A recent study aimed to determine whether there is a significant difference in the prevalence of systemic diseases in either patients referred for periodontal care compared to a general practice population, or patients with periodontitis of varying severity (Georgiou *et al* 2004). Charts of 1000 adult patients were selected from 4 clinics including:

- 1 University Dental School Admissions Clinic.
- 2 University Dental School Periodontics Clinic.
- 3 Private Periodontal Practice.
- 4 Private General Dental Practice.

The prevalence of medical conditions was evaluated using validated self-reported health questionnaires. Periodontal condition was assessed from the most recent relevant radiographs in the files. From these data it was found that periodontal patients had a higher prevalence of systemic diseases compared to the general practice population. Bronchitis, hepatitis and rheumatoid arthritis were most prevalent in patients with advanced

periodontitis. Patients with periodontitis also took more medications and were more likely to suffer from multiple conditions compared to the general dental population.

There is no doubt that this area of periodontics will continue to be very important. While the causative nature of periodontal disease with other systemic conditions is an attractive proposal, there is still considerable work to be done before this can be proven. In order for causality to be shown seven key aspects need to be considered, these are listed in Table 3.

In order for causality of a disease to be proven the following need to be addressed:

- Consistency of association
- Strength of association
- Time sequence correct
- Specificity of associations
- Dose-response effect
- Biological plausibility
- Experimental support

Table 3. Disease causality

Recognizing that implant therapy is an integral part of periodontics

With the development of osseointegration as a predictable means of ensuring clinical success for dental implants came the need for ongoing care and maintenance much the same as for the natural dentition (Lang and Nyman 1994). In this context periodontists became an integral part of the implant team. With further refinements in clinical protocols and implant design, single tooth replacement in addition to larger, more complex multiple tooth replacement became established. Periodontists, through their interest and expertise in soft and hard tissue biology and clinical management, became logical service providers for this type of treatment (Mombelli and Lang 1994). Over

the past two decades periodontists have contributed very significantly to the evidence base upon which implant protocols are now based. Implant dentistry (particularly the surgical placement, subsequent maintenance and management of peri-implant complications) has become an established part of periodontal training programs worldwide.

There are still many aspects of implant dentistry which require new as well as ongoing research and development. It is interesting to note that periodontists are at the forefront of most, if not all, of these areas. Current issues which periodontists (clinicians and researchers) is currently having a significant impact upon are listed in Table 4. One of the more significant issues facing periodontists is placement of osseointegrated implants in patients already affected by advanced periodontitis (van Steenberghhe 2003).

- Biomaterials and implant surfaces
- Peri-implant complications
- Soft and hard tissue interface
- Diagnostic imaging
- Implant fixture selection and surgical techniques
- Implant maintenance
- Immediate loading
- Implants in periodontally compromised patients

Table 4. Current issues in implantology

Understanding that periodontal regeneration is biologically possible

The ultimate goal of periodontal therapy is the regeneration of the affected tissues to their original architecture and function. In the past periodontists have been obsessed with trying to fill bony defects with all manner of substances and grafting materials in the hope of attaining regeneration. The clinical use of

most of these materials has met with limited success because their use is not grounded in solid, evidence based scientific and clinical research. Hence regenerative treatment of periodontal defects with an agent or procedure requires that each functional stage of reconstruction be grounded in a biologically directed process (Bartold *et al* 2000).

With the advent of the concepts of guided tissue regeneration for periodontal regeneration, came a very significant advance in periodontics. The landmark papers on guided tissue regeneration in the 1980's paved the way for a new era in periodontal treatment (reviewed in Karring *et al* 1993). While not always a very predictable clinical procedure, the concepts of guided tissue regeneration have proven correct and it is reasonable to state that periodontal regeneration is biological possible although, at present, not always clinical attainable.

The reasons for the clinical unpredictability of these procedures are many. The inability to control the rapid apical migration of the junctional epithelium is a prime concern as this significantly limits the amount of root surface available for new connective tissue formation. In a similar manner the inability to adequately seal the healing site by way of a tight epithelial adaptation to the coronal root surface hinders regeneration by leaving the site open to the oral environment during the crucial early phases of wound repair. This lack of an adequate seal also significantly increases the risk of infection of the implanted membrane or regenerative material postoperatively. As detailed above, restriction of regeneration to bone while ignoring regenerative processes in the cementogenic and fibrous compartments has significantly progressed in this area. The inability to precisely define the growth and differentiation factors needed for regeneration has also limited progress. Finally, an important, but largely overlooked issue, is whether regenerative therapy increases the long-term life span of teeth.

Notwithstanding these difficulties, the field has progressed in the past 20 years and new therapies based on our understanding of the molecular and cell biology of the regenerating periodontium are constantly evolving (Bartold *et al* 2000). Tissue engineering of the periodontal tissues is now being explored in animal models and offers considerable potential. With the recent demonstration of mesenchymal stem cell populations the potential to utilize these cells within a tissue engineering construct is under investigation (Shi *et al* 2005).

