CURRENT TRENDS IN PERIODONTAL DIAGNOSIS, DISEASE RECOGNITION AND MANAGEMENT

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P. Mark Bartold, Isao Ishikawa, Nannette Vergel de Dios

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It is with pleasure that I present this, the fifth in our series of Asian Pacific Society of Periodontology Proceedings. This volume presents the full papers and abstracts of poster presentations at the 5th Asian Pacific Society of Periodontology meeting held in Cebu, The Philippines on 1-2 December 2003. As in previous years we are very pleased to acknowledge the significant support from the Procter & Gamble Company and Sunstar Corporation which have made the production of these proceedings possible. In addition, I acknowledge the special support from my co-editors Professor Isao Ishikawa and Dr Nannette Vergel de Dios. Of course these proceedings would not be possible had it not been for the excellent contributions from each of the invited speakers. Finally I would like to acknowledge the special assistance provided by Ms Catherine Offler in the preparation of these Proceedings which required not only preparation of numerous drafts prior to publication but the laborious task of ensuring uniformity in style between all of the manuscripts and the final desktop publishing of each manuscript.

In this volume the theme of *Current Trends in Periodontal Diagnosis, Disease Recognition and Management* is explored with perspectives from a wide variety of counties in the Asian Pacific region. It is interesting to note that despite the vast differences between countries in the Asian Pacific rim there is general consensus that periodontal diagnosis is a critical phase of periodontal treatment. It is also recognized almost universally that periodontal diseases are multifactorial and thus diagnostic protocols must take into account aetiological, environmental, genetic and iatrogenic factors. With regards to future trends in periodontal treatments the topical issues of periodontal systemic interactions, host modulation and periodontal regeneration are discussed.

The APSP continues to develop as an organization of importance within the periodontal community in our region. The efforts of the APSP Executive Council to ensure the viability of this organization is gratefully acknowledged. In addition, our members and those willing to participate in our biennial meetings all contribute to make our organization unique. To all of these people, I say thank you and I hope that this volume continues our proud tradition of producing a quality publication which is an accurate record of our most recent meeting.

P. Mark Bartold
June 2004

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Changing Trends in Periodontal Diagnosis, Disease Recognition & Management
Edited by: PM Bartold, I Ishikawa, N Vergel de Dios

Chapter 1

High Risk Individuals for Periodontitis & Their Genetic Markers

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Introduction

Although microbial factors are essential for the initiation of periodontitis (Haffajee and Socransky 1994), they alone do not predict the presence or severity of the disease. Epidemiological studies have indicated that not everyone is equally susceptible to periodontitis (Beck *et al* 1990). Among the population, there is a group of high-risk individuals who are highly susceptible to periodontitis. Twin analyses by Michalowicz *et al* suggested that 38% to 82% of the population variance for the periodontal measures of the disease may be attributed to genetic factors (Mizhalowicz *et al* 1991, Michalowicz *et al* 1991). Now, the questions which remain to be answered are:

1. Are there any genetic marker(s) for high risk individuals for periodontitis?
2. What kind of gene(s) is associated with increased susceptibility to periodontitis?

Understanding these issues will help us to identify high-risk individuals for periodontitis among the population.

To elucidate the genetic background of chronic periodontitis (CP) in the Chinese, the gene polymorphisms of IL-1 cluster (including IL-1A-889, IL-1B-511, IL-1B+3954, IL-1RN intron 2/VNTR), FcγRIIA, FcγRIIIB, TNFA-308, IL-6, Vitamin D receptor gene (VDR), and their association with chronic periodontitis in a Chinese population were investigated.

Materials and Methods

Selection of subjects

All subjects were of Han nationality, in good general medical health, had at least 14 natural teeth and were non-smokers. Clinical attachment loss (CAL) was measured on 6 surfaces of all remaining teeth. The intra-examiner reliability for reproducibility was calibrated (kappa ≥0.75). Subjects were selected according to dental history, radiographic and clinical criteria (Armitage 1999, Armitage *et al* 2000) and were placed into 4 groups based on their average full-mouth CAL measurements:

Healthy control (periodontal healthy or gingivitis): Mean CAL ≤0.5 mm, no interproximal sites with CAL ≥3 mm. No more than 2 missing teeth with exception of extracted third molars, teeth extracted for orthodontic purpose, teeth lost as a result of trauma or extensive decay.

Initial CP: Mean CAL ≥0.6 mm to 1.6 mm and no interproximal sites with CAL ≥3 mm. No more than 3 missing teeth.

Moderate CP: Mean CAL ≥1.6 mm to 2.4 mm and ≤8 sites with interproximal CAL >3 mm distributed through at least 3 quadrants or at least 6 teeth. No more than 5 missing teeth.

Severe CP: Mean CAL ≥2.5 mm and 1 or more sites in 3 out of 4 quadrants with interproximal CAL ≥5 mm. No more than 14 missing teeth.

Sample collection

One sample of exfoliating epithelial cells was taken from every subject using a cotton swab. One cotton swab was rubbed lightly four times on the subject's inside cheek and left to dry at room temperature overnight, then stored at 4°C for DNA isolation.

Preparation of DNA templates

The surface of the cotton was cut from the buccal swab stem, transferred to an Eppendorf tube, and 200 µl Chelex-100 and 10 µl Proteinase K were added. Then, the standard phenol/chloroform extraction procedure was used. The genomic DNA yield was calculated by spectrophotometry at 260nm.

Analysis of genetic polymorphisms

PCR-RFLP or PCR-SSP procedures were used to analyze blindly for polymorphisms. All PCR reactions performed in 20 µl reaction mix which contained 20mM Tris-HCl, 50mM KCl, 1.6mM $MgCl_2$, 0.35mM each dNTP, 0.6 µl each primer, Taq polymerase 1.25U and DNA template 3 µl. The PCR conditions for different gene loci were justified respectively.

IL-1A –889 (Mc Dowell *et al* 1995)**:** Primers: 5'-AAG CTT GTT CTA CCA CCT GAA CTA GGC-3' and 5'-TTA CAT ATG AGC CTT CCA TG-3' were used. The PCR product (5 µl) was digested overnight at 37°C with 2U Nco I, and the restriction pattern visualized by electrophoresis through a 6% PAGE. The two sizes of the PCR products were 83bp+16bp (allele 1) or 99bp (allele 2).

IL-1B-511 (Pociot *et al* 1992)**:** Primers: 5'-TGG CAT TGA TCT GGT TCA TC-3' and 5'-GTT TAG GAA TC T TCC CAC TT-3' were used. The PCR product (5 µl) was digested with 3U Ava I at 37°C overnight, and the restriction pattern visualized through a 6% PAGE. This gave products of 190bp+114bp (allele 1) or 304bp (allele 2).

IL-1B +3954 (Kornman *et al* 1997)**:** Primers: 5'-CTC AGG TGT CCT CGA AGA AAT CAA-3' and 5'-GCT TTT TTG CTG TGA TCC CG-3' were used. The PCR product (5 µl) was digested with 3U Taq I at 65°C overnight, and the restriction pattern visualized by electrophoresis through a 6% PAGE. This gave products of 12bp+85bp+97bp (allele 1) or 12bp+182bp (allele 2) .

IL-1RN (intron 2) VNTR (Tarlow *et al* 1993)**:** Primers: 5'-CTC AGC AAC ACT CCT AT-3' and 5'-TCC TGG TCT GCA GGT AA-3' were used. Electrophoresis in 8%PAGE was performed following PCR. Allele 1 (4 repeats) was 412bp; allele 2 (2 repeats) was 240bp; allele 3 (3 repeats) was 326bp; allele 4 (5 repeats) was 500bp; and allele 5 (6 repeats) was 595bp.

FcγRIIA (Warmerdamn *et al* 1990)**:** PCR-SSP was used. Primer #1: 5'-ATC CCA GAA ATT CTC,CCA-3' and Primer #2: 5'-ATC CCA GAA ATT CTC CCG-3'; Primer #3: 5'-CAA TTT TGC TAT GGG C-3'. The PCR product (253bp) was visualized by electrophoresis through a 6% PAGE.

FcγRIIIB (Ory *et al* 1989): Primer for NA1: 5‘-CAG TGG TTT CAC AAT GTG AAA-3‘, 5‘-CAT GGA CTT CTA GCT GCA CCG-3‘; Primer for NA2: 5‘-CTC AAT GGT ACA GCG TGC TT-3‘, 5‘-CTG TAC TCT CCA CTG TCG TT-3‘. The PCR products of NA1 was 141bp and NA2 was 169bp and were visualized by electrophoresis through a 6% PAGE.

TNFA -308 (Wilson *et al* 1992)**:** Primers: 5‘-AGG CAA TAG GTT TTG AGG GCC-3‘; 5‘-ACA CTC CCC AT C CTC CCG GCT-3‘. The PCR product (5 μl) was digested with Nco I at 37°C overnight, and the restriction pattern visualized by electrophoresis through a 6% PAGE. This gave the products of 87bp+20bp (allele 1) or 107bp (allele 2).

IL-6 –174 (Zheng *et al* 2000)**:** Primers: 5‘-TTG TCA AGA CAT GCC AAA GTG-3‘; 5‘-TCA GAC ATC TCC AGT TCC TAT A-3‘. The PCR product (5 μl) was digested with Nal III at 37°C overnight, and the restriction pattern visualized by electrophoresis through a 7% PAGE. This gave the products of 246bp+54bp (allele G) or 135bp +111+54bp(allele C).

VDR/BsmI (Morrison *et al* 1994)**:** Primers: 5‘-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3‘; 5‘-AAC CAG CGG GAA GAG GTC AAG GG-3‘. The PCR product (5 μl) was digested with BsmI at 37°C overnight, and the restriction pattern visualized by electrophoresis through a 7% PAGE. This gave the products of 800bp (allele B) or 650bp +150bp(allele b).

VDR/Apal (Bell *et al* 2001)**:** Primers: 5‘-CAG AGG ATG GAC AGG GAG CAA-3‘; 5‘-GCA ACT CCT CAT GGC TGA GGT CTC-3‘. The PCR product (5 μl) was digested with ApaI at 37°C overnight, and the restriction pattern visualized by electrophoresis through a 7% PAGE. This gave the products of 740bp (allele A) or 535bp +205bp(allele a).

VDR/TaqI (Bell *et al* 2001)**:** Primers and PCR conditions are same as for VDR/ApaI. The PCR product (5 μl) was digested with TaqI at 37°C overnight, and the restriction pattern visualized by electrophoresis through a 7% PAGE. This gave the products of 495bp+245bp (allele T) or 495bp +290bp+245bp+205bp (allele t).

All PCR products were stained with silver nitrate. All PCR screening methods used in this study have been extensively validated. 10% randomly selected samples were subjected to an additional PCR procedure operated by another technician in a blinded test. These samples were used as laboratory quality controls to assess the reproducibility of the genotyping.

Statistical analysis

The distributions of the genotypes were calculated as percentage of the study population. The differences of gene frequency between each group were determined by x^2 test. All the analyses were performed with the SSCP statistical package.

Results

Comparison of DNA yields and concordance of genotyping

The average quantity of DNA isolated from one buccal swab was 63.8± 18.7μg, which was sufficient to repeat PCR-based genetic analysis 10 times. When the code was broken for the duplicate samples used for the laboratory quality control, the genotyping results matched in all subjects.

IL-1 genotype distribution

The distribution of the sampled subjects according to age and disease is shown in Table 1. IL-1A-889/Nco and allele 2 was carried by 46% of the subjects, all of them were heterozygous. The frequency of allele 2 in both severe and initial/moderate CP groups was significantly higher than in healthy/gingivitis group ($p < 0.01$) (Table 2).

IL-1B+3954/Taq and allele 2 was carried by 12.9% of the subjects, all of them were heterozygous. The frequency of allele 2 in both severe and initial/moderate CP groups was significantly higher than in healthy/gingivitis group ($p < 0.05$) (Table 3).

IL-1B-511/Ava and allele 2 was carried by 83% of the subjects, 39% were heterozygous and 44% were homozygous. The distribution of allele 2 homozygote was significantly higher in both severe and initial/moderate CP groups than in healthy/gingivitis group ($p< 0.05$) (Table 4).

There were only 3.69% of the subjects carried of the composite genotype of IL-1A-889 allele 2 and IL-1B+3953 allele 2 (Table 5).

There were 42.44% of the subjects carrying the composite genotype of IL-1A-889 allele 2 and IL-1B-511 allele 2. The distribution of this composite genotype was significantly higher in both severe and initial/moderate CP groups than in healthy/gingivitis group ($p < 0.01$) (Table 6).

For the composite genotype of IL-1B+3953 allele 2 and IL-1B-511 allele 2, there was only 9.23% of the subjects carrying this composite genotype. The distribution of this composite genotype was significantly higher in both severe and initial/moderate CP groups than in healthy/gingivitis group ($p < 0.05$) (Table 7).

For gene frequency and distribution of IL-1RN (intron2)/VNTR allele, there was no significant difference for the distribution of the genotypes among the three groups (Table 8).

FcγRIIA, FcγRIIIB and TNFA-308 genotype distribution

The distribution of the sampled subjects according to age, gender and disease is shown in Table 9. The genotype of FcγRIIA R/R131 distributed significantly higher in severe CP group than in the healthy control (Table 10). There was no significant difference for the distribution of the FcγRIIIB genotype between the groups (Table 11). It was also found that the frequency of the composite genotype of FcγRIIA R131 and FcγRIIIB NA2 was

Disease category	Age group (years) ≤29	≥30-39	≥40-49	≥50-59	≥60
Severe CP n = 85	1	1	16	51	16
Initial to moderate CP n = 97	6	5	27	47	12
Healty/Gingivitis n = 89	7	46	36	0	0
Totals n = 271	14	52	79	98	28

Table 1. Distribution of sampled population according to age and disease category

Disease category	Size	Distribution of genotype			Frequency of allele	
		I/I (%)	II/I (%)	II/II (%)	I	II
Severe CP	85	32(37.65)	53(62.35)	0	0.688	0.312
Initial/Moderate CP	97	46(47.42)	51(52.58)	0	0.737	0.263
Healthy/Gingivitis	89	69(77.53)	20(22.47)	0	0.888	0.112

Table 2. Gene frequency and distribution of IL-1A-889/Nco I allele

Disease category	Size	Distribution of genotype			Frequency of allele	
		I/I (%)	II/I (%)	II/II (%)	I	II
Severe CP	85	68(80.00)	17(20.00)	0	0.900	0.100
Initial/Moderate CP	97	83(85.57)	12(14.43)	0	0.928	0.072
Healthy/Gingivitis	89	85(95.51)	4(4.49)	0	0.978	0.222

Table 3. Gene frequency and distribution of IL-1B+3954/Taq I allele

Disease category	Size	Distribution of genotype			Frequency of allele	
		I/I (%)	II/I (%)	II/II (%)	I	II
Severe CP	85	12(14.12)	28(32.94)	45(52.94)	0.306	0.694
Initial/Moderate CP	97	13(13.4)	30(30.93)	54(55.67)	0.289	0.711
Healthy/Gingivitis	89	21(23.6)	49(55.06)	19(21.35)	0.511	0.489

Table 4. Gene frequency and distribution of IL-1B-511/Ava I allele

Disease category	Size	Distribution of genotype		Frequency of allele	
		Genotype + (%)	Genotype - (%)	Genotype +	Genotype -
Severe CP	85	4(4.71)	81(95.29)	0.0235	0.9765
Initial/Moderate CP	97	5(5.15)	92(94.85)	0.0258	0.9742
Healthy/Gingivitis	89	1(1.12)	88(98.88)	0.0056	0.994

Table 5. Gene frequency and distribution of the composite genotype of IL-1A-889 allele 2 and IL-1B+3954 allele 2

Disease category	Size	Distribution of genotype Genotype + (%)	Genotype - (%)
Severe CP	85	50(58.82)	35(41.18)
Initial/Moderate CP	97	49(50.52)	48(49.48)
Healthy/Gingivitis	89	16(17.98)	73(82.02)

Table 6. Distribution of the composite genotype of IL-1A-889 allele 2 and IL-1B-511 allele 2

Disease category	Size	Distribution of genotype Genotype + (%)	Genotype - (%)
Severe CP	85	12(14.12)	73(85.88)
Initial/Moderate CP	97	10(10.31)	87(89.69)
Healthy/Gingivitis	89	3(3.37)	86(96.63)

Table 7. Distribution of the composite genotype of IL-1B+3954 allele 2 and IL-1B-511 allele 2

Disease category	Size	Distribution of genotype I/I (%)	II/I (%)	III/I (%)	IV/I	V/I	Frequency of allele I	II
Severe CP	85	42(49.41)	43(50.59)	0	0	0	0.747	0.253
Initial/Moderate CP	95	60(63.16)	35(36.84)	0	0	0	0.8158	0.1842
Healthy/Gingivitis	87	40(45.98)	47(54.02)	0	0	0	0.7299	0.2701

Table 8. Gene frequency and distribution of IL-1RN(intron2)/VNTR allele

Groups	N	Male(%)	Female(%)	Age range	Mean age
Severe CP	63	26(41.27)	37(58.73)	42-60	55
Initial/Moderate CP	103	27(26.21)	76(73.79)	27-60	52
Healthy/Gingivitis	80	32(40.00)	48(60.00)	35-69	53
Total	246	85(34.55)	161(65.45)	27-69	53

Table 9. Subject Data

Disease category	Size	Distribution of genotype			Frequency of allele	
		H/H (%)	H/R (%)	R/R (%)	H	R
Severe CP	63	4(6.35)	47(74.60)	12(19.05)	0.44	0.56
Initial/Moderate CP	103	13(12.62)	78(75.73)	12(11.65)	0.50	0.50
Healthy/Gingivitis	80	18(22.50)	60(75.00)	2(2.50)	0.60	0.40

Table 10. FcγRIIA genotype distribution and allele frequency

Disease category	Size	Distribution of genotype			Frequency of allele	
		NA1/NA1 (%)	NA1/NA2 (%)	NA2/NA2 (%)	NA1	NA2
Severe CP	63	6(9.52)	57(90.48)	0	0.55	0.45
Initial/Moderate CP	103	10(9.71)	93(90.29)	0	0.55	0.45
Healthy/Gingivitis	80	18(22.50)	62(77.50)	0	0.61	0.39

Table 11. FcγRIIIB genotype distribution and allele frequency

Disease category	Size	Distribution of genotype	
		Genotype + (%)	Genotype - (%)
Severe CP	63	53(84.13)	10(15.87)
Initial/Moderate CP	103	83(80.58)	20(19.42)
Healthy/Gingivitis	80	52(65.00)	28(35.00)

Table 12. Distribution of the composite genotype of FcγRIIA-R131 and FcγRIIIB-NA2

Disease category	Size	Distribution of genotype			Frequency of allele	
		I/I (%)	II/I (%)	II/II (%)	I	II
Severe CP	63	53(84.13)	10(15.87)	0	0.92	0.08
Initial/Moderate CP	103	83(80.58)	19(18.45)	1(0.97)	0.90	0.10
Healthy/Gingivitis	80	70(87.50)	10(12.50)	0	0.94	0.06

Table 13. TNFA-308 genotype distribution and allele frequency

Groups	N	Male	Female	Age range	Mean age
Severe CP	63	26	37	42-60	55
Moderate CP	69	20	49	35-60	52
Initial CP	34	7	27	27-60	52
Healthy/Gingivitis	80	32	48	35-60	53

Table 14. Subject Data

		Distribution of genotype			Frequency of allele	
Disease category	Size	A/A(%)	A/ab(%)	a/a(%)	A	a
Severe CP	63	39(61.8)	12(19.1)	12(19.1)	0.71	0.29
Moderate CP	69	14(20.3)	41(59.4)	14(20.3)	0.50	0.50
Initial CP	34	8(23.5)	21(61.8)	5(14.7)	0.54	0.46
Healthy/gingivitis	80	6(7.5)	31(38.8)	43(53.7)	0.27	0.73

Table 15. VDR/ApaI genotype distribution and allele frequency

		Distribution of genotype			Frequency of allele	
Disease category	Size	B/B(%)	B/b(%)	b/b(%)	B	b
Severe CP	63	0	11(17.5)	52(82.5)	0.09	0.91
Moderate CP	69	1(1.58)	7(11.1)	61(88.4)	0.07	0.93
Initial CP	34	0	3(8.8)	31(91.2)	0.04	0.96
Healthy/gingivitis	80	1(1.2)	7(8.8)	72(90.0)	0.06	0.94

Table 16. VDR/BsmI genotype distribution and allele frequency

		Distribution of genotype			Frequency of allele	
Disease category	Size	T/T(%)	T/t(%)	t/t(%)	T	t
Severe CP	63	57(90.5)	6(9.5)	0	0.95	0.05
Moderate CP	69	60(86.9)	9(13.1)	0	0.93	0.07
Initial CP	34	28(82.4)	6(17.6)	0	0.91	0.09
Healthy/gingivitis	80	71(88.8)	9(11.2)	0	0.94	0.06

Table 17. VDR/TaqI genotype distribution and allele frequency

Disease category	Size	Distribution of genotype			Frequency of allele	
		G/G(%)	G/C(%)	C/C(%)	G	C
Severe CP	63	62(98.4)	1(1.6)	0	0.99	0.01
Moderate CP	69	69(100.0)	0	0	1.00	0
Initial CP	34	34(100.0)	0	0	1.00	0
Healthy/gingivitis	80	79(98.8)	1(1.2)	0	0.99	0.01

Table 18. IL-6-174 genotype distribution and allele frequency

significantly higher in severe CP group than in the healthy control (Table 12). There was no significant difference for the distribution of TNFA-308 genotype between the groups (Table 13).

