PERIODONTICS: BEYOND THE POCKET

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Edited by
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KM. Chung

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Acknowledgements

The 8th International meeting of the Asian Pacific Society of Periodontology was held in Singapore from 29-30 August 2009, immediately preceding the FDI Annual World Dental Congress. The theme for the meeting was "Beyond the Pocket".

Following the opening address by Dr Hui Chee Wah (Chairman of the local organizing committee), over 170 delegates were treated to presentations from renowned experts from 14 countries. In addition, 29 posters were scheduled for presentation. As in the past, awards were made for the best poster presentations and while there were only three winners, the quality of all the posters was of a very high standard.

This volume serves as a record of selected presentations made at this meeting. I am sure you will agree with me that the quality of the material presented is not only very interesting but represents many "cutting edge" concepts and represents an overview of periodontics which is certainly "Beyond the Pocket".

The generous support of our Gold Sponsors: Colgate Oral Care, Johnson & Johnson, Procter & Gamble and Sunstar, in conjunction with the Silver Sponsors: Ai Stoma, Biomet 3i , Nobel Biocare and Straumann is very gratefully acknowledged. Without this important financial support the 8th APSP meeting and the publication of these proceedings would not have been possible. I would like to acknowledge the assistance of my co-editor Dr Chung Kong Mun. As in previous years I also thank the presenters for providing their manuscripts for publication. Finally this publication would not have eventuated had it not been for the excellent and efficient production editing of Ms Catherine Offler.

P. Mark Bartold
May 2010

Invited Participants

Invited Speakers at the 8th Meeting of the Asian Pacific Society of Periodontology:
Top row L to R: Prof I Ishikawa (Japan), Prof PM Bartold (Australia)
Bottom row L to R: Dr C-K Kim (Korea), Dr WC Tan (Singapore), Dr S Ciancio (USA), Dr N Laosrisin (Thailand), Prof E Corbett (Hong Kong), Prof NP Lang (Hong Kong), Dr L Jin (Hong Kong), Dr R Longbottom (New Zealand), Dr J-C Zhang (China), Dr T Van Dyke (USA), Dr T Taiyeb Ali (Malaysia), Dr K Gomi (Japan), Dr R Burkhardt (Switzerland)

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Chapter 1

Ultrasonic devices: Mission possible for subgingival debridement

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Introduction

The primary principle of periodontal therapy is based on constant and good oral hygiene by the patient and adequate mechanical debridement performed by the dentist. Scaling and root planing of periodontal pockets is the most common therapy for periodontitis. However, this treatment modality is time consuming, demands a high level of operator skill and is unpleasant for some patients (Kinane 2005). Furthermore, conventional scaling and root planing is performed in a quadrant or sextant-wise manner with a gap of 1 or 2 weeks between appointments, facilitating the recolonization of treated areas by pathogens residing in untreated pockets and other extra-dental sites (Quirynen and Bollen 1995).

The popularity of conventional scaling of supragingival calculus by hand instruments has declined since it was recognized that calculus can be easily removed by ultrasonic devices. Patients often feel pain during hand instrumentation. The anticipation of pain during treatment or past experiences of pain cause fear and result in avoidance of further visits to the dentist (Kleinknecht *et al* 1973). New ultrasonic instruments and various designs of insert tips are now more commonly used to remove subgingival calcified deposits.

Piezoelectric ultrasonic devices work best at a frequency of 20,000 - 35,000 Hz. This is thought to be more pleasant for the patient, since instead of hammering on the tooth surface, the instrument tip scrapes along it. The reduction of pain during treatment results in greater patient compliance with periodontal treatment and prophylaxis and a better long-term prognosis for maintaining a healthy periodontal status.

The conventional protocol for undertaking mechanical debridement is a quadrant-wise approach employing hand instruments, perhaps supplemented by ultrasonic scalers. Quirynen and colleagues (1995) introduced a modified protocol involving one-stage full-mouth disinfection (FMD), which has been evaluated in a series of studies (Vandekerckhove *et al* 1996, Bollen *et al* 1998, Mongardini *et al* 1999). A later report indicated that this full-mouth treatment approach resulted in superior clinical outcomes and microbiological effects than clockwise quadrant scaling and root planing, irrespective of the adjunctive use of chlorhexidine (Quirynen *et al* 2000). Several studies involving aspects of this protocol have been systematically reviewed. These studies differed, however, in that some involved antiseptic use and some allowed for repeated debridement. Other studies, on the other hand,

failed to demonstrate an advantage of full-mouth scaling (FMS) within a 24 hour time period versus quadrant scaling over a longer time period (Apatzidou and Kinane 2004, Koshy *et al* 2005, Wennström *et al* 2005, Jervøe-Storm *et al* 2006, Quirynen *et al* 2006, Zanatta *et al* 2006). It can be concluded that full-mouth debridement, without antiseptic use, is no less effective than the conventional quadrant-wise protocol, and thus clinicians and patients should feel free to choose which debridement procedure to adopt. A full-mouth approach provides potential savings in patient travel, waiting and treatment times to achieve similar results to the conventional protocol. However, it must be recognized that the outcomes of mechanical debridement depend greatly on the patient oral hygiene practices.

Root surface topography after ultrasonic debridement by SEM study

Adequate root debridement in the treatment of periodontal disease, involving mechanical instrumentation to remove plaque, calculus, and contaminated cementum and dentin, has been proved to be very necessary (Aleo *et al* 1974, Ruben and Shapiro 1978, Waerhaug 1978, O'Leary and Kafrawy 1983, McCoy *et al* 1987). This includes not only procedures aimed at removing dental plaque and calculus from the root surface, but also removing a portion of the root surface itself (Clark *et al* 1968, Wilkinson and Maybury 1973, Garrett 1977, D'Silva *et al* 1979, Lie and Leknes 1985, Coldiron *et al* 1990, Ritz *et al* 1991, Zappa *et al* 1991). Other important considerations in periodontal treatment also include the amount of root surface removed and the roughness of the residual root surface after instrumentation (Lie and Meyer 1977, Ritz *et al* 1991). Gentle mechanical preparation of the root surface appears to be more critical when the surgical techniques are associated with gingival attachment or

advanced regenerative periodontal procedures (Yukna 1992, Trombelli *et al* 1994).

Root surface instrumentation with hand instruments is often difficult and time consuming and requires a substantial amount of physical effort. In addition to hand instruments, various powered instruments are available including sonic and ultrasonic scalers and rotary instruments (Clark *et al* 1968, Lie and Leknes 1985, Ritz *et al* 1991). Several studies evaluated the amount of tooth structure removed mechanically by hand scalers, ultrasonic scalers and air abrasives (Coldiron *et al* 1990, Ritz *et al* 1991, Zappa *et al* 1991, Clark *et al* 1968, Wilkinson and Maybury 1973, Van Volkinburg *et al* 1976, Ritz *et al* 1991, Berkstein *et al* 1987).

Each of these type of instruments have some disadvantages, ranging from decreased tactile sensitivity, uncontrolled damage to the root surface and inadequate edge retention (Allen and Rhoads 1963, Moskow and Bressman 1964, Belting and Spjut 1964, Bye *et al* 1986, Berkstein *et al* 1987, Coldiron *et al* 1990). While all instruments have shown clinical effectiveness, none have proven the most effective in typical clinical situations where short application times are required to remove plaque, calculus, and diseased tooth structure while leaving a relatively smooth root surface (Moskow and Bressman 1964, Jones and O'Leary 1978, Bye *et al* 1986, Coldiron *et al* 1990, Jotikasthira *et al* 1992).

For ultrasonic devices, insert tip design is particularly important. A variety of inserts are designed for area specific shapes and surfaces, of which plain metal and diamond coated surface inserts have been preferentially used in scaling and planing of superficial root surfaces. Diamond-coated ultrasonic inserts have been designed to enhance the speed and efficiency of mechanical root preparation and have been shown to be significantly faster in calculus removal in furcations *in vitro* and periodontal pockets *in vivo* (Scott *et al* 1999,
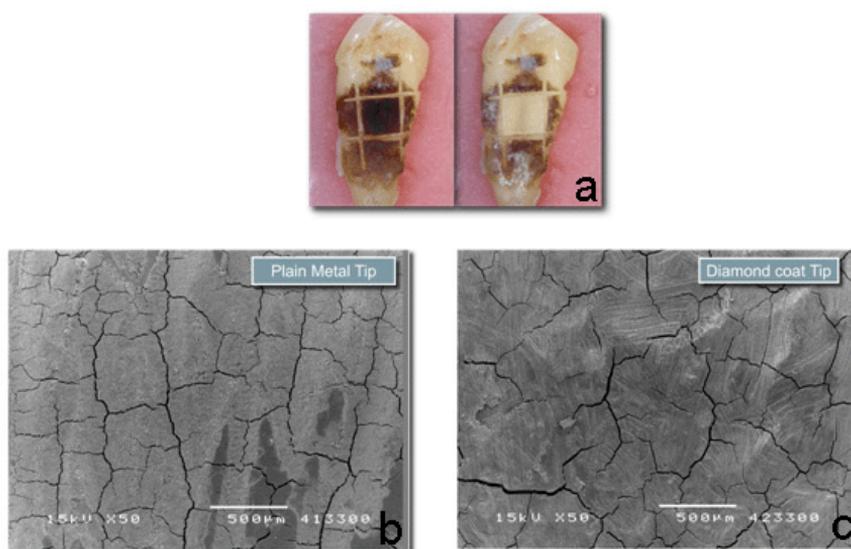
Figure 1. (a) Sub-gingival calculus area in each tooth marked in 3x3 mm, typical SEM image of residual calculus specimen following calculus removal of insert groups in (b) plain metal and (c) diamond-coated.

Yukna *et al* 1997). Numerous studies have investigated root surface pattern on scanning electron microscopy (SEM) following root planning with plain metal and diamond coated ultrasonic insert tips. Lavespere *et al* (1996) showed that use of diamond-coated ultrasonic inserts resulted in greater surface removal and larger residual root surface roughness compared with similarly shaped regular ultrasonic inserts *in vitro*. The microscopic nature of the residual root surface following the use of diamond-coated ultrasonic tips performed by magnetostrictive and by piezoelectric ultrasonic device were reported recently (Vastardis *et al* 2005, Yukna *et al* 2007, Wongsasuluk and Laosrisin 2008). No differences in residual calculus levels have been reported between diamond-coated ultrasonic tips for magnetostrictive usage comparing with piezoelectric ultrasonic devices. The average percentage of residual calculus was 6.3% for diamond-coated inserts and 5.4% for plain metal inserts with no significant difference between the two inserts. However, there was a statistically significant difference in mean time required for scaling,

29.7 seconds for the diamond-coated insert and 91.9 seconds for plain metal inserts (Yukna *et al* 2007).

The effectiveness of diamond-coated and plain metal piezoelectric ultrasonic inserts in calculus removal and effect on various tooth structures have also been compared by Wongsasuluk and Laosrisin (2008). Six periodontal involved teeth were used for evaluating the effectiveness of calculus removal. Sub-gingival calculus areas of 3 mm x 3 mm on each tooth were marked and then randomly debrided either by plain metal or diamond-coated inserts until visually clean. The residual calculus in each area of both insert groups was determined (Figures 1a, b and c). The effectiveness of calculus removal was less in the plain metal group than the diamond-coated group and the time taken was greater (Table 1).

Nine non-periodontal involved teeth extracted for orthodontic purposes were used for evaluating the effect on various tooth structural surfaces. Buccal and lingual surfaces of roots were randomly selected for treatment by the plain metal or diamond-

Insert	Test area (mm2)	Residual Calc area (mm2)	Residual Calc %	Time (sec)	Time/Area (sec/mm2)
Plain metal					
Specimen 1	8.47	1056×10^{-6}	0.012	55.00	6.49
Specimen 2	10.30	1078×10^{-6}	0.015	110.00	10.68
Specimen 3	12.70	458×10^{-6}	0.004	119.00	9.37
Average			0.010		8.85
Diamond coated					
Specimen 1	8.21	2357×10^{-6}	0.029	174.00	21.19
Specimen 2	7.42	3081×10^{-6}	0.042	129.00	17.39
Specimen 3	15.50	3902×10^{-6}	0.025	150.00	9.68
Average			0.032		16.09

Table 1. Mean results for calculus removal effectiveness according to residual calculus per area and time taken by plain metal group and diamond-coated group.

coated group, and divided into areas of 10, 20 and 30 strokes respectively. Each surface was treated either by plain metal or diamond-coated inserts. The effect on the periodontal ligament (Figures 2a and b), cementum (Figures 3b and c) and dentine (Figures 4b and c) surfaces of both inserts in different stroke quantities was determined by soft tissue damage, surface roughness or scratch, and the thickness of remaining cementum was measured by SEM. Periodontal tissue damage and surface roughness or scratch were observed in the diamond-coated group but not in the plain metal group. Finally, the thickness of remaining cementum was slightly greater in the plain metal group than in the diamond-coated group (Figure 5, Table 2). All effects were increasingly observed according to the increasing number of planing strokes. These results indicate the effectiveness of plain metal inserts in ultrasonic root planing on extracted root surfaces.

Subgingival ultrasonic debridement as a therapeutic approach for severe chronic periodontitis: A clinical, microbiological and OPG study

The concept of periodontal debridement was reported by Smart *et al* (1990) as a conservative instrumentation regime of overlapping strokes and light pressure utilized for only a limited time period. The main goal here is to obtain a biocompatible root surface. Intentional removal of tooth structure is not a requirement for periodontal healing and regeneration, as bacterial lipopolysaccharide (LPS) is easily removed from the root surface leading to the development of the full mouth ultrasonic debridement protocol (Nyman *et al* 1986, Goncalves *et al* 2006, Hughes and Smales 1986, Moore *et al* 1986).

An alternative approach to treating chronic periodontitis by diminishing bacterial recolonization in treated sites has been
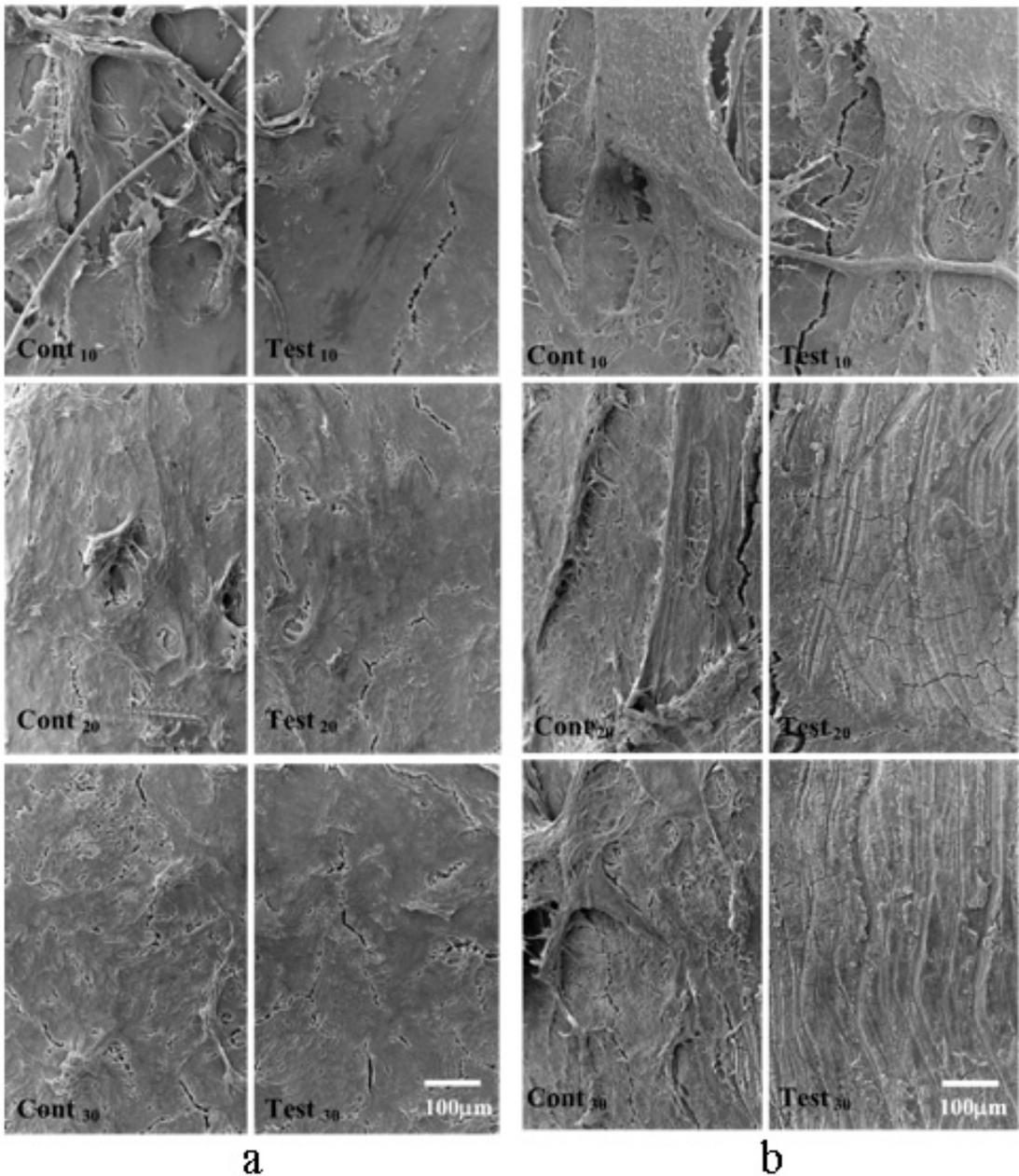
Figure 2. (a) SEM image of the periodontal ligament before (left) and following planing with plain metal inserts (right) at 10, 20 and 30 strokes, (b) SEM image of the periodontal ligament before (left) and following planing with diamond-coated inserts (right) at 10, 20 and 30 strokes.
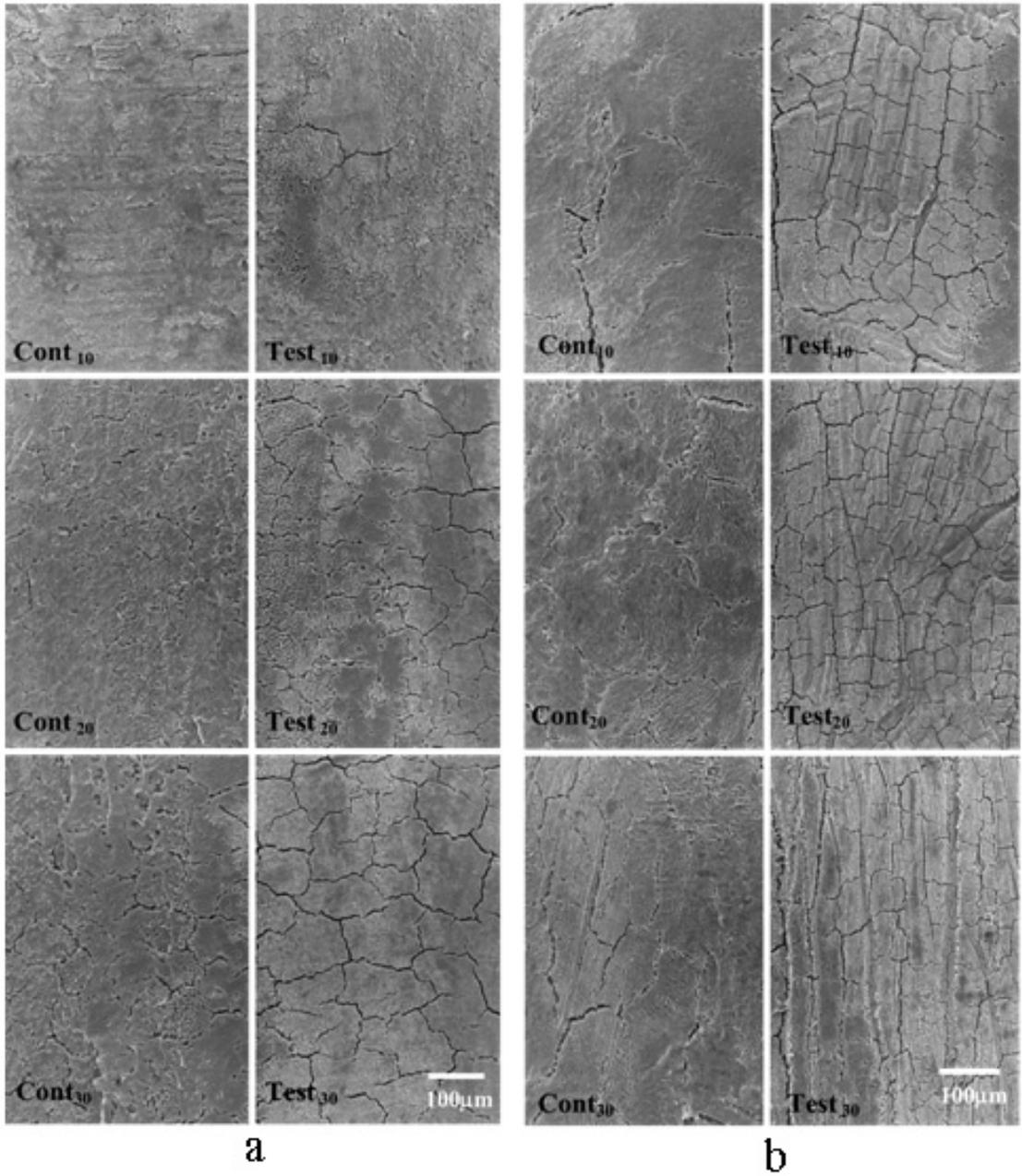
Figure 3. (a) SEM image of cementum before (left) and following planing with plain metal inserts (right) at 10, 20 and 30 strokes, (b) SEM image of cementum before (left) and following planing with diamond-coated inserts (right) at 10, 20 and 30 strokes.
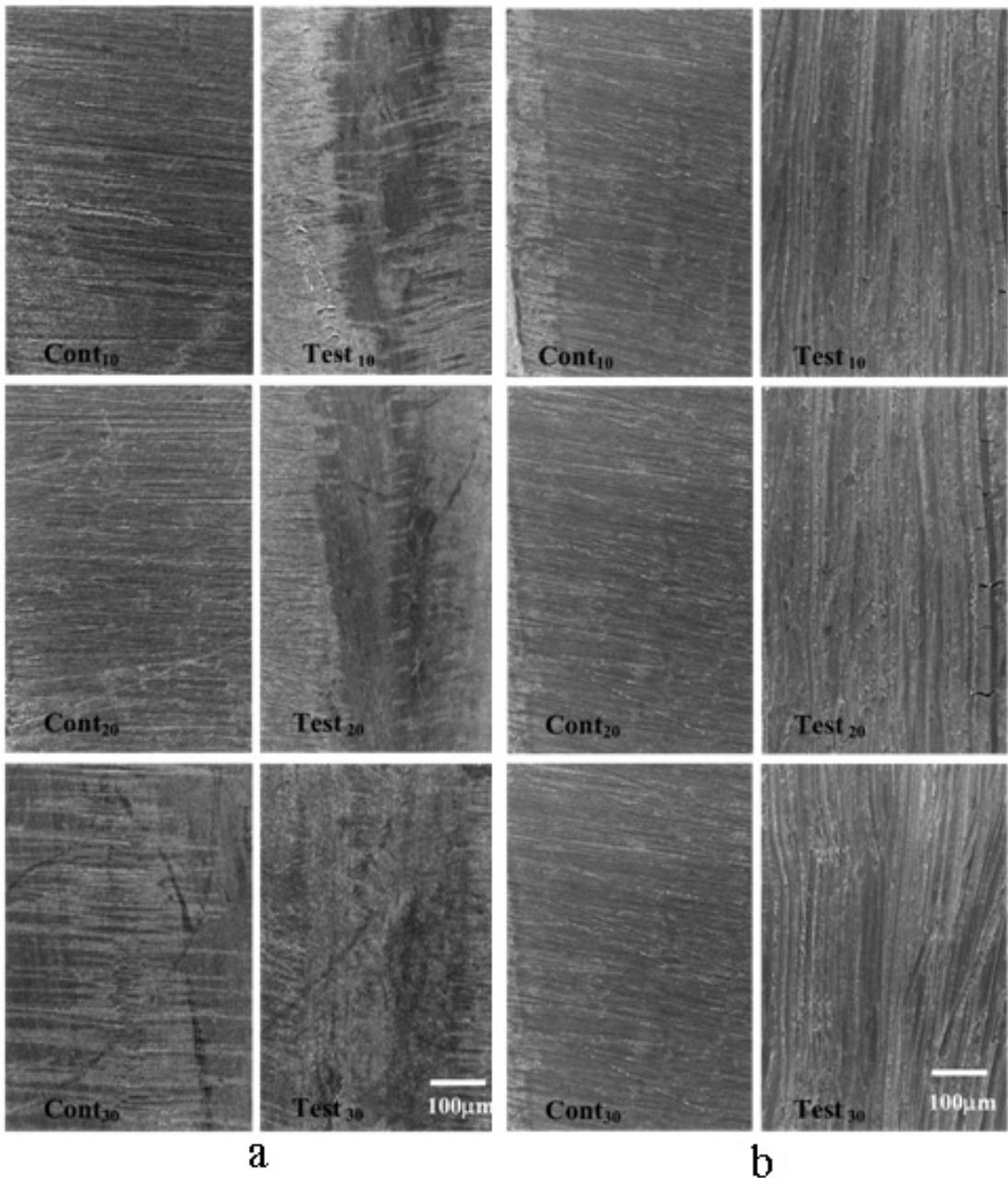
Figure 4. (a) SEM image of dentine before (left) and following planing with plain metal inserts (right) at 10, 20, and 30 strokes, (b) SEM image of dentine before (left) and following planing with diamond-coated inserts (right) at 10, 20, and 30 strokes.
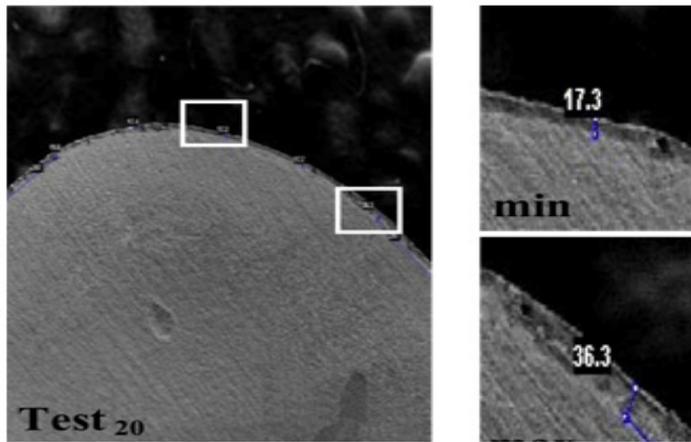
Figure 5. SEM image of the thickness of remaining cementum following planing with plain metal inserts (right) at 20 strokes.