Conclusion

The advances in periodontics in the past 20 years have been substantial. These advances have offered new concepts, paradigms and treatments. While in the past periodontal therapy was largely focussed on plaque control and mechanistic approaches, the new millennium offers a broader scope and has begun to address the goals of controlling the infection, removing predisposing factors, regulating modifying factors and restoring the tissues to their original form and function.

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Abstracts

The following is a record of the Poster Presentations held at the
6th Meeting of the Asian Pacific Society of Periodontology

**Preterm Low Birth Weight and Dental Intervention - Is There Any Link?
Poster Competition - First Place****S. Bala*, S.C. Narula, R.K. Sharma**

(Government Dental College, Rohtak, Haryana, India)

There is compelling evidence that a link exists between PLBW & Periodontitis. Although 25% - 50% of PLBW deliveries occur without any known etiology, there is increasing evidence that infection may play a significant role in preterm delivery. A model explaining the plausible relationship is proposed based upon the concept of infection leading to the cascade of inflammatory reactions associated with preterm labor & periodontal disease. Effect of periodontal intervention on pregnancy outcome in a randomized case-control study of Northern part of India (Haryana) will be presented.

**Periodontal Regeneration Based on Multi-layered Cell Sheet Engineering
Poster Competition - Second Place****M. Gomez Flores*, M. Hasegawa, M. Yamto, T. Okano, I. Ishikawa**

(Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan)

This study was undertaken to investigate the possibility of a novel tissue engineering approach for complicated periodontal tissue structures by layering periodontal ligament (PDL) cells expanded *ex vivo* on temperature responsive cell culture surface.

Human PDL cells were cultured on temperature-responsive cell culture surfaces with and without osteogenic inductor medium. After three weeks the cells were harvested as a single sheet and then layered. The multi-layered PDL cells were verified *ex vivo* and transplanted into immunodeficient rats with dentin blocks.

PDL cell sheets showed a large amount of extracellular matrix and the presence of mineralized nodules. Small amount of mineralized tissue and the attachment of some collagen fibers were confirmed onto the surface of the dentin blocks.

This strategy enables fabrication of viable, transplantable, tissue-engineered periodontal tissue constructs and might be suitable to manage periodontal defects.

Serological Analysis of the Implication of Periodontitis in Vascular Diseases

Poster Competition - Third Place

Y. Chen*, M. Umeda, Y. Huang, Y. Takeuchi, D. Wang, Y. Inoue, T. Iwai, I. Ishikawa

(Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan)

Recent research has associated periodontitis with vascular diseases. The aim of this study is to investigate whether periodontal infection of resulting host immune responses play a role in vascular diseases.

The study population comprised 64 patients with vascular diseases (VD) and 47 control subjects. Periodontal status, serum IgG levels against periodontopathic pathogens and levels of periodontitis – associated inflammatory cytokines were examined. Periodontal status was worse in the VD group. IgG levels against *Porphyromonas gingivalis* and *Treponema denticola*, and inflammatory cytokine levels were significantly higher in the VD group than the control group. This study provides serological evidence that an infection caused by major periodontal pathogens may be associated with vascular diseases.

Synergistic Approach for Optimal Esthetics

A. Uppoor*, D. Naik

(Manipal College of Dental Sciences, Mangalore, India)

Esthetics has long been the domain of restorative dentistry but gingival esthetics have often been compromised. Recently, an entire array of periodontal plastic surgical procedures has contributed to the improvement of facial esthetics. Periodontists working synergistically with prosthodontists will go a long way in achieving optimal esthetics. A series of cases from the Manipal College of Dental Sciences are presented demonstrating the achievement of esthetics through co-operation.

Black Triangles – The Unwanted Outcome of Periodontal Surgery: Methods of Prevention and Correction

K.J. Nisha*, K. Nandakumar

(Department of Periodontics, Government Dental College, Trivandrum, Kerala, India)

Black triangles, or the non-existence of a papilla between two teeth, are one of the notorious drawbacks of periodontal surgical therapy. These black triangles create an unaesthetic appearance when the patient smiles. Once lost, the regeneration of interdental papilla is a very difficult and challenging task to the clinician. This poster presents various case reports demonstrating modified suturing techniques to prevent the loss of interdental papilla and surgical and non-surgical techniques to regenerate the papilla.

Periodontal Manifestation in Florid Osseous Dysplasia – A Rare Case Report

B. Joseph*, K. Nandakumar

(Department of Periodontics, Dental College, Trivandrum, Kerala, India)

A 33 year old female patient reported to the Department of Periodontics for correction of enlarged gingiva and space between the anterior teeth. The gingival enlargement was firm and diffuse with no pocket formation, with grade I mobility and spacing of anterior teeth. Full mouth IOPA xrays showed generalized cotton wool appearance, loss of lamina dura, hypercementosis and blunting of roots. This case was diagnosed as Florid Osseous Dysplasia on the basis of the overall combination of findings. Florid Osseous Dysplasia is a non neoplastic condition characterized by multiple radiopaque lobulated masses and apparent gingival enlargement occurring in two or more quadrants, often in middle aged women. This condition is usually asymptomatic and therefore periodontal maintenance therapy is advised.