VDR and IL-6-174 genotype distribution

The distribution of the sampled subjects according to age, gender and disease is shown in Table 14. It showed that there was a significant over-representation of VDR allele A in all CP groups than in healthy control, the carriage rate of genotype AA was significantly higher in the severe CP group than in the healthy control (61.8% VS 7.5%, $p<0.01$)(Table 15). No significant difference in the distribution of genotype of VDR BsmI, TaqI polymorphism between different groups was observed (Table 16,17). It was showed that no significant differences in distribution of genotype of IL-6-174 were observed among patients and controls. The IL-6 -174 allele C is rare in the Chinese Han nationality (Table 18).

Discussion

In this preliminary study, it was found that genotypes of IL-1A-889 II/I, IL-1B-511II/II, IL-1A-889 allele 2/IL-1B-511 allele 2, VDR/ApaI A/A were significantly associated with severity of chronic periodontitis, and genotypes of IL-1B+3954 II/I, IL-1B+3954 allele 2/IL-1B-511 allele 2, FcgRIIA R/R were also associated with severity of chronic periodontitis, although their distribution in the population were relatively low. According to our data and others, it is believed that an individual may have increased susceptibility to periodontitis due to different factors, genotypes associated with increased susceptibility may be quiet heterogeneous and an individual with increased susceptibility may be influenced by multiple genes. Further studies are being undertaken on more potential candidate genes in large size of samples and with linkage analysis and associate studies in families.

Multifactorial diseases, such as periodontitis, are believed to be due to the combined effect of multiple genes, often interacting over long time periods with environmental factors. For systematic analysis of association of genetic polymorphisms with periodontitis and defining the role of the gentic polymorphisms in the disease pathogenesis, meaningful models to accommodate complexity and diversity of periodontitis due to interactions between genetic and environmental elements are needed.

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Changing Trends in Periodontal Diagnosis, Disease Recognition & Management
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Chapter 2

Current Trends in Periodontal Diagnosis & Disease Recognition in Malaysia

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Introduction

The ability to correctly diagnose and institute effective periodontal therapy is essential to the control of periodontal diseases (PD). Our increased understanding of the etiology and pathogenesis of PD have assisted in improving conventional diagnostic procedures such as visual examination, manual periodontal probing, exudate measurements and radiographic imaging to adapt to current periodontal disease concepts.

Traditional periodontal diagnostic procedures are not precise and only allow retrospective assessment of attachment loss. Considerable improvement and advances have been achieved in improving the accuracy of traditional clinical diagnostic procedures and in developing new diagnostic techniques. Continuing efforts have been directed to improving effectiveness of these diagnostic strategies, which in addition to diagnosis can be extremely useful in evaluating treatment outcomes and patient monitoring for disease recurrence.

Traditionally, periodontal diagnosis includes measurement of probing pocket depths (PPD), and attachment levels (AL) as well as recession with a graduated periodontal probe and visual assessment of patients' gingival tissue for signs of inflammation such as redness, swelling, presence of exudates and bleeding on probing (BOP). Assessment of tooth mobility and plaque levels are additional useful clinical parameters. Radiographic evaluation is vital to identify alveolar bone destruction and to determine disease severity. Additional information which involves demographic data such as age, gender, medical history, history of previous and current periodontal problems are used to tentatively assist in determining clinical form of the disease and possibly activity and prognosis of the disease.

In most cases, these traditional diagnostic methods are sufficient to allow development of an appropriate and effective treatment plan. However, the treatment outcome in a minority of clinical cases may not be as desired and research evidence indicates that conventional diagnostic criteria such as gingival oedema, redness, plaque, bleeding and exudates have fair specificity (71-97%) but poor sensitivity (3-42%) in diagnosing sites or patients with "active" progressive disease (Haffajee *et al* 1983).

Following increased understanding of the latest concepts of the nature, aetiology and pathogenesis of periodontal diseases, diagnostic strategies are being modified to correctly fit current disease concepts. Efforts are currently targetting the development of

new diagnostic tests or procedures to relate pertinent information regarding a patient's particular periodontal disease, disease severity, progression, activity, predictions and response to treatment.

The new strategies in periodontal diagnosis and disease recognition have been categorized as follows:

1. Advances in traditional diagnostic methods:
   a) controlled-force, electronic probes
   b) computer-assisted digitalized subtraction radiography
   c) mobility measuring device e.g. Periotest

2. Detection of periodontopathic organisms
   a) bacteriologic DNA analysis
   b) immunologic-based tests for putative pathogens
   c) microbiologic enzyme assays
   d) Polymerase Chain Reaction (PCR)

3. Assessment of the susceptible host using markers in peripheral blood
   a) Polymorphonuclear leukocytes (PMNLs)
   b) Antibody titres
   c) Monocyte responsiveness to lipopolysaccharides (LPS)

4. Identification of host constituents in gingival crevicular fluid (GCF)
   a) Arachidonic acid metabolites
   b) Host cytokines
   c) Destructive host enzymes
   d) Other inflammatory host markers in GCF

5. Indicators of local physical/metabolic changes
   a) Subgingival temperature
   b) Nuclear medicine techniques (bone scanning)

Advances in traditional diagnostic methods

Controlled-force, electronic probes

The periodontal probe is the most widely used periodontal diagnostic tool for clinically assessing connective tissue destruction secondary to periodontitis. Progression of periodontitis is assessed using sequential examinations to determine attachment loss, indicated by periodontal pocket depth and periodontal attachment level measurements. These measurements are the benchmarks and most frequently used clinical parameters in the evaluation of the periodontal status.

Various types of periodontal probes have been used which include the manual probes like the Williams, North Carolina (Osborn *et al* 1992) and Michigan O probes (Osborn *et al* 1990); the pressure-controlled manual probes (Kalkwarf *et al* 1986, Perry *et al* 1994) and automated electronic probes such as the Florida Probe (Gibbs *et al* 1988, Perry *et al* 1994), Foster-Miller Probe (Jeffcoat *et al* 1986), the Peri-Probe (Vivadent, Schaan, Lichtenstein) and Audio-Probe (ESRO AG, Thalvil, Switzerland). Manual probing results in measurement errors usually associated with probing force (Van der Velden 1979, Mombelli *et al* 1992), probe angulation (Van der Weijden *et al* 1994), periodontal tissue status (Armitage *et al* 1977) and examiners' visual assessment (Magnusson *et al* 1988). To reduce some of these errors, the next generation of probes, automated electronic probes such as the Florida Probe (FP), have been developed.

The FP is aimed at increasing the probing measurement accuracy, reducing operator observational error and recorder error as well as improving standardization of reference points by utilizing the Florida Disk Probe for the measurement of relative attachment levels (RAL) with the occlusal surfaces or incisal

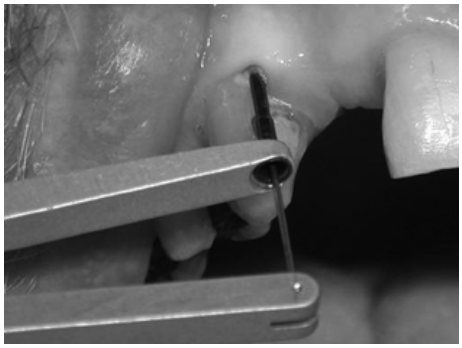

Figure 1. The Florida Pocket Probe in-situ in the periodontal pocket of a patient.

edges as the reference points.

The FP was one of the earliest automated, constant force electronic probes developed (Figure 1). It consists of an autoclavable probe handpiece, foot-switch and computer-interface (Figure 2), which stores captured data. A transducer is used to record the measurements which are transferred electronically to a computer (Figure 3), thereby eliminating transcription errors. Periodontal pocket depth (PPD) is measured with Florida Pocket Probe (FPP) and the Florida Disc Probe (FDP) measures relative periodontal attachment levels (RAL) (Figure 4).

In repeatability studies with manual probes, repeated measurements may vary by as much as 2 mm or more, although most measurements can be repeated to within 1 mm (Glavind and Loe 1967, Badersten *et al* 1981). Repeatability can also be expressed by the standard deviation (sd) of the difference between 2 measurements (Goodson *et al* 1982, Haffajee *et al* 1983, Badersten *et al* 1984). The Florida Probe has exhibited smaller standard deviations than the conventional probes (Magnusson *et al* 1988, Osborn *et al* 1990, Marks *et al* 1991, Yang *et al* 1992).

In a repeatability study of the FP by the author, the distributions of the differences in PPD and RAL measurements are depicted in Table 1 and Table 2 respectively. The differences between repeat PPD and RAL had mean values of -0.04 and -0.05 respectively. The Standard Deviations of the differences between the replicate readings were 0.68 mm for PPD and 0.80 mm for RAL with slightly better repeatability with FPP. It was observed that 88.4% of all PPD measurements and 85.1% of all RAL measurements differed within 1 mm. The distribution of the measurement errors of PPD and RAL measurements as expected had a typical bell-shaped distribution (normal curve).

The distributions of differences between repeat measurements for PPD and RAL for different sites in the dentition is depicted in Table 3 and 4. This was done to investigate if there were any differences on the repeatability outcome between anterior and posterior teeth. The distal surfaces of posterior teeth showed more variability in the repeated PPD measurements. In the case of repeated RAL measurements, greater variability was seen on the buccal and lingual surfaces of posterior and distal surfaces of anterior teeth.

Hence from this preliminary study, adequately repeatable data for clinical use was obtained with the Florida Probe which were comparable to those in other studies for a first-time user (Perry *et al* 1994, Reddy *et al* 1997, Osborn *et al* 1990, Badersten *et al* 1984, Janssen *et al* 1988, Kingsman *et al* 1991, Wang *et al* 1995).

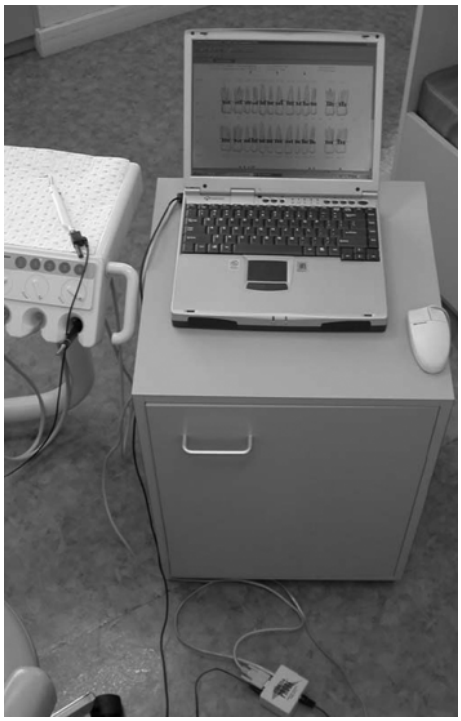

Figure 2. The Florida Probe, consisting of a probe handpiece, computer interface which is connected to a computer laptop, power supply, foot controller and the probe handpiece.

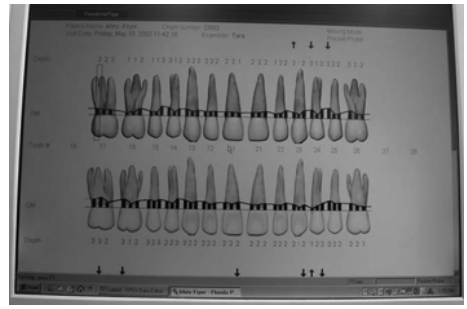

Figure 3. The periodontal measurements electronically recorded and displayed on the computer monitor.

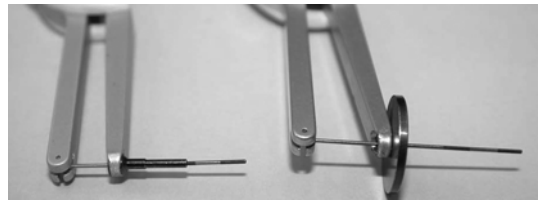

Figure 4. The Florida Pocket Probe (left) and the Florida Disk Probe (right).

Computer Assisted Digital Radiography (CADR)

The levels of alveolar bone are traditionally determined with the long-cone-paralleling technique or with an orthopantomograph. CADR such as subtraction radiography is an improved radiographic technique which is able to diagnose small losses or gains in alveolar bone which may allow immediate intervention of particular active sites. To detect loss of alveolar bone secondary to periodontitis, or gains after regenerative procedures, standardized views of radiographs of the sites separated in time are taken. Changes in as little as 1 – 5% mineral content, or as little as 0.5 mm bone loss along root surface or $<1\ mm^3$ of bone loss (Jeffcoat 1990) can be assessed with consecutive digital images subtracted using developed software. These subtraction images, where bony changes occur, are contrasted or colour enhanced to heighten its readability.

Detection of Periodontal Pathogens

For over 20 years, bacterial culturing has been the primary method of identifying putative pathogens (Socransky and Haffajee 1992, Haffajee and Socransky 1994). However this technique is time consuming and costly, although it remains to be the gold standard for characterizing species and for antibiotic susceptibility testing.

Bacteriologic DNA Analysis

Nucleic acid probes rely on species specific

Mean Difference -0.04	Standard Deviation (SD) 0.68
Measurement Difference	Percentage of sites (%)
≤0.5 mm	58.80
≤1.0 mm	88.41
≤1.5 mm	95.65
≤2.0 mm	99.69
≤2.5 mm	99.90
≤3.0 mm	100.00

Table 1. Distribution of differences (mm) for PPD measurements (with FPP) between the 2 probing sessions for all sites in the dentition

Mean Difference -0.05	Standard Deviation (SD) 0.80
Measurement Difference	Percentage of sites (%)
≤0.5 mm	55.18
≤1.0 mm	85.09
≤1.5 mm	94.10
≤2.0 mm	98.86
≤2.5 mm	99.38
≤3.0 mm	99.69

Table 2. Distribution of differences (mm) for RAL measurements (with FDP) between the 2 probing sessions for all sites in the dentition

genomic sequences for microbial identification. Complimentary oligonucleotide probes are constructed and labeled (Dewhirst and Paster 1991). Subgingival plaque samples collected are enzymatically split into single stranded, denatured DNA fragments. These unknown fragments are then exposed to complimentary, labeled probes and allowed to hybridize to reflect the presence of selected species in the sample

Immunological assays

Specific immunological techniques such as immunoflorescence microscopy or enzyme-linked immunoassay (ELISA) can detect individual bacterial species by using specific labeled antibodies that bind to selected bacterial antigens.

Microbiological enzyme assay

An enzyme which is unique to one or more of selected bacterial species is identified by the detection of the trypsin–like protease produced mainly by *Porphyromonas gingivalis, Tanarella forsythensis* and *Treponema denticola* which hydrolyses benzyl arginine napthlamide (BANA) substrate (Loesche 1992, Loesche *et al* 1990).

Polymerase Chain Reaction (PCR)

PCR involves amplification of a region of DNA flanked by a selected primary pair specific for the target species. Primers to the

	# sites	≤0.5 mm	≤1 mm	≤1.5 mm	≤2 mm	≤2.5 mm	≤3 mm
Posterior							
Distal	178	48.9	84.4	91	98.9	99.4	100
Mesial	178	56.2	88.2	94.4	100		
Buccal	89	73	94.4	98.9	100		
Lingual	89	58.4≤	92.1	98.9	100		
Anterior							
Distal	144	66.7	86.8	97.2	100		
Mesial	144	59	89.6	97.9	100		
Buccal	72	55.6	83.3	91.7	100		
Lingual	72	59.7	93.1	98.6	100		

Table 3. Distribution of differences (% of sites) for PPD measurements for different sites in the dentition

	# sites	≤0.5 mm	≤1 mm	≤1.5 mm	≤2 mm	≤2.5 mm	≤3 mm
Posterior							
Distal	178	59	86	94.4	99.4	99.4	100
Mesial	178	50.6	83.1	91	97.8	99.4	100
Buccal	89	61.8	86.5	94.4	97.8	97.8	98.9
Lingual	89	58.4	84.2	92.1	98.8	98.8	98.8
Anterior							
Distal	144	55.6	87.5	94.4	99.3	99.3	99.3
Mesial	144	47.9	81.9	96.5	98.6	100	
Buccal	72	58.3	86.1	95.8	100		
Lingual	72	55.6	87.5	95.8	100		

Table 4. Distribution of differences (% of sites) for RAL measurements for different sites in the dentition

16S-RNA signature sequences are used. PCR is based on the automated recycling of 3 reactions, namely DNA denaturation, DNA annealing and primary extension. Each reaction is quick and performed under similar conditions except for temperature. Completion of the procedure requires about 30 cycles and PCR displays the best detection limit identifying less than even 10 cells. In multiplex PCR, different bacterial species maybe determined simultaneously. Real-time or Quantitative PCR not only detects, but also quantifies, the target microorganisms.

Assessment of susceptible individuals using peripheral blood markers

Three constituents of peripheral blood have been investigated as markers for host susceptibility, which include polymorphonuclear leukocyte function, circulating antibody levels to bacterial antigens and monocyte responsiveness to bacterial endotoxins.

Polymorphonuclear leukocyte (PMNL)

Chemotactic and phagocytic defects in neutrophil function have been associated with severe forms of PD such as aggressive periodontitis and periodontitis associated with systemic diseases, Down Syndrome, Papillon-Lefevre Syndrome, insulin–dependent diabetes mellitus and cyclic neutropenia (Lavine *et al* 1976, Cianciola *et al* 1977, Clark *et al* 1977). Hence assessing neutrophil function may aid in screening high-risk individuals.

Antibody titers

An elevation in serum antibodies to suspected plaque periodontal pathogens in circulating peripheral blood is associated with increased severity of PD (Caton 1989).

Monocyte responsiveness to endotoxins

It has been hypothesized from results of a study that susceptibility to periodontal destruction could be related to increase host response to Gram-negative bacteria endotoxins (Garrison and Nichols 1989)

Assessment of host biomarkers in gingival crevicular fluid (GCF)

Mediators involved in the destructive host response and byproducts of host tissue metabolism, which may serve as markers of periodontal disease activity, pass into GCF.

These host markers include:

i) Metabolites of arachidonic acid e.g. PGE_2 (Goodson *et al* 1974)
ii) Cytokines e.g. 1L-1α , 1L-1β , 1L-6 (Masada *et al* 1990)
iii) Proteolytic and hydrolytic enzymes of inflammatory cell origin known as Matrix Metalloproteinases (MMP) e.g. Collagenases (Kowashi *et al* 1979), Cathepsins and neutral proteases e.g. elastases, trypsin and chymotrypsin (Lah *et al* 1986).
iv) Other inflammatory host factors e.g. β-Glucuronidase (Bang *et al* 1970), aspartate aminotransferase (Imrey *et al* 1991) and alkaline phosphatase (Ishikawa and Cimasoni 1970).

Many of these markers have a fairly high sensitivity and specificity and hence have potential in assisting prediction of PD progression. For some of these markers chairside assays have been developed and described.

Conclusion

Information obtained from any diagnostic test must be valid and precise for clinical use in patients. The validity of a test for periodontitis reflects its ability to correctly identify individual patients and/or sites with the disease and provide information related to disease classification, activity or susceptibility. The potential of these tests in predicting future periodontal destruction or stability in screening and diagnostics, as well as translating the results to clinical decision-making need to be verified further. Despite our present knowledge and expanding

evidence regarding PD, there are setbacks in the use of these procedures or techniques as diagnostic adjuncts for PD. Although many of these revolutionary diagnostic tools hold promise, most are at present being used in research studies, not yet in day-to-day clinical practice.

In the future, the enormous focus in periodontal diagnostics is expected to further increase and existing techniques improved and refined so as to be easily accessible to virtually all investigators and clinicians.

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Chapter 3

Current Trends in Disease Recognition & Diagnosis - A Perspective From India

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Introduction

In an era of improved understanding of the pathogenesis of periodontal disease, the diagnostic process has never been more central to determining therapeutic methods (Offenbacher & Collins 1993, Armitage 1992). In this age of technological advance, there is no dearth of equipment to assist the periodontal clinician in this task. The multifaceted nature of periodontal disease has clearly laid itself out to be one that is constituted by selective phases of disease activity, a plethora of periodontopathic organisms and consequences that extend even beyond the oral cavity. And yet it is this aspect of periodontal disease that again makes it difficult to diagnose and therefore difficult to treat. In the light of our present understanding of periodontal disease dynamics, the lack of a static diagnostic point of reference therefore, is often one that is felt acutely, by the researcher and clinician alike.

Mounting evidence of the association between periodontal disease and systemic disease (Page 1998, Mealey 1999) has served to make periodontal diagnosis more important that it ever was before. Established findings have shown us the association between periodontal disease and cardiovascular disease (Beck *et al* 1996, Beck *et al* 1998), chronic obstructive pulmonary disease (Scannapieco *et al* 1998, Travis *et al* 1994, Scannapieco & Mylotte 1996), cerebrovascular accidents (Grau *et al* 1995, Syrjanen *et al* 1988), diabetes mellitus (Loe 1993) and pre term low birth weight babies (Dasanayake 1998, Offenbacher *et al* 1996). Some preliminary evidence also exists for pathophysiological similarities between rheumatoid arthritis and periodontal disease as well.