Insert	10 strokes			20 strokes			30 strokes		
	Control	Test	Difference (%)	Control	Test	Difference (%)	Control	Test	Difference (%)
Plain metal									
Specimen 1	34.14	32.00	2.14 (6.27)	28.22	25.38	2.84 (10.06)	23.40	20.14	3.26 (13.93)
Specimen 2	37.16	31.96	5.18 (13.94)	25.70	21.70	4.00 (15.56)	33.30	27.48	5.82 (17.48)
Specimen 3	24.02	22.22	1.80 (7.49)	25.34	21.32	4.02 (15.86)	22.58	18.82	3.76 (16.65)
Average	31.77	28.73	3.04 (9.57)	26.42	22.79	3.63 (13.74)	26.43	22.15	4.28 (16.19)
Diamond coated									
Specimen 1	27.54	22.20	5.34 (19.39)	31.32	24.10	7.22 (23.05)	27.14	16.40	10.74 (39.57)
Specimen 2	39.70	27.44	12.26 (30.88)	36.14	24.90	11.1 (31.10)	38.66	26.30	12.36 (31.97)
Specimen 3	26.36	17.42	8.94 (33.92)	26.38	16.90	9.48 (35.94)	29.40	16.70	12.70 (43.20)
Average	31.20	22.35	8.85 (28.37)	31.28	21.97	9.31 (29.76)	31.73	19.80	11.93 (37.60)

Table 2. Mean results for thickness of remaining cementum (μm) for the plain metal and diamond-coated insert groups after 10, 20 and 30 strokes.

proposed (Quirynen and Bollen 1995). Fundamentally, a full-mouth disinfection (FMD) approach consisting of scaling and root planing of all pockets in two visits within 24 hours in conjunction with adjunctive chlorhexidine treatment was proposed. This approach was subsequently evaluated in a series of studies by the same research group (Vandekerckhove *et al* 1996, Bollen *et al* 1998, Mongardini *et al* 1999). A later report indicated that this full-mouth treatment approach resulted in superior clinical outcomes and microbiological effects than clockwise quadrant scaling and root planing, irrespective of the adjunctive use of chlorhexidine (Quirynen *et al* 2000). More recent studies, however, failed to demonstrate any advantage of full-mouth scaling (FMS) within 24 hours versus quadrant scaling (Apatzidou and Kinane 2004, Koshy *et al* 2005, Wennstrom *et al* 2005, Jervøe-Storm *et al* 2006, Quirynen *et al* 2006, Zanatta *et al* 2006).

It has been observed that the completion of scaling and root planing within a short time frame seems to have a beneficial effect in the treatment of moderate and severe periodontitis (Quirynen *et al* 2006). The effect of this approach on the quantity of periodontopathogens and on host-immune inflammatory response in comparison with the standard procedure has been examined (Del Peloso Ribeiro *et al* 2008). One session of full-mouth periodontal debridement with a time limit of 45 minutes in the test group resulted in a similar clinical, microbiological and immunological outcomes when compared to standard scaling and root planing and therefore may be assumed as a viable approach to deal with severe chronic periodontitis.

Recently, a systematic review has been conducted to address the issue of full-mouth treatment concepts, which may have great influence on clinical practice (Eberhard *et al* 2008). This review aimed to compare the clinical effects of conventional scaling and root planing by hand curette and FMD and FMS approaches for the treatment of chronic periodontitis. It showed that the FMD approach resulted in a modest additional reduction of probing depth compared with the conventional treatment for sites with an initial probing depth of 5-6 mm in single-rooted teeth. It may be questioned whether this minor difference in results can justify the extensive use of chlorhexidine over a period of several months. All three interventions can result in improvements in clinical treatment of periodontitis. Additional improvements from FMD are inconsistent across tooth types and initial pocket depths. Therefore, no recommendation regarding additional benefits can be made on the basis of clinical data to date. The decision to choose one nonsurgical periodontal therapy over another should involve patient preference and convenience of the treatment schedule.

Chlorhexidine is not the only antiseptic solution that can control subgingival biofilm. The use of several kinds of antiseptic solution as an adjunct to ultrasonic debridement has received great consideration. Essential oils which have been shown to possess an anti-microbial effect may be a potential candidate for such treatment. The clinical safety and efficacy of essential oil solutions used as coolant during piezoelectric root debridement in chronic periodontitis patients was investigated (Thongsiri and Laosrisin 2008). Eighty chronic periodontitis patients presenting with at least four teeth with a probing pocket depth of ≥ 5 mm and bleeding on probing were selected and randomly assigned to groups using either essential oil solution or water as a coolant during ultrasonic full mouth debridement. Clinical safety and outcomes, including probing depth, clinical attachment level, bleeding on probing, plaque index, and gingival inflammation index were assessed. Real-time PCR was used for

| | Average depth | | Initial pocket depth group | | | |
| | | | 5-6 mm | | > 6 mm | |
	control (n=80)	test (n=80)	control (n=51)	test (n=47)	control (n=29)	test (n=33)
Baseline	6.06 ± 1.13	6.23 ± 1.20	5.35 ± 0.48	5.32 ± 0.47	7.38 ± 0.49	7.52 ± 0.57
6 weeks	3.80 ± 1.24	4.10 ± 1.18	3.45 ± 1.08	3.62 ± 0.97	4.41 ± 1.27	4.79 ± 1.11
12 weeks	3.58 ± 1.19*	3.77 ± 1.18*	3.16 ± 0.97*	3.21 ± 0.91*	4.31 ± 1.20*	4.58 ± 1.06*

Table 3. Mean and standard deviation of probing depth (PD) before and after treatment.
* Statistically significant differences from repeated measures ANOVA
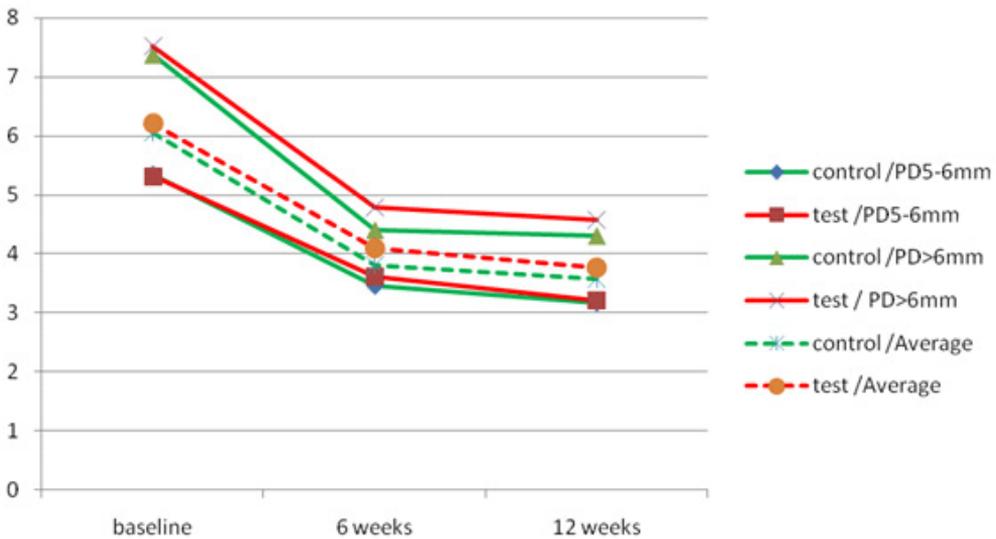
Figure 6. Pocket depth (PD) at various examination intervals

quantitative analysis of *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola*. Enzyme-linked immunosorbent assay permitted the detection of osteoprotegerin (OPG) in gingival crevicular fluid (GCF). All parameters were evaluated at baseline and at 3 and 6 months after treatment.

The clinical safety test revealed that there were no significant adverse effects of essential oil solution when used as coolant during root debridement. Significant reductions in mean probing depths (Figure 6, Table 3), mean clinical attachment levels (Figure 7, Table 4), and mean BOP were noted in weeks 6 and 12 when compared to the baseline values in both groups. However, the differences of clinical outcomes between the two study groups were not statistically significant.

In both groups the detection frequencies of *P. gingivalis, T. forsythia and T. denticola* were markedly decreased in week 6 but slightly rebounded at week 12 when compared to the baseline (Table 5). In addition, the levels of *P. gingivalis, T. forsythia and T. denticola* (Figures 8 - 10) in the test group were

| | Average depth | | | Initial pocket depth group | | |
| | | | | 5-6 mm | | > 6 mm |
	control (n=80)	test (n=80)	control (n=51)	test (n=47)	control (n=29)	test (n=33)
Baseline	6.37 ± 1.82	6.68 ± 2.09	5.47 ± 1.35	5.64 ± 1.58	8.03 ± 1.21	8.15 ± 1.84
6 weeks	4.84 ± 1.95	5.24 ± 2.03	4.22 ± 1.76	4.57 ± 1.89	5.93 ± 1.79	6.18 ± 1.88
12 weeks	4.66 ± 1.97*	4.93 ± 2.07*	4.02 ± 1.63*	4.26 ± 1.79*	5.79 ± 2.04*	5.91 ± 2.08*

Table 4. Mean and standard deviation of clinical attachment level (CAL) before and after treatment.
* Statistically significant differences from repeated measures ANOVA
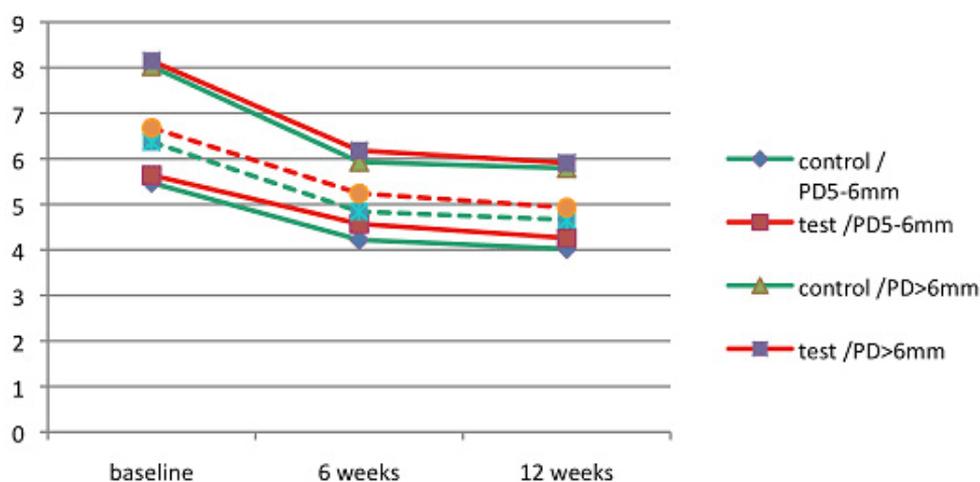
Figure 7. Clinical attachment levels (CAL) at various examination intervals

significantly lower than in the control group (p <0.05). The median value of OPG concentration in GCF were significantly increased from 1.91 (pg/µl) at baseline to 3.29 (pg/µl) and 3.55 (pg/µl) after 6 and 12 weeks in the experimental test group (p <0.05). No significant difference was observed the in control group (Figure 11) (Sirisoontorn and Laosrisin 2009).

Within the limits of this study, it can be concluded that the use of essential oil solution as coolant during piezoelectric root debridement was safe and significantly

reduced the number of *P. gingivalis, T. forsythia* and *T. denticola* but did not result in a significant change in clinical parameters when compared to water. This treatment protocol has potential as an alternative strategy to improve periodontal health due to increased OPG concentration in GCF.

Discussion and conclusion

Periodontal therapy with up to date ultrasonic devices directly affects the environment of the tooth. The efficiency of a

| | | *P. ging* N(%) | | *T. forsyth* N(%) | | *T. dent* N(%) | |
		control (n=80)	test (n=80)	control (n=80)	test (n=80)	control (n=80)	test (n=80)
Baseline	*Detectable*	80(100)	78(97.5)	71(88.75)	74(92.5)	52(65)	55(68.75)
	Undetectable	0(0)	2(2.5)	9(11.25)	6(7.50)	28(35)	25(31.25)
6 weeks	*Detectable*	68(85)	55(68.75)	37(46.25)	59(73.75)	17(21.25)	34(42.5)
	Undetectable	12(15)	25(31.25)	43(53.75)	21(26.25)	63(78.75)	46(57.5)
12 weeks	*Detectable*	73(91.25)	67(83.75)	46(57.5)	58(72.5)	25(31.25)	31(38.75)
	Undetectable	7 (8.75)	13(16.25)	34(42.5)	22(27.5)	55(68.75)	49(61.25)

Table 5. Number of *Porphyromonas gingivalis, , Tannerella forsythia* and *Treponema denticola* detected sites in control and test group at times of examination
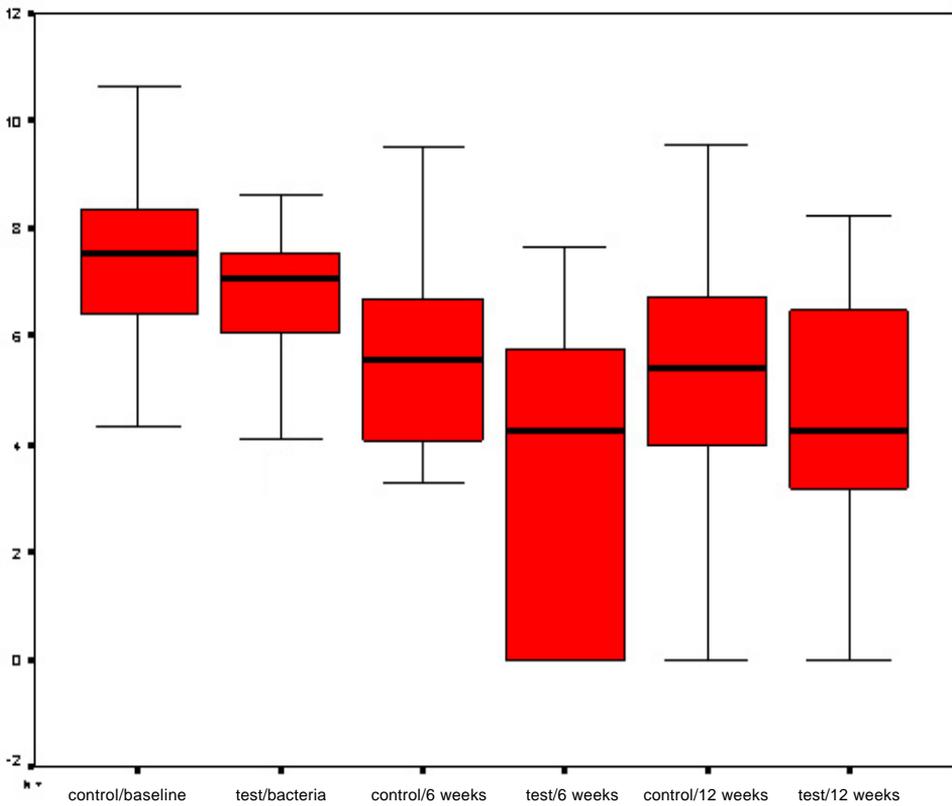
Figure 8. Box plot graph of number DNA of *Porphyromonas gingivalis* (Log_N) at various intervals
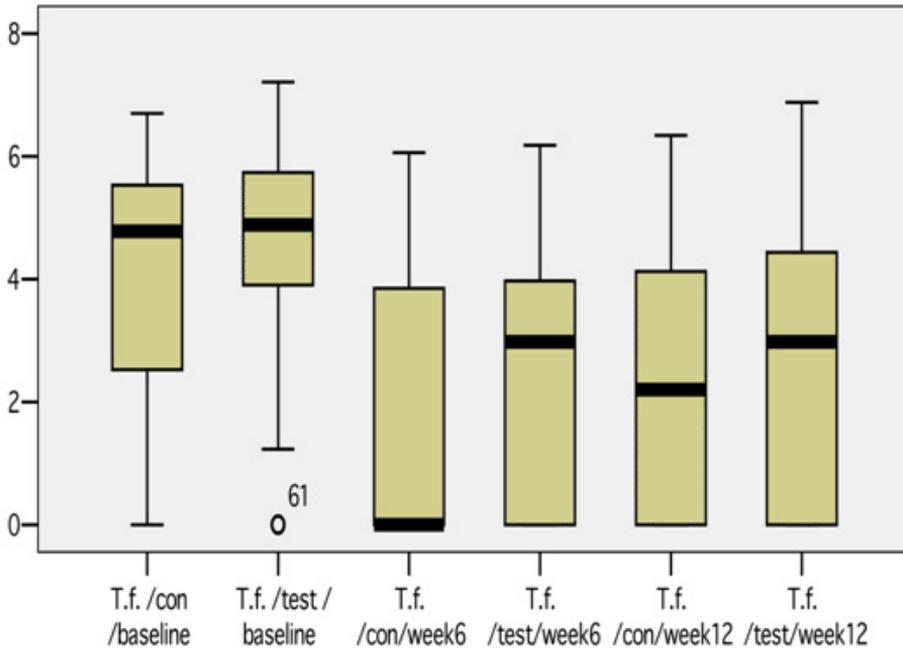
Figure 9. Box plot graph of number of DNA of *Tannerella forsythia* (Log_N) at various intervals
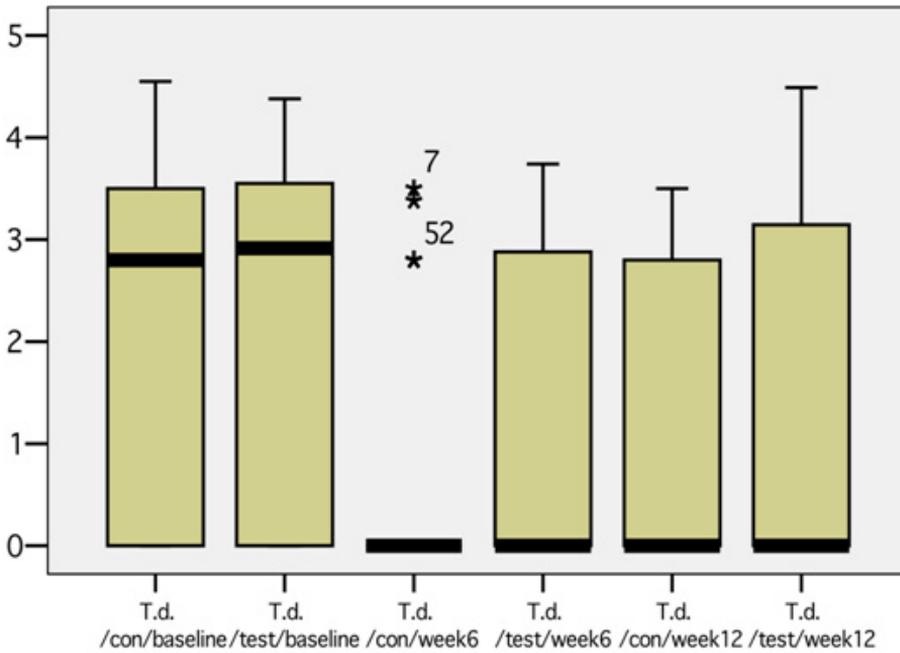
Figure 10. Box plot graph of number of DNA of *Treponema denticola* (Log_N) at various intervals