Efficacy of Chithra Calcium Phosphate Cement Graft in the Management of Periodontal Osseous Defects

J.B. Rajesh, K. Nandakumar

(Department of Periodontics, Government Dental College, Trivandrum, Kerala, India)

In an independent research and development attempt, the Biomedical Technology wing of Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum has developed a unique bioactive calcium phosphate cement (Chitra-CPC) for use in the management of periodontal osseous defects.

The principal of CPC is a cementing action of acidic and basic calcium phosphate compounds on wetting with an aqueous medium. The cement powder contains tetra calcium phosphate (TTCP) and dicalcium phosphate dihydrate (DCPD) particles of size in range 100 μ m, mixed in equimolar ratio. The wetting medium used was distilled water with Na₂HPO₄. Mixing of these in suitable proportions gives a self-setting mass. The chemical phase of the set mass depends on the net Ca-P ratio in the ingredients and when this is adjusted to 1.67, hydroxyapatite, the basic bone mineral with the chemical formula Ca₁₀(PO₄)₆(OH)₂ will result.

This present study is designed to evaluate the regenerative potential of Chitra calcium phosphate cement when used in the management of periodontal osseous defects.

A Study to Evaluate Tooth Mobility in Menstruating and Non-Menstruating Women

P. Mishra*, P.P. Marawar, G. Byakod

(Department of Periodontics, Rural Dental College, Loni, Maharashtra, India)

Hormonal variation in puberty and menstruation tends to accentuate the existing clinical signs and symptoms of gingivitis. Oral health care needs during these stages are unique which require the clinician to have a better understanding of the changes occurring in the tissues at these specific times.

The aim of this study is to determine the precise difference in mobility of teeth in menstruating and non-menstruating females using a mobilometer.

Diabetes Mellitus and the Periodontium – Case Report

R.B. Pushpalatha

(M.S. Ramaiah Dental College & Hospital, Bangalore, India)

The impact of systemic conditions on oral health is well recognized. The periodontium is an end organ similar in many ways to other end organs such as the skin and the glomerulus of the kidney. In this regard, conditions commonly affecting various end organs throughout the body like diabetes mellitus, may also influence the periodontium.

Modern epidemiologic methods used in large populations have clearly established that diabetes is a risk factor for periodontal disease. While diabetes significantly impacts the periodontium, evidence also suggests the potential for periodontal infection to adversely affect glycemic control in diabetics.

This poster depicts the classic two-way relationship between diabetes mellitus and the periodontium with the help of case reports.

Familial Aggregation of Aggressive Periodontitis – A Case Report of Siblings Affected

S. Janitha

(M.S. Ramaiah Dental College and Hospital, Bangalore, India)

Aggressive periodontitis comprises a group of rare often severe, rapidly progressing forms of periodontitis often characterized by an early age of onset and distinctive tendency to aggregate in families. It is widely recognized that siblings of patients with aggressive periodontitis frequently also suffer from periodontitis. Almost 50% of siblings are affected by aggressive periodontitis. Familial aggregation suggests genetic etiology. However, families also share many aspects of common environmental factors like diet, nutrition, exposure to pollutants, and aggregation of certain micro-organisms in the families. Twin studies performed concluded that genetics form the basis for familial aggregation of aggressive periodontitis.

This poster presents information on the pedigree of the family and how this affected their clinical, radiographic and laboratory findings and the treatment given to siblings affected by aggressive periodontitis.

Matrix Disrupter – A Creative Plaque Disrupting Device

M. Parvez*, S. Hedge, K.S. Rajesh

(Department of Periodontics, Yenepoya Dental College, Mangalore, India)

Over the years, time and money have been invested in designing oral health care products to marry form and function. Products are available in all shapes, colours and sizes, promising to perform better than the rest. These have changed to fit the times, and are likely to continue to evolve.

This poster is a blueprint of a new mechanical oral hygiene device equipped with multiple oral hygiene aids, designed with the help of specialized 3D Software.

Evaluation of the Efficacy of Taurine as an Antioxidant in the Management of Patients with Chronic Periodontitis

S. Lakshmi Sreee

(R.M. Dental College and Hospital, Annamalai University, Tamil Nadu, India)

This study evaluated the antioxidant property of Taurine in the management of chronic periodontitis patients. Periodontal status in ten chronic periodontitis patients was assessed in terms of gingival index, plaque, index, probing pocket depth and clinical attachment level prior to and after administration of Taurine. The oxidative stress present in the gingival tissue and blood (measured using the test TBARS) and the antioxidants namely GSH (reduced glutathione) and GPX (glutathione peroxidase) were also estimated before and after administration of Taurine. The changes in the biochemical parameters were significant statistically where as the changes in the clinical parameters were insignificant.