Diagnosis is best understood in terms of the consequences of periodontal disease and the parameters affected by the process. It is the consequence of disease that we seek to measure, not only as our indicator of the presence and extent of disease but also as the therapeutic end point of success. In these terms, it becomes easier to classify diagnosis into clinical, radiological, microbiological and biochemical methods.

The value of a diagnostic test is largely defined by its ability to predict disease accurately. A test can give varied results that range from a true positive to a true negative or a false positive to a false negative. Depending on the co-relationships that a certain diagnostic test subscribes to, it is possible to determine the predictive value of a given test (Hill 1971, Sackett *et al* 1991). The ability of a diagnostic test to read as truly positive in the actual presence of disease is regarded as the sensitivity of a test. On the other hand, the

ability of a diagnostic test to read as a true negative in the actual absence of disease, is regarded as its Specificity (Hill 1971, Sackett *et al* 1991). These parameters can be evaluated by an objective estimation of the number of true/false positives or true/false negatives that result from the use of a particular diagnostic method. As an extension of these parameters, it is then possible to calculate the relative or absolute risk of a certain factor and obtain odds ratios that are a more objective representation of diagnostic evaluation. Diagnostic tests need to be understood in these terms and it is the onus of research to address these issues before expecting clinical application.

Clinical methods

Clinical methods of periodontal diagnosis have routinely relied on the periodontal probe as being an indicator of the primary consequence of periodontal disease i.e., the loss of periodontal attachment. Periodontal probing suffers from inherent limitations nevertheless and issues concerning probing like probing force, probing angle, the resistance of an inflamed periodontium to probing technique and the reproducibility of repetitive assessment (Badersten *et al* 1984), remain. These problems have been addressed to some limited extent by the use of constant force electronic (Clark *et al* 1992) or computer controlled probes like the Florida probe. But these probes offer considerable difficulty in terms of practicality of use and economic affordability.

The idea of having fiber optic devices at the end of investigative instruments is not new to medicine. Medical specialties have made extensive use of such instruments to reach inaccessible anatomic locations in the body and indeed have gone further by using such devices for non-invasive surgery as well. Undoubtedly some of the limitations of periodontal therapy such as visual and physical access to the disease-involved site may be addressed by the incorporation of such technologies into investigative devices that are small enough to enter the periodontal structures. The Perioscope is one such device that uses fiber optic technology to illuminate the periodontal pocket, offering a magnified and clear view of the root surface and inaccessible areas such as trifurcations and bifurcations.

This technology can be used to detect subgingival calculus remnants, ulcerated sulcular epithelium, cemental perforations and the like. SEM studies (Armitage & Christie 1973) of teeth involved in more aggressive forms of periodontitis have provided key information about cemental abnormalities, particularly at the cementodentinal junction (Tamamoto 1999) and also extensive resorption lacunae that characterize the entire length of the root surface. It is possible that some of these findings, have implications for the refractory nature of periodontitis in some individuals and the clinical diagnosis of such abnormalities may be made more possible by devices like the Perioscope.

Clinical diagnosis also needs to keep in mind the implications that arise from the multifactorial nature of periodontal disease. While there is much attention paid to the relationship between periodontal and endodontic disease, the implications of restorative dentistry are less understood by the periodontist and the restorative dentist alike. The explosion of restorative materials, calls for greater attention to the interaction with the periodontium like never before. And while periodontal investigation of biocompatibility is not discounted, iatrogenic periodontal abuse from restorative abnormalities has nevertheless to be contended with.

The sacrosanct area of the biologic width (Vacek 1994) and its relevance to periodontal integrity and restorative longevity is probably most neglected. While fixed prosthodontics has

evolved to accommodate this key periodontal concept, routine interproximal dentistry continues to violate biologic principles (Yuodelis *et al* 1973) many times. Accommodating the periodontium to receive a restoration and the involvement of the periodontist in restorative dentistry are concepts that must become clinical reality. Equally, orthodontic movement is focused so much on the root surface, that the associated changes in soft tissue are often overlooked. The periodontium must not be loaded beyond the call of routine orthodontic principles.

Localised periodontal conditions associated with developmental abnormalities are not commonplace but are however a pertinent diagnostic implication the practitioner should be aware of. The prevalence of defects such as *dens in dente* (Roland 1979, Ruprecht *et al* 1987), enamel hypoplasia, the palatogingival groove etc. may also vary between different ethnic populations and data is limited in this regard. The ability of some of these defects to harbor local etiologic factors and provide a nidus for accumulation of bacteria is what makes them relevant. Early recognition of these factors by thorough clinical examination can serve to prevent such situations in some cases.

Radiological Methods

The advancements in radiography and imaging probably represent one of the most fascinating aspects of diagnostic technology. Routine roentgenograms have expanded to include larger areas of the facial structure and the use of orthopantomograms, coaxial images and computerized navigation of tomograms have become common. These techniques undoubtedly offer a larger picture of the effect of periodontal disease on osseous structures and have contributed tremendously to radiographic diagnosis. The use of computer algorithms to generate spiral computerized tomographic images of osseous structures in 3-D is however new, at least in terms of its application for the diagnosis of periodontal disease. A simulated anatomic dissection offers a perspective that is unprecedented in the form of these imaging techniques. Bone can be viewed three dimensionally and the bidimensional limitations of the routine radiograph are easily a thing of the past.

Older radiographic techniques such as subtraction radiography (Grondahl & Grondahl 1983) and CADIA (Bragger *et al* 1988) were not accessible to the general dental practitioner for several reasons. Today direct digital radiography, either through the use of CMOS sensors or phosphor plates, offers a better opportunity to understand the consequence of periodontal disease on osseous density. Colorization of the 256 gray scales that constitute the spectrum of a black and white image has also made the interpretation of such images more contemporary. The elimination of radiographic magnification error in these techniques by calibration, has also served to make objective measurement possible. Computerized 3D reconstructions of these images also yield valuable, if only somewhat limited information. The benefits of decreased radiation with these techniques have also made their routine use in examination more realistic. It is now possible to show a patient 3D reconstructions of the alveolar structure and there is no doubt that these technologies go a long way in educating the patient and enabling him to accept periodontal treatment. Their use in maintenance and longitudinal assessment is also naturally obvious.

Microbiological Methods

Microbiological methods of periodontal diagnosis do not easily lend themselves to routine use in the hands of the general dental practitioner. The nature of investigation rarely

helps chair side use and this has contributed to their limited application. But periodontal disease is nevertheless bacteriologically mediated and therefore microbiologic diagnosis must form a mainstay of diagnostic investigation.

Microscopy of any nature is rarely definitive in its identification of periodontopathic organisms. The availability of specific immunologic staining reagents in some cases however makes such techniques continually viable. Such reagents do exist for a variety of periodontopathic organisms such as *P. gingivalis*, *P. intermedia*, *T. denticola*, and *A. actinomycetemcomitans*.

Culture methods (Slots 1986) continue to have some limited application as well. The use of selective and non-selective media can serve to isolate certain periodontopathic organisms and the relevance of such methods to testing antibiotic sensitivity in refractive forms of periodontitis continues. Quantification of microorganisms with some of these techniques is also possible in less equipped laboratories by the use of colony counting devices that are computerized and fairly efficient.

DNA probes (Grench *et al* 1986) based on a genomic understanding of bacterial microstructure are a more predictable, but expensive, method. DNA probes are available for *A. actinomycetemcomitans*, *P. intermedia* and *P. gingivalis* and these are well identified in genomic libraries that are commercially available. The degree of accuracy in organism identification is what makes the use of these techniques potent. The use of extended methodologies such as PCR (Griffen *et al* 1992) will also come into use in the future.

Enzymes, derived from either the host or the microbes, can also form useful markers for diagnosis. A trypsin-like enzyme (Loesche 1986) present in *T. denticola* and *P. gingivalis* is absent from at least 60 other subgingival plaque organisms. This enzyme can be detected by the hydrolysis of the trypsin substrate benzoyl-DL-arginine naphthylamide (BANA) (Loesche 1985) with almost 88% accuracy. This forms the basis for a chromogenic chairside test that is user friendly and potentially useful in the hands of even a general dental practitioner.

Biochemical methods

Biochemical diagnosis revolves primarily around the estimation of one or more of four key products that are released as part of the pathogenic process of periodontal disease. These are usually either host derived enzymes (Loesche 1986), bacteriological products, inflammatory mediators or tissue breakdown products. Many of the studies that have identified techniques for objective estimation of these products revolve around the collection of gingival crevicular fluid. Techniques for collection (Brill 1969) of this fluid and its limited availability in quantity have however served to limit such applications to research. The translation to clinical use continues to be defiant and this is perhaps one reason why biochemical diagnosis does not find routine clinical application.

Salivary biochemistry on the other hand lends itself to far more clinical relevance and recent studies estimating CD14 levels and the like, serve to reinforce its potential. Biochemical assays exist for a variety of enzymes like aspartate aminotransferase (Chambers *et al* 1991), β-glucuronidase, alkaline phosphatase (Chapple *et al* 1994), dipeptidyl peptidase, elastase and cathepsins (Cox & Eley 2003). Inflammatory mediators like Prostaglandin E_2 (Offenbacher *et al* 1986) and cytokines of various types (Masada *et al* 1990) can also be estimated in saliva, gingival crevicular fluid or tissue samples. None of these techniques have however become commercially available as yet. Other salivary assays for the salivary peroxidase system and

salivary antioxidants (Moore *et al* 1994) hold promise too.

Biochemical assays for other markers in serum, like C reactive protein, also seem to offer potential for use.

Genetic tests for polymorphisms (Greenstein & Hart 2002, Kinane & Hart 2003, Greenstein & Hart 2002) in the IL-1 gene cluster are a new direction and economic limitations continue to be a stumbling block here as well. Variability between ethnic subpopulations also implies the need for more data, before the objective transition to a diagnostic assay of risk predictability can be made.

Conclusion

It seems that clinical diagnosis will continue to be the cornerstone of periodontal disease recognition even with its inherent limitations. A recent position paper of the American Academy of Periodontology substantiates this (AAP Position Paper on Diagnosis of Periodontal Disease). To quote from the paper: "Despite our increased understanding of the etiology and pathogenesis of periodontal infections, the diagnosis and classification of these diseases is still based almost entirely on traditional clinical assessments". To arrive at a periodontal diagnosis the dentist must rely upon such factors as: 1) presence or absence of clinical signs of inflammation 2) probing depths 3) extent and pattern of loss of clinical attachment and bone 4) patient's medical and dental histories; and 5) presence or absence of miscellaneous signs and symptoms, including pain, ulceration and amount of observable plaque and calculus.

There is no doubt that disease recognition has important implications for interventional therapy. The nature of periodontal disease itself is a hurdle but not a limiting factor to much needed research in this area.

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Changing Trends in Periodontal Diagnosis, Disease Recognition & Management
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Chapter 4

The Diagnosis of Periodontal Diseases in the Periodontal Clinic, Dental Hospital, University of Indonesia

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Introduction

The Periodontal Clinic is part of the Dental Hospital, Faculty of Dentistry, University of Indonesia. As a public hospital we provide specialist dental treatment as well as manage referrals from other dental services. It is one of the specialist dental care units in Jakarta that provides public services to a population of over 10 million people.

Periodontal treatment in our clinic is performed by students under the supervision of nine staff from the Department of Periodontology. Undergraduate students in the 7^{th} to 10^{th} semesters of their program are responsible for treatment in the clinic. Treatment consists of basic to complex procedures which have been selected for undergraduate or clinical postgraduate students.

The diagnosis of periodontal lesions is determined by clinical and radiographic examination and classified based on the Classification of American Academy of Periodontology International Workshop 1999. Until the year 2002 we referred to the classification of the AAP 1993 to diagnose periodontal lesions, where early onset periodontitis was termed rapidly progressive periodontitis & localized juvenile periodontitis. However, commencing in 2003 we modified the clinical diagnosis of periodontal lesions to follow the classification of AAP 1999, with some modification based on clinically evidenced conditions.

The average social status of most patients is low to middle class level, predominantly from the low class. Statistically it would appear very difficult to treat periodontal problems in an ideal manner.

Diagnosis of periodontal lesions

To determine the diagnosis of periodontal lesions, clinical examinations and conventional radiographs are utilized. In cases where periodontal problems correlated to systemic diseases or aggressive periodontitis, laboratory tests were performed. The diagnosis and disease recognition are divided into two groups: gingivitis and periodontitis.

We classify periodontal diseases based on a modification of the Classification of the American Academy of Periodontology (AAP) 1999 (Armitage 1999), as follows:

- Gingivitis
  - Puberty gingivitis
  - Gingival overgrowth
  - Necrotizing ulcerative gingivitis

- Chronic periodontitis
  - Localized
  - Generalized

- Aggressive periodontitis
  - Generalized aggressive periodontitis
  - Localized aggressive periodontitis

- Periodontitis as a manifestation of systemic diseases, such as periodontitis associated with Diabetes Mellitus

- Periodontitis associated with endodontic lesions

- Development or acquired deformities and conditions such as occlusal trauma

The periodontal diagnosis should be first determined by the presence of disease; the type, the extent, the distribution, the severity and the pathologic processes (Newman *et al* 2002). With very limited facilities in our clinic, the examination commences with analysis of the case history and identification of clinical signs and symptoms using conventional dental, bitewing and panoramic radiographs. Clinical indications such as, probing pocket depth, tooth mobility, loss of attachment and recession are measured to define the amount of periodontal destruction.

To diagnose periodontal lesions we classify chronic periodontitis as localized and generalized forms, which were formerly known as adult periodontitis or chronic adult periodontitis. It is generally considered to be a slowly progressing disease (Kinane *et al* 2001). We still use the term "generalized form of chronic periodontitis" to refer to slowly progressing periodontitis.

The distribution of periodontal lesions in our clinic is shown in Table 1. The data were taken from 274 patients in the Periodontal Clinic in the year 2002.

It is shown that the most prevalent form is localized chronic adult periodontitis (61.68 %) a result similar to the US National survey showing that the highest prevalent form of periodontitis in the adult population among individuals 30 years and older was chronic periodontitis (Kinane *et al* 2001).

Based on clinical evidence, the progression of chronic periodontitis can be modified by local factors such as overhanging dental restorations, food impaction caused by tooth form and position, environmental factors such as smoking habits and stress. This is supported by Kinane (1999), stating that the localized form of peridontitis was found to be related to local factors, and site involvement was less than 30% (Kinane 1999). The attachment loss may vary from slight (1 to 2 mm) to moderate (3 to 4 mm) or severe ($\geq$ 5 mm). The amount of destruction was consistent with local factors and supra- and sub-gingival calculus was frequently found. With increased age the distribution of the periodontal disease also increased. This finding is supported by Axelsson (2002), in that the prevalence of periodontal disease is strongly related to age and the susceptibility to periodontitis was found to increase with age (Axelsson 2002).

Referring to the previous classification, that early onset periodontitis includes Rapidly Progressive Periodontitis (RPP), in our experience and observation of clinical evidence we have grouped RPP into 2 categories/types.

Type 1: when patients aged 30–35 years visit the clinic with moderate to severe periodontal inflammation, with some tooth mobility or loss of one or two teeth, with no evidence of other local factors such as malposition, traumatic occlusion, food impaction and with conditions unlikely related to systemic disease

Type 2: when patients aged 20–30 years

Age group	CAP		Aggressive Periodontitis		Juvenile Periodontitis	Periodontitis + DM
	Generalized SPP	Localized	RPP I	RPP II		
15-29	2	37	3	2	1	-
30-44	14	71	8	8	-	1
45-64	56	49	3	-	-	3
≥ 64	4	12	-	-	-	-
	76 (27.74%)	169 (61.68%)	14 (5.11%)	10 (3.65%)	1 (0.46%)	4 (1.46%)

Table 1. Periodontal lesions distribution per age group in Periodontal Clinic, Faculty of Dentistry, University of Indonesia (2002)

visit the clinic with severe periodontal inflammation, generalized bone destruction with severe tooth mobility and significant tooth loss.

In both categories the amount of microbial deposits is usually inconsistent with the disease activity. Due to limited facilities and the high expense in doing so we do not identify the bacterial pathogen. However, in our experience prescribing Amoxycilline and Metronidazole gives a very good response before further treatment is needed.

Several clinical signs such as increase in pocket depth and clinical attachment loss, loss of alveolar crest and the presence of bleeding on probing are important for prognosis of the disease.

By assaying levels of alkaline phosphatase in gingival crevicular fluid (GCF ALP), Dewi Nurul (2003) found that RPP type I and II can be differentiated.

The distribution of localized Juvenile Periodontitis in our clinic is 0.36%, a figure nearly equal to the study in Saudi Arabia where the prevalence of LJP was reported to be 0.42% (Albandar & Rams 2002). We found however, that if the LJP affected patient was under the age of 30 the onset of localized aggressive periodontitis usually happens around puberty (Hormand 1979).

We classify LJP as an aggressive type of periodontitis which is different from the aggressive periodontitis type I and type II. Clinical evidence showed that the inflammation in LJP patients is less than seen in RPP or aggressive periodontitis. The color and texture of gingiva look normal, but there is increased severity in attachment and alveolar bone loss. Bone destruction is found more around the incisors and first molars than other areas. Occasionally it becomes unclear as to whether the disease is Aggressive Periodontitis or LJP. More recently, diseases with characteristics of LJP have been renamed as localized aggressive periodontitis (Armitage 1999).

The distribution of periodontitis associated with systemic disease is 1.46%. Periodontitis associated with Diabetes Mellitus (DM) is the most common form found in our clinic. To distinguish between generalized aggressive periodontitis and periodontitis associated with

systemic disease such as DM, blood tests have been performed to analyze blood glucose levels. We found that the synergistic effects of plaque accumulation and host response in DM patients may increase periodontal destruction. According to our study of 30 DM type II patients, there is no significant relationship between blood glucose levels and periodontal disease status.

Other studies related to periodontal diseases in our clinic

A requirement for clinical postgraduate education students, besides certain skill capacities, is to carry out studies relating to clinical therapy. Unfortunately, we have very few students in the Periodontics Program compared to other programs such as orthodontics, oral surgery and prosthodontics (Prayitno 2001).

Examples of studies which have been carried out in 2002:

- The analysis of correlation of the Antigen HLA – class I (A, B, C) with Rapidly Progressive Periodontitis type I. This study found that a genetic immune factor had played an important role in the pathogenesis of rapidly progressive periodontitis. It showed a high frequency of distribution of HLA – A9, A11 and A24 in 10 RPP patients.
- The effectiveness of diluted chlorhexidine 0.2 % 1:1 for Gingivitis patients and evaluation of its discoloration of teeth. It was found that diluted CHX 1:1 was effective on gingivitis and there was no significant difference in tooth discoloration between rinsing regimens on seventh days (Rosemlita 2003).
- The clinical effect of subgingival application of metronidazole gel 25% mixture and providone–iodine 10 % as an adjunct to scaling and root planing in chronic periodontitis patients. This study showed a significant difference in reduction of pocket depth and attachment gain in each group before and after application (Suwandi 2003).
- The effect of irrigation Tetracycline HCl 10 % solution after scaling and root planing in chronic periodontitis patients with pocket depths of 4–6 mm. The results showed that there were significant reductions in probing pocket depth and loss of attachment at test sites compared to control sites (Natalina 2003).

Conclusion

Clinical examination, conventional radiographs and blood tests are standard examinations we performed to determine the diagnosis of periodontal lesions. The most prevalent type periodontal disease in our clinic is Chronic Adult Periodontitis, therefore attention should be paid to prevent the progression of periodontal disease as early as possible which is in line with the government's policies to concentrate on prevention and promotion programs. Based on our clinical evidence we are hesitant to rename LJP as localized aggressive periodontitis. We still subsclassify aggressive periodontitis into type I and type II, where in type I the destruction and progression is less than in type II and is related also to age group.

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Changing Trends in Periodontal Diagnosis, Disease Recognition & Management
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Chapter 5

Current Trends in Periodontal Diagnosis & Disease Recognition - A Perspective from the USA

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Introduction

Arriving at an accurate diagnosis is one of the most important tasks a prudent clinician has to carry out as this is what a treatment plan will be based upon. It is therefore not surprising to see the amount of time a periodontist allots in examining a patient. One usually starts with responsibly gathering and/or updating medical, dental and other pertinent histories of the patient. After this process, several diagnostic techniques are utilized to acquire the necessary data to successfully determine the patient's disease category. Despite the tremendous increase in current knowledge with regards to the etiology and pathogenesis of periodontal diseases, the classification and diagnosis of periodontial diseases are still largely based on traditional clinical assessments (Armitage 1995, Armitage 1996).

A clinical diagnosis of periodontitis is still based on clinical attachment loss and bone loss. This information reflects past periodontal destruction but does not provide any information regarding current disease activity or susceptibility to further periodontal destruction. Most clinicians assign a diagnosis of "periodontitis" to inflamed sites with clinical and radiographic bone loss. This diagnosis is made under the assumption that such sites are at increased risk for disease progression and thus would require active periodontal therapy. Here lies the reason behind the continued quest for developing advanced diagnostic tests.