| | Average depth Mean±SD | | Initial pocket depth group | | | |
| | | | 5-6 mm Mean±SD | | > 6 mm Mean±SD | |
	control (n=60)	test (n=60)	control (n=36)	test (n=34)	control (n=24)	test (n=26)
Baseline	3.08 ±3.01	2.25 ±2.31	3.03 ±3.03	2.08 ±2.11	3.16 ±3.05	2.47 ±2.58
6 weeks	2.25 ±2.37	2.27 ±1.74	2.47 ±2.52	2.40 ±1.63	2.10 ±2.24	2.10 ±1.89
12 weeks	1.83 ±1.95	1.94 ±1.91	1.99 ±1.97	2.66 ±1.90	1.59 ±1.94	1.01 ±1.48

**Table 6.** Means and standard deviation of OPG level of control and test group depend on the depth of pocket at times of examination

| | Average depth Mean±SD | | Initial pocket depth group | | | |
| | | | 5-6 mm Mean±SD | | > 6 mm Mean±SD | |
	control (n=60)	test (n=60)	control (n=36)	test (n=34)	control (n=24)	test (n=26)
Baseline	2.95 ±3.20	2.47 ±3.64	3.29 ±3.64	2.92 ±4.53	2.44 ±2.39	1.88 ±1.89
6 weeks	6.97 ±10.82	4.60 ±5.15	8.14 ±12.33	5.23 ±4.94	5.85 ±9.76	3.77 ±5.38
12 weeks	7.96 ±19.26	5.49 ±7.00	10.47 ±23.49	8.20 ±7.99	4.20 ±9.33	1.95 ±1.87

Table 7. Means and standard deviation of OPG concentration of control and test group dependant on the depth of pocket at times of examination

new ultrasonic technique in the complex treatment of periodontal diseases was determined. The completion of scaling and root planing with specific inserts within a short period of time seems to have a beneficial effect in the treatment of moderate and severe periodontitis. Future studies should investigate and confirm these findings in clinical settings and evaluate the short and long-term effects of these insert tips used during periodontal surgery on periodontal healing.

The treatment of periodontal disease with ultrasonic root debridement using adjunct antiseptic or antibiotic for controlling bacterial infection has an expected better clinical outcome. Previous studies demonstrated that using ultrasonic root debridement with 0.5% PVP-iodine did not have any additional anti-microbiological effect when compared to ultrasonic debridement alone (Leonhardt *et al* 2007). Another study investigated effects of systemic administration of amoxicillin and metronidazole during the full-mouth ultrasonic debridement of patients with severe chronic periodontitis. No improvement in the microbiologic or immunologic outcome was observed with the adjunctive use of systemic amoxicillin and metronidazole (Ribeiro *et al* 2009). However, our study showed that use of an essential oil solution had additional microbiological and biological effects confirmed by OPG levels (Thongsiri and
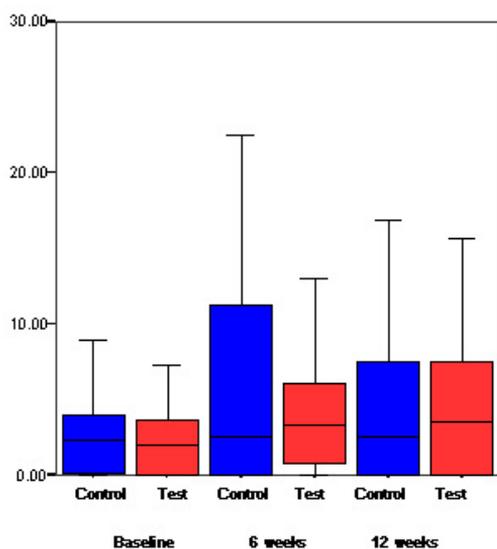
Figure 11. Box plot graph shows Median, Maximum, Minimum, Percentiles 25 and Percentiles 75 of OPG concentration (pg/µl) per site between control and test group at times of examination
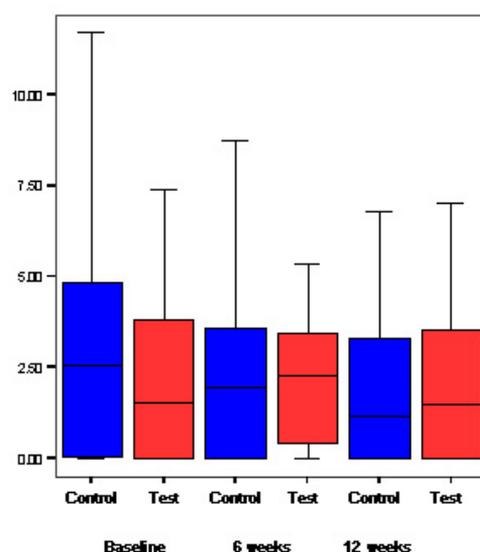
Figure 12. Box plot graph shows Median, Maximum, Minimum, Percentiles 25 and Percentiles 75 of OPG level (pg/site) per site between control and test group at times of examination

Laosrisin 2008, Sirisoontorn and Laosrisin 2009). Since these studies did not show a significant clinical improvement when compared to using water ultrasonic root debridement itself may have sufficient effectiveness for treating chronic periodontitis.

Even if there are numerous advantageous of using ultrasonic root debridement, adverse effects in some patients should be considered. For example, increased serum IL-6 and reduced serum-soluble thrombomodulin have been demonstrated more frequently after single-visit full-mouth mechanical debridement than quadrant-wise mechanical debridement (Ushida 2008). It seems that the stronger transient effect on systemic vascular endothelial functions of full-mouth ultrasonic debridement might increase the risk of pathogenesis of cardiovascular disease or increased severity of disease in those patients. Moreover, using ultrasonic devices as a full mouth treatment requires the patient to attend

less often. Personal oral hygiene instruction should be more emphasised at the first dental visit.

Although this treatment regimen for severe periodontitis is often successful, the reduced healthy periodontium is still unable to mechanically withstand occlusal loads of the remaining teeth. Strengthening these compromised teeth with simple chairside splinting techniques allows the dentist the opportunity to test the healing potential, without committing the patient to complex and expansive therapy and also provides alternative options for further treatment: either preserving periodontally involved teeth or planning implants with a better prognosis in a healed environment.

References

Aleo JJ, De Renzis FA, Farber PA, Varboncoeur AP. The presence and biologic activity of

cementum-bound endotoxin. *J Periodontol* 1974;45:672-675.

Allen EF, Rhoads RJ. Effect of high speed periodontal instruments on the tooth surface. *J Periodontol* 1963;34:352-356.

Apatzidou DA, Kinane DF. Quadrant root planing versus same-day full-mouth root planing. I. Clinical findings. *J Clin Periodontol* 2004;31:132-140.

Berkstein S, Reiff RL, McKinney JF, Killoy WJ. Supragingival root surface removal during maintenance procedures utilizing an air-powder abrasive system or hand scaling. An *in vitro* study. *J Periodontol* 1987;58:327-330.

Belting CM, Spjut PJ. Effects of high-speed periodontal instruments on the root surface during subgingival calculus removal. *JADA* 1964;69:578-584.

Bollen CM, Mongardini C, Papaioannou W, *et al*. The effect of a one-stage full-mouth disinfection on different intra-oral niches. Clinical and microbiological observations. *J Clin Periodontol* 1998;25:56-66.

Bye FL, Ghilzon RS, Caffesse RG. Root surface roughness after the use of different modes of instrumentation. *Int J Perio Restor Dent* 1986;6:36-41.

Clark SM, Grupe HE, Mahler DB. The effect of ultrasonic instrumentation on root surfaces. *J Periodontol* 1968;39:135-137.

Coldiron NB, Yukna RA, Weir J, Caudill RF. A quantitative study of cementum removal with hand curettes. *J Periodontol* 1990;61:293-299.

Del Peloso Ribeiro E, Bittencourt S, Sallum EA, *et al*. Periodontal debridement as a therapeutic approach for severe chronic periodontitis: a clinical, microbiological and immunological study. *J Clin Periodontol* 2008; 35: 789-798.

D'Silva IV, Nayak RP, Cherian KM, Mulky MJ. An evaluation of the root topography following periodontal instrumentation – a scanning electron microscopic study. *J Periodontol* 1979;50:283-290.

Eberhard J, Jervøe-Storm P-M, Needleman I, *et al*. Full-mouth treatment concepts for chronic periodontitis: a systematic review. *J Clin Periodontol* 2008; 35: 591-604.

Garrett JS. Root planing: a perspective. *J Periodontol* 1977;48:553-557.

Goncalves PF, Gurgel BC, Pimentel SP, *et al*. Effect of two different approaches for root decontamination on new cementum formation following guided tissue regeneration: a histomorphometric study in dogs. *J Periodont Res* 2006;41:535-540.

Hughes FJ, Smales FC. Immuno-histochemical investigation of the presence and distribution of cementum-associated lipopolysaccharides in periodontal disease. *J Periodont Res* 1986;21:660-667.

Jervøe-Storm PM, Semaan E, Al Ahdab H, *et al*. Clinical outcomes of quadrant root planing versus full-mouth root planing. *J Clin Periodontol* 2006;33:209-215.

Jones WA, O'Leary T J. The effectiveness of *in vivo* root planing in removing bacterial endotoxin from the roots of periodontally involved teeth. *J Periodontol* 1987;49: 337-342.

Jotikasthira NE, Lie T, Leknes KN. Comparative *in vitro* studies of sonic, ultrasonic and reciprocating scaling instruments. *J Clin Periodontol* 1992;19:560-569.

Kinane DF. Single-visit, full-mouth ultrasonic debridement: a paradigm shift in periodontal therapy? *J Clin Periodontol* 2005;32:732-733.

Kleinknecht RA, Klepac RK, Alexander LD. Origins and characteristics of fear of dentistry. *JADA* 1973;86:842-848.

Koshy G, Kawashima Y, Kiji M, *et al*. Effects of single-visit full-mouth ultrasonic debridement versus quadrant-wise ultrasonic debridement. *J Clin Periodontol* 2005;32:734-743.

Lavespere JE, Yukna RA, Rice DA, LeBlanc DM. Root surface removal with diamond-coated ultrasonic instruments: an *in vitro* and SEM study. *J Periodontol* 1996;67:1281-1287.

Leonhardt A, Bergström C, Krok L, Cardaropoli G. Microbiological effect of the use of an ultrasonic device and iodine irrigation in patients with severe chronic periodontal disease: a randomized controlled clinical study. *Acta Odontol Scand* 2007;65:52-59.

Lie T, Leknes KN. Evaluation of the effect on root surfaces of air turbine scalers and ultrasonic instrumentation. *J Periodontol* 1985;56:522-531.

Lie T, Meyer K. Calculus removal and loss of tooth substance in response to different periodontal instruments. A scanning electron microscope study. *J Clin Periodontol* 1997;4:250-262.

McCoy SA, Creamer HR, Kawanami M, Adams DF. The concentration of lipopolysaccharide on individual root surfaces at varying times following *in vivo* root planing. *J Periodontol* 1987;58:393-399.

Mongardini C, van Steenberghe D, Dekeyser C, Quirynen M. One stage full- versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. I. Long-term clinical observations. *J Periodontol* 1999;70:632-645.

Moore J, Wilson M, Kieser JB. The distribution of bacterial lipopolysaccharide (endotoxin) in relation to periodontally involved root surfaces. *J Clin Periodontol* 1986;13, 748-751.

Moskow BS, Bressman E. Cemental response to ultrasonic and hand instrumentation. *JADA* 1964;68:698-703.

Nyman S, Sahed G, Ericsson I, *et al*. Role of "diseased" root cementum in healing following treatment of periodontal disease. An experimental study in the dog. *J Periodont Res* 1986;21:496-503.

O'Leary TJ. Kafrawy AH. Total cementum removal: a realistic objective? *J Periodontol* 1983;54:221-226.

Quirynen M, Bollen CML. The influence of surface roughness and surface free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* 1995;22:1-14.

Quirynen M, De Soete M, Boschmans G, *et al*. Benefit of "one-stage full-mouth disinfection" is explained by disinfection and root planing within 24 hours: a randomized controlled trial. *J Clin Periodontol* 2006;33:639-647.

Quirynen M, Mongardini C, De Soete M, *et al*. The role of chlorhexidine in the one-stage full-mouth disinfection. Long-term clinical and micro-biological observations. *J Clin Periodontol* 2000;27:578-589.

Ribeiro Edel P, Bittencourt S, Zanin IC, *et al*. Full-mouth ultrasonic debridement associated with amoxicillin and metronidazole in the treatment of severe chronic periodontitis. *J Periodontol.* 2009;80:1254-1264

Ritz L, Hefti AF, Rateitschak KH. An *in vitro* investigation on the loss of root substance in scaling with various instruments. *J Clin Periodontol* 1991;18:643-647.

Ruben MP, Shapiro A. An analysis of root surface changes in periodontal disease a review. *J Periodontol* 1978;49:89-91.

Scott JB, Steed-Veilands AM, Yukna RA. Improved efficacy of calculus removal in furcations using ultrasonic diamond-coated inserts. *Int J Periodont Restor Dent* 1999;19:355-361.

Sirisoontorn I, Laosrisin N. Level of osteoprotegerin in GCF after using essential oil solution as coolant during piezoelectric ultrasonic root debridement. Masters Periodontology thesis. Srinakharinwirot University, Bangkok, 2009.

Smart GJ, Wilson M, Davies EH. Kieser JB. Assessment of ultrasonic root surface debridement of residual endotoxin levels. *J Clin Periodontol* 1990;17:174-178.

Thongsiri C, Laosrisin N. Clinical safety, clinical and microbiological change after using essential oil solution as coolant during piezoelectric ultrasonic root debridement. Masters Periodontology thesis. Srinakharinwirot University, Bangkok, 2008.

Trombelli L, Schincaglia G, Checchi L, Calura G. Combined guided tissue regeneration, root conditioning, and brin-bronectin system application in the treatment of gingival recession. A 15-case report. *J Periodontol* 1994;65:796-803.

Ushida Y, Koshy G, Kawashima Y, *et al*. Changes in serum interleukin-6, C-reactive protein and thrombomodulin levels under periodontal ultrasonic debridement. *J Clin Periodontol.* 2008;35:969-975.

Van Volkinburg JW, Green E, Armitage GC. The nature of root surfaces after curette, cavitron and alpha-sonic instrumentation. *J Periodont Res* 1976;11:374-381.

Vandekerckhove BN, Bollen CM, Dekeyser C, *et al*. Full-versus partial-mouth disinfection in the treatment of periodontal infections. Long-term clinical observations of a pilot study. *J Periodontol* 1966;67:1251-1259.

Vastardis S, Yukna RA, Rice DA, Mercante D. Root surface removal and resultant surface texture with diamond-coated ultrasonic inserts: an *in vitro* and SEM study. *J Clin Peridontol* 2005;32:467-473.

Waerhaug J. Healing of the dento-epithelial junction following subgingival plaque control II: As observed on extracted teeth. *J Periodontol* 1978;49:119-134.

Wennström JL, Tomasi C, Bertelle A, Dellasega E. Full-mouth ultrasonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontitis. *J Clin Periodontol* 2005;32:851-859.

Wilkinson RF, Maybury JE. Scanning electron microscopy of the root surface following instrumentation. *J Periodontol* 1973;44:559-563.

Wongsasuluk Y, Laosrisin N. Root surface after planing with plain metal and diamond coated ultrasonic inserts*: an in vitro and SEM study*. Masters Periodontology thesis. Srinakharinwirot University, Bangkok, 2008.

Yukna RA. Clinical human comparison of expanded polytetrauoroethylene barrier membrane and freeze-dried dura mater allografts for guided tissue regeneration of lost periodontal support. I. Mandibular molar Class II furcations. *J Periodontol* 1992;63:431-442.

Yukna RA, Scott JB, Aichelmann-Reidy ME, *et al*. Clinical evaluation of the speed and effectiveness of subgingival calculus removal on single-rooted teeth with diamond-coated ultrasonic tips. *J Periodontol* 1997;68:436-442.

Yukna RA, Vastardis S, Mayer ET. Calculus removal with diamond-coated ultrasonic inserts *in vitro*. *J Periodontol* 2007;78:122-126.

Zanatta GM, Bittencourt S, Nociti FH Jr, *et al*. Periodontal debridement with povidone-iodine in periodontal treatment: short-term clinical and biochemical observations. *J Periodontol* 2006:77;498-505.

Zappa U, Smith B, Simona C, *et al*. Root substance removal by scaling and root planing. *J Periodontol* 1991;62:750-754.

Chapter 2

The efficacy of full mouth scaling and root planing under systemically administered Azithromycin

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Introduction

Periodontal disease is a bacterial infection caused by periodontopathic bacteria. Elimination of periodontal pockets, where bacteria proliferate, is the gold standard of periodontal treatment. However, it has become clear that health of the periodontium can be achieved by alteration of intrapocket bacterial flora into stable non-pathogenic bacterial flora. In order to obtain stable bacterial flora approaches such as mechanical plaque control via scaling and root planing have been considered. Reports have shown that bacterial flora cannot be changed only by scaling and root planing (Alaluusua 1991, Von Troil-Linden 1995, Quirynen *et al* 1996, Quirynen *et al* 2001, Greenstein and Lamster 1997, van Steenbergen 1997). Conventional scaling and root planing is usually performed in several appointments in a quadrant-wise or sextant-wise manner and usually takes 1 to 3 months to complete the full mouth treatment depending on the frequency of scaling and root planing. It has been reported that periodontal pathogens detected in the peri-implant sulcus of periodontally diseased patients have the same genotype of pathogenic bacteria collected from periodontal pockets of diseased natural teeth (Sumida 2002). This indicates that periodontopathic bacteria can translocate within the same oral cavity. When treatment is lengthy, bacteria in untreated pockets may be transmitted to the previously treated sites and it is possible that periodontal disease may recur. Thus, bacterial flora do not change by mechanical plaque control alone.

Another method of changing intrapocket bacterial flora is the use of antibiotics or antibacterial agents. The formation of biofilms in pockets can limit the effects of chemical agents. In order to destroy the biofilm with chemical agents, long term administration is required, however long term antibiotic therapy is not acceptable. If no improvement in pocket depths is obtained, formation of periodontopathic bacterial flora will recur. Therefore bacterial flora does not change through chemotherapy alone.