A Clinical Evaluation of the Use of Demineralized Bone Matrix and Bioactive Collagen Membranes in Peri Implant Bone Regeneration

V. Chadha*, V.S. Battu, G. Rajan

(Private Practitioner, Chandigarh, India)

The objective was to evaluate the clinical efficacy of the adjunctive use of Chlorhexidine Chip (CHX) following scaling and root planing (SRP) in treatment of chronic periodontitis.

A randomized, blinded, split-mouth study was designed. A total of 16 patients, both men and women, aged between 30 and 60 years with chronic periodontitis with at least 2 sites having 5 to 8 mm pockets that bled on probing were enrolled into the study. A tooth was selected as a target and its parameters of probing depth, attachment loss, gingival index, and plaque index were recorded. Each site was randomly assigned to one of two treatments – SRP + CHX or SRP alone. All parameters were recorded at baseline, 1 month, 3 months, and 6 months. Results showed the reduction of PD and gain of attachment at 6 months in the group of SRP plus CHX (3.94 mm, 2.83 mm) were significantly higher than those in group of SRP alone (1.39 mm, 1.89 mm) ($P < 0.001$).

It was concluded that the chlorhexidine chip is an effective adjunct to SRP for use in patients with chronic periodontitis.

Tissue Engineering – Revolutionizing Periodontics Towards Greater Horizons

N.K. Sowmya, T.S. Sriniva

(Bapuji Dental College & Hospital, Davangere, Karnataka, India)

True regeneration is at the forefronts of research in periodontics. Many treatment modalities have been developed to achieve complete regeneration. Tissue engineering is one of the latest treatment modalities used to achieve this “elusive” goal. This poster presents the case reports of materials used in periodontal regenerative treatment modalities which work on the principle of tissue engineering. This poster depicts the use of Emdogain, Pepgen P-15 & PRP with Bio-Oss used in periodontal intra-bony osseous defects.

Buccal and Coronal Bone Augmentation Using Forced Eruption and Buccal Root Torque - A Case Report

V. Priya Darshini

(Bapuji Dental College & Hospital, Davangere, Karnataka, India)

The following case report described the buccal and coronal bone augmentation around an irretrievable tooth using forced eruption and buccal root torque for immediate non-sub merged implant placement. Biodynamic orthodontic modeling modifies the osseous foundation for implant placement. A mandibular left second premolar with degree 2 mobility, severe buccal bone resorption and interproximal angular bony defects was subjected to forced eruption and buccal root torque. Five months after this process, the tooth was displaced 15 mm coronally and the root apex faced buccally. Buccal and coronal bone augmentation and soft tissue enlargement were evident at re-entry surgery. This technique enabled proper implant placement in a situation where the bone was compromised.

Ridge Augmentation with Dense Hydroxyapatite on Resorbable Suture (Permaridge) Matrix

S.R. Krishna*, P. Talreja

(Department of Periodontics & Implantology, Bapuji Dental College & Hospital, Karnataka, India)

The atrophic edentulous ridge represents one of the greatest challenges faced in routine practice. The endosseous dental implant is a proven, predictable and practical method of treating the edentulous maxilla and mandible and providing enhanced stability and retention for a full arch prosthesis. Many of these patients for various reasons are not candidates for endosseous implants. Presented here is a method of ridge augmentation with dense hydroxyapatite beads linked in a resorbable suture matrix to prevent particle migration during healing. This technique may be performed with minimally invasive surgery providing long term prosthetic support.

Psychological Stress and Oral Health

R. Jain, R Vidhyasagar*

(M.R. Ambedkar Dental College, Bangalore, India)

Gingivitis and periodontitis are inflammatory diseases primarily caused by an interaction between a specific group of micro-organisms colonizing the oral sites and the host. Various systemic factors like genetic background, diabetes mellitus, etc. alter the host response to local factors and may predispose the individual to rapid periodontal destruction. Recently, psychosocial factors such as stress have also been identified as one of the risk indicators for periodontal disease. Stress acts on the hypothalamic-pituitary-adrenal axis, increasing the release of noradrenalin. This stimulated release of Cortisol and noradrenalin has a suppressive action on the immune system, inhibiting the action of PMNs and antibodies and stimulating the release of prostaglandins and proteases. Academic stress is considered as a naturally occurring stressor of high ecologic values. This study was designed to evaluate the effects of academic stress on the gingival tissues in a group of 35 dental students during an academic exam and one month after the exams. Clinical parameters like simplified oral hygiene index, bleeding index and gingival index were evaluated and information was collected regarding oral hygiene measures and use of tobacco products.