The parameters that are currently used, such as pocketing and bleeding on probing, have inherent shortcomings in determining risk for further attachment loss, and in the case of probing depths, there is also the question of accuracy. Although Lang (Lang *et al* 1986) reported that sites that bled on probing at several visits had a higher probability (30%) of losing attachment than those that bled at one visit or did not bleed, well-controlled studies failed to demonstrate a significant correlation between bleeding on probing and other clinical signs and subsequent loss of attachment (Badersten *et al* 1985, Haffajee *et al* 1983). The periodontal probe is still the most widely used tool for the assessment of clinical attachment loss. Unfortunately, it carries problems in sensitivity and reproducibility. Probing is very subjective since it can be affected by several factors such as degree of tissue inflammation, probing technique, force, size of the probe, angle of insertion and precision of the probe calibration (Listgarten *et al* 1976).

Over the past decade, several developments have occurred in the diagnosis of periodontal disease, the most noticeable of which is the change in the classification of the disease. This manuscript highlights some important aspects of the revised classification. The reader is referred to the Annals of Periodontology for a more detailed discussion of each category, the revisions and the rationale for the changes (American Academy of Periodontology 1999).

Revised Classification of Periodontal Diseases and Conditions (1999 International Workshop)

The revised classification of periodontal diseases includes seven general types of plaque-induced periodontal diseases namely; gingivitis, chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic diseases, necrotizing periodontal diseases, abscesses of the periodontium and periodontitis associated with endodontic lesions. It has a detailed classification of gingival diseases and lesions that are either plaque-induced or not primarily associated with dental plaque. This section also includes other gingival lesions and disorders that affect the gingiva.

The term "Adult Periodontitis" was replaced with "Chronic Periodontitis" mainly in order to eliminate the restrictive use of age of onset as a major factor in determining diagnosis. In the new classification, the nonspecific term "Chronic Periodontitis" is characterized by the fact that it is most prevalent in adults, but can also occur in children (Papapanou 1996). It is said to progress in a slow to moderate rate (Loe *et al* 1986, Papapanou *et al* 1989), but may have periods of rapid progressions (Socransky *et al* 1984, Jeffcoat and Reddy 1991). It is consistent with a variable microbial pattern and can be associated with local predisposing factors as well.

"Early-onset periodontitis" was discarded since it is assumed that one has temporal knowledge of when the disease started. In addition, the age by which an "adult" is defined in the old classification is considered arbitrarily chosen. It is therefore recommended that a diagnosis of "Aggressive Periodontitis" should be based on clinical, radiographic, historical and laboratory findings. It is characterized by the following: rapid attachment loss and bone destruction, generally clinically healthy individuals, familial aggregation and the amounts of microbial deposits are inconsistent with the severity of periodontal destruction (American Academy of Periodontology 1999). Although the microbial etiology of the early onset syndromes has been primarily associated with *A. actinomycetemcomitans* (Slots *et al* 1980), recent studies have indicated that differences exist among various ethnicities (Lee *et al* 2003, Dogan *et al* 2003, Takeuchi *et al* 2003).

Advances in Traditional Diagnostic Methods

The increased understanding in the etiology and pathogenesis of periodontal diseases underscores the importance of advancing diagnostic techniques. Since the most common means of determining clinical attachment loss is through probing, attempts have been made to improve its accuracy and reproducibility. One of which is the development of the computer linked, controlled-force electronic periodontal probes. Although electronic probes offer the advantage of controlled insertion forces, automatic recording of data into a computer (Greenstein 1997, Armitage 1996) and better resolution (Jeffcoat and Reddy 1991), there are still several drawbacks associated with it. Electronic probing requires more time to use, is more costly, can be uncomfortable and may

potentially underestimate deep probing depths (Perry *et al* 1994). It has also been reported that in order to improve reproducibility of clinical measurements, a double pass method (measuring each site twice) should be utilized (Osborn *et al* 1990).

Significant advances have also been made in radiographic imaging to aid in periodontal diagnosis. Conventional radiographs have been shown to routinely underestimate the amount of bone loss (Greenstein, 1997) It also requires 30-50% bone mineral resorption before changes can be detected by routine radiographic examination (Jeffcoat and Reddy 1991). The introduction of subtraction radiography to the dental field, allowed detection of changes in bone density as low as 5%. Both hardware and software have been improving and will continue to do so in attempts to correct some of the problems initially associated with subtraction radiography such as; subtle differences in contrast, projection geometry and other repeatability errors (Hausmann *et al* 1994). The use of digital radiography in periodontal diagnosis has tremendous potential especially since studies have demonstrated an 80% agreement between probing and radiographic methods in identifying sites that have lost attachment (Jeffcoat 1992, Hausmann *et al* 1994).

Supplemental Diagnostic Tests

Supplemental diagnostic tests have been developed and investigated since there is an enormous potential in using the results to successfully identify therapeutic targets, monitor the response to therapy, identify sites at high risk for progression and to assist in determining a patient specific recall. As of to date, these tests can be used to detect the presence of: substances associated with putative pathogens, host derived enzymes, tissue breakdown products and inflammatory mediators. There are numerous examples of supplemental diagnostic tests, however, this paper will only mention some of the more commonly used clinical tests.

The most common means of identifying the microbial composition of plaque samples is through bacterial culturing. The main advantage of this method is that one can obtain relative and absolute counts of the cultured species. It is also the only *in vitro* method able to assess for antibiotic susceptibility of the microbes. The microbial composition of subgingival plaque has always been a point of interest in the diagnosis and treatment planning for periodontal disease. It has been demonstrated that progressing periodontitis is associated with certain periodontopathogenic bacteria (Machtei *et al* 1997). However, a clinician should not rely on the microbial testing alone to determine diagnosis and individualized treatment, since it has also been determined that the presence or absence of specific bacteria cannot discriminate subjects from different disease categories (Mombelli *et al* 2002). In addition, another study concluded that the presence of putative pathogens can only predict future attachment loss in only 20% of the sites. Their absence was considered a better predictor of no further attachment loss than their presence was of disease progression (Wennstrom *et al* 1987).

Another means by which bacterial identification can be accomplished is through an enzymatic test such as the BANASCAN. This enzymatic assay provides a rapid chair side test (15 minutes) to detect *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythensis* (formerly *Bacteroides forsythus).*

Other supplemental diagnostic tests are designed to provide information regarding the ongoing inflammatory process. One example is the Periocheck, which tests for collagenase as a marker of inflammation. Compared with healthy sites, locations with gingivitis or

periodontitis had higher levels of collagenase (Larivee *et al* 1986). However, there are no data showing a relationship between the level of collagenase and progressive periodontitis. In addition, this test cannot differentiate between gingivitis and periodontitis.

One of the most comprehensively studied host derived enzyme associated with periodontal disease is aspartate amino transferase (AST), which is released by dead or dying cells. It has been widely utilized to detect heart damage after a myocardial infarction as well liver damage during hepatitis. Gingival crevicular fluid samples taken from sites with severe gingival inflammation demonstrated a marked increase in AST levels (Chambers *et al* 1991). It is however, unable to discriminate between sites with severe inflammation but with no attachment loss from sites that are losing attachment. It remains to be seen whether this test offers some advantage over existing clinical measures of disease (Magnusson *et al* 1996, Persson *et al* 1995).

It has been well documented that susceptibility to periodontal diseases is highly variable and depends on host responses to pathogens (Tonetti 1994, Ishikawa *et al* 1997, Offenbacher 1996). Thus, attempts have been made to develop a reliable test for host-based susceptibility. The only test for host susceptibility that is available to practitioners is a genetic test for polymorphisms in the interleukin gene cluster (PST) (Kornman *et al* 1997). Approximately 30% of Caucasians are positive for a composite genotype of IL-1A and IL-1B polymorphisms. People who test positive for this composite genotype are said to be at increased risk of the following: bleeding on probing (Lang *et al* 2000), severe chronic periodontitis (Kornman *et al* 1997), tooth loss (McGuire and Nunn 1999), and attachment loss after therapy (De Sanctis and Zucchelli 2000) or increased secretion of IL-1β (Pociot *et al* 1992). However, other studies have shown conflicting results (Cattabriga *et al* 2001, Papapanou *et al* 2001, Ehmke *et al* 1999, Mark *et al* 2000). The prevalence of this genotype also varies among different populations (Armitage *et al* 2000), thus may be of little value in determining the risk for susceptibility to periodontitis.

Conclusion

Despite the astounding improvements in both traditional and supplemental diagnostic techniques, the majority of clinicians still rely heavily on basic clinical and radiographic assessments gathered from using a periodontal probe and conventional radiographs. Recent developments in diagnostic techniques, without a doubt, have significant potential and scientific merit. However, several of these advancements still require definition of their clinical utility and cost-effectiveness in order to promote a more widespread use. Addressing these issues and continuing the quest for diagnostic techniques that will not only provide clinicians with current periodontal disease status but also future risk of periodontal breakdown that are both patient and site-specific, will have a tremendous impact on the way we diagnose periodontal diseases and ultimately, how we develop our treatment plan.

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Chapter 6

Periodontal Disease Risk Management: Smoking, The Patient Controlled Modifiable Risk

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Introduction

In many ways we define periodontal diseases as being the body's response to plaque, yet work in the last two decades has emphasised that while dental plaque is necessary for the disease process to begin, it is not by itself sufficient to cause disease in all subjects. Periodontal diseases require a combination of pathogenic plaque at an appropriate environmental location (e.g. the dento-gingival junction) in a susceptible host. It could be said that most oral diseases have these requirements, however the clinical entity of disease only occurs in the rare situation that all these factors occur together at the same time. An understanding of these fundamentals have allowed Page and Kornman (Page & Kornman 1997) to describe the currently accepted paradigm of periodontal pathogenesis which combines the knowledge that the interactions between the host, the environment and the aetiology are not just in one direction, but are at least two way, if not more complex exchanges.

Diagnosis and management of periodontal disease

The complexities of the pathogenesis of the periodontal diseases has helped make sense of the ironic diagnostic feature that while our traditional diagnostic tools (probing, radiographs etc.) are good at measuring the extent of disease that has occurred in the past they provide little, if any, information about the current level of disease activity (i.e. actual loss of connective tissue attachment) or predicting future disease progression (Hirschfield & Wasserman 1978). The disease process (i.e. activity) is a rare event even in susceptible individuals, and as with the treatment of many chronic diseases, there has been a move towards managing the risk of disease progress as well as dealing with the presenting acute exacerbations. Lang and Tonetti (Lang & Tonetti 1996, Lang & Tonetti 2003) have settled on 6 objective parameters of risk that have been validated for use in determining the level of SPT required for periodontal patients. The resulting *spider web* diagram currently encompasses the percentage of sites with bleeding on probing (BOP), the absolute number of sites with probing depths of 5mm, the number of teeth lost, the ratio of percentage bone loss to age, the presence of systemic/genetic factors (eg diabetes) and the level of smoking. It is quite possible that with further knowledge, more factors will come to be added to this mix, however the current evidence supports only these factors at present.

Clinicians are well aware that that

successful management of periodontal disease relies not only on their own skill as clinicians but also on the ability of the patient to participate in appropriate home care measures or failing this, frequent professional care. There is only one parameter of risk identified by Lang and Tonetti (Lang & Tonetti 2003) which is modifiable by the patient. While the measurement of percentage of sites with BOP relies on effective oral hygiene to allow the assessment to be made *in the absence of marginal gingivitis,* the actual BOP score relates to the effectiveness of the subgingival debridement. Thus it is only smoking that can be truly altered by the patient to make a positive outcome towards management of their disease progression risk.

Effects of smoking on periodontal disease

The actual mechanisms of the deleterious effects of smoking on periodontal disease are yet to be fully elucidated however many aspects are understood. Smoking can be thought to act on the host via effects on the vasculature, cells and the repair potential and possibly also on the flora (although this effect may be opportunistic due to host changes). The principal vascular effect is peripheral vasoconstriction (Clark *et al* 1981, Danielsen *et al* 1990). Smoking also has deleterious effects on fibroblasts (Raulin *et al* 1988) and in particular PMNs (Bridges *et al* 1977, Kenney *et al* 1977, Kinane & Chestnutt 2000), to say little of the detrimental inflammatory effect on immunoglobulin and cytokine production. (For reviews see Johnston 1999, Kinane & Chestnutt 2000.)

There have been many studies linking smoking to increased prevalence of periodontal disease. Early studies were hampered by poor design and control of other factors such as plaque control however more recent work has clearly shown a consistent association with smoking leading to a 200-600% increased risk of having periodontitis depending on the study population (Kinane & Chestnutt 2000). Further close analysis suggests smokers have greater attachment loss, furcation involvements, and deep pockets and more calculus than non-smokers. There is also some evidence to suggest a dose response effect such that smoking more cigarettes per day and for more years results in greater periodontal destruction. Young adults who smoke seem to be particularly at risk of showing periodontal disease early in life.

Smokers versus non-smokers

Periodontal treatment is not as effective in smokers as non-smokers. This is not to say that treatment is completely ineffective but rather than it is not as effective (Ah *et al* 1994, Machtei *et al* 1998, Preber *et al* 1995). This is true of both surgical and non-surgical approaches. Of considerable concern however is that of patients considered refractory to usual treatment, approximately 90% are reported to be smokers (MacFarlane *et al* 1992). In summary, smokers have more periodontal disease which is of greater severity than non-smokers and do not respond as well to treatment. There is some indication that the level of smoking is influential on the level of risk involved. It should be noted that smoking in and of itself does not cause periodontitis but it does have significant detrimental effects in patients who are otherwise susceptible to developing periodontitis.

Cessation of smoking

The best news for patients is that smoking cessation appears to be quite beneficial (Haber 1994), and because of the cumulative nature of periodontal disease destruction ultimately ex-smokers will present with similar disease prevalence to never smokers (Kraal *et al*

1997). Smoking cessation is never easy however members of the dental team are well suited to assisting patients in quitting their smoking habits. It is well known that the longer the face to face interaction with a patient the greater the impact on cessation. Members of the dental team often have more time and more follow-up visits (particularly in periodontal patients) than in medical settings to discuss smoking cessation. The 4-A's of ask, advise, assist and arrange are often advised for use in the health care setting and are based largely on observational studies suggesting these methods are effective in helping patients to stop smoking. (To date it appears that only pharmacologic agents have been fully evaluated in controlled trials). There have been few published reports of the use of this method in the dental setting although anecdotal evidence suggests that it is helpful. Our own group is evaluating a modified version of this method of helping patients in a dental setting, however we have yet to evaluate the results.

Smoking education and prevention campaigns

The problem of smoking in the Asia-Pacific region is very large with smoking rates ranging from almost 40% (in places like Bangladesh, Cambodia and China) to about 15% (eg. Singapore). In many countries of the region smoking is a largely male activity (e.g. in China, Korea, Tonga more than 60% of adult males smoke) although there is significant country variation. Some significant increases in female smoking rates are also seen in some cases (e.g. Nauru, New Zealand, Australia and Japan) (http://nationmaster.com 2004). Of the World Health Organisation (WHO) health regions in the world, the Western Pacific has the most rapid uptake of smoking, with one in three of all the cigarettes smoked in the world being smoked in China and at the current rate one in five tobacco related deaths in the world occurs in the WHO Western Pacific region. In the last 2 decades the Western Pacific and Southeast Asian Regions have experienced increases in tobacco consumption, and of particular concern is the increased rate of uptake amongst children and young adults (David 2000). Individual country summaries are available online from the WHO as are the results of the Global Youth Tobacco Survey (http://tfi.wpro.who.int/gyts.asp 2004) which highlight the disturbing trend of smoking uptake in adolescents, offers of free cigarettes from tobacco companies and the large proportion of youth smokers who are trying to stop smoking.

While health education campaigns are not without their worth, the evidence to date would suggest that on a population basis, effective methods of reducing smoking prevalence are to provide no government support for the tobacco industry and to enforce comprehensive tobacco control laws (e.g. preventing sale to children and banning advertising and sponsorship). However the most effective smoking prevention programme appears to be raising the cost of cigarettes via taxes (David 2000). To date, both in Australia and Malaysia, tobacco company sponsored youth prevention programs have been instituted however these appear to be largely public relations exercises to ingratiate the companies to governments and the public to limit or reduce taxes and advertising restrictions.

The WHO holds "World No Tobacco Day" each year on May 31, in an effort to focus attention on tobacco issues in health and in particular the Tobacco Free Initiative (http://www.who.int/tobacco/en/ 2004). Each year focuses on a theme such as second-hand, passive smoking or the 2003 theme of tobacco free film and fashion (http://www.who.int/tobacco/areas/communications/events/wntd/en 2004). Much of the understanding of the

link between tobacco use and product placement in Hollywood produced movies has been compiled by Stanton Glantz at the University of California (Glantz 2004). Recent findings have indicated that in adolescents of non-smoking parents, 52.2% of smoking initiation could be attributed to exposure to smoking in movies (Dalton *et al* 2003). The 2004 theme relates to poverty and tobacco use, highlighting the high costs of tobacco products particularly for those of little means, which is particularly applicable in many parts of the Asia Pacific region.

Conclusion

Clinicians have a number of roles to play. Firstly there is need to act as non-smoking role models within communities and act as true healthcare professionals. We need to acknowledge that smoking is an additive health issue and not just a social choice. It is the responsibility of oral healthcare workers to ask patients about their smoking, discuss the detrimental effects it has on their oral health and to encourage them to quit their habit. There is a need to have appropriate resources or referral lines for patients who express an interest in smoking cessation and finally as individuals and as part of professional groups we should reinforce the need for effective tobacco control with governments.

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Chapter 7

The Taming of the Host - Host Modulation in Periodontitis

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Introduction

One of the significant periodontal findings in the last century was the discovery that plaque is the cause of periodontal disease. The realization that microbial plaque organized into biofilms clarified why antibiotics alone have limited long-term effect in the treatment of periodontitis. Biofilms are defined as "matrix enclosed bacterial populations, adherent to each other and/or to surfaces" (Costerton *et al* 1994). Biofilms are communities of bacteria that evolved to permit survival of the community as a whole. Because of its numerous characteristics such as differing pH, oxygen tension, primitive circulatory system and the presence of matrices, bacteria in the biofilm may have up to 1000 times decreased susceptibility to antibiotics compared to planktonic grown bacteria. Because of this unique nature of biofilms, mechanical plaque control remains the main mode of treatment of periodontal disease.

Lately the focus was changed when investigators began to document the host's contribution to disease pathogenesis. With the realization that plaque is necessary, but alone is not sufficient in the progression of periodontal disease, more emphasis was put on the role of host mediators in the pathobiology of the disease. With this current realization of a link between host response and periodontal disease pathogenesis, it is intuitive that pharmaceutical inhibition of host response pathways may be an adjunctive strategy for treating periodontal disease (Paquette and Williams 2000).

In essence, high levels of interleukin-1 (IL-1), matrix metalloproteinase (MMPs), prostaglandins (PGE) and all other pro-inflammatory mediators are found in disease. (Figure 1) (Page 1998). In health, tissue inhibitor of MMPs, interleukin antagonists and interleukin-10 are the predominating mediators (Page 1998). The concept of mediating the host is focused on trying to decrease the high level of mediators that are found in periodontitis and also to increase or promote the levels of the mediators that are normally found in health.

Matrix Metalloproteinases (MMPs)

Because the clinical manifestation of periodontal disease involves attachment and bone loss, which histologically involves connective tissue destruction, the blocking of connective tissue mechanisms is a viable strategy for management of periodontal disease. Collagenases, elastase and acid proteases are the enzymes that digest and

Disease		Health
High	1L-1β, TNG–α, INF-γ, PGE-$_2$, MMPs	Low
Low	IL-4, IL-10, TGF-β, IL-ra, TIMPs	High

Table 1. Essence of Pathogenesis of Periodontitis (Page RC 1998)

destroy the matrix of the gingival tissue and periodontal ligaments. There appear to be multiple opportunities to block MMPs, including blocking production of MMPs, blocking production of pro-enzyme and activating inhibitors. Some of the inhibitors involve action in removing Zn++ and Ca++, which are essential to the active sites of MMPs.

The initial demonstration that tetracycline antibiotics can inhibit host-derived matrix MMPs, by a mechanism independent of the antimicrobial properties of the drugs, was performed by Golub and co-workers (Golub *et al* 1983). In 1987, Golub *et al* described the new chemically modified tetracyclines (CMTs) which are devoid of antibacterial activity but retain their anticollagenase property. Because chemically modified tetrcyclines are not yet approved for human use, the majority of the human clinical trials have involved the use of 20 mg or 50mg doxycycline (Periostat). In multiple clinical studies conducted using sub-antimicrobial dose doxycycline (SDD), there has not been a difference noted in the resistance level or composition of the oral flora (Walker *et al* 2000, Thomas *et al* 2000).

Several series of double-blind, placebo controlled clinical trials of three, six and nine months' duration on SDD have shown some degree of efficacy for these medications (statistically) based on prevention of attachment loss, pocket depth reduction as well as inhibition of collagenase activity (Golub *et al* 1994, Crout *et al* 1996, Golub *et al* 1997, Caton *et al* 1999, Caton *et al* 2000).