Quirynen *et al* (1995) introduced one-stage full mouth disinfection (FMD), which uses mechanical plaque control and chemotherapy combined in order to eliminate periodontopathic bacteria from the oral cavity in a short time frame. In FMD, full mouth scaling and root planing is performed in two visits within 24 hours using chlorhexidine. There have been many reports that FMD results in good clinical outcomes (Quirynen *et al* 1995, Quirynen *et al* 1998, Quirynen *et al* 1999, Quirynen *et al* 2000, Mongardini 1999). However there are also reports that

FMD resulted in no better results than conventional scaling and root planing alone (Apatzidou and Kinane 2004a, Apatzidou and Kinane 2004b). Systematic reviews have concluded that FMD does not provide clinically relevant advantages over conventional scaling and root planing (Lang *et al* 2008, Ebwrhard 2008). Furthermore, FMD has several disadvantages such as length of time for completion of scaling and root planing (over 2 hours), frequently induced pyrexia after treatment and the questionable effectiveness of chlorhexidine.

However, the concept of FMD, (scaling and root planing and chemotherapy combined) for the removal of periodontopathic bacteria from the oral cavity in a short time is an acceptable form of treatment. Therefore we planned full mouth scaling and root planing whilst reducing oral bacteria through systemically administered antibiotics. Azithromycin (AZM) was selected as the antimicrobial agent due to its long half-life and good tissue penetration. Additionally, AZM is preferentially taken up by phagocytes and so its concentration in infected tissues will be higher than in non-infected sites (Gladue 1989, Foulds 1990, McDonald and Pruul 1991, Schentag and Ballow 1991). It has stronger antibiotic effects for gram-negative microorganisms compared to other macrolides. It has been demonstrated that AZM might be retained in inflamed gingiva for almost over a week and is effective against periodontal disease-related bacteria (Gomi 2007a). It may also result in a shift to a healthy flora consisting of nonpathogenic bacteria.

The effect of full mouth scaling and root planing using azithromycin

34 adult subjects (16 males, 18 females, average age 48.2 ± 11.5) with severe chronic periodontitis were selected. After tooth brushing instruction and scaling, they were then randomly allocated to either the test group (17 subjects: 8 males and 9 females, average age 45.4 ± 14.3) or control group (17 subjects: 8 males and 9 females, average age 51.0 ± 8.8). In the test group, full mouth scaling and root planing was performed in conjunction with systemically administered AZM, and in the control group, conventional scaling and root planing was performed in 4 to 6 sessions with an interval of about 1 week (Figure 1). Probing pocket depth, clinical attachment level, Gingival Index (GI) and bleeding on probing were measured. The total number of cultivated microorganisms and black-pigmented rods were counted after 7 days anaerobic
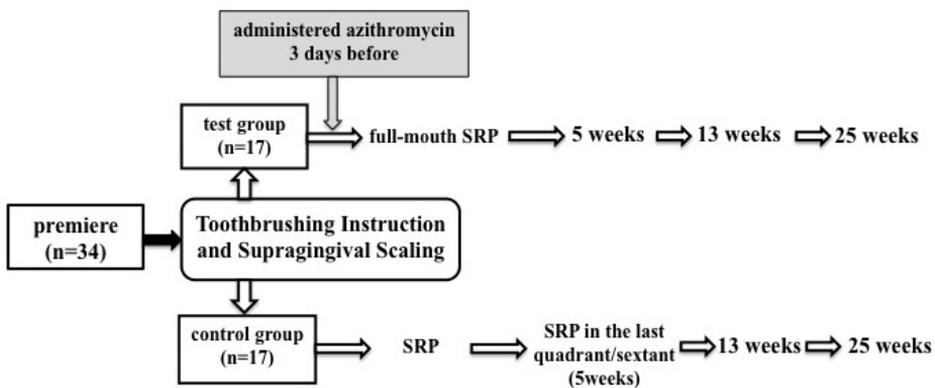
Figure 1. Flowchart of the experimental schedule. 34 patients were randomly divided into test and control groups after toothbrushing instructions and supragingival scaling.
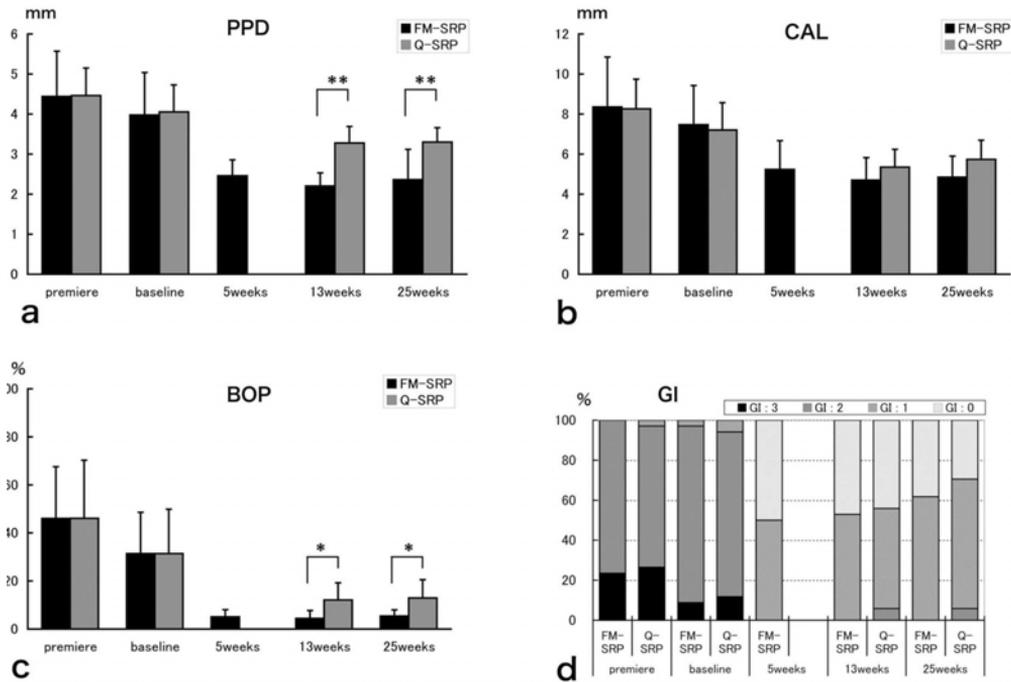
Figure 2. Changes in clinical parameters. a: probing pocket depth, b: clinical attachment level, c: bleeding on probing, d: gingival index (**P < 0.001, *P < 0.01)

incubation. Six periodontopathic bacteria (*T. forsythensis*, *T. denticola*, *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia* and *P. nigrescens*) were identified in each subgingival sample by conventional PCR method.

The body temperature was checked with a thermometer placed in the maxilla immediately before full mouth scaling and root planing, the next morning and for the control group, after the first treatment.

Clinical evaluation

Significant differences were found in probing pocket depth, clinical attachment gain and ratio of bleeding on probing between full mouth scaling and root planing and conventional scaling and root planing (Figure 2). Full mouth scaling and root planing showed improvement of each clinical

parameter at early stages and maintenance of these standards long term.

Bacteriological evaluation

Black pigmented rods were not detected in the test group until 13 weeks after full mouth scaling and root planing (Figure 3). The total bacterial number of black pigmented rods were reduced to half in the control group one month after baseline but relapsed to the baseline levels 13 weeks after treatment. When examined by PCR all strains of periodontopathic bacteria demonstrated a tendency to decrease with time in the test group, but *P. gingivalis*, *T. forsythensis* and *P. intermedia* tended to reappear in the control group 13 weeks after baseline. It was assumed that recolonisation of bacterial flora occurred in periodontal pockets in the test group, because the ratio of total bacterial number to
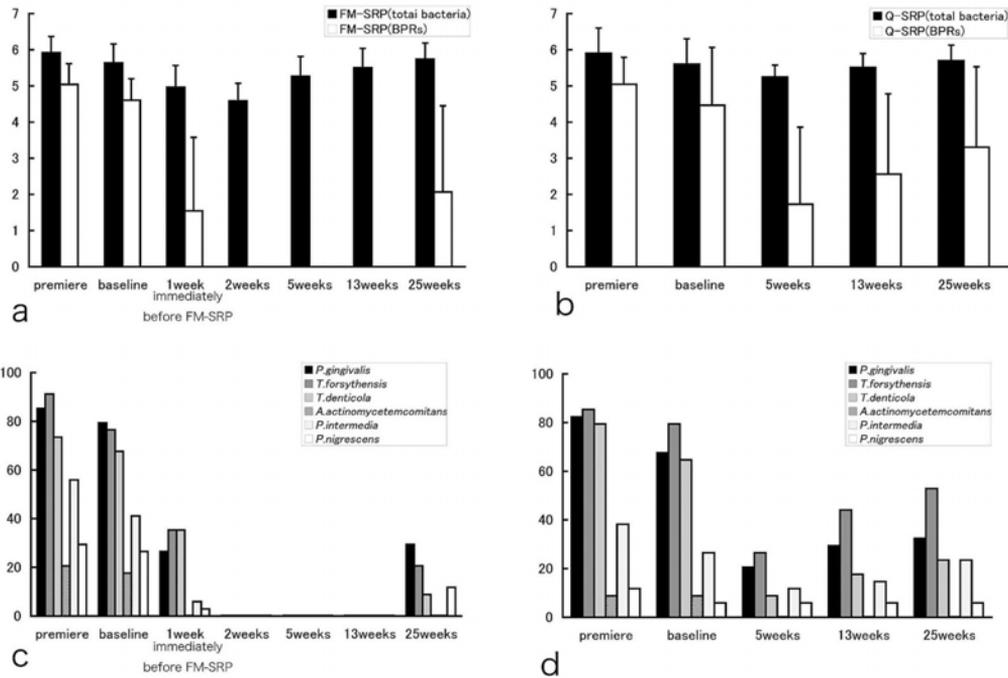
Figure 3. Bacteriological evaluation. Changes in total cultivated bacteria and black-pigmented rods in test group (a) and control group (b). The detection rate of six periodontopathic bacteria in test group (c), and control group (e).
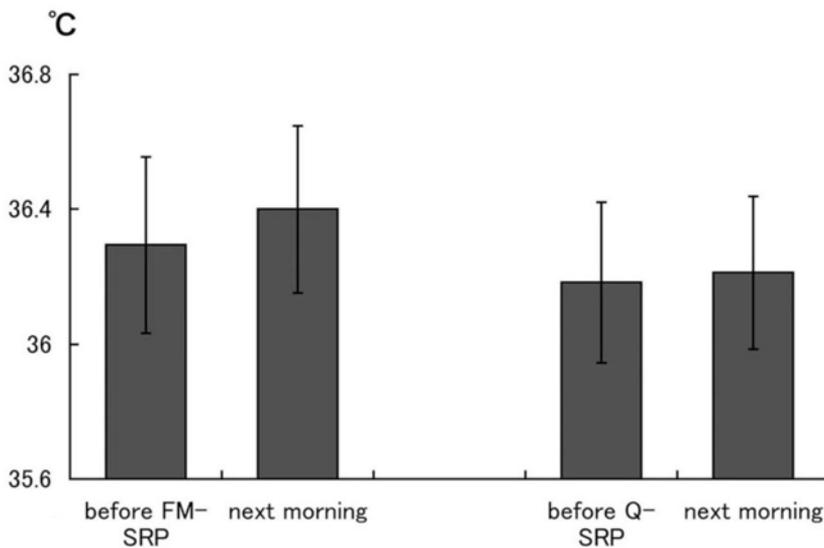
Figure 4. Changes in body temperature. Body temperature in the morning before scaling and root planing and next morning.

number of BPRs was reduced. In contrast, it is thought that bacterial re-infection arises from the untreated sites in the control group.

Body temperature

The body temperature of test subjects in the morning before full mouth scaling and root planing and next morning was 36.30±0.24 °C and 36.40±0.24 °C, respectively (Figure 4). In the control group the corresponding values in the morning before treatment and in the morning after the treatment of the first part were 36.18±0.26°C and 36.21±0.23°C, respectively. There were no statistically significant differences between the groups.

Case report

A 34 year old Japanese female presented with extensive periodontal destruction. A diagnosis of aggressive periodontitis was made. At the first visit, there were redness and swelling of the gingiva, and formation of a periodontal abscess. Significant alveolar bone resorption was observed. The average probing

Figure 5a. Oral photographs and X-ray photographs at base line.

Mobility		1	1	1	1	0	1	3	2	0	0	2	2	0	0	
CAL		5 3 5	5 4 4	5 5 7	7 3 5	4 2 4	7 4 7	6 3 5	5 3 10	7 2 7	8 3 4	4 3 5	5 3 6	6 3 5	5 3 4	
PPD		4 3 7	7 4 5	7 3 8	8 2 6	5 2 8	8 4 8	7 2 4	4 3 12	7 2 8	9 2 4	4 3 7	7 3 8	8 3 4	4 2 4	
		5 5 7	4 3 8	9 5 5	5 4 6	6 5 7	8 4 8	4 4 4	4 12 10	7 6 6	6 8 4	5 6 4	6 6 4	6 3 5	5 4 7	
upper	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
lower	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
PPD	5 3 2	3 2 8	6 2 4	4 3 4	8 8 4	3 3 5	6 2 5	5 2 4	6 2 5	6 5 5	3 2 4	6 6 5	5 3 5	4 4 6	6 3 4	3 3 5
	5 2 3	4 3 8	8 3 4	4 2 3	8 2 2	2 2 7	7 4 8	8 6 7	7 4 10	10 10 7	6 3 6	7 2 5	5 2 6	8 4 8	8 2 4	2 2 4
CAL	4 3 3	3 3 5	6 3 3	3 3 3	6 2 2	2 2 5	5 4 6	6 4 5	5 4 7	7 7 5	5 3 5	5 2 4	4 2 4	6 3 6	6 2 3	2 2 3
Mobility	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

The average PPD:4.9mm, CAL: 4.4mm, BOP: 78.9%

Figure 5b. Periodontal chart (probing pocket depth, CAL, Mobility).

pocket depth was 4.9 mm, CAL was 4.4 mm and the ratio of bleeding on probing was 78.9% (Figures 5a and 5b). After tooth brushing instruction and supra-gingival scaling, full mouth scaling and root planing in combination with systemically administered AZM was performed.

One month after full mouth scaling and root planing, improvement of gingival inflammation and recession of gingival margin and significant reduction of probing pocket depth and improvement of bleeding on probing were observed (Figure 6).

Three months after treatment, the periodontium had healthy keratinized gingiva. Average probing pocket depth was reduced to 2.0 mm and bleeding on probing was also extremely improved to a level of 0.6%. Clinical attachment level was 3.3 mm with a 1.1 mm attachment gain compared to baseline (Figures 7a and 7b).

Two years after full mouth scaling and root planing, the periodontium was observed to still be in a healthy condition (Figure 8).

Difference between FMD and full mouth scaling and root planing using azithryomycin

Systematic reviews (Lang *et al* 2008, Ebwrhard 2008) have shown no difference between full mouth disinfection and conventional scaling and root planing. However, good clinical and bacteriological results were obtained with full mouth scaling and root planing using AZM in our study. Therefore, the question arises, what is the difference between FMD and full mouth scaling and root planing using AZM? Several reasons may account for this. For example, carrying out full mouth scaling and root planing whilst controlling bacteria by antibiotic administration. Alternatively, the characteristics of AZM, such as phagocyte delivery and long half-life may be of significance.

The average PPD: 2.4mm, CAL: 3.3mm, BOP: 2.4%

Figure 6. One month after full mouth scaling and root planing using azithromycin.

Figure 7a. Oral photographs and X-ray photographs at 3 month after full mouth scaling and root planing.

Figure 7b. Periodontal chart (probing pocket depth, CAL, Mobility).

Mobility		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
CAL		3 2	4 3 3	3 4 3	5 5 3	4 3 2	3 4 4	5 5 3	4 4 3	5 4 2	5 5 3	3 3 3	4 4 3	4 3 3	4 4 3	4
PPD		2 2 2	3 2 2	2 2 2	2 2 1	2 2 1	2 3 2	2 3 1	2 2 2	2 2 2	2 2 2	2 2 1	2 2 2	2 2 2	2 2 2	2
		2 2 3	2 2 2	3 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 3	2 2 2	2 2 2	3
upper	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
lower	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
PPD	3 2 2	2 2 2	2 2 3	2 2 2	2 2 2	2 2 2	1 2 2	1 2 2	1 2 1	2 2 1	2 2 1	2 2 3	2 2 2	2 2 2	2 2 3	
	3 2 2	2 2 3	3 2 2	2 2 2	1 2 2	1 2 2	2 3 2	2 2 1	2 3 2	2 2 1	2 2 1	2 2 2	2 3 2	3 2 2	2 2 2	
CAL	3 2 2	3 2 3	2 2 2	3 2 2	4 2 2	2 2 4	4 2 3	5 4 4	3 5 5	5 3 3	4 4 2	3 3 2	3 5 3	5 4 4	2 3 2	2 3
Mobility	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
										The average PPD: 2.2mm, CAL: 3.4mm, BOP: 1.2%						

Figure 8. Two yeas after full mouth scaling and root planing using azithromycin.

Additional effects of azithromycin

When AZM was used in conjunction with full mouth scaling and root planing, gingival recession occurred more than with the use of other antibiotics. AZM has been used for treatment of cyclosporin gingival overgrowth (Gomez 1997, Citterio 2001, Tokgoz 2004). From these reports, AZM seems to have the effect of gingival reduction. Kim *et al* (2008) reported that AZM may improve cyclosporine A-induced gingival overgrowth by blocking cyclosporine A induced cell proliferation and collagen synthesis and by activating MMP-2 in gingival fibroblasts of persons with cyclosporine A-induced gingival overgrowth.

Conclusion

It has been demonstrated that full mouth scaling and root planing using systemically administered AZM was a clinically and bacteriologically useful basic periodontal treatment for severe periodontitis (Gomi 2007b). Pre-medication with systemic antibiotics decreased the total number of bacteria and inhibited bacteremia. The efficiency in reforming the intra-pocket bacterial flora was not only due to antimicrobial activity, long half-life and good tissue penetration of AZM, but also the accumulation of AZM in phagocytes at the inflamed site and this acts similar to a self-delivery drug system. Additionally, AZM has the effect of inhibiting collagen synthesis and activating MMP-1,2 causing pocket reduction. A shift in the bacterial flora is responsible for the inflammatory improvement and decrease in the periodontal pocket depth.

This method of combining full mouth scaling and root planing and AZM has two major merits. The first is to be able to achieve pocket reduction in the early stage of treatment. It is proposed that pocket reduction was caused by the effects of AZM itself, not only inflammatory regression. The second advantage is to be able to maintain the periodontium for a long time by obtaining stable bacterial flora. Future periodontal treatment is undertaken to control the bacterial flora using an appropriate drug which has good antimicrobial and additional effects.

References

Alaluusua S, Asikainen S, Lai CH. Intrafamilial transmission of Actinobacillus actinomycetemcomitans. *J Periodontol* 1991;62:207-210.

Apatzidou DA, Kinane DF. Quadrant root planing versus same-day full mouth root planing. II. Microbiological findings. *J Clin Periodontol* 2004a;31:141-8.

Apatzidou DA, Kinane DF. Quadrant root planing versus same-day full mouth root planing. I. Clinical findings. *J Clin Periodontol* 2004b;31:132-40.

Citterio F, Di Pinto A, Borzi MT, *et al*. Azithromycin treatment of gingival hyperplasia in kidney transplant recipients is effective and safe. *Transplant Proc* 2001;33:2134-2135.

Ebwrhard J, Jervøe-Storm PM, Needleman I, *et al*. Full-mouth treatment concepts for chronic periodontitis. A systematic review. *The Cochrane Library* www.thecochranelibrary.com (Accessed 12 December 2009)

Foulds G, Shepard RM, Johnson RB. The paramacokinetics of azithromycin in human serum and tissue. *J Antimicrobial Chemotherapy* 1990;25(Suppl A):73-82.

Gladue RP, Bright GM, Isaacson RE, Newborg MF. In vitro and in vivo uptake of azithromycin (CP-62, 993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. *Antimicrob Agents Chemother* 1989;33:277-282.

Gómez E, Sánchez-Nuñez M, Sánchez JE, *et al*. Treatment of cyclosporine-induced gingival hyperplasia with azithromycin. *Nephrol Dial Transplant* 1997;12:2694-2697.

Gomi K, Yashima A, Iino F, *et al*. Drug concentration in inflamed periodontal tissues after systemically administered azithromycin. *J Periodontol* 2007a;78:918-923.

Gomi K, Yashima A, Nagano T, *et al*. Effects of full mouth scaling and root planing in conjunction with systemically administered azithromycin. *J Periodontol* 2007b;78:422-429.

Greenstein G, Lamster I. Bacterial transmission in periodontal diseases: a critical review. *J Periodontol* 1997;68:421-431.

Kim JY, Park SH, Cho KS, *et al*. Mechanism of azithromycin treatment on gingival overgrowth. *J Dent Res* 2008;87:1075-1079.

Lang NP, Tan WC, Krähenmann MA, Zwahlen M. A systematic review of the effects of full mouth debridement with and without antiseptics in patients with chronic periodontitis. *J Clin Periodontol* 2008;35(Suppl 8):8-21.

McDonald PJ, Pruul H. Phagocyte uptake and transport of azithromycin. *Eur J Clin Microbiol Infect Dis* 1991;10:828-833.

Mongardini C, van Steenberghe D, Dekeyser C, Quirynen M. One stage full versus partial-mouth disinfection in the treatment of chronic adult or generalized early onset periodontitis. I. Long-term clinical observations. *J Periodontol* 1999;70:632-645.