Effect of Aspirin Intake on Periodontal Status

S. Swamy*, F. Tarannum

(M.R. Ambedkar Dental College, Bangalore, India)

Periodontitis is an inflammatory disease, which results from complex interactions between plaque micro-organisms and the host immune system. PGE₂ is the predominant inflammatory mediator implicated in the pathogenesis of periodontal disease whilst destruction of alveolar bone is attributed to PGE₂ in association with other proinflammatory cytokines. Aspirin, an anti-inflammatory drug is widely used by the middle aged and elderly population because of its benefits in preventing inflammation, coronary artery disease and peripheral vascular disease. Aspirin has inhibitory effects on cyclo-oxygenase metabolites including PGE₂ and this has lead to many studies on the effects of aspirin and other NSAIDs on periodontal diseases. Since patients with various cardio-vascular disorders are on long-term low dose aspirin therapy, this study aims to evaluate the periodontal status in these patients. It is a case-control study assessing probing pocket depth, clinical attachment loss and bleeding on probing in patients on aspirin therapy (n=100) as compared to those who are not taking any anti-inflammatory drug (n=100).

Effect of 0.2% Tempered Chlorhexidine as an Antiplaque Agent

S. Ebenezer, Md. Jalaluddin, S.K. Nair

(M.R. Ambedkar Dental College, Bangalore, India)

Epidemiological studies [Loe et al 1965] as well as clinical research [Page and Schroeder 1982] concluded that plaque is the primary etiological factor in gingival inflammation. The mainstay of primary and secondary prevention of periodontal disease is the control of supragingival plaque. As an adjunct to mechanical tooth cleaning various antimicrobial mouthrinses can be used. The most common is 0.2% chlorhexidine gluconate, the first clinically effective mouth rinse that inhibited supragingival plaque formation and thus the development of gingivitis as well as caries. Konig J et al – 2002 concluded from his study that 0.2% tempered chlorhexidine was more effective in inhibiting plaque. However, insufficient studies have been conducted on this subject to be able to provide conclusive results. The aim of this study is to evaluate the efficacy of 0.2% tempered (45° C) chlorhexidine (n=25) as antiplaque agent over 0.2% cold (25° C) chlorhexidine (n=25).

Investigation of Periopathic Bacterial Transmission From Periodontal Pockets to Peri-Implant Sulci

H. Ogawa*, T. Ohshima, F. Kawasaki, K. Gomi, N. Maeda, T. Arai

(School of Dental Medicine, Tsurumi University, Yokohama, Japan)

The aim of this study was to investigate the transmission of four periopathic bacterial (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*) from natural teeth to implants in the same patient.

Bacterial samples were obtained from 113 implants and 92 natural teeth of 50 partially edentulous patients and cultured. The bacterial strains from the same individuals were compared by arbitrarily primed polymerase chain reaction (AP-PCR). In more than 70% of the patients, the same strains existed both in the natural teeth pockets and the implants sulci. These results strongly suggested that the bacterial transmission occurred from the remaining natural teeth to peri-implant sulci.

Effects of Fractionated Enamel Proteins on Human Periodontal Ligament (HPDL) Cells Differentiation**T. Nagano*, S. Oida, S. Suzuki, A. Kakegawa, K. Gomi, T. Arai, M. Fukae**

(School of Dental Medicine, Tsurumi University, Yokohama, Japan)

The purpose of this study was to identify osteogenic factors in porcine enamel extract. Enamel proteins were separated by size exclusion chromatography into 25 fractions, which were tested for ST2 and HPDL cells. Fraction 10~16 reduced ALP activity in ST2, but enhanced it in HPDL cells. The enhanced ALP activity was completely blocked by TGF- β inhibitors. We demonstrated that fraction 13 can induce promoter activity of the PAI-1 gene. These results show that the osteoinductive activity of enamel extracts on HPDL cells is mediated by TGF- β 1, and suggest that HPDL cells and osteoblast-like cells respond differently to TFG- β 1 stimulation.

The Antimicrobial Effect of Porcine Amelogenins**M. Ebisawa*, T. Ohshima, T. Nagano, K. Gomi, M. Fukae, N. Maeda, T. Arai**

(School of Dental Medicine, Tsurumi University, Yokohama, Japan)

The purpose of this study was to evaluate the antimicrobial effect of amelogenins, which are main component of enamel proteins, used for periodontal regeneration clinically. Twenty-five kDa porcine amelogenin and its derivatives were extracted from immature enamel and purified to be homogenous. Their antimicrobial effects to oral pathogenic microorganisms were examined as MBC. The 25kDa porcine amelogenin and its derivatives showed significant antimicrobial effect against *Candida albicans*, while they showed low antimicrobial effect against *Porphyromonas gingivalis*. Amelogenins have antimicrobial properties against oral pathogenic micro-organisms.

Comparative Evaluation of CO₂ Laser and Radiosurgery as Techniques in the Treatment of Gingival Hyperpigmentation - A Clinical, Histological and Subjective Patient Evaluated Study Followed Over Six Months

R.P. Thakur

(Government Dental College and Hospital, Mumbai, India)

The need for esthetics and the demand for a pleasing smile has made individuals conscious of dark patches of pigmentation in the facial aspect of the anterior gingival region, which can be strikingly apparent during smiling and speech. This study tested the efficacy of CO₂ laser and radiosurgery for gingival depigmentation. The study was a randomized, split mouth comparison clinical trial carried over six patients in twelve sites. The parameters considered were Dummett's Pigmentation Index, area of pigmentation and an evaluation of inflammation and pain. A patient questionnaire was performed. Biopsies were taken pre-operatively and after six months for histological analysis. This study shows that both CO₂ laser and radiosurgery were successful treatment modalities for gingival depigmentation.