With regards to MMP inhibition, Golub and colleagues (Golub *et al* 1990) demonstrated that a two-week regimen of SDD reduced collagenase level in the gingival crevicular fluid (GCF). However subsequent studies using SDD therapy adjunctive to routine scaling and root planing indicated that after cessation of SDD a rebound of collagenase activity was noted, suggesting that continuous drug therapy over a period of several months appears to be necessary for maintaining collagenase levels to near normal (Golub *et al* 2001, Ashley *et al* 1999).

One of the clinical studies of Periostat that was pivotal for the attainment of U.S. Food and Drug Seal of Approval was by Caton and co-workers in 2000. This study involved a total of 190 subjects with a treatment period of nine months. This study found statistically significant differences in the mean per-patient average attachment level with Periostat plus SRP compared with placebo plus SRP, with the treatment group having 0.38 mm better attachment gain. The clinical significance of 0.38 mm attachment gain after 9 months in the SDD group vs. placebo can be questioned (1.55 mm SDD vs. 1.17 mm placebo). The mean decrease in pocket depths (1.68 for SDD vs 1.20 mm for placebo) can be also be achieved by SRP alone in numerous studies. These studies, together with all other clinical

trials, should be interpreted with respect to their clinical relevance. Clinicians should evaluate the results cautiously with practicality and long-term benefit in mind. The suggestion that SDD should be routinely used as an adjunct to scaling and root planing in the treatment of chronic periodontitis should also be questioned with the knowledge that the majority of periodontal disease responds only to routine mechanical debridement.

Bisphosphonates

Bisohosphonates are non-biodegradable analogs of pyrophosphate that have a high affinity for calcium phosphate crystals. It has been observed that when osteoclasts phagocytize bone crystals containing bisphosphonates, osteoclastic activity is inhibited. Alendronate and tiludronate are some of its various forms. Biphosphonates are powerful inhibitors of bone resorption. Although their mechanism of action has not yet been fully elucidated, it is thought that they inhibit osteoclasts from resorbing bone by interfering with their metabolic activity (ion transport) (Sato *et al* 1991). Various forms such as Alendronate and tiludronate are being used clinically to treat metabolic bone diseases in humans such as Paget's disease, hypercalcemia and osteoporosis (O'Doherty *et al* 1992, Thiebaud *et al* 1988, Thiebaud *et al* 1990).

Numerous studies have demonstrated some benefit in the management of periodontal disease exposure to bisphosphonates. Non-human primate models of periodontitis have been widely used to examine the effects of this potential therapeutic agent. Weinreb *et al* (1994) studied the histomorphometrical effects of Alendronate on bone loss caused by experimental ligature–induced periodontitis in an animal model. Bisphosphonates were administered via intravenous infusion of saline solution (control) or alendonate (0.05 mg/kg and 0.25 mg/kg IV every 2 weeks for 16 weeks). Result showed that alendronate at 0.05 mg/kg significantly reduced bone loss associated with experimental periodontitis. In contrast, the higher dose of alendronate (0.25 mg/kg) was almost ineffective in blocking periodontal bone loss. The same biphasic response was observed in the 1992 Brunsvold *et al* study. The authors pointed out that the reason for this was unclear.

A combination study has been described by Llavaneras and co workers (2001) where a combined chemically modified doxycycline (CMT-8 given orally) and bisphosphonate (clodronate given subcutaneously) were used. The result showed no significant reduction in alveolar bone loss observed in CMT-8 and clodronate alone, but combination of sub-optimal CMT-8 and clodronate "normalised" elevated MMPs and reduced bone loss.

The unusual dose response pattern observed in numerous studies, where the lower dose produced better inhibition of bone loss than the higher dose, should point out that attempts to intervene in complex regulatory mechanisms should proceed with caution.

Although bisphosphonates are being used clinically to treat metabolic diseases such as Paget's disease, hypercalcemia and osteoporosis; the high dose used in the animal studies to control periodontitis is equivalent to 20-fold the dose proposed for the treatment of human osteoporosis. Therefore caution should be taken in translating the animal results to the clinical setting.

Prostaglandins

The synthesis and release of prostaglandins is one of the first host immunoinflammatory pathways implicated in periodontal disease. Prostaglandin is a potent mediator of bone resorption. The relationship of increased levels

of PGE_2 to periodontal disease is well-established. PGE levels are associated with disease severity at individual sites and at patient level and the highest level of prostaglandin tends to be in actively progressing sites (Williams *et al* 1990, Seymour *et al* 1988, Offenbacher *et al* 1986, Offenbacher *et al* 1992).

PGE_2 is produced primarily in the tissues via cyclooxygenase enzymes. Cyclooxygenase 1 (COX-1) is produced constituvely and is important for tissue homeostasis. In other words, COX-1 maintains "housekeeping" functions such as gastric cytoprotection and vascular and renal homeostasis. Cyclooxygenase 2 (COX-2) however, appears to be activated in inflammation and contributes to the increase in PGE_2 in periodontitis. In periodontal disease, monocytes and fibroblasts produce PGE_2 in response to activation by interleukin 1a (IL-1a), tumor necrosis factor a (TNF-a) and lipopolysaccharides (LPS).

In 1971, Vane and co-workers made a landmark discovery that NSAIDs blocked cyclooxygenase as their mechanism of action, thus inhibiting prostaglandin production. NSAIDs though, inhibit both COX-1 and COX-2 pathways, thereby also inhibiting the "housekeeping" functions of COX-1, causing renal and gastric side effects.

Numerous animal studies from the mid-70s to the present have shown some benefit in using NSAIDs in reducing bone resorption and inflammation. NSAIDs such as indomethacin, flubiprofen and naproxen were used, administered either orally or topically (Nyman *et al* 1979, Williams *et al* 1984, Williams *et al* 1988, Howell *et al* 1991). Human clinical studies also showed some degree of benefit from using NSAIDs in the prevention of bone loss in periodontitis (Feldman *et al* 1983, Williams *et al*, 1988, Jeffcoat *et al* 1991). Because of the numerous side-effects associated with NSAIDs they cannot be used long-term, particularly for the specific control of periodontitis.

Although there is plenty of evidence from pre-clinical and clinical studies indicating inhibition of periodontal disease progression, the difference with placebo or antibiotic counterparts are rarely clinically dramatic (-/+ 1-2 mm in attachment gain). We do not know much about dosages of NSAIDs to be employed in human studies. It is not clear if we can rely on NSAIDs dosages used in the treatment of pain associated with rheumatoid arthritis to slow alveolar bone loss in periodontitis. Would increasing the dosage to attenuate the anti-inflammatory properties outweigh the potentially harmful side-effects (renal toxicity, gastric ulceration, internal bleeding etc)? Well-controlled longitudinal studies in humans on the effect of NSAIDs on periodontal disease progression, with tooth retention as the indication of success, are needed.

COX-2 Inhibitors

As mentioned above, NSAIDs block both COX-1 and COX-2, therefore inhibiting the beneficial "housekeeping" functions by COX-1. An exclusive COX-2 inhibitor was developed to prevent this from happening. Numerous studies published to date have focused on the efficacy of COX-2 inhibitors for treating acute pain, osteoarthritis and rheumatoid arthritis, with lower incidence of gastrointestinal adverse events than patients taking NSAIDs. COX-2 inhibitors (meloxicam, celecoxib, etc) have potencies for COX-2 that are 10-1000 fold higher than COX-1 in inhibiting enzymes associated with inflammation rather than those associated with homeostasis.

Bezerra *et al* (2000) studied Indomethacin (NSAID) and Meloxicam (COX-2 inhibitor)

Procedures	Pocket Depth Reduction (mm)	Attachment Gain (mm)
Scaling & Root Planing (SRP)	1.2 - 2.2 mm	0.55 - 1.33 mm
Open Flap Debridement	2 - 3 mm	1 - 1.5 mm
Tetracycline Fibre/SRP	1 - 2 mm	1.1 - 1.2 mm
Chlorhexidine Chip/SRP	0.95 mm	0.65 mm
Periostat/SRP	1 - 1.5 mm	0.9 - 1.22 mm
NSAID/SRP	1.5 - 2 mm	0.82 - 1.6 mm

Table 2. Comparison of different treatment modalities

in experimental periodontitis in rats. Their results showed that NSAIDs inhibited bone resorption and inflammation in a dose-dependent manner (0.5, 1 and 2 mg/kg), in a similar degree as COX-2 inhibitors, but with associated gastric and hemorrhagic lesions in rats. Hence, selective COX-2 inhibitors may exhibit a better clinical risk/benefit ratio than classical NSAIDs.

Interleukin-1 (IL-1)

IL-1 has been strongly associated with the pathogenesis of periodontitis (Williams 1990, Genco 1992, Gemmel *et al* 1997). Elevated levels in gingival crevicular fluid are associated with more severe periodontal disease. By binding specifically to their complimentary receptors, IL-1 with tumor necrosis factor trigger signaling events leading to tissue destruction. IL-1 also directly activates osteoclasts resulting in bone resorption. To maintain balance in health, IL-1 has endogenous inhibitors. The IL-1 inhibitor is structurally similar to IL-1 however it triggers no response when it binds to the IL-1 receptor (Dinarello 1996). IL-1 is potently induced by gram-negative bacteria through LPS production. It is mainly secreted by macrophages but can also be released from blood platelets, fibroblasts and endothelial cells. IL-1 directly stimulates bone resorption, induces PGE2 and MMPs release from fibroblasts and monocytes thereby perpetuating further destruction (Dinarello 1996, Pfizenmaier *et al* 1996).

A few studies have looked at the potential for pharmacotherapeutics in inhibiting IL-1 by the use of its inhibitor (IL-1 receptor antagonist). Graves and co-workers (1998) demonstrated that the conversion from gingivitis to periodontitis in experimental periodontitis cases is characterized by the progressive movement of the inflammatory front toward alveolar bone and that application of IL-1 and TNF blockers inhibited this progression. Delima and co workers (2001) used experimental periodontitis in a monkey model treated with the IL-1 receptor antagonists. The experimental group showed reduction in connective tissue destruction by 51% and reduction in bone loss by 91%. The potential of this IL-1 receptor antagonist in treating human periodontitis should be investigated. Although early studies on risk/benefit of injecting IL-1 and TNF antagonist in animal model already showing some side-effects such as relative deficiency of macrophages in immune defense (Yang *et al* 1994).

What are the other fields of medicine doing?

The concept of host mediated destruction is also a topic of interest in other fields, particularly chronic inflammatory conditions such as rheumatoid arthritis (RA). RA and periodontitis have a very similar pathobiology (Mercado *et al* 1998, 2003). With the realization that an imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines exists also in the pathogenesis of RA, emerging therapies are focusing on the inhibition of pro-inflammatory cytokines and destructive proteases. Littman *et al* 1994 demonstrated that Tenidap (Cox-2 inhibitor) reduces bone resorption and cartilage degradation in RA patients. Combination therapy of CMT (MMP-inhibitor) and Flubiprofen synergistically inhibited severe bone destruction in arthritic rats (Greenwald *et al* 1992). Synovial Injection of IL-1 ra versus placebo in a multi-center double-blind study in 175 RA patients; result showed significant improvement in test patients in terms of bone erosion, pain and clinical symptoms of RA (Campion *et al* 1996).

Conclusion

Looking at Table 2, where various treatment modalities are compared, it appears that the conventional mechanical debridement is still not far off, or even sometimes better, when compared to other treatment modalities such as chemotherapy or even host modulating agents. In general at this stage, clinicians should still try to eliminate the bacterial challenge using conventional therapy before administering systemic drugs whose objective is to alter the host response. It is important to keep in mind that drug administration is still not a substitute for professional debridement and proper personal hygiene. It can still be considered that repeated debridement might achieve a larger improvement in clinical parameters than one episode of root planing plus SDD, which needs to be used for several months.

Significant advances in the study of host modulating agents in the treatment of periodontal and other chronic inflammatory diseases have been made in the last century. However, we still have gaps in our knowledge. Further studies on the genetic make-up and detailed studies on the pathophysiology of all the cells involved in the development of the disease are needed. As these drugs are developed, the safety of the people who are going to use them, their affordability and ease of use and of course the relative improvements that will happen after using these medications should be considered before administering them. These host modulating drugs, although not indicated for majority of the periodontitis patients, may someday make a difference in those patients who are highly susceptible to aggressive forms of the disease.

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Chapter 8

Treatment Strategies in Periodontal Disease Management in Korea

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Introduction

The management strategy for periodontal disease in any community depends on the disease epidemiology and social environment for health care. Therefore, this paper will focus on the demand for, and the provision of, periodontal care, together with the current status and trend of periodontal disease management in Korea.

Demand for periodontal management

Epidemiology of periodontal disease

In Korea, recent epidemiological studies on periodontal disease were done in 1995 and 2000, based on clinical examinations and the Community Periodontal Index of Treatment Need (CPITN) system.

As shown in Table 1, periodontitis increasingly appears in the adult population over the age of 30. A prevalence of periodontitis of 31 to 35% was reported in the adult population. The prevalence of periodontal disease, including gingivitis in the adult population, is higher than 80%.

Demand for periodontal care

The need for periodontal care is determined by the prevalence and severity of disease in any population. Therefore, analysis of epidemiology data for the adult populations will be required to evaluate the quantity of the demand for periodontal health care services.

As is apparent in Table 2, Korean society is aging. The percentage of the young population is shrinking slowly and the percentage of adults over 30 years old continues to increase from 43.8% in 1990 to 53.0% in 2000. It is expected to be 61% in 2010, and 66% in 2030. This indicates the potential for an increase in the number of people suffering from periodontal disease. This is because periodontitis usually appears after the age of 30 years.

The numbers of people with periodontal disease is roughly estimated at 1/3 of the population over the age of 30 (Annals by the Ministry of Health and Welfare in Korea 1995, MOHW Health Resource Division 2002, KIOHS 2000). Therefore the number of people with periodontal disease in 1990 and 1995 was approximately 6.2×10^6 and 7.3×10^6 respectively, and increased to 8.8×10^6 in 2002. The rise in the proportion of elderly people over the age of 65, from 5.13 to 7.13%, during this period also increased the need for complex periodontal care, as periodontal disease increased in both severity and prevalence with age (Table 3). An epidemiological study (Moon et al 1995) examined the prevalence of

Author	Method	Age of population	Prevalence Gingivitis	Periodontitis	Evaluation
Korean Dental Association (1989)	Randomized population	3-65 30-65	29.7	31.0	CPITN
Moon et al (1995)	Randomized population	total 30~	32.8 55.7	15.5 32.3	CPITN
KIOHS (2000)	Randomized population	total 30~	32.4 59.6	11.8 33.8	CPITN

Table 1. Prevalence of periodontal diseases in Korea

	1975	1980	1985	1990	1993	1995	1997	2000	2002	2005	2010
Total ($x10^3$)	35,280	38,124	40,806	42,869	44,194	45,093	45,991	47,275	48,062	48,785	49,123
0-9 y	25.56	22.27	19.14	16.44	15.00	14.88	14.56	14.99	14.72	14.08	12.96
10-19 y	25.31	23.56	21.81	19.52	18.50	17.12	16.25	14.70	13.86	6.37	6.89
20-24 y	8.71	10.74	10.47	10.13	10.25	9.74	8.91	8.17	8.25	7.67	6.12
25-29 y	7.19	8.06	10.04	10.09	9.51	9.54	9.67	9.19	8.43	7.77	7.36
30-34 y	12.07	6.62	7.57	9.64	9.90	9.53	9.09	9.00	9.17	8.76	7.47
35-44 y	11.42	11.69	11.80	12.88	14.45	15.71	16.81	17.52	17.43	17.12	16.65
45-54 y	7.37	8.09	9.32	9.77	9.89	10.00	10.15	11.20	12.20	13.93	15.86
55-64 y	4.62	5.16	5.56	6.41	7.13	7.57	7.98	8.11	8.22	8.52	9.78
65+ y	3.45	3.83	4.28	5.12	5.51	5.89	6.37	7.13	7.74	8.65	9.94
73 y ($x10^3$)	11,721	13,488	15,720	18,786	20,728	21,966	23,154	25,037	26,312	27,991	30,226

Table 2. Population trend and proportion ratio by age group in Korea

periodontal disease in each age group of the adult population. The proportion of periodontally healthy people was 27 % of the 25 to 29 year old group and decreased to less than 1% of the 55 to 64 year group. The proportion of people suffering from severe forms of the disease was 1.7 % of the 30 to 34 year old age group and increased to more than 13 % of the 55 to 64 year old age group.

	Age	0 Healthy	1 Bleeding	2 Calculus	3 Shallow Pocket	4 Deep Pocket
1995	25-29	27.3	2.4	67.2	3.3	0.1
	30-34	19.3	3.5	64.8	10.6	1.7
	35-44	17.3	3.0	60.1	15.2	4.4
	45-54	9.1	2.9	50.8	31.0	5.4
	55-64	0.7	1.3	38	41.3	13.3
	65 over	2.5	0.3	23.2	40.3	12.7
	>30 yr	**12.06**	**2.58**	**53.10**	**25.55**	**6.70**
	Total popln.	**14.63**	**2.55**	**55.45**	**21.78**	**5.59**
2000	25-29	16.83	11.22	66.43	5.06	0.46
	30-34	11.12	10.59	64.20	12.90	1.18
	35-44	9.67	8.78	58.83	18.48	4.25
	45-54	5.46	5.66	55.03	27.57	6.29
	65-74	3.77	7.24	42.60	37.59	8.80
	75 over	4.97	10.01	40.82	36.69	7.52
	>30 yr	**6.54(2.22)**	**7.64(1.11)**	**52.00(1.89)**	**27.34(0.67)**	**6.49(0.10)**
	>65 yr	**4.16(2.58)**	**8.14(0.77)**	**42.02(1.69)**	**37.30(0.84)**	**8.38(0.12)**
	Total popln.	**14/56(3.13)**	**11.65(1.10)**	**50.97(1.34)**	**18.48(0.36)**	**4.32(0.06)**

(1995 report cited from Moon HS, J Korean Dent Assoc 6:351, 2000 report cited from KIOHS report)

Table 3. Periodontal conditions by age group as a percentage of subjects by highest CPI score and mean number of sextants affected (in parenteses) in 2 studies in 1995 and 2000

Age group (years)	Prop. in total popln.(%)	Complex treat in each group (%)	Complex treat in whole popln (%)	Perio patients in total popln (%)
25-29	9.19	0.46	0.042274	0.51
30-34	9.0	1.18	0.1062	1.27
35-44	17.52	4.25	0.7446	3.98
45-54	11.2	6.29	0.70448	3.79
55-64	8.11	10.42	0.844251	3.72
65+	7.13	8.38	0.599494	3.26
Total popln	$47,275 \times 10^3$		**3.04%** ($1,437 \times 10^3$)	16.53% ($7,814 \times 10^3$)
>30 y	$25,037 \times 10^3$			**33.83%** ($7,573 \times 10^3$)
Periodontal pt	$8,345 \times 10^3$			

Table 4. Estimation of patient population needing complex periodontal care in 2000

Provision of periodontal care in Korea

General dentists

In Korea, the first school of dentistry opened in 1922 and the School of Dentistry at Seoul National University first held lectures in 1946. Today, 11 dental schools have about 760 graduates every year. This results in approximately one dentist for every 4480 people in 1990, 2,990 in 1997 and 2,618 in 2000. The patient care ratio has continued to improve with the ratio of adults to dentists being 1,956 in 1990, 1,429 in 1997, and 1,389 in 2000. Fortunately many general practitioners are interested in the periodontal field, resulting in considerable specialized periodontal care being provided these days. The importance of public service dentists at the community level cannot be overemphasized. Since 1984, dentists graduating from college have been required to do 3 years of community service, delivering community oral health education and other prevention-oriented services, instead of military service. Approximately 1,300 public dentists and 1,200 dental hygienists worked in the community in 1997.

Periodontists

Postgraduate courses in Periodontics have been offered since 1957 at Seoul National University. Similar courses are now offered at 11 dental schools and 5 hospitals. Approximately thirty periodontal specialists graduated every year during the 1990s. The numbers of qualified periodontists in Korea were 140 in 1990, 402 in 2000, and 521 in 2003. The Korean Academy of Periodontology (KAP) listed 780 practicing periodontists in 1997 and 1,200 in 2002, which constitutes approximately 5% of the total number of practicing dentists.

As shown in Table 5, the ratio of the adult population to periodontists was 39,300 in 1990 to 28,450 in 2000, which is 10-times greater than the ratio of total population/general dentists. The number of periodontitis patients per periodontist was 9,483 in 2000, being 3 times higher than the population to dentist ratio.