Quirynen M, Bollen CML, Vandekerckhove BNA, *et al*. Full- vs. partial-mouth disinfection in the treatment of periodontal infection: Short-term clinical and microbiological observations. *J Dent Res* 1995;74:1459-1467.

Quirynen M, De Soete M, Dierickx K, van Steenberghe D. The intra-oral translocation of periodontopathogens jeopardizes the outcome of periodontal therapy. A review of the literature. *J Clin Periodontol* 2001;28:499-507.

Quirynen M, Mongardini C, van Steenberghe D. The effect of a 1-stage fullmouth disinfection on oral malodor and microbial colonization of the tongue in periodontitis. A pilot study. *J Periodontol* 1998;69:374-382.

Quirynen M, Mongardini C, Pauwels M, *et al*. One stage full- versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. II. Long-term impact on microbial load. *J Periodontol* 1999;70:646-656.

Quirynen M, Mongardini C, de Soete M, *et al*. The role of chlorhexidine in the one-stage full mouth disinfection treatment of patients with advanced adult periodontitis. Long-term clinical and microbiological observations. *J Clin Periodontol* 2000;27:578-589.

Quirynen M, Papaioannou W, van Steenberghe D. Intraoral transmission and the colonization of oral hard surfaces. *J Periodontol* 1996;67:986-993.

Schentag JJ, Ballow CH. Tissue-directed pharmacokinetics. *Am J Med* 1991;12:5-11.

Sumida S, Ishihara K, Kishi M, Okuda K. Transmission of periodontal disease-associated bacteria from teeth to osseointegrated implant regions. *Int J Oral Maxillfac Implants* 2002;17:696-702.

Tokgöz B, Sari HI, Yildiz O, *et al*. Effects of azithromycin on cyclosporine-induced gingival hyperplasia in renal transplant patients. *Transplant Proc* 2004;36:2699-2702.

Von Troil-Linden B, Torkko H, Alaluusua S, *et al*. Periodontal findings in spouses. A clinical, radiographic and microbiological study. *J Clin Periodontol* 1995;22:93-99.

van Steenbergen TJ, Bosch-Tijhof CJ, Petit MD, Van der Velden U. Intra-familial transmission and distribu*tion of Prevotella i*ntermedia and *Prevotella nigrescens*. *J Periodontal Res* 1997;32:345-350.

Chapter 3

The relationship between periodontitis and coronary heart diseases, level of total cholesterol, low-density lipoprotein and triglyceride in Health Service Centres in Indonesia

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Introduction

Many recent studies have reported that cardiovascular disease (CVD) is the main cause of death and disability. It is estimated that in 2020 heart disease will exceed infectious diseases as the main cause of death. Coronary heart disease occupies the highest rank and accounts for approximately 7.2 million deaths every year in both developing and developed countries (World Health Organization 2002). Based on The Indonesian Household Health Survey 2005 it was found that deficiencies in the circulation system are the leading cause of death with cardiovascular diseases causing 26.4% of all deaths (Department of Health Indonesia 2006). The Indonesian Household Health Survey 2001 found that oral diseases were the highest occurring, with 60% of the population experiencing dental caries or periodontal diseases (Department of Health Indonesia 2001). A survey in 2002 at the Department of Periodontology Faculty of Dentistry University of Indonesia stated that the prevalence of chronic periodontitis in 274 patients was 61.68% (Syafril 2004).

Recent evidence has shown there is a possible relationship between periodontal diseases and Coronary Heart Disease (CHD) (Genco *et al* 2002, Persson *et al* 2003, Buhlin *et al* 2003, Spahr *et al* 2006). According to Genco *et al* (2002) periodontal infection is a risk factor for heart diseases. Most risk factors for periodontal diseases are also risk factors for CVD (Beck *et al* 1998, Matilla *et al* 2000). Poor oral health is associated with coronary heart diseases and provides evidence that inflammation could play an important role in this association (Montebugnoli *et al* 2004, Emingil *et al* 2000, Beck *et al* 1996). Other studies have stated that there is a correlation between calculus accumulation and Type 2 Diabetes Mellitus with CHD (Kemal *et al* 2000). Persson *et al* (2003) stated that there was a relation between alveolar bone damage and heart infarctions.

Several studies have reported that periodontitis is connected with hyperlipidaemia (Moeintaghavi *et al* 2005, Cutler *et al* 1999, Losche *et al* 2000). Buhlin *et al* (2003) stated there was a significant relation between periodontitis and the level of low HDL. CHD is caused by atherosclerosis or obstruction of the coronary artery by plaque atheroma. Atherosclerosis is a calcification and deposition of calcium and fat in the media tunica of the coronary artery which causes

destruction of the endothelial wall. It is known that the blood cholesterol and Low Density Lypoprotein (LDL) are risk factors for CVD. It is well recognised that Gram-negative bacteria are one of the causes of periodontitis. *P gingivalis and Streptococus sanguis* have been found in plaque atherosclerosis (DeStefano *et al* 1993). Infection with Gram-negative bacteria results in the release of various cytokines such as Interleukin-1β (IL-1β) and Tumor Necrosis Factor-α (TNF-α) which play a role in changing fat metabolism and may result in hyperlipidaemia (Feingold *et al* 1992). Both have also been shown to play a role in the progression of atherosclerosis (Kinane 1998, Katz *et al* 2001). Analysis of the total cholesterol, LDL and triglyceride levels of individuals with periodontal disease has shown higher levels than in healthy individuals (Loesche *et al* 2000, Posch Machado *et al* 2005). The Third National Health and Nutrition Examination Survey (NHANES III) showed that there was a weak correlation between periodontal condition and total cholesterol (Wu *et al* 2000). From the National Cholesterol Education Programme Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adult Treatment 2001 it was shown that high triglyceride, low high-density lipoprotein (HDL) increase the risk factors for CHD (Cynthia 2006). Most of the risk factors for periodontal diseases are also as risk factors for CVD (Beck *et al* 1998). The mechanisms of the relationship between periodontitis and hyperlypidaemia are still unclear as to whether periodontitis-related hyperlipidaemia is one of the risk factors for CHD.

The purpose of this study was to evaluate the relationship between periodontitis and Coronary Heart Disease, the Level of total cholesterol, low density lipoprotein and triglycerides.

Materials and methods

A case control study was carried out in Bekasi Public Hospital West Java Indonesia. The study was approved by the Ethics Committee, Faculty of Dentistry University of Indonesia.

Consecutive sampling was performed to determine patients with Coronary Heart Disease who attended the Cardiac Clinic Bekasi Public Hospital, that were a match with the inclusion criteria. The test group consisted of 26 male subjects with clinically confirmed CHD, and the control group consisted of 23 male subjects without CHD. All subjects were aged between 38 and 69 years. The diagnosis of Coronary Heart Disease in the test group was determined by a Cardiologist based on a standard 12 impulse electrocardiogram or coronary angiography. Exclusion criteria for the selection of CHD subjects consist of the presence of Diabetes Mellitus, and other systemic diseases, CHD patients with a previous history of stroke and those with a smoking habit. All subjects gave informed consent. All subjects completed a written questionnaire relating to their health and smoking habits. Standardized clinical periodontal status was determined using the Plaque Index of Sillness and Loe, Papillary Bleeding Index of Muhleman, Calculus Index of Ramfjord, Pocket Depth and Loss of Attachment from cemento enamel junction using a Hu-Freidy probe.

Blood samples were taken and the level of total cholesterol at least <200 mg/dl, LDL level of at least <160 mg/dl, and triglyceride level of at least <160 mg/dl were determined using a Hitachi 917 analyzer (Roche AG Diagnostics, Mannheim, Germany).

Statistical analysis was carried out using Fisher Exact Test and Pearson Chi-Square Test to describe the correlation of periodontal condition, total cholesterol, LDL, and triglyceride in CHD and non-CHD patients.

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Chronic Periodontitis	CHD	Non-CHD	Total
Mild	7 (14.29%)	18 (36.73%)	25 (51.0%)
Moderate	16 (32.65%)	5 (10.20%)	21 (42.9%)
Severe	3 (6.12%)	0 (0.0%)	3 (6.12%)
Total	26 (53.06%)	23 (46.94%)	49 (100%)

Table 1. The distribution of severity of chronic periodontitis in CHD and non-CHD patients

Results and discussion

Fisher Exact test showed that there was a significant correlation between the severity of periodontitis in CHD and non-CHD patients, p=0,001 (Table 1). In 26 CHD patients 32.65% had moderate periodontitis, and 6.12 % had severe periodontitis. In comparison, in 23 non-CHD patients, 36.73 % had mild periodontitis, and no severe periodontitis was recorded. This study showed that there was a possible relationship between periodontal diseases and Coronary Heart Disease (CHD) similar to other previous studies (Genco *et al* 2002, Persson *et al* 2003, Buhlin *et al* 2003, Spahr *et al* 2006). Genco *et al* (2002) showed that periodontal infection is a risk factor of heart diseases (Syafril 2004).

These results are similar to the study by Persson *et al* (2003), in which it was stated that there was a relationship between alveolar bone damage and heart infarctions. In this study alveolar bone damage was detected by periodontal pocket depth and loss of attachment.

Fisher Exact Test

The results in Table 2 demonstrate that there was no significant difference in Plaque Index between periodontitis patients with CHD and Non CHD (p=0.065) however most of the subjects had a Plaque Index Score >2, in both periodontitis patients with CHD 42.86 % and non CHD 24.49%. This recent study is contradictory with another study in which poor oral health was associated with CHD (Montebugnoli *et al* 2004). This may suggest that the socio-economic status and education of the subjects were almost similar between CHD and Non CHD patients. Age, which varied among the subjects, is also an important variable on periodontal condition and CHD relationship (Matilla *et al* 2000, DeStefano *et al* 1993). The number of the subjects also has an impact in the results obtained.

There was no significant difference of PBI between periodontitis patients with CHD and non CHD (p=0.448). The score of PBI in both CHD and non-CHD patients approximately ≤1. This may suggest that most cases of chronic periodontitis are do not always have increased papillary bleeding, and this condition probably reflects a long standing or low grade of inflammation and fibrosis of the marginal tissues (Novak and Novak 2006). Other reasons for the inconsistency of findings might related to differences in age, sex,

	CHD	Non-CHD	Total	P
Plaque Index				0.065
≤2.00	5 (10.2%)	11 (22.45%)	16 (32.65%)	
>2.00	21 (42.86%)	12 (24.49%)	33 (67.35%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	
Calculus Index				0.033
≤2.00	1 (2.04%)	3 (6.12%)	4 (8.16%)	
>2.00	25 (51.02%)	20 (40.82%)	45 (91.84%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	
Papillary Bleeding Index				0.448
≤1.00	23 (46.94%)	18 (36.74%)	41 (83.67%)	
>1.00	3 (6.12%)	5 (10.20%)	8 (16.33%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	

Table 2. The difference of the relationship of Plaque Index, Calculus Index and Papillary Bleeding Index in periodontitis patients with CHD and non-CHD

variation of periodontal parameter, oral status, and CHD condition of subjects (Genco *et al* 2002).

A significant difference in Calculus Index was noted between periodontitis patients with CHD patients and non CHD (p=0.033). This result is similar to a previous study that showed that Calculus Index in Diabetic Mellitus Type 2 with CHD is higher than Non-CHD, and this probably correlated with coronary artery calcification in CHD and dental calculus accumulation (Kemal *et al* 2000).

As presented in Table 3 no significant difference of total cholesterol (p= 0,572) and LDL (p= 0,448) was noted between periodontitis patients with CHD and Non-CHD. However, there was significant difference in triglyceride levels between periodontitis patients with CHD and non-CHD (p=0.039). Some of the CHD subjects were using medication to control their blood lipids, as noted in the questionnaire. However, triglyceride levels are difficult to decrease without an exercise or diet programme. Other studies have reported that total cholesterol, LDL and triglyceride in periodontal disease patients show higher levels of lipids than in

	Periodontitis with CHD	Periodontitis and Non-CHD	Total	P
Total Cholesterol				0.572*
≤200 mg/dl	16 (32.7%)	12 (24.5%)	28 (57.1%)	
>200 mg/dl	10 (20.4%)	11 (22.4%)	21 (42.9%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	
Low-Density Lipoprotein				0.448**
≤160 mg/dl	23 (46.9%)	18 (36.7%)	41 (83.7%)	
>160 mg/dl	3 (6.1%)	5 (10.2%)	8 (16.3%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	
Triglyceride				0.039**
≤160 mg/dl	14 (28.6%)	19 (38.8%)	33 (67.3%)	
>160 mg/dl	12 (24.5%)	4 (8.2%)	16 (32.7%)	
Total	**26 (53.06%)**	**23 (46.94%)**	**49 (100%)**	

Table 3. The difference of the relationship of total cholesterol, low-density lipoprotein and triglyceride in periodontitis patients with CHD and non-CHD (* Pearson Chi-Square, ** Fisher Exact Test)

healthy individual (Cutler *et al* 1999, Losche *et al* 2000, Posch Machado *et al* 2005). According to the Third National Health and Nutrition Examination Survey (NHANES III) the correlation between periodontal condition and total cholesterol is weak (Wu *et al* 2000). However high triglyceride, low high-density lipoproten (HDL) will increase the risk factor for CHD (Cynthia 2006).

As presented in Table 4 in severe chronic periodontitis, triglyceride levels ranged between 179-642 (mean ± sd: 365 ± 244.547) and the range of the level of total cholesterol was from 172–283 (mean ± sd : 213 ± 60.918). These levels were higher than the range of these lipids in mild and moderate periodontitis. These results are similar to Moenintaghavi *et al* (2005) which found that there was a relationship between the level of cholesterol in blood and periodontitis, but it is not yet definitively known whether periodontitis increases the level of lipid serum as a risk factor for CHD. Further study is needed to determine the relation between CHD and periodontitis in different criteria and in larger number of subjects.

Chronic Periodontitis	Total Triglyceride	Min-Max (Mean ±SD) Low Density Lipoprotein	Total Cholesterol
Mild	56-309 (130.04 ± 66.039)	50-214 (131.08 ± 37.814)	96-303 (200.56 ± 41.898)
Moderate	45-279 (150.38 ± 70.861)	59-175 (116 ± 39.290)	116-300 (190.71 ± 39.29)
Severe	179-642 (365 ± 244.547)	34-131 (73 ± 51.215)	172-283 (213 ± 60.918)

Table 4. Mean values, standard deviation of total cholesterol, LDL and triglyceride in chronic periodontuits with CHD and non-CHD patients

Conclusion

In this study there was a possible relationship between the severity of periodontitis and CHD. There was a significant difference in the Calculus Index between periodontitis patients with CHD and non-CHD, however there were no significant differences both in Plaque Index and Papilla Bleeding Index between periodontitis patients with CHD and those in the non-CHD group.

There was a significant difference in the level of triglycerides between periodontitis patients with CHD and non-CHD, however there were no significant difference in both total cholesterol and LDL level between periodontitis with CHD and non-CHD patients. Further study is needed with different criteria, in larger number of subjects and more control of confounding factors.

References

Beck J, Offenbacher S, Williams R, *et al*. Periodontitis: A risk factor for coronary heart disease*? Ann Periodontol* 1998;3:127-141.

Beck JD, Garcia R, Heiss G, Vokonas P. And Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67:1123-1127.

Buhlin K, Gustafsson AA, Pockley GA, *et al*. *J European Heart* 2003;24:2099-2107.

Cutler CW, Shinedling EA, Nunn M, *et al*. Association between periodontitis and hyperlipidaemia: Cause or effect*? J Periodontol* 1999;70:1429-1434.

Cynthia RS. The marker of inflammation, oxidative stress and endothelial dysfunction in metabolic syndrome. *Forum Diagnosticum. Prodia Diagnostic Educational Services* 2006;2:2-11.

Department of Health, Republic of Indonesia. Indonesian Health Profile 2001. Jakarta 2002.

Department of Health, Republic of Indonesia. Indonesian Health Profile 2005. Jakarta 2006.

DeStefano F, Anda RF, Kahn HS, Williamson DF,Russel CM. Dental disease and risk of coronary heart disease and mortality. *BMJ* 1993;88-306.

Emingil G, Buduneli E, Aliyev A, *et al*. Association between periodontal disease and acute myocardial infarction. *J Periodontol* 2000;71:1882-1886.

Feingold KR, Staprans I, Memon RA, *et al*. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: Low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J Lipid Research* 1992;33:1765-1776.

Genco R, Offenbacher, Beck J. Periodontal disease and cardiovascular disease. Epidemiology and possible mechanism. *J American Dental Association* 2002;133:14-22,

Katz J, Chaushu G, Sharabi Y. On the association between hypercholesterolemia, cardiovascular

disease and severe periodontal disease. *J Clin Periodontol* 2001;28:865-868.

Kemal Y, Oemardi M, Prasetyo S. The correlation between periodontal and coronary artery disease status in type 2 Diabetes Mellitus patients. In: *Progress of Periodontal Research and Practice in Asian Pacific Countries.* Bartold PM, I. Ishikawa, M Sirirat, eds. Asian Pacific Society of Periodontology, 2000. p 96-101.

Kinane DF. Periodontal disease contributions to cardiovascular disease: An overview of potential mechanism. *Annals Periodontology* 1998;3:127-141.

Loesche W, Karapetow F, Pohl A, Pohl C, Kocher T. Plasma lipid and blood glucose levels in patients with destructive periodontal disease. *J Clin Periodontol* 2000;27:537-541.

Matilla KJ, Asikaenan S, Wolf J, *et al.* Age, dental infection, and coronary heart disease. *Journal of Dental Research* 2000;79:756-760.

Moeintaghavi A, Ardakani HA, Ardakani MT, Tabatabale I. Hyperlipidemia in patients with periodontitis. *J Contemp Dent Pract* 2005;3:78-85.

Montebugnoli L, Servidio D, Miaton RA, *et al.* Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factor. *J Clin Periodontol* 2004;31:25-29.

Novak MJ, Novak KF. Chronic Periodontitis. In Carranza's Clinical Periodontology, 10 Edition. Klokkevold PR, Newman MG, Takei HH, Carranza F A, eds. Saunders, 2006. p 494-499.

Persson RG, Ohlsson O, Pettersson T, Renvert S .Chronic periodontitis, a significant relationship with acute myocardial infarction. *J European Heart* 2003;24:2108-2115.

Posch Machado AC, de Souza Quirino MR, Costa Nascimento LF. Relation between chronic periodontal disease and plasmatic level of triglyceride, total cholesterol and fractions. *Braz Oral Res* 2005;19:284-289.

Spahr A, Klein E, Khuseyinova N, *et al.* Periodontal infections and coronary heart disease. Role of periodontal bacteria and importance of total pathogen burden in the coronary event and periodontal disease (CORODONT) Study. *Arch Intern Med* 2006;166:554-559.

Syafril Y. The diagnosis of periodontal disease in the Periodontal clinic, Dental Hospital, University of Indonesia. In: *Changing Trends in Periodontal Diagnosis, Disease recognition and Management.* Bartold PM, I Ishikawa, N Vergel de Dios, eds. Asian Pacific Society of Periodontology, 2004. p 34-38.

World Health Organization. *The Word Health Report 2002.* http://www.who.int/whr/en. Accessed 16 July 16 2003.

Wu T, Trevisan M, Genco RJ, Falkner KL, *et al.* Examination of the relation between periodontal health status and cardiovascular risk factors: Serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. *Am J Epidemiology* 2000;151:273-282.

Chapter 4

A preliminary study on common genetic risk factors between periodontitis and diabetes

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Introduction

Recent studies have suggested that chronic inflammation plays an etiologic role in the development of insulin resistance (Nishimura *et al* 2007, Vozarova *et al* 2002). Periodontitis, as a common chronic inflammation, has been shown to be related to the clinical indexes, complications, and mortality of diabetes (Saremi *et al* 2005, Struch *et al* 2008, Garofalo 2008). An exaggerated host response, presenting as elevated inflammatory cytokines, is a mechanism shared by both periodontitis and type 2 diabetes mellitus (Nishimura *et al* 2007).

Cyclooxygenase-2 (COX-2) is a key enzyme in the production of prostaglandins, which are critical mediators in cytokine stimulation. Prostaglandins, especially PGE_2, are well known for their role in bone resorption which leads to tooth loss in periodontitis (Noguchi *et al* 2007). In diabetics the glucose-induced insulin secretion by pancreaticβ-cell is also reduced by PGE_2. The COX-2 gene is located on 1q25.2, a region which has been proved to be linked with early onset type 2 diabetes in Pima Indians (Konheim *et al* 2003). Some common single nucleotide polymorphisms (SNPs) in the COX-2 gene have been identified as risk factors for multiple inflammatory diseases and cancers, as well as periodontitis and type 2 diabetes (Gao *et al* 2007, Ferguson *et al* 2008, Sanak *et al* 2005, Shi *et al* 2008, Ho *et al* 2008, Konheim *et al* 2003).

IL-6 is a multifunctional cytokine with a central role in host defense. Three single nucleotide polymorphisms (SNPs) at positions -174 (rs1800795), -572 (rs1800796), and -597 (rs1800797) of the IL6 promoter may result in inter-individual variation in IL6 transcription and expression (Terry *et al* 2000). Several studies have reported that the SNPs of IL-6 are associated with the progression of periodontal disease or diabetes (Hamid *et al* 2005, Illig *et al* 2004, Brett *et al* 2005, Holla *et al* 2004).