Possible Association Between Preterm Low Birth Weight and Early Periodontitis – A Case Control Study

V.S. Eligar

(Department of Periodontics, K.L.E.S. Dental College, Belgaum, Karnataka, India)

According to many studies generalized periodontitis can be a risk factor for preterm low birth weight. A case control study was carried out to examine if early, localized periodontitis could be a risk factor for adverse pregnancy outcome. Post partum women without any systemic diseases were included into the study. A pre term birth case was defined if a patient had a threatening premature labor during pregnancy, preterm premature rupture of membranes and/or the weight of the newborn was <2499 gm. Control women had delivery after the 37th gestational week and newborn weight was more than 2500 gm. The socio economic status and periodontal status was recorded. Smoking, alcohol and drug consuming subjects were excluded from the study. The data was statistically analyzed with results still forthcoming.

Finite Element Method in Periodontics – Looking Beyond the Obvious

M. Taneja*, I. Gupta*, K.L. Vandana

(College of Dental Sciences, Davangere, Karnataka, India)

Finite Element Method (FEM) is a computer assisted application of a mathematical model to study the stress and displacement pattern in structures after application of a load. In dentistry FEM is used in the fields of prosthodontics, orthodontics & implantology, but it has not been observed in the periodontal literature so far. Periodontal disease is influenced by traumatic occlusal forces and the study of normal occlusion and trauma from occlusion is one of the areas of FEM application in periodontics. Various animal models, photoelastic and strain gauge methods have shown inadequate information on comparison. This poster presents a study of stress analysis in the periodontium using FEM.

Attachment Loss in a Case of Pendred Syndrome - Incidental or Constitutive?

D. Sharma

(Government Dental College, Bangalore, India)

Pendred Syndrome (PS) is a rare inherited autosomal recessive disorder with an iodine organification defect of thyroxine hormone. The constitutive features of PS include sensorineural hearing loss, classically congenital, and goitre. No cases have been described with periodontal findings. This poster described a 21 year old female patient of PS with localized extensive attachment loss involving mandibular incisors without significant associated local factors. Hence, impairment of primary host immune response was suspected and the possibilities of neutrophil defects (in degranulation and chemotaxis) were investigated. The results were discussed in the presentation along with the management of such rare cases.

Primary Tuberculous Gingival Enlargement - A Rare Entity

B.V. Karthikeyan

(Goverment Dental College, Bangalore, India)

Primary gingival tuberculosis is an extremely rare and forgotten entity. It commonly manifests as an ulcer. This presentation highlights an unusual finding, primary tuberculosis, manifesting as gingival enlargement, which is reported for the first time in the literature. Diagnosis was based on histopathological features, immunological investigation of antibodies against Mycobacterium tuberculosis and PCR assay. Antituberculous therapy administered for six months resulted in complete resolution. It also emphasizes that we, as periodontists, need to be aware of this possibility and include tuberculosis in the differential diagnosis of gingival enlargements so that we can play of role in the early detection of tuberculosis.

Pachyonychia Congenita With Unusual Dental Findings – A Rare Case Report

C. Nagaraja

(Department of Periodontics, Goverment Dental College, Bangalore, India)

Pachyonychia congenita (PC) is a rare form of hereditary palmoplantar keratoderma. The most characteristic finding of the affected patient is the marked subungual hyperkeratosis with thickening of the distal parts of the nails. The oral findings include leukokeratosis of the tongue, buccal mucosa and palate, angular stomatitis, presence of natal and neonatal teeth, periodontitis and severe dental caries.

This poster represents a case report of PC associated with unusual dental findings like hypercementosis of posterior teeth, multiple retained deciduous roots, multiple talons cusps in patient with a poor periodontal condition and oral malodor and discusses the management of the same.

Plasma Cell Granuloma of Gingiva - A Case Report

P. Devi

(Department of Periodontics, Government Dental College, Bangalore, India)

An exceedingly rare case of gingival plasma cell granuloma is reported in a 40 year old female patient which presented as an exophytic mass in the gingiva, clinically resembling a traumatic fibroma. Histopathological findings revealed dense sheets of plasma cell infiltrate. Immunohistochemistry for kappa and lambda light chains showed polyclonal staining pattern confirming a diagnosis of plasma cell granuloma. Clinical features are usually suggestive of traumatic fibroma (benign) but histopathologically, in the early stages it closely resembles plasmacytoma (malignant). This poster highlights the need to biopsy such lesions to rule out potential neoplasms regardless of clinical impression and/or perceived surgical success.

Role of Platelet-Rich Plasma in Periodontal Regeneration - Fact or Fiction?