	Population			Dentist				Periodontist			
	Pop>30 ($\times 10^3$)	PD pt ($\times 10^3$)	Comp tx need ($\times 10^3$)	No	Pop /dentist	Pop >30 /dentist	PD pt /dentist	No	Pop>30 /perio	PD pt /periotx	Comp /perio
1975	11,721	3,907	1266	2,512	14,045	4,666	1,555	141	83,120	27,709	8,267
1980	13,488	4,496	1258	3,549	10,742	3,800	1,267	204	66,110	22,039	6,167
1985	15,720	5,240	1346	5,375	7,592	2,924	975	310	50,710	16,903	4,343
1990	18,786	6,262	1414	9,606	4,480	1,955	652	478	39,300	13,100	2,959
1995	21,957	7,322	1497	13,668	3,300	1,562	536	680	32,304	10,768	2,291
1997	23,154	7,720	1544	15,370	2,990	1,429	502	780	29,684	9,897	1,945
2000	25,037	8,345	1638	18,026	2,610	1,388	463	880	28,450	9,483	1,772
2002	26,312	8,770	1751	19,724	2,420	1,334	445	1200	20,880	6,960	1,359
2005	27,991	9,330	1866	22,274	2,180	1,256	409				
2010	30,226	10,075	2014	26,524	1,870	1,139	380				

Table 5. Population trend per dentist and periodontist in Korea

Assuming that the average periodontist treats 200 cases of severe destructive periodontitis per year, it would take more than 50 years to treat all current patients. Considering that at least 17 % of these patients may have recurrent diseases or refractory forms (Hirschfeld & Wasserman 1978, McFall 1982), there is an obvious abundance of disease that requires treatment. It would be impossible for periodontists to manage all the patients with periodontal disease. Therefore, periodontists need all the help they can get from the general dental practitioners in order to control periodontal disease.

Strategies to manage the estimated large patient population should include general and public service dentist serving as both primary periodontal care providers and in the delivery of primary preventive periodontal care.

Current status of periodontal management in Korea

much interest in research in periodontal regeneration. Some periodontists have also been involved in osseointegrated implants since around 1991 and the importance of soft tissue management has been advocated for the long-term maintenance of implants.

Public awareness regarding periodontal disease and its effect on systemic health has increased recently. Greater public awareness and increased expectations among patients have led general dentists to make more referrals and better diagnosis. Some general dentists carry out periodontal care themselves and regularly refer patients to periodontists for GTR, implant surgery and periodontal plastic surgery.

Although the prevalence of periodontitis and the demand for periodontal care is as high as 35%, the proportion of periodontally managed cases reported to the Medical Insurance Management Center's was only 4.69% of the cases treated in dental clinics and hospitals in 1995. This is despite the fact that severe periodontal disease exists in more than 10% of the population. However, reports from University Dental hospitals indicated that periodontal care comprises 31.0% of all dental care done in the University Dental hospital. This means that periodontal care is mainly performed in University Dental hospitals and is rarely done in local dental clinics.

According to one report (Ryu 1996), the scope of periodontal care was extended to surgery by more than 50% of the periodontists and to subgingival curettage in 75% of general dentists. He reported that 25% of dentists had an active attitude towards periodontal disease, although more than 50% of them often change their attitude so as to minimize the amount of periodontal care. This attitude was attributed to frequent surveillance and investigation by insurance management centers and to the inadequate fees paid by these centers. General practitioners and even periodontists were not inclined to provide adequate periodontal care to the patient because of the current situation of the medical insurance system which controls and supervises periodontal care and the inadequately scheduled fee for periodontal care. Therefore, in order to provide adequate treatment for periodontal patients, the treatment fees and surveillance systems must be optimized.

A survey (Chung 2003) was recently performed on the attitudes of dentist and periodontists to periodontal patients (Table 6a–c). It was found that the frequency of periodontal care is increasing. This study compared different methods of periodontal care between the general dental practitioner and periodontists. It is encouraging that younger dentists are doing an increasing amount of periodontal care.

Areas of dental care	Prop. of perio pt	Prof of perio care	Prop of perio income
Perio care	48.6 (8.9)	48.4 (8.7)	39.4 (8.6)
Total care	31.6 (4.8)	28.7 (4.7)	17.6 (4.6)
No Response	28.1(2.9)	24.5 (2.8)	17.2 (2.8)

Table 6a. Proportion of periodontal practice amongst daily dental care according to main area of dental care

Areas of dental care	Scaling & OP	Curettage	Plaque control education	Perio Flap	Bone Graft	GTR	Furcal Therapy
Perio care	87	87	75	87	88	75	88
Total care	99	97	93	85	73	48	82
No Response	92	93	86	68	48	32	58

Areas of dental care	Perio-Pros	Perio-Ortho	G. graft /MG surg	Maint. Care	Perio Esthetics	Dental Implant
Perio care	19	37	81	87	81	93
Total care	70	24	68	93	67	75
No Response	45	13	36	63	48	63

Table 6b. Percentage of dentists practicing periodontal care

Suggestions for periodontal disease management

Role of General dentists and periodontists in the management of periodontal disease

A realistic approach to the management of periodontal disease on a large scale would be for periodontal care to be carried out by general dental practitioners. General dental practitioners should be able to recognize gingivitis and early periodontitis before it has progressed to the advanced stages. General dentists should share the responsibility for the early detection and treatment of periodontal disease, by diagnosing periodontal disease and distinguishing the different types of patients, based on what level of periodontal therapy they require. For the early detection and treatment, every oral examination should include an evaluation of the periodontium. To simplify the periodontal examination including probing into pocket, general practitioners are recommended

Dependent Variable Frequency of practice/w	Perio care	Total care	No response
Scaling	13.7 (3.2)	14.3 (1.9)	7.3 (2.0)
TBI	35.4 (3.1)	9.2 (1.9)	7.8 (2.0)
Recall	13.3 (4.3)	9.0 (2.6)	9.3 (2.8)
Occlusal therapy	2.9 (2.4)	3.5 (1.5)	5.0 (1.6)
Flap	4.7 (1.1)	3.2 (0.7)	2.0 (0.7)
Bone graft	1.8 (0.5)	1.6 (0.3)	0.6 (0.3)
GTR	1.3 (0.3)	0.9 (0.2)	0.5 (0.2)
Furcal therapy	1.0 (0.3)	1.1 (0.2)	0.5 (0.2)
Gingival graft	0.7 (0.2)	0.7 (0.1)	0.4 (0.1)
Dental implant surgery	1.8 (0.5)	1.2 (0.3)	1.3 (0.3)

Table 6c. Frequency of practicing periodontal care (per week) by respondants according to area of dental care

to use PSR (Periodontal Screening & Recording) which is an adaptation of the Community Periodontal Index of Treatment Needs (CPITN). For screening, the dentition is divided into sextants. A probe with a ball end 0.5 mm in diameter and a color-coded area extending from 3.5 to 5.5 mm and a gentle probing is recommended. At least six areas in each tooth should be examined. For each sextant with one or more teeth or implants, only the highest score is recorded.

Referral system

In periodontics, one of the hallmarks of good practice is the ability to customize an appropriate treatment plan for the patient. According to Fetner (1994), there are three types of patients, based on the level of periodontal therapy required:

1) patients who don't require surgery (early periodontitis with no furcation involvement should be treated by the general practitioner who has sufficient skills in nonsurgical therapy),

2) patients who may require surgery and

3) patients who will require surgery. The two latter types of patients are referred to periodontists with the data concerning their original condition diagnosis, charting, completed procedure, charting at re-evaluation, plaque score, oral hygiene aids and patient motivation.

Referrals to periodontists are common in Korea. In Chonnam National University Hospital, 4.5% of all dental outpatients and almost 33% of the periodontal patients have been referred by general dentists. The referred patients are managed with additional treatment by periodontists to achieve their health, functional, and aesthetic goals.

Role of Periodontists in the management of periodontal disease

Referred patients who have advanced or aggressive periodontitis could be managed with

additional treatment by periodontists to achieve their health, functional, and esthetic goals. The choices are highly individualized and depend upon a complete oral and periodontal examination. Thus a full range of treatment alternatives is available for a wide spectrum of disease severity and patient circumstances.

Periodontists are able to concentrate on the complex types of periodontal care such as surgical pocket therapy, regenerative therapy such as bone grafting and GTR, plastic reconstructive surgery, and interdisciplinary therapy, transplantation and dental implant treatment.

Conclusion

Due to the rapid increase in the elderly population (>65y) in our aging society, an increase in the number of people suffering from periodontal disease requires the cooperation between general dentists and periodontists. Periodontists are able to concentrate primarily on the complex treatment of advanced or severe form of periodontitis. Periodontists should provide periodontal care including an interdisciplinary approach to saving teeth even though implant therapy has become one of the routine modes in replacing missing teeth. Implant therapy should be the last mode of periodontal therapy, because the tooth given by nature is the best.

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Chapter 9

Periodontal Risk Assessment & Prognosis: Current Status & Future Development - A Perspective From Hong Kong

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Introduction

Global risk factors and risk indicators for periodontal diseases have recently been overviewed (Albandar 2002). The case for risk, the probability that an event will occur in the future, versus association, found in large scale epidemiological studies, is explored. Risk factors and risk indicators discussed as acting globally include: geographic region, oral hygiene level, smoking, diabetes mellitus, age, gender, race-ethnicity, genetic factors, bacterial specificity, viruses, host response factors, socio-economic status, osteopenia and osteoporosis, psychological factors and local factors. This review concludes that periodontal disease is clearly a multi-factorial disorder. Periodontitis is thus a multi-factorial disease with microbial dental plaque as its initiator and perpetuator. Dental plaque is a necessary factor for periodontitis.

Periodontal Risk in Asians

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. In 1978, Löe and co-workers reported on the rate of periodontal destruction before the age of 40 years in Sri Lankan male Tamil tea plantation workers (Löe *et al* 1978 a&b). On the basis of the mean data this report showed fairly even progression of destruction across the Sri Lankan subjects and destruction greater in the Sri Lankan males than in Norwegian male students and academics. However, in 1986 a recalculation of the rates of progression based on mesial attachment loss and tooth loss in these Sri Lankan males aged 16 – 46 years allowed for the identification of three subgroups within the studied subjects on the basis of periodontal destruction, which were labeled 'no progression, moderate progression and rapid progression' subgroups (Löe *et al* 1986). A subsequent analysis, on the basis of data collected in that study, which tried to identify what was associated with risk for periodontal destruction in these Sri Lankan tea plantation workers, found that age, gingival index and calculus index were all associated with destruction but that plaque index, tobacco smoking and betel-quid chewing were not (Neely *et al* 2001). It may well be that this study did not capture data about each subject which would have allowed for a comprehensive assessment of risk associated for the periodontal destruction encountered.

Risk for periodontal destruction in Asians has recently been reviewed as part of an overview of periodontal conditions in Asia and Oceania (Corbet *et al* 2002). Longitudinal studies into periodontal disease behaviour have been conducted in many Asian countries besides the well-known study in Sri Lanka (Löe *et al* 1978a), in Indonesia (Timmerman *et al* 1998, Timmerman *et al* 2000), in Japan (Lindhe *et al* 1989) and in China (Baelum *et al* 1997, Suda *et al* 2000). In Indonesia, the longitudinal study being conducted found age, amount of subgingival calculus and subgingival presence of *Actinobacillus actinomycetemcomitans* to be associated with attachment loss in adolescents. In Japan, the longitudinal study associated calculus, age and previous disease experience with attachment loss (Haffajee *et al* 1991). In China, age, gender (with males being worse than females), pre-existing periodontitis and percentage of teeth involved, and certain periodontopathogenic bacteria exceeding thresholds have all been associated with periodontal destruction (Baelum *et al* 1997, Papapanou *et al* 1997, Suda *et al* 2000). The overview concluded that not much particular to Asians with respect to risk has been uncovered, save an association with dental calculus.

Dental calculus, in terms of its risk association for periodontal destruction poses some interesting considerations, particularly among Asians in whom its high prevalence in large amounts has led to easy study. Calculus in Asians is not always associated with periodontal inflammation or pockets (Takahashi *et al* 1988, Holmgren and Corbet 1990, Peng *et al* 1990). There is evidence that tooth brushing alone will improve periodontal conditions despite the persistence of dental calculus (Gaare *et al* 1990). The removal of calculus alone has no effect on periodontal conditions (Lembariti *et al* 1998). Hence its role as a true risk factor for periodontal destruction is difficult to comprehend and it should be considered to be associated with periodontitis perhaps as a sequel, when there is large amount of subgingival calculus.

Periodontal Risk: A Perspective

An overview of periodontal risk factors has recently been published (Nunn 2003). This overview noted that distinctions between terms used in descriptions of risk associations are not always clear, nor is it clear how a clinician makes use of such information. This overview defines a risk factor as 'any characteristic, behaviour or exposure with an association to a particular disease', noting that the relationship is not necessarily causal in nature (Brownson and Pettiti 1998). Yet the global review of periodontitis and risk (Albandar 2002) in a different issue of the same journal had defined a risk factor as a distinctive characteristic or exposure that increases the possibility of developing periodontitis or leads to measurable periodontal attachment loss. The overview of Nunn (2003) allows that a risk factor that cannot be modified is often referred to as a (risk) determinant (Genco 1996). To differentiate clearly between those risk associations for which it is possible to intervene, to reduce the risk, and those which are immutable to change, e.g. age, race, genetics, gender, is very useful in practice and for many medical conditions is the basis of the medical model. For instance, those males with a risk determinant for cardio-vascular disease due to their fathers having had cardio-vascular disease are generally encouraged to modify those modifiable risk factors for cardio-vascular disease such as diet and exercise, whereas they can do nothing about the cardio-vascular history of their fathers.

Having a known risk determinant shapes

the preventive and perhaps therapeutic approaches in more stringently controlling those modifiable risk factors, while the risk determinant is immutable. In the same way, levels of plaque control can be improved in those with genetically determined risk to periodontal disease, so as to reduce the risk of periodontal destruction. A risk indicator was defined by Nunn (2003) as 'a potential risk factor identified to be associated with disease from case-control or cross sectional studies'. A risk marker was defined by Nunn (2003) 'as a risk factor that can be used to predict the future course of disease'. Whereas Albandar (2002) drew the distinction between factors which have a true risk-modifying effect and those which are associated with periodontitis, which he considered to be risk indicators.

The overview of Nunn (2003) considers a range of risk factors which have been studied, and grouped these as follows: subject determinants such as age and race, social and behavioural factors such as tobacco smoking, socio-economic status, nutrition and psychological factors, systemic factors such as diabetes mellitus, drugs, HIV, genetic factors considering twins and genotype polymorphisms, tooth factors such as anomalies, crowding, restorations, fractures etc, microbial risk factors, featuring the holy triumvirate of *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis* (formerly *Bacteroides forsythus*) and *Porphyromonas gingivalis* (Zambon 1996) and concludes with an introduction to emerging risk factors to do with systemic disease periodontal disease interrelationships.

Periodontal Risk Levels Combined in Clinical Risk Assessment

It is apparent therefore from the overview of Nunn (2003) that periodontal risk factors can act at multiple levels: at the subject level, at the tooth level and even at the specific site level. Risk factors acting these different levels can be grouped to give subject risk assessments and examples of this approach have been recently reported for prediction of future periodontal status (Page *et al* 2002, Page *et al* 2003) and in the management of supportive periodontal therapy for periodontitis patients (Lang & Tonetti 2003). It has been suggested that a systematized approach to subject risk assessment is more consistent than subjective expert opinion in clinical and periodontal decision-making (Persson *et al* 2003). The periodontal risk calculator (PRC) has been developed and tested in the United States of America and the Periodontal Risk Assessment (PRA) in Europe. If an Asian country has data on risk associations for periodontal destruction in its own citizens, it would probably be prudent for periodontal researchers in that country to construct and test the validity of a risk assessment model for clinical use in that country. Such an approach has been adopted in Hong Kong on the basis of data from epidemiological studies conducted in Hong Kong and Southern China and on longitudinal studies of periodontitis patients in Hong Kong.

Future Directions

Developments in the understanding of the pathogenesis of periodontitis have shed light on possible future directions in research and clinical applications (Page *et al* 1997). In contrast to a unidirectional approach to the study of risk factors, e.g. studies of subgingival periodontopathogens in dental plaque biofilms, the emerging trend of studying both periodontopathogens and the host response they evoke heralds the future trends. We first looked at granulocyte elastase activity in gingival crevicular fluid (GCF), a marker of intracrevicular PMN activity (Lamster 1997),

in relation to the presence of subgingival periodontopathogens in subjects with untreated chronic periodontitis as an attempt to improve risk evaluation through studying dynamic bacteria-host relations (Jin *et al* 1999). This study showed that low elastase activity in GCF was coincident with low prevalence of the target species, while a wide variation of elastase activity existed among the untreated periodontitis sites with similar co-infections of *B. forsythus, P. gingivalis, P. intermedia* and *T. denticola*, suggesting the local host inflammatory response to the bacterial challenge in untreated periodontal pockets is diverse based on both subject level and site level within the subjects. This study was then expanded to investigate multiple interrelated markers of host response evoked by the presence of various periodontopathogens, which suggested that shifts in host-bacteria interactions may reflect different phases of the inflammatory response and therefore indicate a range of periodontal disease activity levels, despite the presence of the periodontopathogens (Jin *et al* 2000). This investigative approach is being applied not only in untreated chronic periodontitis but also in relation to the response to treatment efforts. We found that IL-8-related granulocyte elastase activity in GCF was related to the change in infection patterns of subgingival periodontopathogens following scaling and root planing. Varying initial IL-8 levels in GCF and a corresponding shifting change of granulocyte elastase activity in GCF may characterize the different short-term treatment responses (Jin *et al* 2002). In a recent study, we evaluated the dynamics of host response marker in gingival crevicular fluid under various periodontal conditions in subjects with healthy periodontium and those with gingivitis and chronic periodontitis (Jin *et al* 2003). This study showed that patterns of dynamic changes in GCF flow and elastase activity varied under different periodontal conditions. Assessment of both static GCF and flow GCF may allow better insight into the dynamic change of the target components in GCF. The markers of host response studied have recently been expanded to others which are strongly linked to bacteria-host interactions with promising results, such as soluble CD14 (Jin and Darveau 2001) and membrane-bound CD14 (a lipopolysaccharide receptor) (Jin *et al* 2004).

The simultaneous study of a battery of host response markers and subgingival periodontopathogens evoking these responses signals future approaches for combined risk profile assessment beyond what is offered from the history taking, clinical and radiographic examination. Our ongoing research efforts focus on testing for bacteria, intrinsic GCF components and messenger RNA/protein expression in adjacent healthy and diseased periodontal tissues, which may lead to the development of new diagnostic strategies for identification of high-risk individuals and sites particularly susceptible to periodontal destruction thus enabling targeted prevention and better control of periodontitis. Also the future seems to hold the possibility of screening, diagnostic and monitoring tests which are based on co-biomarkers and genetic markers for periodontal diseases utilizing both oral fluid (e.g. GCF and saliva) and serum as testing samples. In addition to models for risk assessment being constructed and tested, 'Bio-assessment' models are being constructed based on our data for later testing and validation.

Conclusion

On the basis of what is already known about risk for periodontal destruction as a component of the multi-factorial disease like

periodontitis for which microbial dental plaque is a necessary factor for its initiation and perpetuation, it is possible to construct clinically useful risk assessment models. The future holds the hope that by testing for inflammatory mediators and their antagonists; matrix metalloproteinase and their tissue inhibitors; genotypes and phenotypic expression; lipopolysaccharides and host pattern recognition receptors including lipopolysaccharide-binding protein, CD14, IL-1 receptor/toll-like receptor superfamily; along with further studying the microbial biofilm, bio-assessment can be combined with clinical risk assessment for better prevention and treatment of and supportive care for periodontitis in its various forms.

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Chapter 10

Systemic Diseases & Periodontal Pathogens

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Introduction

Periodontitis is one of the most common infectious diseases of humans. Periodontitis results from chronic exposure of the periodontium to dental plaque, especially subgingival plaque, which contain mostly gram-negative anaerobic bacteria. The associated tissue destruction results from the various toxic bacterial products and host responses. Considering the chronic nature of the disease, bacterial toxins and the local and systemic host responses involved, it is reasonable to argue that periodontitis may influence systemic health. Observational studies indicate periodontal infection as a risk factor for pre-term low birth weight and systemic conditions like cardiovascular disease.

Over the past 10 years, several studies have been published pointing towards a relationship between periodontal disease and various systemic disorders or diseases. Here we present data from our laboratory, as well as from available literature on the link between peripheral vascular disease (PVD) and *Helicobacter pylori* associated gastritis to oral microbiota.

This paper discusses the biological possibility for a link between periodontal infection and systemic disease. It has become increasingly clear that the oral cavity can act as the site of origin for dissemination of pathogenic organisms to distant body sites.

Periodontal pathogens in oral cavity, aortic aneurysm and atherosclerotic blood vessels

Epidemiological studies show periodontal patients have a higher risk (odds ratio: 1.5 - 2.0) of fatal cardiovascular diseases (CVD) than periodontally healthy subjects. A positive and significant correlation has been shown between periodontal infections and heart disease including CVD and stroke.