Most investigations have focused on the association between polymorphisms and either of these diseases. Little is known if these functional SNPs play roles in the relationship between type 2 diabetes and periodontitis, except for a few studies carried out in Pima Indians (Emrich *et al* 1991). A recent cross-sectional survey has claimed that diabetic patients with interleukin-1 (IL-1) genotypes have an increased risk for periodontitis in a Caucasian population, suggesting the potential role of gene polymorphisms in the development of these two diseases (Struch *et al* 2008). However, more rigorous studies, with various regions and ethnicities are

needed. For this purpose, three common SNPs (rs689466, rs20417, rs5275) in COX-2 , and three SNPs (rs800795, rs1800796, rs1800797) of IL6 were investigated to assess the associations among gene polymorphisms, diabetes and periodontitis in a Chinese population.

Material and methods

Study population

The study protocol was approved by the Ethics Committee of Southern Medical University and informed consent was obtained from each individual. Patients with type 2 diabetes were selected from the endocrinology department of Zhujiang Hospital (attachment hospital of Southern Medical University), and subjects with or without chronic periodontitis were from the department of periodontology of Guangdong Provincial Stomatological Hospital. Patient history including age, gender, body mass, height, ethnicity, education years, insurance, tooth brushing habit, and smoking status were obtained from each subject. Smoking status was classified as "never smoked" and "ever smoked". The body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters. The exclusion criteria included periodontal systemic therapy in the previous three months, cardiovascular diseases, and women in pregnancy. The prescribed medications taken by diabetic patients were recorded.

Clinical examination and diagnosis

Type 2 diabetes patients were diagnosed by physicians according to the diagnosis criteria (WHO 1999). The periodontal examinations were performed by two periodontists whose homogeneity had been checked (kappa value >0.7). Six index teeth

or their substitutes were checked, and the clinical parameters including probe depth (PD), attachment loss (AL), plaque index (PL), and bleeding index (BI) were recorded at six sites for each teeth. Diagnosis was made based on these clinical parameters according to the criteria defined by the American Academy of Periodontology in 1999. In general, subjects having a mean attachment loss more than 4 mm and at least two molars with probing depth more than 5 mm were diagnosed with moderate to severe chronic periodontitis, while subjects with probing depth less than 3 mm or attachment loss less than 2 mm were identified as periodontal healthy controls (including mild periodontitis and gingivitis). In the diabetic patients group, the edentulous patients and those with lost index teeth were diagnosed as severe chronic periodontitis so that a certain part of diabetic patients could be included. To minimise misdiagnosis, the cause of lost teeth was required to be have been periodontal disease, which was confirmed verbally by the patients themselves.

Genotyping

A buccal swab was obtained from each subject. DNA was extracted by Chelex-100 (Sigma, USA) as previously described by Duan *et al* (2001). Genotyping was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) methods. The primers and PCR conditions are shown in Table 1. The primer sequences for COX-2 could be related to the human COX-2 gene sequence (GeneBank accession number D28235.1). PCR-RFLP for IL-6 was undertaken (Fernandez-Real *et al* 2000, Seow *et al* 2005, Komatsu *et al* 2005). A total of 8 µl of PCR product was digested with the appropriate restriction endonuclease (New England Biolabs). The digested fragments were electrophoretically separated on 3%

	Primer sequence	PCR condition	Digest condition
Rs689466	F:ccctgagcactacccatgat R:gcccttcataggagatactgg	95°C 5 min; 32 cycles; 94°C 30 sec; 58°C 30 sec; 72°C 40 sec	PvuII 37°C overnight
Rs20417	F:ccgcttcctttgtccatcag R:ggctgtatatctgctctatatgc	95°C 5 min; 32 cycles; 94°C 30 sec; 58°C 30 sec; 72°C 40 sec	AciI 37°C overnight
Rs5275	F:gaaattttaaagtactttgat R: cttttacaggtgattctaccc	94°C 5 min; 32 cycles; 94 °C 30 sec; 46°C 30 sec; 72°C 20 sec;	BclI 50°C overnight
Rs1800795	F: tga cttcagctt tactctttgt R:ctgattggaaaccttattaag	94°C 3 min; 35 cycles; 94°C 30 sec; 52°C 30 sec; 72°C 20 sec	NIa III 37°C overnight
Rs1800795	F: gagacgccttgaagtaactg R: aaccaaagatgttcgaactga	94°C 3 min; 35 cycles; 94°C 30 sec; 52°C 30 sec; 72°C 20 sec	BsrB I 37°C overnight
Rs1800097	F: ctcctctaagtgggctgaag R: caagcctgggattatgaaga	94°C 3 min; 35 cycles; 94°C 30 sec; 52°C 30 sec; 72°C 20 sec	Fok I 37°C overnight

Table 1. Primer sequences, PCR conditions and restriction enzymes

agarose (Spain) gel containing goldview (Dingguo, Beijing, China) and observed under ultraviolet light. The images were stored digitally for further analysis. All the PCR procedures and restriction were performed with a negative control. To confirm the results of the PCR-RFLP, the PCR products of two or more samples in every genotype were randomly selected to sequence directly (Invitrogen, China).

Statistical analyses

All analyses were performed with Statistical Analysis System software (V.13.0 SAS Institute). Hardy-Weinberg equilibrium was tested to compare the observed and expected genotypes frequencies in control groups. The demography characters were compared with one-way ANOVA (for continuous variables) and x^2 test (for categorical variables) among groups. The distribution of genotypes and allele frequencies among groups were also compared with x^2 test. When there was a statistical significance, the odds ratios (ORs) and 95% confident intervals (CIs) were calculated by univariate and multivariate logistic regression analyses adjusting for age, gender, smoking status, BMI, tooth brushing habit, education and insurance. The criteria for significance were set at $p < 0.05$ and the p value for multiple testing (Bonferronni correction) was also corrected.

Results

Results of polymorphisms of Cyclooxygenase-2 (rs689466, rs20417 and rs5275)

The general characteristics are shown in Table 2. Significant differences were found for age, smoking status, BMI, tooth-brushing habits, education, and insurance ($p < 0.05$).

Hardy-Weinberg equilibrium were established in both control and case groups for rs689466 and rs20417, but the genotype distribution for rs5275 deviated from Hardy-Weinberg equilibrium in the control group (p

	T2DM (G1) (n = 46)	T2DM+CP (G2) (n = 76)	CP (G3) (n = 158)	Control (G4) (n = 112)	p value
Gender (F/M)	24/22	39/37	74/84	64/48	0.423
Age	57.93±10.46	61.74±9.37	50.51±7.83	51.11±8.39	<0.001
Smoking (N/Y)	37/9	57/19	104/54	92/20	0.016
BMI*	25.15±3.19	23.88±3.84	22.48±3.21	23.02±3.23	<0.001
Toothbrush (1/2)	32/14	31/45	102/56	83/29	<0.001
Education (1/2)	30/16	64/12	70/88	53/59	<0.001
Insurance (Y/N)	38/8	60/16	92/66	63/49	<0.001
Onset age*	50.73±10.33	55.66±10.22	-	-	0.021
Duration*	7.32±6.30	5.94±5.35	-	-	0.615

Table 2. General characteristics of subjects
Values are shown as mean±SD. Smoking status: N: never smoker; Y: ever smoker. BMI: *with several missing data. Tooth brushing: 1: ≥twice/day; 2: ≤once/day. Education: 1: ≤9 years; 2: >9 years. Insurance: Y: with insurance; N: with no insurance. Onset, duration: * compared with independent samples t test

= 0.04).

The distribution of genotypes and allele frequencies are shown in Table 3. For rs689466, the distribution of both genotye (AA+GA) and allele frequency (A allele) were found to be higher in G3 than in G4, and there was also a more prevalent distribution of the A allele in G2 compared with G4. The allele frequency of rs689466 was significant in G3 (p_c = 0.018) when the p value was corrected for multiple testing it only showed a marginal significance in G2 (p_c = 0.051). For rs20417, all differences were non-significant after the p value was corrected. No significance for rs5275 was found.

The interactions of rs689466 genotypes with diabetes are shown in Table 4. Diabetic subjects with the AA+GA genotype had an increased risk of periodontitis compared with those carrying the GG genotype (OR 2.130, 95% CI 1.104-4.108, p = 0.024), but the adjusted OR was reduced and non-significant

(OR 1.704, 95% CI 0.778-3.733, p = 0.183). Among the traditional risk factors, age, smoking status, BMI, education and insurance were found to be confounding factors in the multivariate logistic regression mode (p <0.05, data not shown).

Results of polymorphisms of Interleukin-6 (rs 1800795, rs 1800796, rs 1800797)

The general characteristics of subjects are shown in Table 5. The genotypes at three loci were in Hardy-Weinberg equilibrium (each P value >0.05). Only 3 haplotypes were observed out of 4 possible haplotypes (Table 6). Two major haplotypes (GGG and GCG) accounted for more than 99% of three haplotypes were observed.

The locus, IL-6-174, showed very low allele frequency (Table 7). In 481 subjects (11 failed genotyping), only two GC heterozygotes

	T2DM (G1) (n = 46)	T2DM+CP (G2) (n = 76)	CP (G3) (n = 158)	Controls (G4) (n = 112)	
rs689466					
GG	11 (23.9)	15 (19.7)	27 (17.1)	33 (29.5)	
GA	20 (43.5)	33 (43.5)	79 (50.0)	55 (49.1)	
AA	15 (32.6)	28 (36.8)	52 (32.9)	24 (21.4)	
p value	G1 vs G4 0.328	G2 vs G4 0.053	G3 vs G4 0.022	G1 vs G2 0.827	G2 vs G3 0.639
G	42 (45.6)	63 (41.5)	133 (42.1)	121 (54.0)	
A	50 (54.4)	89 (58.5)	183 (57.9)	103 (46.0)	
p value	G1 vs G4 0.520	G2 vs G4 0.017*	G3 vs G4 0.006**	G1 vs G2 0.520	G2 vs G3 0.895
rs20417					
GG	42 (91.3)	72 (94.7)	149 (94.3)	97 (86.6)	
GC	4 (8.7)	4 (5.3)	9 (5.7)	15 (13.4)	
CC p value	0 (0) G1 vs G4 0.410	0 (0) G2 vs G4 0.070	0 (0) G3 vs G4 0.029*	0 (0) G1 vs G2 0.458	G2 vs G3 0.892
G	88 (95.6)	148 (93.4)	307 (97.2)	209 (93.3)	
C	4 (4.4)	4 (2.6)	9 (2.8)	15 (6.7)	
P value	G1 vs G4 0.425	G2 vs G4 0.077	G3 vs G4 0.033	G1 vs G2 0.466	G2 vs G3 0.894
rs5275					
TT	28 (60.9)	50 (65.8)	111 (70.3)	78 (69.6)	
TC	17 (37.0)	26 (34.2)	43 (27.2)	27 (24.1)	
CC	1 (2.2)	0 (0)	4 (2.5)	7 (6.25)	
p value	G1 vs G4 0.185	G2 vs G4 0.578	G3 vs G4 0.290	G1 vs G2 0.583	G2 vs G3 0.490
T	73 (79.4)	126 (82.9)	265 (83.9)	183 (81.7)	
C	19 (20.6)	26 (17.1)	51 (16.1)	41 (18.3)	
p value	G1 vs G4 0.629	G2 vs G4 0.766	G3 vs G4 0.510	G1 vs G2 0.489	G2 vs G3 0.792

Table 3. Distributions of genotypes and allele frequencies of rs689466, rs20417 and rs5275
Values represent as numbers (%), *marginal significant after p value was corrected ($p_c = 0.051$), **significant after p value was corrected ($p_c = 0.018$)

T2DM	genotype	CP(n)	Control(n)	OR (95%CI)	p-value	OR[a] (95%CI)	p-value
no	GG	27	33	1		1	
no	GA+AA	131	79	2.027 (1.135-3.621)	0.017	1.727 (0.927-3.217)	0.085
yes	GG	15	11	1.667 (0.658-4.222)	0.281	1.380 (0.463-4.115)	0.563
yes	GA+AA	61	35	2.130 (1.104-4.108)	0.024	1.704 (0.778-3.733)	0.183

Table 4. ORs of rs689466 genotype and diabetes and its interaction
OR[a], adjusted for age, gender, smoking status, BMI, tooth brushing habit, education, and insurance
CI, confidence interval

Variable	Control (n=135)	T2DM (n=88)	CP (n=159)	CP&T2DM (n=110)	P value
Gender(male/female,N)	42/93	24/64	83/76	46/64	<0.001*
Smoking(No/Yes,N)	114/21	79/9	108/59	86/24	<0.001*
Stress(No/Yes,N)	100/35	57/31	75/84	74/36	<0.001*
Age(years, mean±SD)	51.60±9.56	59.65±10.44	51.84±8.69	62.03±10.14	<0.001**
BMI(kg/m², mean±SD)	22.76±3.98	24.22±5.47	22.34±2.79	24.06±3.54	<0.001**
Missing teeth(N, mean±SD)	1.01±2.09	1.42±2.81	2.09±2.70	3.83±3.74	<0.001**
PD(mm, mean±SD)	2.05±0.39	1.81±0.34	3.25±0.87	2.30±0.72	<0.001**
CAL(mm, mean±SD)	0	0	4.86±1.36	3.4±0.96	<0.001***
BOP(%, mean±SD)	0	0	69.38±32.67	61.27±36.81	0.078***

Table 5. General Characteristics of Subjects (n=492)
Differences in subject numbers are due to missing data. N = number.
BMI = body mass index (calculated as body weight divided by body height squared), PD = probing pocket depth, CAL = clinical attachment loss, BOP = bleeding on probing (percentage of sites with bleeding on probing).
* P value was obtained using $\div^2$ test. ** P value was obtained using one way ANOVA. *** P value was obtained using t-test. P values less than 0.05 were considered significant.

were found, and others were GG homozygotes. In the locus IL-6-597, no A-containing variant was detected (Table 7). The two loci were not analyzed statistically because their allele frequencies were too low in the population. IL-6 haplotypes were not further analyzed because they had almost the same distributions as the IL-6-572 allele frequencies.

The genotype and allele data of the locus IL-6-572 are summarised in Table 6. In terms of its genotype distribution and allele frequencies, the crude comparison among 4 groups showed a significant difference (P = 0.030 and 0.013, respectively). Specifically, the CC genotype frequency in type 2 diabetes or chronic periodontitis group (61.4% or 67.9%) was lower than the control group (71.6%), and chronic periodontitis and type 2 diabetes group shared the lowest frequency (54.5%). A similar trend could be seen in the allele frequencies.

Multiple comparisons (Table 7) between chronic periodontitis and type 2 diabetes group and control group revealed two significant P values and OR values in the CC versus GC+GG model, namely a CC genotype distribution and C-allele frequency of IL-6-572 (P = 0.006, OR = 0.475, 95%CI: 0.279-0.808 and P = 0.002, OR = 0.502, 95%CI: 0.319-0.788, respectively). In other multiple comparisons in the same model, no significant P value was observed (Table 7). In addition, crude and multiple comparisons of other two models (GG versus CC and GG versus GC+CC) did not show a significant P value (data not shown). The trend demonstrated that CC genotype and C-allele of IL-6-572 might be a protective factor for chronic periodontitis combined with type 2 diabetes. However, further logistic regression analysis with adjustment for age, gender, BMI, smoking and stress did not support the result of a CC genotype (Table 8, P = 0.058, OR= 0.523, 95%CI: 0.268-1.022).

As shown in Table 8, several significant risk factors were observed in the logistic regression analysis. BMI, age and stress may be risk factors of chronic periodontitis, type 2 diabetes or chronic periodontitis combined with type 2 diabetes.

The final outcome of periodontal disease is the loss of teeth. Despite the large variability of the number of teeth, further indications came from comparison of the number of missing teeth with respect to the IL-6-572 genotype inside each group (Figure 1). Inside chronic periodontitis and type 2 diabetes group or chronic periodontitis group, there was a significant difference (P = 0.012 or 0.021) between the number of missing teeth of different genotypes (CC versus GG+CG). Additionally within the type 2 diabetes group or control group, no similar significant difference was observed (data not shown). The results indicated that the chronic periodontitis patients carrying the G-containing genotype were more likely to lose teeth.

Discussion

The specific function of COX-2 in the formation of prostaglandins makes COX-2 a good candidate gene in both periodontitis and diabetes. The main findings in the present study were that the variant A allele of rs689466 may increase the risk of chronic periodontitis for subjects with type 2 diabetes.

The variant A allele was observed as a risk factor for several diseases, and functional analysis proposed a mechanism that the A allele of rs689466 creates a C-MYB site which is a promoter activator (Zhang *et al* 2005). In this study, unexpectedly the A allele frequencies of rs689466 were observed as being more prevalent in all case groups, and significance was found in G2 ($p_c = 0.051$) and G3 ($p_c = 0.018$) compared with G4, but not in G1 ($p_c = 1.560$). It seems that the A allele carriers are more susceptible to chronic

Haplotype 1 GGG(%)	Haplotype 2 GCG(%)	Haplotype 3 CGG(%)
178 (18.8)	768 (81.0)	2 (0.2)

Table 6. IL-6 promoter haplotypes and their frequencies in a Chinese population

	Control (n=135)	T2DM (n=88)	CP (n=159)	CP&T2DM (n=110)	P value
-174 GC					
GG(%)	132	85	156 (99.4)	106 (99.1)	
GC(%)	0	0	1 (0.6)	1 (0.9)	
CC	0	0	0	0	
-597 GA					
GG	133	88	157	108	
GA	0	0	0	0	
AA	0	0	0	0	
-572 GC					
GG (%)	2 (1.5)	2 (2.3)	3 (1.9)	7 (6.4)	
GC (%)	36 (26.9)	32 (36.4)	48 (30.2)	43 (39.1)	
CC (%)	96 (71.6)	54 (61.4)	108 (67.9)	60 (54.5)	0.030*
C-allele (%)	228 (85.1)	140 (79.5)	264 (83)	164 (74.1)	0.013**

-572 GC	T2DM vs. Control	CP vs. Control	CP&T2DM vs T2DM	CP&T2DM vs CP	CP&T2DM vs Control
CC vs GC+GG					
***P value	0.110	0.491	0.335	0.026	0.006
OR	0.629	0.838	0.756	0.567	0.475
(95%CI)	(0.355-1.112)	(0.507-1.385)	(0.427-1.336)	(0.343-0.936)	(0.279-0.808)
C-allele vs G-allele					
****Pvalue	0.130	0.499	0.203	0.784	0.002
OR	0.682	0.858	0.735	0.942	0.502
(95%CI)	(0.415-1.122)	(0.549-1.339)	(0.458-1.182)	(0.612-1.449)	(0.319-0.788)

Table 7. Genotype and allele data for the -174, -572 and -597 of IL-6 promoter in study participants Differences in subject numbers are due to missing genotypes. OR = odds ratio. CI = confidence interval. Vs. = versus. * P value for genotype distribution among 4 groups was obtained using $\div^2$ test in a model (CC vs. GC+GG). ** P value for allele frequency among 4 groups was obtained using $\div^2$ test. ***P values for genotype distribution of multiple comparisons were obtained using $\div^2$ test in a model (CC vs. GC+GG). **** P values for allele frequency of multiple comparisons were obtained using $\div^2$ test. * and ** P values less than 0.05 were considered significant. *** and **** P values less than 0.008 were considered significant.

		T2DM vs Control	CP vs Control	CP&T2DM vs T2DM	CP&T2DM vs CP	CP&T2DM vs Control
Gender	P value	0.791	0.056	0.131	0.937	0.14
	OR (95%CI)	1.117 (0.494-2.524)	1.882 (0.983-3.6)	0.577 (0.283-1.177)	1.016 (0.411-2.511)	0.564 (0.264-1.206)
Age	P value	<0.001	0.106	0.467	<0.001	<0.001
	OR (95%CI)	1.109 (1.07-1.15)	1.026 (0.995-1.058)	0.988 (0.958-1.02)	0.889 (0.849-0.931)	0.886 (0.853-0.919)
BMI	P value	0.072	0.181	0.899	<0.001	0.072
	OR (95%CI)	1.067 (0.994-1.145)	0.949 (0.879-1.025)	0.996 (0.929-1.067)	0.796 (0.702-0.902)	0.92 (0.839-1.008)
Smoking	P value	0.818	0.249	0.201	0.088	0.849
	OR (95%CI)	0.878 (0.29-2.658)	1.574 (0.728-3.403)	0.524 (0.195-1.41)	2.404 (0.879-6.574)	0.912 (0.354-2.353)
Stress	P value	0.02	<0.001	0.748	0.048	0.006
	OR (95%CI)	2.328 (1.14-4.754)	3.048 (1.76-5.281)	1.114 (0.575-2.159)	2.232 (1.005-4.954)	0.361 (0.175-0.745)
IL-6-572	P value	0.218	0.998	0.438	0.291	0.058
	OR (95%CI)	1.545 (0.773-3.09)	0.999 (0.569-1.754)	0.779 (0.414-1.465)	0.652 (0.295-1.442)	0.523 (0.268-1.022)

Table 8. Logistic regression analyses for associated risk factors of chronic periodontitis and type II diabetes in a Chinese population
Independent variables: gender (dichotomous, male = 1, female = 0), age and BMI (continuous), smoking (dichotomous, yes = 1, no = 0), stress (dichotomous, yes = 1, no = 0), IL-6-572 (dichotomous, CC = 1, GC+GG = 0). P values less than 0.05 were considered significant.

periodontitis rather than type 2 diabetes, indicating the effect of this genetic polymorphism was different in these two diseases. From Table 4, it was observed that there was a potential interaction between the genotypes and type 2 diabetes, and the risk of chronic periodontitis was increased when considering both two factors. However, confounding factors such as age, smoking, BMI and tooth brushing should be considered, as the ORs changed over 10% after adjustment, which indicated the effects of these confounding factors should be potentially important (Shen *et al* 2006). Besides the functional effect of this variant allele, other studies have shown the risk effect on asthma and colorectal cancer (Shi *et al* 2008, Tan *et al* 2007), which are similar to our study. Therefore the conclusion that the A allele is a risk factor and the effect of this polymorphism on type 2 diabetes and chronic periodontitis may be different.