S. Pai

(Department of Periodontics, Government Dental College, Bangalore, India)

Platelet-rich plasma (PRP) is a component of blood in which the platelets are concentrated in a limited volume of plasma. Platelets contain many growth factors, including Platelet-derived growth factor, insulin-like growth factor and transforming growth factor-beta, which enhance wound healing and help to induce tissue repair and regeneration. Today, PRP represents the technique available for clinicians to apply tissue stimulation principles in periodontal regenerative procedures. This poster demonstrates the successful use of PRP combined with other regenerative techniques in the repair of intrabony defects, furcations and cyst cavities.

A Comparative Study Between Bioresorbable and Non-Resorbable GTR Membranes in Three Walled Defects

P.K. Singh

(Department of Periodontics, P.M.N.M. Dental College & Hospital, Bagalkot, India)

The objective of conventional reconstructive periodontal therapy is the predictable regeneration of periodontium at the sites of previous periodontal breakdown. Periodontal wound healing has resulted in the development of a treatment modality known as “Guided Tissue Regeneration” based on the principle of guiding the proliferation of the various periodontal tissue components during healing following periodontal surgery.

First generation GTR device have been non-resorbable which calls for a second surgical procedure. This was avoided when bio resorbable devices became available, however they have some disadvantages such as early resorption.

In the present clinical study a comparison of the use of these two methods on patients was made.

Clinical Efficacy of a Novel Collagen Membrane of Fish Origin in the Management of Periodontal Intrabony Defects

S. Anila*, K. Nandakumar

(Department of Periodontics, Dental College, Trivandrum, India)

Numerous animal and human studies have documented the efficacy of guided tissue regeneration. We evaluated a new bioabsorbable collagen membrane of fish origin (synthesized from type 1 collagen from air bladder of fresh water fish *Sacchobronchur opiocephalus*) in the management, alone and in combination with hydroxypatite graft and platelet rich plasma, in two\three walled intrabody defects. The effect of the therapy was evaluated by assessing clinical attachment gain and the position of marginal gingiva at the time of surgery and 12 months after surgery. It was concluded that the use of the matrix barrier resulted in reduced probing depths, pronounced gain of clinical attachment and improved periodontal treatment outcome.

Varied Gingival Neoplastic Presentations of the Dreadful AIDS - Case Series**D. Deepa*, J. A. Kumar**

(Bapuji Dental College & Hospital, Davangere, India)

Oral lesions are common in individuals with HIV infections. Early recognition & treatment of these oral lesions may reduce morbidity.

Diagnosis is made from biopsy, for these lesions may mimic other forms of swelling or ulceration. Patients with HIV disease have an increased risk of developing infections like lymphoma & other types of neoplasms. Their presence is an indication of immunodeficiency and predicts the progression of HIV disease.

Here, we report a series of highly malignant diffused lesions of gingiva with unique histopathological features, reported to our department.

Efficacy of a Local Drug Delivery System Using 8% Tetracycline Hydrochloride (Periodontal Plus AB) in the Treatment of Chronic Periodontitis Evaluated by a DNA Probe**B. Fernandes**

(College of Dental Surgery, Mangalore, India)

Several methods such as bacterial culturing, direct microscopy, immunodiagnostic methods, enzymatic methods and deoxyribonucleic acid (DNA) probe technology have been employed for the detection of putative pathogens in subgingival samples. Some of these have been strictly used for research purposes, whereas others have been adapted or modified for clinical use.

This poster depicts a study conducted to evaluate the efficacy of 8% tetracycline hydrochloride (Periodontal Plus AB) as non-surgical therapy in the treatment of chronic periodontitis. A DNA probe (DMDx) was used to assess the qualitative and quantitative changes in the microflora over a period of 6 months.

Guided Tissue Regeneration Using Collagen Membrane - A Practical Approach in the Management of Gingival Recession

A. Madhukant*, S. Hedge, K.S. Rajesh

(Department of Periodontics, Yenepoya Dental College, Mangalore, India)

The concept of guided tissue regeneration in the treatment of gingival recession has shown promising results and is gaining clinical acceptance. Recent research has focused upon utilization of a bio-absorbable collagen membrane in guided tissue regeneration (GTR).

16 systematically healthy patients with Miller Class I or II gingival recession defects were randomly treated by coronally advanced flap, alone or in combination with a bio-absorbable collagen membrane (8 patients each), and followed up for a period of six months.

This poster depicts the clinical applicability of a type I collagen membrane (HealiguideTM) in the treatment of localized gingival recession and the clinical results obtained.

Effect of Periodontal Treatment on Monocyte Functions in Periodontitis Patients

D. Hormdee*, T. Nagasawa, G. Koshy, M. Kiji, R. Yashiro, H. Nitta, I. Ishikawa

(Department of Hard Tissue Engineering, Tokyo Medical & Dental University, Tokyo, Japan)

Inflammatory cytokines, including IL-6 and TNF- α , play important roles in the pathogenesis of periodontitis-associated systemic diseases and monocytes are major source of these cytokines. The aim of this study was to evaluate the effect of periodontal treatment on the production of IL-6 and TNF- α by circulating monocytes. 36 systematically healthy subjects diagnosed with chronic periodontitis were treated with ultrasonic debridement. Clinical parameters and peripheral blood samples were obtained before treatment (baseline), immediately, 1, 3 and 6 months after treatment (follow-up). Production of IL-6 and TNF- α immediately after were significantly greater than those at baseline. Production of both cytokines at baseline was significantly greater than those at follow-up. The present study suggested that periodontal inflammation might activate circulating monocytes, and such activation might be reduced by the periodontal therapy.