Numerous studies indicate the association between oral bacteria and cardiovascular diseases (Haraszthy *et al* 2000, Okuda *et al* 2001, Stelzel *et al* 2002). Therefore, we undertook the task of detecting periodontopathic bacterial DNA in peripheral vascular diseases and comparing the detection to that of levels in the oral cavity. 32 patients with PVD were selected. Periodontal status of these patients is presented in Figure 1. Informed consent was obtained from each subject. Saliva samples were collected before the surgery and aortic lesions removed by surgeon were examined. Arterial wall samples were homogenized and then the DNA of periodontal pathogens was extracted from the homogenized samples by High Pure PCR Template Preparation Kit® (Roche). The 16S

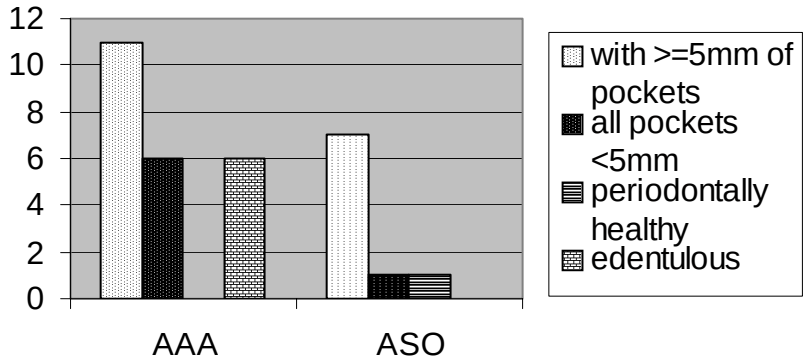

Figure 1. Periodontal status and AAA and ASO patients

P. gingivalis positive		24/32	75.0%
AAA	Lesion	17/23	75.0%
	Arterial wall	15/21	71.4%
	Thrombus	8/9	88.9%
ASO	Lesion	7/9	77.8%
T. Denticola positive		20.32	62.5%
P. intermedia positive		11/32	34.4%
C. rectus positive		4/32	12.5%

Figure 2. The detection frequency of periodontopathic bacteria in aortic lesions examined by PCR

rRNA-based PCR detection of periodontal pathogens was performed in abdominal aortic aneurysm (AAA) lesions and arteriosclerosis obliterans (ASO) lesions resected by surgery (Ashimoto *et al* 1996). Our results showed quite high detection rates of periodontal pathogens in the examined AAA and ASO samples (Figure 2). Particularly, *P. gingivalis* was detected in 75.0% of examined aortic lesions by PCR method. Bacterial enzymes, *P. gingivalis* gingipains, can stimulate platelet aggregation and thrombus formation (Lourbakos *et al* 2001). These bacterial components may feature in the co-aggregation process of atherosclerosis and formation of aortic aneurysm.

According to our data, PVD patients whose saliva was positive for *P. gingivalis* and *T. denticola* showed higher detection rates for these bacteria in the examined aortic lesions, while negative patients showed a lower detection for these bacteria in the lesions. This may be an important finding to indicate the close relationship of periodontal pathogens and cardiovascular diseases.

History of gastritis or peptic ulcer	Subjects	Positives	Rates of positives (%)
With	45	18	40.0
(subjects with *H. pylori* in stomach or duodenum)	(28)	(13)	(46.4)
Without	12	2	16.7
Total numbers	57	20	35.1

Figure 3. Detection frequencies of *H. pylori* in the oral cavity (nested PCR)

	Subjects	Positives	Rates of positives (%)
Patients with pockets <4 mm	11	1	9.1
Patients with pockets ≤4 mm	17	7	41.1

Figure 4. Prevalence of *H. pylori* in the dental plaque of patients with the bacterium present in stomach or duodenum

Detection of *Helicobacter pylori* in the Oral Cavity of Periodontitis Patients by PCR Method

Helicobacter pylori is a spiral, microaerophilic, Gram-negative, motile bacterium with polar-sheeted flagellae. It has been associated with the development of peptic ulcers and gastric cancer (Henshall & Warren 1984). Although it may be transmitted through the oral cavity, it is unknown whether the oral cavity acts as a permanent reservoir for this bacterium (Bernander *et al* 1993, Banatvala *et al* 1993, Hammar *et al* 1992, Krajden *et al* 1989, Nguyen *et al* 1993, Shimada *et al* 1994). *H. pylori* infection is more prevalent in developing countries than in developed countries. Its presence is always associated with chronic active gastritis, and eradication of the bacterium is always followed by resolution of gastritis. Nearly all patients with duodenal ulcer disease have *H. pylori* gastritis, and ulcer relapse is exceptional after *H. pylori* eradication. *H. pylori*-infected persons have an increased risk of developing intestinal-type, but not undifferentiated, gastric adenocarcinoma.

In this study we used nested polymerase chain reaction (PCR) to clarify whether the oral cavity acts as a reservoir for *H. pylori* (Mapstone *et al* 1993). The existence of *H. pylori* in the oral cavity was determined by nested PCR in 57 subjects and by culture method in 18 subjects. The presence of periodontopathic bacteria was also determined by 16S rRNA-based PCR method. Although *H. pylori* was rarely detected in the oral cavity by culture technique, it was frequently detected (35.1%) by nested PCR in the oral cavity, especially among periodontitis patients who had the bacterium in the gastrointestinal tract (46.4%) (Figure 3). Among the subjects who harboured *H. pylori* in the stomach or duodenum, 41.2% of patients with periodontal pockets > or = 4 mm and 9.1% of subjects without pockets showed *H. pylori* in dental plaque, although a statistically significant difference was not observed (Figure 4). One patient who had periodontal pockets retained *H. pylori* in the oral cavity even after eradication of the bacterium from the stomach and duodenum. Most (8/10) of the patients who had *H. pylori* in dental plaque harboured *Bacteroides forsythus* in their oral cavities. These results suggest that close attention should be given to periodontitis patients who harbour *H. pylori* in the oral cavity.

Conclusion

These studies add to the existing evidence that periodontal pathogens may contribute to a variety of systemic diseases. Furthermore, we suggest that the oral cavity may act as a reservoir for *H. pylori* infection (Umeda *et al* 2003). At present the major concern in periodontal disease management is to prevent the progression of local tissue destruction. Nevertheless, the above discussion and others of its kind points out the impact of periodontal disease and oral microbiota on systemic health. Further investigations are needed to clarify the relationship between periodontal disease and systemic diseases. This in turn will determine if periodontal disease management will help prevent systemic conditions like CVD, PVD, gastric carcinoma and pre-term low birth weight infants.

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Chapter 11

Trends in Periodontal Repair & Regeneration

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Introduction

In the new millennium the ultimate goal of periodontal therapy is 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.

Since the periodontium is a complex arrangement of at least four different tissues, namely cementum, periodontal ligament, alveolar bone and gingiva, to try to regenerate these tissues by focusing solely on bone is not going to produce satisfactory outcomes. Indeed, to fill a periodontal defect with a substance that has no relevance to the next functional stage of reconstruction is irrational (Bartold *et al* 2000). Interestingly these concepts are not new. As early as 1980 it was recognized that since granulation tissue from bone has the potential to induce root resorption and ankylosis, the rationale of favoring bone growth with the use of bone transplants was considered to be highly questionable (Karring *et al* 1980). Later this same group commented that ignorance of the contribution of the various tissue compartments in periodontal wound healing may explain the widespread use of bone transplantation in the treatment of intrabony defects (Karring *et al* 1984). More recently it has been noted that as a profession we have become obsessed with filling holes in bone rather than studying the natural healing processes (Becker & Becker 1999). 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).

Bone Fillers

Various types of bone grafts have been investigated to determine their ability to stimulate new bone formation. Of these the following have been studied in detail:

1. Alloplastic materials which are generally synthetic filler materials.
2. Autografts which are grafted tissue from one site to another in the same individual.
3. Allografts of tissue between individuals of the same species but with different genetic composition.
4. Xenografts which consist of grafted materials between different species.

Alloplastic Materials	
Ceramics	Hydoxyapatite
Polymers	Bioglass
Autografts	
Cortical bone	Cancellous bone
Bone marrow	Iliac crest
Allografts	
Demineralized freeze dried bone	Freeze dried bone
Xenografts	
Deproteinized bovine bone	

Table 1. Grafting materials used in periodontal intrabony pockets

Examples of these are provided in Table 1.

Treatment of intrabony periodontal defects has often focused only on the bony defect and this has lead to the use of a number of grafting materials to stimulate bone repair. Allografts and alloplastic materials, while convenient for the filling of defect volume, have little osteoinductive activity. Autogenous bone grafts are thought to be osteoinductive *in vivo* but are still of limited value for inducing periodontal regeneration since their abilities to induce new cementum and periodontal ligament are limited. Although utilization of various grafting materials may result in some gain in clinical attachment levels and radiographic evidence of bone fill, careful histologic assessment usually reveals that these materials have little osteoinductive capacity and generally become encased in a dense fibrous connective tissue (Figure 1). In addition, the problematic junctional epithelium still seems to form between the graft and tooth surface in spite of the placement of a graft (Dragoo *et al* 1973, Moskow *et al* 1979). Thus, these materials do not fulfill our criteria as useful periodontal regenerative materials.

Periodontal Wound Healing

It is now well accepted that periodontal wound healing is far more complex than either soft tissue or bone healing (Bartold & Narayanan 1998). Since the periodontium is comprised of four different tissues its healing and repair dictates that both hard and soft tissues must be able to heal in tandem. In most cases the epithelial cells of the gingival tissues proliferate faster than the underlying connective tissues and this results in the formation of a long junctional epithelium which significantly impedes any potential regeneration of cementum, periodontal ligament or alveolar bone.

Guided Tissue Regeneration

By the mid 1980's the concepts of guided tissue regeneration (GTR) had been espoused and developed. Based on sound scientific research, for the first time it seemed that periodontal regeneration was clinically possible (Gottlow *et al* 1986, Karring *et al* 1993, Nyman *et al* 1982). This procedure was based on the premise that the periodontal

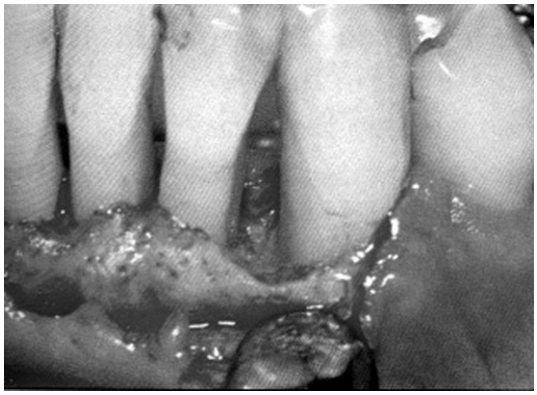
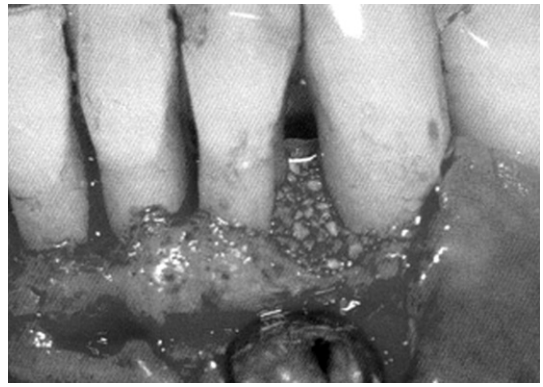


Figure 1A



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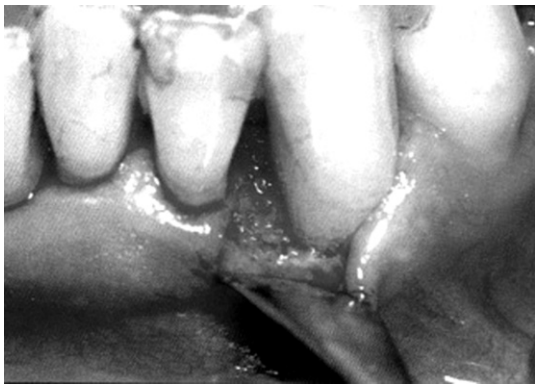

Figure 1C

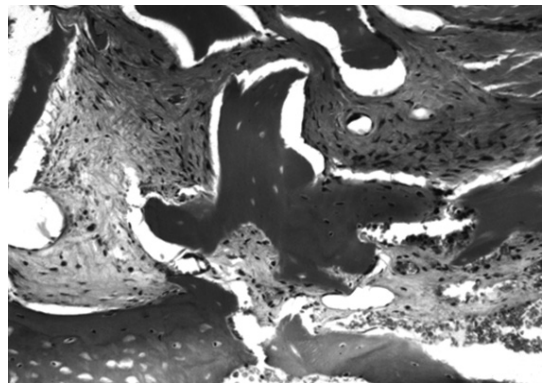

Figure 1D

Figure 1. Fill of periodontal defect with Demineralized Freeze Dried Bone Allograft
A. Bony defect
B. Bony defect filled with DFDBA
C. "Bone Fill" at re-entry
D. Histological appearance of "Bone Fill"
Note no new bone, only DFDBA particles surrounded by fibrous connective tissue.

ligament contained all of the progenitor cells required for the formation of bone, cementum and periodontal ligament. Through repopulation of the wound site by the progenitor cells, periodontal regeneration could be induced. This procedure became widely accepted as a clinical procedure (Karring *et al* 1993, Nakae *et al* 1991). Unfortunately, recent clinical evaluations have indicated that the clinical improvements obtained by this procedure are small and highly variable (Bratthal *et al* 1998, Pontoriero & Lindhe 1995, Wallace *et al* 1994). A common finding upon reentry for removal of nonresorbable membranes is soft granulation tissue with little or no evidence of osseous fill of the defect (Figure 2). Whether this tissue goes on to form osseous tissue, periodontal ligament and new cementum is questionable. In addition, histological studies have questioned the principle relies of exclusion of epithelium and gingival connective tissue cells,

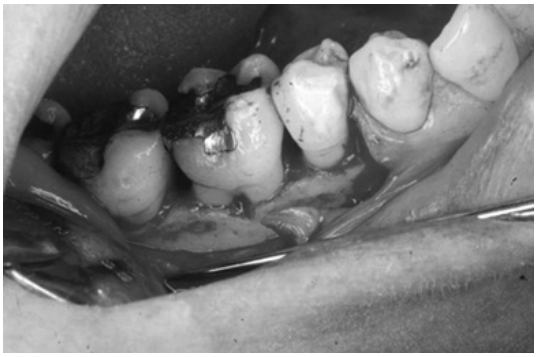

Figure 2A

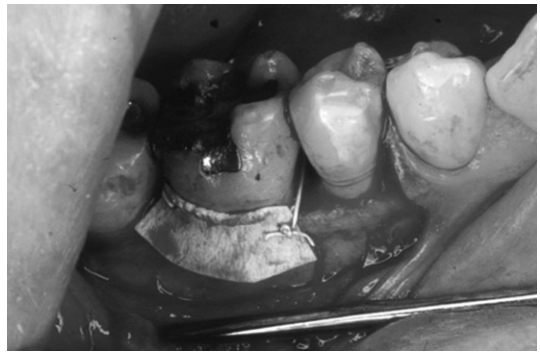

Figure 2B

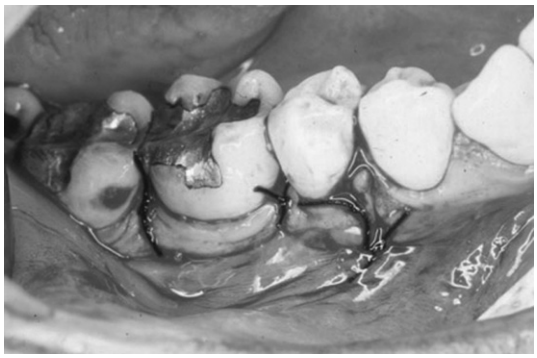

Figure 2C

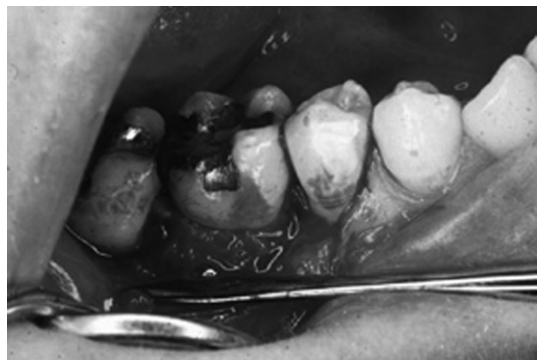

Figure 2D

Figure 2. Guided Tissue Regeneration
A. Grade II molar furcation defect
B. ePTFE GoreTex membrane placed over defect
C. Membrane sutured into site and covered with mucoperiosteal flap
D. Appearance of regenerated tissues at time of membrane removal
Note presence of granulation tissue only. There is no evidence clinically of new bone formation at the time of membrane removal.

by demonstrating downgrowth of epithelial cells between the membrane and tooth surface.

The development of infection following placement of the barrier membrane is also a factor in determining successful GTR outcomes (Demolon *et al* 1994, MacDonald *et al* 1998, Tempro & Nalbandian 1991). This finding, as well as the difficulty in sealing the healing tissue from the oral environment, are significant impediments to successful GTR outcomes. If the wound site could be closed (as happens for guided bone regeneration) and infection by oral bacteria controlled then one would expect that the regeneration process following GTR would be far more predictable.

In summary, development of the GTR procedure has promoted many studies into the development of therapeutic procedures based on fundamental biological principles. However because of its limited clinical predictability it is more of historic interest than clinical value.

Name	In Vitro Studies	In Vivo Studies
bFGF	yes	yes
BMP's	yes	yes
EGF	yes	no
EMDOGAIN*	yes	yes
FGF	yes	yes
IGF-1	yes	yes
PDGF	yes	yes
TGF-β	yes	no

* Not strictly a growth factor but is believed to work in a similar fashion to growth factors, differentiation facts and the morphogenetic proteins.

Table 2. Growth factors used for periodontal regeneration

Problems Associated With Periodontal Regeneration

From the above it is apparent that a number of important observations have been made with regards to the outcomes of attempts to regenerate periodontal tissues. Firstly it is apparent that one of the most predictable regenerative therapies is the treatment of three wall intrabony defects. Whether these heal just as well without any aids is now open to question. Secondly numerous studies have concluded that while many of the commercial preparations of allogenic and alloplastic bone fillers have a long and safe history of use, they are primarily osteoconductive and probably have little role to play in periodontal regeneration. Thirdly, barrier membranes provide short-term evidence of improving class II furcation defects but for class III defects they are very unpredictable. Finally, and perhaps most importantly, to date there is no good evidence that regenerative therapies increase the long-term life span of teeth.

Reasons for Periodontal Regeneration Failure To Date

Many of the currently available methods for periodontal regeneration (membranes and bone fillers) still fail to result in predictable clinical outcome for a variety of reasons. First and foremost is our inability to control the rapid proliferation and apical migration of the junctional epithelium which prevents the regeneration of new cementum, periodontal ligament and alveolar bone. The inability to protect and seal the healing site from the oral environment, in order to decrease the likelihood of infection and contamination by unwanted tissues such as junctional epithelium, is also a significant problem which has not been satisfactorily overcome. As detailed above, another important reason why periodontal regeneration has been elusive relates to our tendency to principally focus on bone and at the same time often disregarding the importance of also stimulating other components of the periodontium, such as cementum and periodontal ligament. Finally,

and possibly most importantly, a major reason for periodontal regeneration failure is our inability to precisely define the growth and differentiation factors needed for regeneration.

Growth & Differentiation Factors For Regeneration

A case for a role for growth and differentiation factors in periodontal regeneration comes from many studies. In particular, studies concerning periodontal development and wound healing have clearly implicated specific roles for these factors in controlling cell behavior and extracellular matrix synthesis. Recently, studies have investigated application of a variety of growth and differentiation factors to root surfaces to stimulate cell repopulation of periodontal defects and subsequently induce regeneration. Because of their regulatory effects on immune function, and on the proliferation and differentiation of cells from the epithelium, bone and soft connective tissues, growth factors are an attractive group of agents to target for potential wound regeneration studies. A list of various growth factors currently under *in vitro* and *in vivo* investigation are shown in Table 2. The most effective of these are PDGF and IGF-I, which have been reported to enhance regeneration in beagle dogs and monkeys (Lynch *et al* 1991, Rutherford *et al* 1993). The bone morphogenetic proteins (BMP) also show potential for stimulating bone and cementum regeneration (Kuboki *et al* 1998, Ripamonti & Reddi 1994). From our own studies we have identified roles for differentiation factors such as growth hormone and growth factors such as PDGF and IGF in regulating cell proliferation, cell attachment, matrix synthesis and clonal selection of cells.

In more recent times methods of transducing cells to overexpress growth factors such as PDGF and subsequent reimplantation into periodontal defects have been investigated as a novel means of local delivery of growth factor to periodontal defect sites (Anusaksathien *et al* 2004).

To date there have been too few controlled long-term studies to allow any substantial comments about the use of growth factors for periodontal regeneration for humans. Although they show some promise, it is likely that difficulties will be encountered. The stability of the tissues that are to be formed under the influence of these factors will also pose problems. In addition, how these agents will be delivered to the site and isolated from the oral environment to allow their action within a "closed healing environment" remains unsolved. Finally, we do not fully understand the differentiation capabilities of the periodontal cells and what the precise target cells that are to be modulated by these factors. This last issue has led to a number of studies focused on specific cell populations within the periodontium (McCulloch *et al* 1991).

Clonal Selection of Cells

Following the recognition that there are many subsets and subpopulations of cells residing within the periodontal tissues it became necessary to study these cells in more detail (Ivanovski *et al* 1991). A significant advance was made with the ability to clone and expand cells from the normal and regenerating periodontal tissues (Figure 3).

These studies now pave the way for exciting developments in the fields of gene manipulation and tissue engineering. Gene manipulation of cells enables one to insert genes into cells and thus have a means of delivery to a site of cells which express certain growth factors or matrix components which are critical to the next phase of tissue regeneration.