There are many diversities on the C allele of rs20417 in various ethnicities, some suggest a protective effect (Ho *et al* 2008, Papafili *et al* 2002, Orbe *et al* 2006) while others suggest a risk effect (Konheim *et al* 2003, Szczeklik *et al* 2004). In this study, the variant C allele of rs20417 was more prevalent in G4 compared with the other case groups, but no significance was found referring to the corrected p value (p_c >0.05). However, the protective effect of the C allele could not be ignored for its functional effect on the promoter activity, and the result in the present study may be due to the relatively small sample size (Papafili *et al* 2002).

No significant difference was found for rs5275, interestingly, the Hardy-Weinberg equilibrium was not detected in this control group either. It was assumed that the hospital-based study still has its limits on selection bias (Berkson bias). Although the recruited subjects were aged from 40 to 80, most of the controls were under 60 years (data not shown).

This may also lead to the non-equilibrium. However, a trend that the prevalence of the CC genotype was more prevalent in G4 compared with the other groups could still be observed. The protective role of the C allele was also reported in other diseases (Shen *et al* 2006, Park *et al* 2006, Hu *et al* 2005). From the data in Table 1, it can be surmised that subjects with periodontitis and diabetes have a larger frequency of cigarette smoking, less years of education, and less frequency of tooth brushing. In fact, the older age of diabetic subjects may lead to these differences, for instance, the older generation did not have as much education as the younger generation had, and the awareness of oral health is also lacking. Therefore, these data might not represent true variables in this analysis.

A different genetic background in Chinese and Caucasians was found with respect to the allele frequencies of the IL-6 promoter SNPs. The allele frequencies of IL-6-174 and IL-6-597 reported in Caucasian (0.40-0.47) (Terry *et al* 2000, Holla *et al* 2004) were much higher than those found in this study (data not shown). On the other hand, the frequency of the C allele of IL-6-572 in Caucasians (0.03-0.1) was much lower than those of the Chinese population (0.74-0.85). Our results are similar to Korean and Japanese studies (Park *et al* 2003, Kitamura *et al* 2002, Zhai *et al* 2001). High frequency population-specific alleles are particularly useful for mapping genes responsible for disease susceptibility (Stephens *et al* 2001). The SNP of IL-6-572 may be a useful marker for the association study in Asians.

In multiple comparisons, IL-6-572 genotypes distribution and allele frequencies revealed that the CC genotype and C-allele might be a protective factor of chronic periodontitis combined with type 2 diabetes. After adjustment for age, gender, BMI, smoking and stress the result became a borderline trend in terms of IL-6-572

genotypes distribution. A possible explanation is that our subjects were not matched on age, gender and smoking (Table 1, p <0.001). Therefore, subjects matched for age, gender and smoking status should be selected in similar studies hereafter.

The IL-6-572 C-allele as a protective factor of diseases was seldom reported in previous studies. Holla *et al* (2004) first observed that IL-6-572 polymorphism may be one of the protective factors in the development of chronic periodontitis. It is interesting that the result of association between the number of missing teeth and IL-6-572 genotype (Figure 1) supports the conclusion of the IL-6-572 C-allele as a protective factor.

The viewpoint above is somewhat in contrast to those published by Ferrari *et al* (2003) who found that carriage of the C-allele at IL-6-572 was associated with increased IL-6 biological activity in a Caucasian population. The reason for the discrepancy is unclear, but different population and genetic backgrounds may be reasons (Holla *et al* 2004). In previous gene polymorphisms studies, the discrepant results were observed on many occasions among different populations, for example IL-1 gene polymorphisms (Laine *et al* 2002, Meisel *et al* 2003, Rogers *et al* 2002). One deficiency of the present study is that plasma IL-6 levels were not measured in this Chinese population. Further studies are required to interpret the discrepancy.

In the present study, the C-allele frequency of IL-6-572 in chronic periodontitis group was lower than the control group, but without significant difference. The lowest frequency was observed in chronic periodontitis and type 2 diabetes group with a significant difference between chronic periodontitis and type 2 diabetes group and control group. This phenomenon seems to indicate that IL-6-572 polymorphism is a modifying factor which does not directly influence the incidence of
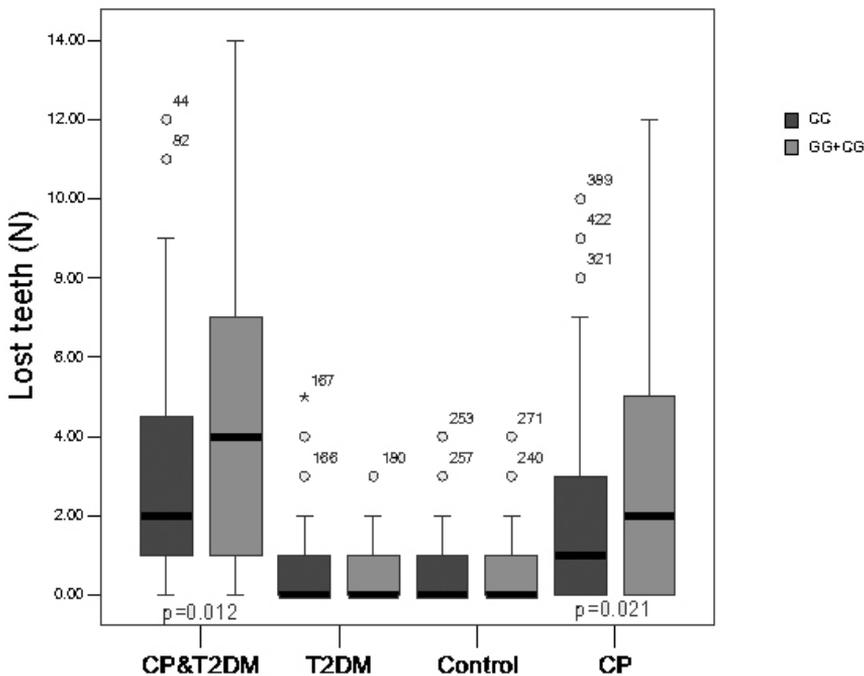
Figure 1. Box plots show association between IL-6-572 different genotypes and the numbers of lost teeth in each group. P value was obtained using T-test.

periodontitis in the population without type 2 diabetes but reduces the possibility of periodontitis complications in diabetes patients. To some extent this verified the hypothesis that the promoter SNPs of IL-6 might be associated with the progression of periodontitis aggravated with diabetes.

In conclusion, it showed that the variant A allele of rs689466 appears to be a strong risk predictor for severe chronic periodontitis, and this risk was increased for the diabetic subjects carrying the A allele. However, further studies with more rigid control of confounding factors are needed. There is not enough evidences to confirm that the gene polymorphism at IL-6-572 is one of potential protective factors of chronic periodontitis combined with type 2 diabetes for the present, but the IL-6-572 genotype and allele distributions are undoubtedly unique to subjects with chronic periodontitis and type 2 diabetes in a Chinese population.

References

Brett PM, Zygogianni P, Griffiths GS, *et al*. Functional gene polymorphisms in aggressive and chronic periodontitis. *J Dent Res* 2005;84:1149-1153.

Duan H, Zhang J, Huang P, Zhang Y. Buccal swab: A convenient source of DNA for analysis of IL-1 gene polymorphisms. *Huaxi Kouqiang Yixue Zazhi* 2001;19:11-13.

Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. *J Periodontol* 1991;62:123-131.

Ferguson HR, Wild CP, Anderson LA, *et al*. Cyclooxygenase-2 and inducible nitric oxide synthase gene polymorphisms and risk of reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2008;17:727-731.

Fernandez-Real JM, Broch M, Vendrell J, *et al*. Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes* 2000; 49:517-520.

Ferrari SL, Ahn-Luong I, Garnero P, *et al*. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. *J Clin Endocrinol Metab* 2003;88:255-259.

Gao J, Ke Q, Ma HX, *et al*. Functional polymorphisms in the cyclooxygenase 2 (COX-2) gene and risk of breast cancer in a Chinese population. *J Toxicol Environ Health* 2007;70:908-915.

Garofalo GS. Relationships between diabetes mellitus and periodontal disease: Current knowledges and therapeutic prospects. *La Clinica terapeutica* 2008;159:97-104.

Hamid YH, Rose CS, Urhammer SA, *et al*. Varistions of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* 2005;48:251-260.

Ho YP, Lin YC, Yang YH, *et al*. Cyclooxygenase-2 Gene-765 single nucleotide polymorphism as a protective factor against periodontitis in Taiwanese. *J Clin Periodontol* 2008;35:1-8.

Holla LI, Fassmann A, Stejskalova A, *et al*. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. *J Periodontol* 2004;75:30-36.

Hu Z, Miao X, Ma H, *et al*. A common polymorphism in the 3'UTR of cyclooxygenase 2/prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. *Lung Cancer* 2005;48:11-17.

Illig T, Bongardt F, Schopfer A, *et al*. Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 Diabetes. *J Clin Endocrinol Metab* 2004;89:5053-5058.

Kitamura A, Hasegawa G, Obayashi H, *et al*. Interleukin-6 polymorphism (-634C/G) in the promoter region and the progression of diabetic nephropathy in type 2 diabetes. *Diabet Med* 2002;19:1000-1005.

Komatsu Y, Tai H, Galicia JC, *et al*. Interleukin-6(IL-6) -373 A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. *Tissue Antigens* 2005; 65:110-114.

Konheim YL, Wolford JK. Association of a

promoter variant in the inducible cyclooxygenase-2 gene (PTGS2) with type 2 diabetes mellitus in Pima Indians. *Hum Genet* 2003;113:377-381.

Laine ML, Farre MA, Garcia-Gonzales MA, *et al*. Risk factors in adult periodontitis: Polymorphism in the interleukin-1 gene family (in Dutch). *Ned Tydschr Tandheelkd* 2002;109:303-306.

Meisel P, Siegemund A, Grimm R, *et al*. The interleukin-1 polymorphism, smoking, and the risk of periodontal disease in the population-based SHIP study. *J Dent Res* 2003;82:189-193.

Nishimura F, Iwamoto Y, Soga Y. The periodontal host response with diabetes. *Periodontol 2000* 2007;43:245-253.

Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. *Periodontol 2000* 2007;43:85-101.

Orbe J, Beloqui O, Rodriguez JA, *et al*. Protective effect of the G-765C COX-2 polymorphism on subclinical atherosclerosis and inflammatory markers in asymptomatic subjects with cardiovascular risk factors. *Clinica Chimica Acta* 2006;368:138-143.

Papafili A, Hill MR, Brull DJ, *et al*. Common promoter variant in cyclooxygenase-2 represses gene expression: Evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 2002;22:1631-1636.

Park BL, Lee HS, Kim YJ, *et al*. Association between interleukin 6 promoter variants and chronic hepatitis B progression. *Exp and Mol Med* 2003;35:76-82.

Park JM, Choi JE, Chae MH, *et al*. Relationship between cyclooxygenase 8473T>C polymorphism and the risk of lung cancer: A case-control study. *BMC Cancer* 2006;6:70.

Rogers MA, Figliomeni L, Baluchova K, *et al*. Do interleukin-1 polymorphisms predict the development of periodontitis or the success of dental implants? *J Periodont Res* 2002;37:37-41.

Sanak M, Szczeklik W, Szczeklik A. Association of COX-2 gene haplotypes with prostaglandins production in bronchial asthma. *J Allergy Clin Immunol* 2005;116:221-223.

Saremi A, Nelson RG, Tulloch-Reid M, *et al*. Periodontal disease and mortality in type 2 diabetes. *Diabetes Care* 2005;28:27-32.

Seow A, Ng PKD, Choo S, *et al*. Joint effect of asthma/atopy and an IL-6 gene polymorphism on lung cancer risk among lifetime non-smoking Chinese women. *Carcinogenesis* 2005;27:1240-1244.

Shen J, Gammon MD, Terry MB, *et al*. Genetic polymorphisms in the cyclooxygenase-2 gene, use of nonsteroidal anti-inflammatory drugs, and breast cancer risk. *Breast Cancer Res* 2006;8:R71.

Shi J, Misso NL, Kedda MA, *et al*. Cyclooxygenase-2 gene polymorphisms in an Australian population: Association of the -1195G > A promoter polymorphism with mild asthma. *Clin Exp Allergy* 2008;38:913-920.

Stephens JC, Schneider JA, Tanguay DA, *et al*. Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001;293:489-493.

Struch F, Dau M, Schwahn C, *et al*. Interleukin-1 gene polymorphism, diabetes, and periodontitis: Results from the Study of Health in Pomerania (SHIP). *J Periodontol* 2008;79:501-507.

Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. *J Allergy Clin Immunol* 2004;114:248-253.

Tan W, Wu J, Zhang X, *et al*. Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. *Carcinogenesis* 2007;28:1197-1201.

Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem* 2000;275:18138-18144.

Vozarova B, Weyer C, Lindsay RS, *et al*. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002;51:455-461.

Zhai RH, Liu G, Yang C, *et al*. The G to C polymorphism at -174 of the interleukin-6 gene is rare in a southern Chinese population. *Pharmacogenetics* 2001;11:699-701.

Zhang X, Miao X, Tan W, *et al*. Identification of functional genetic variants in cyclooxygenase-

Chapter 5

Clinical periodontal practice: The Philippine scenario

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Introduction

Trends in the clinical practice of dentistry, particularly in Periodontics, have undergone many changes in the last ten years. A conceptual shift of periodontal disease being regarded as not merely an oral health problem but as having an impact on systemic health has emerged. This interest in the possible systemic effects of periodontal disease has subsequently led to a shift in treatment goals. Research is now focused on investigating factors that affect disease progression and how these can be modified to promote early intervention (Dave *et al* 2004).

It is hypothesized that inflammation creates a triangular interaction between obesity, diabetes and periodontal disease, with obesity being considered a risk factor for both type 2 diabetes and periodontal disease, and with diabetes also increasing the risk for periodontal disease (Genco 2006).

The key role of inflammation in cardiovascular conditions, particularly in atherosclerosis, has been established through 20 years of research. Inflammation is widely believed to be the underlying factor in the formation of atheromatous plaques, which can increase in size and may eventually rupture, causing heart attack or stroke (Libby 2006).

The correlation of certain systemic conditions, most especially diabetes and cardiovascular diseases, to the periodontium (and vice versa) cannot be overemphasized. Current research has heightened these associations (Ryder 2007, Paquette 2007) and formed the basis for the evolution of the role of dentists from mere oral care providers to healthcare providers (Di Matteo 2005). The dental practitioner currently plays an active role in the prevention and control of certain systemic conditions and may be in the first line of determining their possible existence. According to Dr F Panagakos in an interview reported in *Inside Dentistry*, the dentists' role has evolved from being limited to repair of damage to the tissues of the oral cavity to the proactive prevention of diseases (Di Matteo 2005). He adds that dentists should participate more often in improving the systemic health of our patients. Embracing this role involves thoroughness whilst investigating the patient's medical background as well as assessment of the oral cavity. General dentists are, at the least, expected to be able to accurately diagnose the periodontal condition, thoroughly educate their patients and provide appropriate referrals (Di Matteo 2005).

The last two decades have also brought the understanding that periodontal regeneration, while biologically possible, is not always clinically attainable (Bartold

2006).There are many aspects in regenerative procedures where treatment outcomes cannot be predicted due to the many factors involved in the process which cannot be precisely defined and controlled. There is also no evidence that regenerative procedures increase the life span of teeth (Bartold 2004).

A better understanding of the principles of osseointegration, together with recent developments in implant design and placement techniques has made implant therapy a valid alternative to maintaining teeth affected with advanced periodontal disease. Patients and dentists now view the replacement of teeth lost from oral infections differently, with the introduction of better and more affordable implant systems (Chung 2006).

In 1999, during the 3rd Asian Pacific Society of Periodontology Meeting in Bangkok, Thailand, it was reported that the clinical practice of periodontics in the Philippines was not well–developed (Vergel de Dios *et al* 2000). As of 1999, there were only five practitioners who had obtained formal postgraduate training in periodontics (Master's degree or at least two years full time training). Given a population of 88 million and a limited number of dentists with training in periodontics, it would be advantageous for the populace if general dentists were able to perform basic periodontal procedures for disease control.

In 2009 we once again examined the progress of periodontal practice in the Philippines. Keeping in mind the theme of this 8th APSP Meeting; "Periodontics: Beyond the Pocket", the authors decided to assess if indeed periodontal practice in the Philippines had advanced and progressed to the level beyond infection or disease control. The aims of this study were to:

1. determine the present status of periodontal practice in the Philippines
2. determine what periodontal procedures other than infection or disease control are being performed by dental practitioners in the Philippines
3. assess the level of awareness of dental practitioners regarding recent dental concepts and developments particularly on systemic health conditions that may be associated with periodontal disease
4. compare data gathered in 1999 with the results of this study.

Materials and methods

Sample population

Convenience sampling was utilised for this study. Random sampling was the intended method however this was not possible due to Philippine Dental Association policy changes which did not allow the authors access to the database of the general membership of the association. The sample population consisted mostly of general dental practitioners who were recruited to answer the survey questions during regional dental conferences, dental seminars, and other dental forums conducted in the different areas of the Philippines.

Data collection

The survey questionnaires were distributed to attendees of dental conferences held from March 2009 to July 2009.

The survey questionnaires consisted of eighteen questions formulated to determine the range of periodontal diagnostic and treatment procedures which the respondents provide in their clinical practices, as well as questions on treatment practices for patients with systemic illnesses. Other variables that were considered were dentists/respondents' profile and practice demographics.

The data was then encoded and analysed using Statistical Package for the Social Sciences (SPSS) Version 15.

Location/Area	Frequency	Percent
Greater Manila Area	215	40.5
North Central Luzon	90	16.9
South Luzon	76	14.3
Visayas	111	20.9
Mindanao	39	7.3

Table 1. Area distribution of sample population

Type of Practice	Frequency	Percent (2009)	Percent (1999)
General Practice	464	87.4	85.8
Limited to Specialty	17	3.2	12.8
Associate Practice/ Partnership	31	5.8	1.4
No Response	19	3.6	

Table 2. Practice profile of sample population

Results

1500 survey questionnaires were distributed to Filipino dentists attending regional and area conferences. Only 531 questionnaires returned were found to be valid and were considered for analysis.

Table 1 shows the geographical areas the survey responders came from. The Philippines is composed of three main islands: Luzon, Visayas and Mindanao. The smallest percentage of responders came from Mindanao. While the island of Mindanao is the second largest, dental conferences in Mindanao were cancelled because of security threats from terrorist attacks and armed conflicts during the period of data collection.

Respondents' profile

391 respondents were female, while 107 were males. Two respondents did not indicate their gender. The dominance of female practicing dentists in the Philippines had long been recognized and was previously reported in the 1999 survey (Vergel de Dios *et al* 2000). The ratio is approximately 1:4.

Survey respondents were mostly General Practitioners (comprising 91% of respondents) while 3.2% of respondents claimed they have limited their dental practices to their respective specialties. The rest (5.8%) were employed as associates or engaged in a partnership (Table 2). This profile is similar to the findings ten years ago (Vergel de Dios *et al* 2000).

The percentage of dentists who had been in practice for 11 to 15 years (20.5%) remained the same as that reported in the 2000
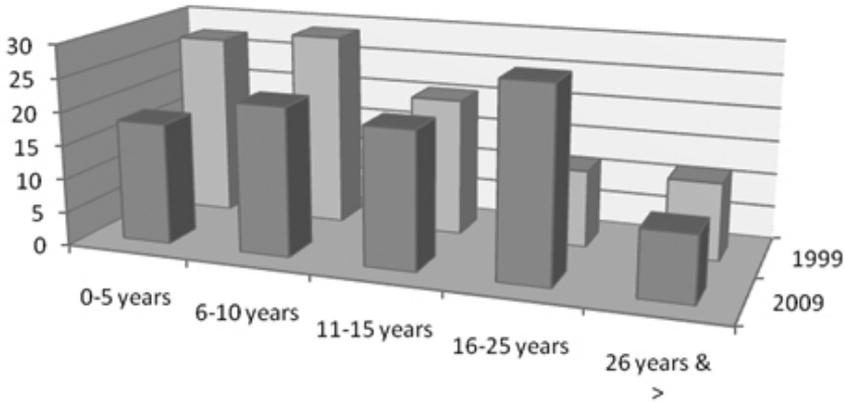
Figure 1. Distribution of dentists according to length of years in practice

survey. The largest proportion of respondents (28.43%) was those who had been in dental practice for 16 to 25 years (Figure 1).

Most dentists (52.9%) reported they work around 8 hours per day. Less than 10% (7.9%) work 10 hours per day and nearly the same amount (7.21%) will spend 4 hours or even less in practice (Table 3).

About two thirds (73.1%) of respondents have urban or city practices. The rest hold practices in rural areas (17.9%) and smaller percentage (6.2%) in suburban areas or outside the city limits.

Periodontal practice

More than half of respondents reported that 25% of their practice is devoted to providing periodontal treatment procedures. About a quarter of total respondents said that 50% of their practice is allocated to providing periodontal treatment. Only 1.3% of respondents have limited periodontal practices. 22 (4.1%) out of 531 respondents did not answer this item (Figure 2).