Management of An Isolated and Advanced Gingival Recession in a Maxillary Premolar – A Case Report**A.A. Salman**

(Department of Periodontics, Tamilnadu Goverment Dental College & Hospital, Chennai, India)

Isolated and advanced gingival recession is one of the complex mucogingival problems to treat. A 35 year old female with gingival recession score of F4 – 9* (Smith RG – 1997) in tooth number 14, with buccal root tip projecting into oral cavity reported to the Government Dental College, Chennai. Endodontic management of the non-vital 14 was performed. Intraoperatively, the protruding buccal root tip was smoothed and thorough root planning with tetracycline root conditioning was done. A laterally repositioned pedicle from 15, 16 region was used to cover the recession. The donor area of 15, 16 was covered with a FGG from the palate. A six month follow up examination showed good coverage of the recession.

Stem Cell Properties of Human Periodontal Ligament Cells**K. Nagatomo*, M. Komaki, K. Noguchi, S. Oda, I. Ishikawa**

(Department of Hard Tissue Engineering, Tokyo Medical & Dental University, Tokyo, Japan)

The stem cell properties of periodontal ligament (PDL) cells are not fully understood. The purpose of this study was to investigate whether human PDL cells possessed stem cell properties including stem cell marker expression, self-renewal and multi-potency. PDL cells were sparsely plated and staining of STRO-1, FACS analysis for stem cell markers, proliferation assay for self-renewal and differentiation assay for multi-potency were performed. PDL cells proliferated, made colonies derived from a single cell and expressed stem cell markers. Some colonies showed positive staining for STRO-1 and differentiated into adipocytes and osteoblasts. Our findings indicate that PDL cells have crucial stem cell properties, self-renewal and multi-potency.

Production of BMP-2 by Human Periodontal Fibroblasts Stimulated with TGF- β

R. Yashiro*, T. Nagasawa, M. Kiji, D. Harmdee, G. Koshy, H. Nitta, I. Ishikawa

(Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan)

Transforming growth factor (TGF- β) is abundant in the bone matrix. Bone morphogenetic protein (BMP-2) is essential for bone formation. The presented study was performed to evaluate the regulatory effects of TGF- β on BMP-2 production by human periodontal ligament cells (PDL). PDL was stimulated with TGF- β , with or without various additives. BMP-2, mRNA expression was quantified by real-time PCR. BMP-2 production was measured using ELISA. BMP-2 mRNA expression and BMP-2 production were augmented by TGF- β in PDL. PKC inhibitors suppressed TGF- β -induced BMP-2 production in PDL, whereas activators enhanced it. These results suggest that PDL may contribute to bone and cementum formation by producing BMP-2 in response to TGF- β through the PKC pathway.

Chronic Periodontitis with Gingiva Hyperplasia

R.M. A. Ferriols

(Private Practitioner, Cubao Quezon City, Philippines)

The treatment of a patient afflicted with chronic periodontitis with gingival hyperplasia and having class II division II occlusion was presented. Treatment involved surgical removal of root fragments, extensive reduction of probing depths through scaling and root planning, gingivectomy of fibrotic gingival enlargement, filling of carious teeth and continual motivation to improve oral hygiene practices. Orthodontic alignment took the longest time due to major loss of bone in the area. A prosthesis was constructed after retainers were removed. A synergistic interdisciplinary treatment approach contributed to the remarkable transformation in physical appearance as well as in intra-oral condition of the patient.

Multiple Epiphyseal Dysplasia/Bilateral Genu Valgum with Localized Aggressive Periodontitis - A Case Report

G. Sivaram,* K. Bharadwaj

(Private Practitioner, Chennai, India)

A case report of an 18 year old female patient who was diagnosed with multiple epiphyseal dysplasia with bilateral genu valgum and was referred for the management of bleeding gums. She was then diagnosed with localized aggressive periodontitis and treated by surgical periodontal therapy for pocket elimination and splinting. This case report attempts to examine the link between the two stated conditions and also the incidence of such cases.

Assessment of Patient Recapitulation of Post Surgical Instructions

S. Kakarala*, M.G.S. Prasad

(Bangalore Institute of Dental Sciences, Bangalore, India)

Post surgical instructions undoubtedly play a very important role in reducing patients anxiety and stress after surgical procedures. The presented study consisted of two groups of twenty patient. Subjects in group I were given verbal instructions alone and whereas group II received both verbal and written instructions. The information provided by the patient is tabulated and analyzed. Results showed that the patients who received both verbal and written instructions were able to remember and follow the instructions better than those with verbal instructions only, thereby increasing patient compliance.