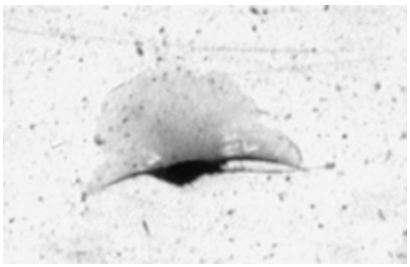

Figure 3a

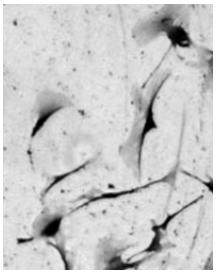

Figure 3b

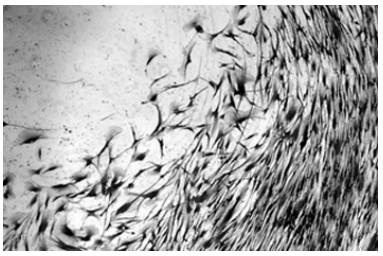

Figure 3c

Figure 3. Clonal expansion of periodontal ligament cells.
A. Single Cell Clone.
B. Cells expanding from cloned cells.
C. Large scale culture from single cell clone

Tissue Engineering

Tissue engineering is an evolving field of science aimed at developing techniques for the fabrication of new tissues to replace damaged tissues and is based on principles of cell biology, developmental biology and biomaterials science (Narem & Sambanis 1995, Vacanti *et al* 1991, Reddi 1998).

To produce an engineered tissue there are several key elements which must be considered. These include appropriate levels and sequencing of regulatory signals, the presence of responsive progenitor cells and an appropriate extracellular matrix or carrier construct. Ongoing advances in cell biology, and development of biodegradable polymer constructs have allowed successful tissue engineering of cartilage, bone and related tissues. Preliminary studies have indicated that periodontal ligament and bone cells can be transplanted into periodontal sites with no adverse immunologic or inflammatory consequences (Lang *et al* 1998, Malekzeh *et al* 1998, Van Dijk *et al* 1991).

Periodontal regeneration involves the recruitment of progenitor cells which can differentiate into committed regenerative cells, proliferation of these cells, and finally synthesis of new connective tissues. A tissue engineering strategy for periodontal regeneration would involve use of specialized periodontal cells grown within an artificial scaffold and subsequent implantation into a periodontal defect (Figure 4). In doing so, the need for recruitment of cells to the site is negated and the predictability of the outcome may be enhanced.

The requirements for successful tissue engineering (including periodontal tissue engineering) can be divided into two main areas (Bartold *et al* 2000, Brekke & Toth 1998). The first set of requirements are engineering issues such as biomechanical properties of the scaffold, architectural geometry and space maintaining properties. The second group of requirements relate to the biological functions of the engineered matrix including cell recruitment, permission of neovascularization and delivery of the requisite morphogenetic, regulatory and growth factors for tissue regeneration. Many of these specific features are listed in Table 3 and illustrated in Figure 5.

Directions for the Future

Nanotechnology will undoubtedly impact significantly on the fledgling field of periodontal tissue engineering.

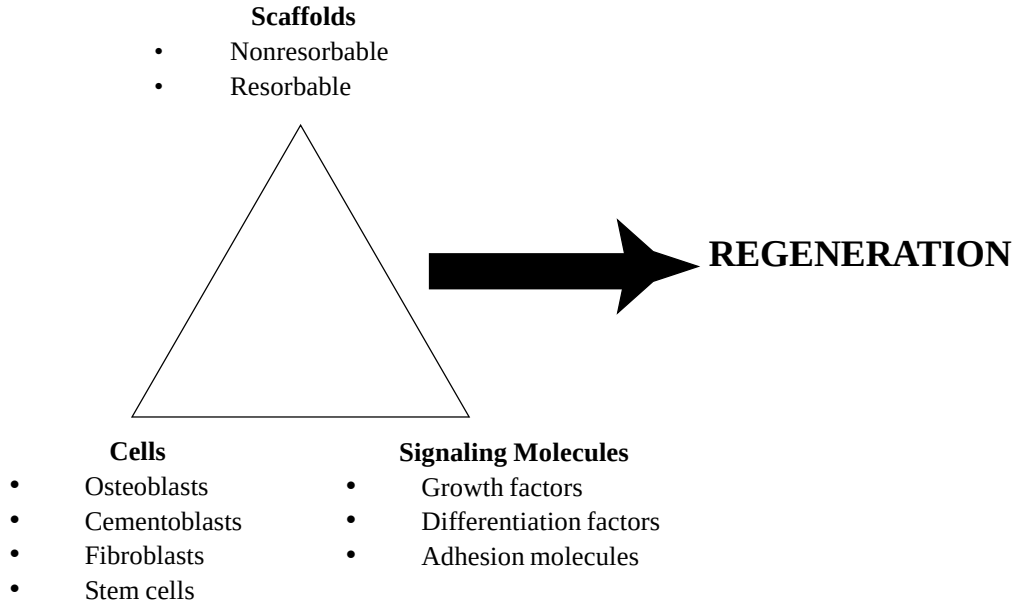

Figure 4. Requirements for successful tissue engineering

Biomechanical Features
Space maintenance
Barrier or exclusionary features
Biological functions
Biocompatibility
Incorporation of cells
Incorporation of instructive messages

Table 3. Requirements for successful tissue engineering

Nanotechnology is the science of engineering at the individual molecular level to produce materials of hitherto unthought of properties. Already self-assembly systems have been described and fabricated which mimic many features of the extracellular matrix. For example, nanostructured fibrous scaffolds reminiscent of extracellular matrix can be constructed using the pH-induced self-assembly of a peptide-amphiphile. After cross-linking, the fibers are able to direct mineralization of hydroxyapatite to form a composite material in which the crystallographic c axes of hydroxyapatite are aligned with the long axes of the fibers. This alignment is the same as that observed between collagen fibrils and hydroxyapatite crystals in bone (Hartgerink *et al* 2001). Similarly, self-assembling biomaterials with molecular features designed to interact with cells and scaffolds for tissue regeneration have been reported (Hwang, *et al* 2002)

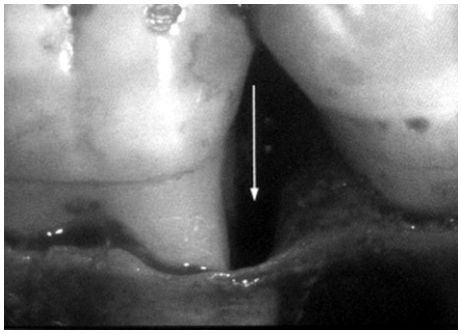

Figure 5A

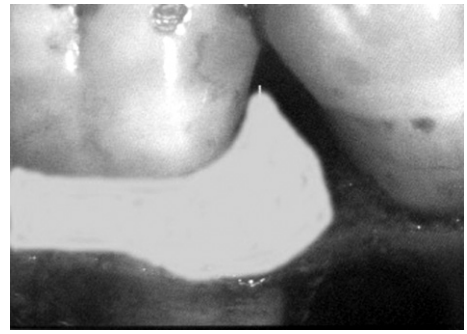

Figure 5B

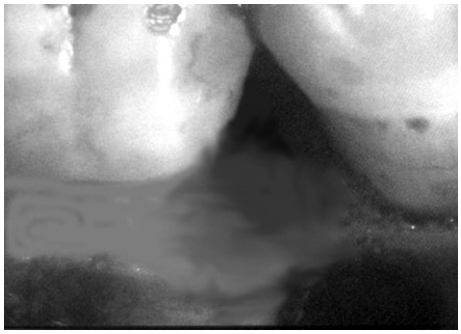

Figure 5C

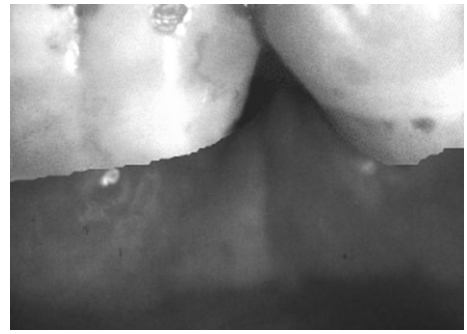

Figure 5D

Figure 5. Simulated Outcome of Periodontal Tissue Engineering.
A. Periodontal defect
B. Defect filled with tissue engineering scaffold seeded with regenerative cells
C. Scaffold and cells covered by sealing mucoperiosteal flap
D. New bone and other periodontal tissues filling original periodontal defect
Note: This figure is a computer generated schematic proposal of the outcomes of tissue engineering and does not represent a real life clinical outcome.

Conclusion

In light of the above it is clear that we as periodontists cannot achieve our goal of predictable periodontal regeneration alone. The field is so very complex that it will require the combined efforts of scientists and clinicians alike. It should be apparent that we will need to enlist the help from microbiologists to help control infection of the tissue engineered constructs and immunologists to understand how the host will react to these newly implanted materials and ensure immunologic safety. Matrix biologists, molecular biologists will be required to produce constructs of biologic significance and together with biomaterials engineers and nanotechnologists produce constructs with all of the necessary cells and instructional messages. Finally pharmacologists and toxicologists will be required to further help with the assessment of the materials and their fate within the tissues once implanted.

There is no doubt that the field of tissue engineering is a new and exciting field for periodontics. Nonetheless a large amount of

work is still required to complete this task with fundamental research being needed in cell biology, molecular biology and gene manipulation. Once the constructs have been developed, further challenges lie ahead in how to control the delivery of these agents. Surgical and anti-infective procedures will need to be developed to help protect them within the harsh environment of a donor site. How to maintain the regenerated tissues in a fully functional state will also pose special challenges. Finally, after all of the above is achieved, the last remaining hurdle will be to prove that such procedures do indeed enhance the longevity and survival rate of the treated teeth.

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Abstracts

The following is a record of the Poster Presentations held at the 5th Meeting of the Asian Pacific Society of Periodontology

Guided Bone Regeneration Technique for Optimal Implant Placement

C. Kyu-Tae
(Department of Periodontics, Samsung Medical Center, Korea)

The success of implants are influenced by the bony conditions of the recipient site. As the success rates of implants have been high in recent years, methods to modify bony conditions of the recipient site have been developed in order to install implants with proper esthetics and function. Adapting the principles of guided tissue regeneration, a guided bone regeneration (GBR) technique was developed to promote selective proliferation of osteoblasts with the insertion of a membrane. The membrane increases the proliferation of epithelial cells and fibroblasts and makes space for bone regeneration.

The indications for GBR are fenestration defects, dehiscence defects, localized ridge augmentation and immediate implant placement following extraction. The method for GBR is classified as a simultaneous and staged approach. To reduce surgery and healing times a simultaneous approach is recommended. However initial stability, prosthetic location and size of defect must be considered.

Regulation of Matrix Metalloproteinase-3 Production by Prostaglandin E_2 in Interleukin-1β-stimulated Human Gingival Fibroblasts

S.M.P.M. Ruwanpura*, K. Noguchi, I. Ishikawa
(Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan)

PGE_2 exerts its biological effects on cells through specific prostaglandin receptors, called EP_1, EP_2, EP_3 and EP_4. Matrix metalloproteinases (MMPs) including MMP-3 (stromelysin-1) are a family of zinc endopeptidases that play key roles in extra cellular matrix turnover. Recent studies have shown that gingival crevicular fluid MMP-3 levels are associated with periodontal disease progression. The aim of this study was to investigate whether PGE_2 regulated interleukin (IL)-1β-induced MMP-3 secretion in human gingival fibroblasts (HGF).

HGF were obtained from periodontally healthy and diseased gingival tissues, and designated H-HGF and P-HGF, respectively. The cells were stimulated with buffer or IL-1β in the presence or absence of indomethacin, PGE_2, 17 phenyl-trinor PGE_2 (EP_1 agonist), butaprost (EP_2 agonist) and ONO-AE1-329 (EP_4 agonist), alone or in combination. Conditioned medium was collected and analyzed for PGE_2 and for MMP-3 levels.

IL-1β induced a significant increase in MMP-3 and PGE_2 production in H-HGF. In the cells, indomethacin significantly increased IL-1β-induced MMP-3 production, whereas IL-1β-induced PGE_2 was completely inhibited. Exogenous PGE_2 inhibited IL-1β-induced MMP-3 production. The EP_2 and EP_4 agonists reduced IL-1β-induced MMP-3 secretion, whereas the EP_1 agonist increased it. On the other hand, in P-HGF, indomethacin significantly decreased IL-1β-induced MMP-3 production. In the cells, exogenous PGE_2 and the EP_1 agonist enhanced IL-1β-induced MMP-3 production, whereas EP_2 and EP_4 agonists showed little or no effect.

From these data, we suggest that IL-1β-induced MMP-3 production is differently regulated in H-HGF and P-HGF. Regulation of MMP-3 levels via EP receptors by PGE_2 in HGF may control tissue breakdown in periodontal diseases.

Recipient of Best Poster Presentation Award - First Place

Accelerated Bone Healing After Er:YAG Laser Irradiation

A. Pourzarandian*, **H. Watanabe**, **A. Aoki**, **I. Ishikawa**
(Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan)

The aim of this study was to analyze the early healing process of bone tissue irradiated by Er:YAG laser and compare it with that treated by mechanical drilling and CO_2 laser. Er:YAG laser has a potential for hard tissue cutting due to its high ability of ablation and less thermal damage.

Twenty-four male Wistar rats were subjects in this study. The calvarial bone of rats was exposed and straight grooves were prepared by Er:YAG laser, CO_2 laser and mechanical drill. Four rats were sacrificed at each of six time intervals: 10 minutes, 6 and 24 hours, 3, 7, and 14 days post-surgery and sections were prepared for light and transmission electron microscope (TEM) observation.

Unlike the mechanical bur and CO_2 groups, early inflammatory cell infiltration adjacent to the irradiated bone surface increased fibroblastic reaction, and revascularization were more pronounced in the Er:YAG laser irradiated tissues. A cell-rich granulation tissue with fibroblasts and osteoblasts was predominant in Er:YAG 7-day specimens. Histopathological analysis of 14-day specimens in Er:YAG group also exhibited significantly higher new bone formation compared with the CO_2 laser and the mechanical bur groups ($p<0.001$). The early bone healing process following Er:YAG laser was found to be superior than that of CO_2 laser and mechanical bur. No adverse effects were observed on the bone tissue after Er:YAG laser irradiation. The present study indicates osseous resection with Er:YAG laser may be more advantageous compared with a mechanical bur or CO_2 laser.

Recipient of Best Poster Presentation Award - Second Place

Periodontal Mesenchymal Cells Differentiation by Porcine Enamel Extracts

T. Nagano[1]*, S. Oida[1], T. Iwata[2], S. Suzuki[1], T. Tanabe[1], Y. Ogata[3], K. Gomi[1], M. Fukae[1], T. Arai

([1]Tsurumi University, Japan, [2]Graduate School, Tokyo Medical Dental University, Japan, [3]Nihon University, Japan)

The purpose of this study was to investigate osteogenic factors in fractionated porcine enamel proteins. Porcine enamel proteins were separated into 4 fractions (fr.1 - fr.4) by gel filtration chromatography. These four fractions, as well as exogenous growth factors (BMP-2 and TGF-β1) were tested for osteogenic activities on human periodontal ligament (HPDL) cells in vitro. Osteogenic activities were assessed by alkaline phosphatase (ALP) activitiy, mitogenic assay, arizarin red staining, calcium content assay and RT-PCR for osteogenic marker proteins. In HPDL cells, osteoinductive activities was enhanced by TGF-β1 and fr.3 and ALP activity was blocked in both cases by anti-TGF-β antibody. Furthermore, we showed using a dual-luciferase reporter assay that the plasminogen activator inhibitor type-I (PAI-1) promoter activity, which was normally up-regulated by TGF-β stimulation was induced by fr.3. These results indicate that the enamel matrix contains TGF-β which may control the periodontal mesenchymal cells differentiation.

Evaluation of Alveolar Bone Resorption Among Different Fimbriae Type *Porphyromonas Gingivalis* Infection in Hamsters

N. Shibukawa*, D. Kato, N. Maeda, T. Arai
(Tsurumi University, Yokohama, Japan)

Porphyromonas gingivalis, one of the causative bacteria for periodontitis, has many virulence-associated factors, including fimbriae, hemagglutinin, protease and lipopolusaccharides. Recently, fimbriae genes (*fin A*) were classified into 5 different types (Type I-V).

The purpose of this study was to determine the distribution of *P. gingivalis* which had different fimbrae genes in periodontitis patients and compare alveolar bone resorption on the animal model among the different fimbriae types. Type II fimbriae was the most common, followed by Type IV. The activity of alveolar bone resorption on the animal model in Type I had the highest levels. These results suggest that the pathogenicity of *P. gingivalis* differs in the isolates but not in the fimbriae types.

PCR Detection of Selected Periodontal Pathogens in Filipinos With Chronic Periodontitis

S.E. Poco*, F. Nakazawa, M.C. Magno-Dino, E. Hoshino
(Niigata University, Faculty of Dentistry, Japan)

The aim of this study was to identify the culture-different strains of a recently proposed assaccharolytic anaerobic gram-positive rod (AAGPR), *Mogibacterium*, by specific PCR primers which were designed based on the sequence analysis of their 16S rDNA. Out of the 41 primer combinations examined, two were found to be genus-specific for *Mogibacterium* (247F-567R and 247F-809R) and produced a band with an appropriate size of ca 300 bases and 550 bases respectively when used with the DNA of the 5 *Mogibacterium* species but not with other AAGPR species. Thirteen (40.6%) of the 32 subgingival plaque samples from 10 chronic periodontitis patients (1st time visit, pocket depth > 6 mm) yielded positive to *Mogibacterium* by the developed PCR method. *P. gingivalis* was detected in 87.5% of the subgingival plaque samples while *A. actinomycetemcomitans* was detected in 37.5% of samples.

Recipient of Best Poster Presentation Award - Third Place

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

N. Surathu[1], A. Deshpande[1], D. Arunachalam[2]*, S. Gunasekaran[3], V. Rayapati[3]
[1] Department of Periodontics, Saveetha Dental College and Hospitals, Chennai, India, [2] KGF Institute of Dental Sciences, Karnataka, India, [3] Encoll Corporation, USA and Advanced Biotech Products, India

The importance of having a stable osseointegrated environment around an implant is critical to implant longevity and success. The quantum of available bone is but one aspect of this requirement due to implants making functional demands on the bone which support them. This naturally demands a certain quality of bone as well.

In scenarios where there is a deficiency in bone or the quality of bone is suspect even if the quantum seems adequate the periodontist is faced with a challenging situation. Occasionally the periodontist may have to consider implant placement secondary to osseous regenerative procedures or even abort an implant treatment plan.

Several solutions have been offered over the years with regards to osseous grafting. The identification of successful techniques and materials have remained elusive at best and the periodontist has gambled with several solutions. Consistent predictability and treatment longevity have defied many of these attempts and we have sought better alternatives on the basis of our understanding of bone healing for some time now. Since Hegedus first placed an autograft in 1924 we have come full circle and are again examining options in autogenous grafting as potential solutions for predictable regenerative therapy. A detailed grasp of the cascade of biochemical events that contribute to wound healing and regeneration has also refocused interest on some of the techniques that evolve autogenous bone. In addition, the advent of tissue engineering has contributed to accelerating the regenerative process as well. At last we seem to be getting closer to our goal, by an effective clinical technique combination, that harnesses these advances.

This presentation will focus on the success of autogenous grafting in combination with the use of a new demineralized bone matrix (Osseograft DMBM) and a new bioactive collagen membrane (Healiguide) for use in implantology. A few case reports are presented to illustrate the effective use of these materials.

An Assessment of the Use of a New Bioactive Resorbable Collagen Membrane & Demineralized Bone in the Treatment of Class II Furcation Defects: A Comparative Clinical Study

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Collagen has found innumerable applications in dentistry in the form of membranes, gels, fibrillar local drug delivery devices and extraction socket implantable devices. The use of collagen in guided tissue regeneration, particularly because of its resorbability, has been extensively documented. Studies have also demonstrated that the adjunctive use of osseous grafts in guided tissue regeneration may be an advantage.

The present study assessed the efficacy of use of a new bioactive collagen membrane (Healiguide) which is claimed to enhance the pace and quality of the regenerative process, in comparison to a commercially available membrane (BioMend, Centerpulse, USA) in the treatment of Class II periodontal furcation defects. The study also assessed a new osseous xenograft consisting of demineralized bone (Osseograft DMBM) in the treatment of Class II periodontal furcation defects.

Results at 12 months indicated that there were significant probing depth reductions, from baseline, for the control group and all three tests ($P<0.007$ - Group 1, $P<0.005$ - Group II, $P<0.005$ - Group III and $P<0.005$ - Group IV), however the differences between groups were not significant. Changes in gained attachment level were also significant in all groups whilst not being significant between groups.

The results suggest that guided tissue regeneration using collagen membranes is an effective technique for the treatment of Class II furcation defects. The use of bioactive membranes may however accelerate the regenerative process and offer an additional advantage over currently available commercial collagen membranes. The additional use of xenogeneic osseous graft, while not significantly affecting treatment outcomes, is nevertheless recommended.