Periodontal diagnosis

In the past, examination of the periodontium was not routinely integrated into

	Frequency	Percent
No response	15	2.8
10 hours	42	7.9
8 hours	281	52.9
7 hours	38	7.2
6 hours	69	13
5 hours	48	9
4 hours or less	38	7.2
Total	**531**	**100**

Table 3. Distribution of dentists according to hours devoted to practice

the basic mouth examination conducted by Filipino dentists. The focus was mainly on caries detection and searching for non-restorable teeth. To determine if this practice of overlooking the periodontal tissues had changed over the last ten years, we included six items addressing this issue in the survey questionnaire. The results of the question as to whether periodontal probing was being done as part of routine mouth examination had the following results: four respondents
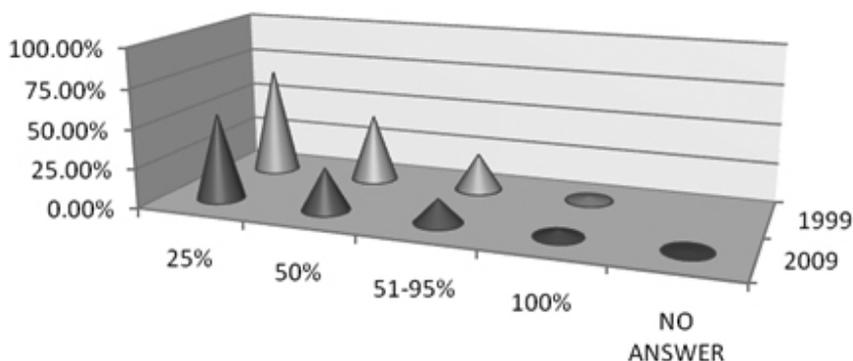
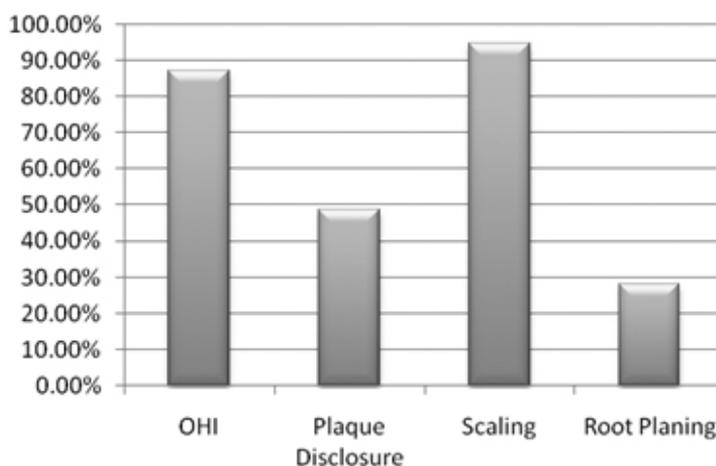**Figure 2.** Percentage of practice devoted to periodontics

Figure 3. Periodontal procedures undertaken

(0.86%) did not answer this item, of the 302 (56.9%) who answered that they perform periodontal probing, only 33 (10.92%) would perform periodontal probing on all new cases while the rest (89.08%) would probe only when there are symptoms of periodontal disease present or when patients complain of discomfort in gingival areas.

It is interesting to note that 66.7% of Filipino dentists surveyed will not proceed with periodontal treatment without preoperative radiographs. However, only 36 (55.4%) of those who require preoperative radiographs would take them routinely for all new patients diagnosed with periodontitis. The rest (44.6%) will perform radiographic

examination only when there is existing symptoms such as tooth mobility and/or patient discomfort or pain. The remaining 168 or 31.6% of dentists surveyed however will still proceed with administration of periodontal treatment even without the aid of radiographs.

Periodontal treatment procedures

Similar to the findings in the 1999 survey, the majority of dentists (94.16%) perform scaling and polishing. 86.6% of respondents conduct chairside oral hygiene instruction. A mere 27.87% would perform root planing, slightly better than the 20.2% reported in the

1999 survey (Figure 3).

446 out of the 506 dentists surveyed reported that they have a mechanically driven (ultrasonic) scaler in their clinics. However, of the 148 dentists who claim to perform root planing only 106 of them reported owning periodontal curettes.

Only 508 of among the 531 respondents answered the item on what periodontal surgical procedures they perform in their clinical practices. Of these, 36.9% stated they perform periodontal surgery. Of the types of surgical procedures undertaken, 79.6% perform resective procedures such as gingivectomy, 44.3% do access surgery, 8% carry out regenerative procedures and 10.1% do implant therapy. Another 8% perform surgical procedures other than the ones mentioned in the survey.

In relation to the question on crown lengthening, 30 respondents (5.6%) did not respond to this item. Among the 501 dentists, 168 (33.53%) perform this procedure, primarily for prosthetic and restorative reasons. However, a large percentage (60.4%) of the dentists surveyed, do not perform any of the listed surgical procedures. The reasons given were lack of training (63.86%),

inadequate materials and instruments (32.40%), lack of skill (21.8%), no cases that warrant surgery (13.71%) and lastly, surgery not being an adhered treatment concept (4.6%) (Figure 4). It is noteworthy that 73.3% of the respondents refer patients to periodontists and/or qualified dentists.

With regards to treating patients who have systemic conditions, 187 out of the 371 respondents (50.4%) would treat diabetic patients while 53 (14.2%) would perform periodontal therapy on an anaemic patient. Only 15.6% of dentists will manage patients with atherosclerosis while a smaller 12.6% of dentists who answered this item will manage the dental problems of someone with liver cirrhosis.

328 of dentists surveyed (61.77%) selected fear of complications as a reason for their unwillingness to treat patients with systemic conditions. There were 147 (33%) responses to lack of medical supplies and equipment to handle possible complications and 101 (22.7%) replies that indicated inadequate understanding of the appropriate management as to why dentists surveyed would refuse treatment on patients with known systemic conditions.
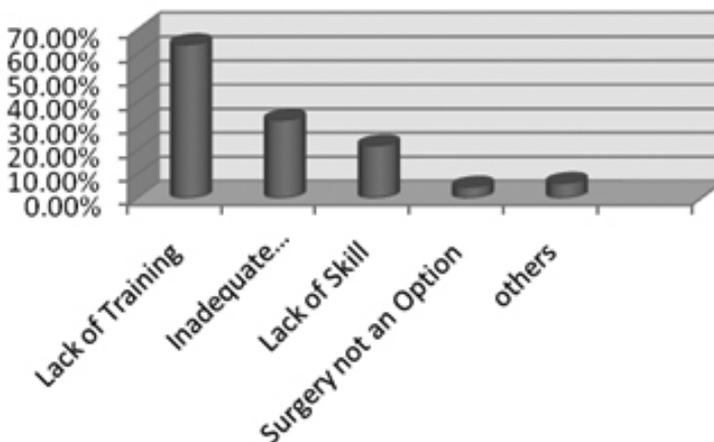

Figure 4. Reasons for not performing periodontal surgery

Periodontal disease/systemic condition interrelationship

6.6% of the 531 dentists did not reply to this item. Among the 496 who answered, 80.2% believe that diabetes affects periodontal disease progression. 46.3% indicated a recognition of the influence on pregnancy on periodontitis. Only 16.9% noted the association of atherosclerosis with periodontitis.

Supportive care

Of the 531 respondents, 42 did not answer this question. Of the 489 who replied, 472 (96.5%) prescribe regular visits to their patients for maintenance care while only 17 (3.4%) do not recall their patients regularly.

Discussion

This study looked into the ten-year progress of periodontal practice in the Philippines. It was also undertaken to find out if practice styles had changed from merely managing or controlling oral infections to looking beyond the periodontal pocket and addressing other patient concerns like aesthetics and implant therapy. At present, there are 13,500 registered members of the Philippine Dental Association (PDA 2008) with approximately 17,500 total practicing dentists in the Philippines (Professional Regulation Commission 2000). Of these, only 1,750 are in the public health care system (Monse and Yanga-Mabunga 2007). The number of Filipino dentists who had acquired Postgraduate training in periodontics increased only slightly since 1999. There is now one holder of a PhD in Microbiology (Periodontology), four have obtained Master's degrees in periodontics, four have two years training in periodontics, whilst seven underwent training courses in periodontics of a one year or less duration.

The results showed that there is a lack of development in terms of periodontal treatment practices. The majority of Filipino dentists surveyed were general practitioners would devote only about 25% of their clinic hours to performing periodontal procedures, very similar to the findings in 1999 (Vergel de Dios *et al* 2000). Of the different surgical procedures, resective procedures such a gingivectomy remain of the most common in dentists surveyed. Regenerative procedures and implant therapy on the other hand have remained low. Major reasons cited for not doing periodontal surgery were the lack of training, lack of skill and inadequate access to the proper instruments and materials. The absence of postgraduate programs and course offerings in periodontics until the late 1990's may be a big determinant in this finding. The first postgraduate offering in periodontics commenced in 1998 by a private university but this had ceased operations in 2002 from a lack of qualified teachers. Two other private universities, the University of the East and the Centro Escolar University are now offering Masters degree programs in periodontics, orthodontics and prosthodontics. However, these only commenced in 2004 and 2008 respectively. The only dental specialty that seems to be well developed in the Philippines is orthodontics. There are about 150 practicing orthodontists who either have postgraduate degrees or had undertaken training programs that are certified by the Philippine Board of Orthodontics. The state university, University of the Philippines only offers Orthodontics in their Graduate School. Skill development and training of other specialities is not readily available therefor dentists need to go abroad to study, most of the time remaining there to work upon completion of their studies.

Periodontal treatment involves making complex decisions and planning the appropriate treatment procedure. Thus, patient

and dentist-related factors should be taken into consideration as well as the range of treatment procedures that are available. Dentist factors include the education, skill and interest of the practitioner as well as their mode of practice (Jeffcoat 1993). Patient-related factors on the other hand, such as cost, preference and convenience are sometimes less considered when choosing treatment alternatives (Grembowski *et al* 1988). Currently the average Filipino worker earns a daily wage of US$8.30. As such, it is expected that the average patient will prioritise basic needs (e.g. food, shelter) over oral health care. In addition, about 40% of the Philippine population live in poverty, earning less than US$0.75 a day. For this grossly disadvantaged group, any health care would be deemed as outright expensive and unaffordable (Philippine National Statistics Office 2009).

Notably, the majority of respondents came from the group of dentists who had been in practice for 16-25 years and most probably did not have the benefit of receiving information on current paradigm shifts and conceptual changes in the areas of disease susceptibility, genetics and disease-modifying risk factors while they were studying. Unless this group were active in attending continuing education courses there could be a lack of knowledge and skill in newer treatment concepts. Dental training programs should consider incorporating new information such as the use of prognostic risk factors (Kornman 2001).

Most of the respondents in this study are from areas outside of the Greater Manila Area. 73.1% of respondents claimed they hold their practices in an urban centre or a capital city, similar to the findings in Australia where 70% of practicing dentists and in Finland where 91% of dentists in the private sector hold their practice in a capital city, however by Western standards these centres or cities in the Philippines are not truly urbanized (Schwarz 2006, Heinikainen *et al* 2002). To update themselves on what is current and further develop their skills, Filipino dentists will have to travel to Metro Manila where training institutions are located or at best out of the country. In addition, the dentist has to consider all other expenses that would be incurred for the duration of the training, including procurement of sophisticated equipment and updated materials.

Implant therapy and regenerative procedures such as tissue regeneration, bone and connective tissue grafts, are still not well subscribed treatment modalities. Whilst in the United States a decline in the prevalence rate of dental caries and in the mean number of decayed, missing, or filled (DMF) teeth is observed in every age and income groups, the prevalence rate of dental caries in the Philippines has remained at a high 90% in the last two decades (Maas 2006, Monse and Yanga-Mabunga 2007). We can only surmise then that disease control still constitutes the main focus of dental practices nationwide whether in periodontics or in Restorative Dentistry.

Oral diseases have long been recognized as a low priority in most health care delivery systems and the Philippine dental health care system is no exception. Health policies should address the continued high prevalence of dental caries and other oral infections particularly in undeveloped countries (Van Palenstein and Benzian 2007). We need to support the advocacy put forward by the World Health Organization that when planning for national health care systems, it can no longer be accepted that the mouth is separate from the rest of the body.

Acknowledgments

References

Bartold PM. Future Directions in clinical periodontics. In: *Research Advances and Clinical Practice in Periodontics: Bridging the Gap.* Asian Pacific Society of Periodontology 2006: pp 103-111.

Bartold PM. Trends in periodontal repair and regeneration. In: *Current Trends in Periodontal Diagnosis, Disease Recognition and Management.* Asian Pacific Society of Periodontology 2004: pp 79-88.

Chung KM. Contemporary clinical techniques for enhanced periodontal prognosis. In: *Research Advances and Clinical Practice in Periodontics: Bridging the Gap.* Asian Pacific Society of Periodontology 2006: pp 90-94.

Dave S, Batista Jr EL, Van Dyke TE. Cardiovascular disease and periodontal diseases: Commonality and causation. *Compendium of Continuing Dental Education in Dentistry* 2004;25:26-37.

Di Matteo A. How much do we know? *Inside Dentistry* 2005;39-52.

Genco RJ. The three-way street. Oral and whole body health. *Scientific American* 2006:18-22.

Grembowski D, Milgrom P, Fiset L. Factors influencing dental decision making. *J Public Health Dent* 1988;48:159-167.

Heinikainen M, Vehkalahti M, Murtomaa H. Periodontal treatment practices of Finnish dentists. *J Clin Periodontol* 2002;29:1101-1106.

Jeffcoat M. When to treat: When to refer. *International Dental Journal* 1993;43:185-191.

Kornman KS. Patients are not equally susceptible to periodontitis: Does this change dental practice? *J Dent Educ* 2001;65:777-784.

Libby P. Heart Health in the Inflammation Age. Oral and Whole Body Health. *Scientific American* 2006:12-16.

Maas WR. Access to care-What can the United States learn from other countries? *Comm Dent Oral Epidemiol* 2006;34:232-240.

Monse B, Yanga-Mabunga S. Oral health needs of Filipino children: The Results of the 2006 National Oral Health Survey. *Developing Dentistry* 2007;8:7-9.

Pacquette DW, Brodala N, Nichols TC. Cardiovascular disease, inflammation and periodontal infection. *Periodontol 2000* 2007;48:113-126.

Philippine Dental Association (PDA). Personal communication. August 2008.

Philippine National Statistics Office. http://www.census.gov.ph/data (Accessed 30 November 2009)

Ryder MI. The influence of smoking on host responses in periodontal infections. *Periodontology 2000* 2007;43:267-277.

Schwarz E. Access to oral health care - an Australian perspective. *Community Dent Oral Epidemiol* 2006;34:225-231.

Van Palenstein HW, Benzain H. New momentum for oral health worldwide depends on all of us. *Developing Dentistry* 2007;8:2-3.

Vergel de Dios NV, Serraon, AP, Mabunga SY. Progress of periodontal research and practice in the Philippines. In: *Progress of Periodontal Research and Practice in Asian Pacific Countries.* Asian Pacific Society of Periodontology 2000; pp 62-73.

Chapter 6

Periodontal screening and management: The foundation of general dental practice

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Introduction

Periodontal diseases are the most commonly occurring yet unusual infections in humans due to the anatomically unique dento-gingival structure and the nature of pathogenic plaque biofilm infection (Jin 2008a). Periodontitis is characterized by bacteria-induced inflammatory destruction of tooth-supporting tissues and alveolar bone, and it remains the most common cause of tooth loss in adults worldwide. Although the presence of bacteria is essential, it is insufficient for disease to occur nor is it directly responsible to the severity of the diseases. The disease severity is dependent upon a dynamic equilibrium of bacteria-host interactions which are significantly influenced by various genetic, epigenetic and environmental factors in a susceptible host (Page *et al* 1997, Kornman 2008, Gomez *et al* 2009). A range of risk factors which have been studied include subject determinants, social and behavioural factors, systemic factors, genetic factors, tooth factors, and microbial risk factors (Nunn 2003). Emerging evidence demonstrates that periodontal diseases are serious infections significantly affecting not only oral health, but also potentially exert profound effects on general health, e.g. cardiovascular diseases, cerebral infarction (or stroke), diabetes, pre-term birth, and aspiration pneumonia (Jin *et al* 2003, Pihlstrom *et al* 2005, Jin and Wang 2007). Recent studies suggest that control of periodontal infection through non-surgical treatment could reduce the levels of systemic inflammation and improve the endothelial function in patients with severe periodontitis (Mattila *et al* 2002, Ide *et al* 2003, D'Aiuto *et al* 2005a, D'Aiuto *et al* 2005b, Offenbacher and Beck 2005, Seinost *et al* 2005, Tonetti *et al* 2007). Recent findings on the association of chronic periodontitis with increased levels of circulating endothelial progenitor cells further support the potential link between periodontal inflammation and endothelial function (Li *et al* 2009).

Over the past two decades, substantial progress has been made in understanding the etiology and pathogenesis of periodontal diseases and in developing various innovative approaches in periodontal practice, which could be well reflected in recognition of dental plaque as biofilms (Jin 2008b, Darveau *et al* 1997). Risk assessment has been incorporated into clinical practice and innovations in non-surgical periodontal therapy, host modulation therapy, periodontal tissue engineering and regeneration (Page *et al* 2002, Page *et al* 2003, Preshaw 2008, Sanz and Teughels 2008, Palmer and Cortellini 2008). Cost-effective periodontal maintenance care has been

developed (Gaunt *et al* 2008). New knowledge and technical advances in periodontics provide exciting opportunities and challenges for general dental practitioners (GDPs). Committed GDPs should therefore update themselves on the clinical management philosophy for evidence-based dental practice, which is fundamentally important for successful management of periodontal diseases, long-term maintenance of periodontal health and a successful general dental practice.

This chapter first describes briefly the impact of uncontrolled periodontal disease on patients and the high risk for disease progress and other severe consequences if dental and implant treatments are performed without proper periodontal care. Then, the importance of preventive strategies in management of periodontal patients is emphasized, through periodontal screening and recording, assessment and control of risk factors for early disease recognition and timely intervention by formulation of a comprehensive, well-sequenced treatment planning. In delivering treatments, a reinforced measure of 'periodontal clearance' prior to proceeding with other dental treatments is elaborated. The paper concludes with a brief review of the current professional guidelines on appropriate management of periodontal patients through an effective teamwork.

Periodontal disease as an emerging risk in general dental practice

In general dental practice periodontal patients usually present with a wide range of oral problems and the treatment of these problems is a unique challenge to GDPs. The impact of disease on an affected individual is increasingly apparent and becomes more significant with progression of the disease; from gingival recession with dentine hypersensitivity at a relatively early stage,

towards tooth mobility, pathological migration, and eventually tooth loss, thereby affecting chewing and speech functions, aesthetics, psychological aspects and quality of life as well as increasing financial burden (Jin 2008a). It is evident that periodontal care is a fundamental component of general dental practice, as without sufficient control of periodontal diseases other dental treatment may be greatly compromised or ultimately fail (Pihlstrom 2001). Dental implants have now become an increasingly widely accepted treatment option for replacing missing teeth (Van der Weijden *et al* 2005, Ong *et al* 2008). Scientific evidence suggests that increased individual susceptibility to periodontitis may have a corresponding increased risk to peri-implant infection and decreased success rate of implants (Hardt *et al* 2002, Karoussis *et al* 2003, Van der Weijden *et al* 2005). Periodontitis patients seem to experience more implant loss and complications around implants than non-periodontitis patients (Ong *et al* 2008). Periodontal patients may often miss the opportunity for preventative maintenance, leading to symptom-driven appointments at a relatively late stage. On the other hand, it has been reported that GDPs usually show a limited interest in treatment of periodontal diseases and in many instances appropriate referrals may be compromised due to various factors (Ong 1990, Buckley 1993, Lee et al 2009). Taken together, it is conceivable that there are potentially significant risks incurred by clinical negligence and other factors. It has been reported that in recent years, a number of dentally-related law-suit cases have arisen either from periodontal negligence litigation or poor clinical outcomes, such as inappropriate crown and/or bridge work, detrimental orthodontic treatment and implant failure occurring in susceptible periodontal patients where prior control of periodontal disease or follow-up supportive care was

absent (Zinman 2001).

Key issues in management of periodontal patients

In recent years, the science of periodontology and its impact on periodontal practice is rapidly changing and this presents exciting opportunities and challenges for GDPs (Pihlstrom 2001). It is clear that evidence-based dentistry and preventative care strategies should be incorporated in critical decision-making of clinical practice. A number of translational and clinical studies in preventive strategies, risk assessment and control of risk factors have been conducted and the relevant knowledge has been increasingly translated into daily practice by GDPs. In clinical practice, the focus of prevention shifts from prevention of inception to prevention of disease progression, so-called secondary prevention, which is highly cost-effective in that the debilitating or disfiguring effects of the diseases can be minimized (Hancock and Newell 2001). In this regard, early recognition of disease, control of risk factors and timely treatments with a followed up long-term regular supportive and maintenance care would be the key elements for successful management of periodontal patients in a general dental practice.

In the clinical management of periodontal patients, the following key issues should be addressed:
- consider oral health within the patients' overall health
- have an holistic and patient-centered approach
- understand the patients' needs and expectations
- recognise oral (periodontal) conditions and their manifestations
- identify the origins and causes of the observed conditions and diseases
- determine the therapeutic objectives

- select the appropriate treatment modalities including multidisciplinary approaches
- establish an individualised appropriate treatment plan with correct sequence (Baehni and Giovannoli 2004).

In addition, informed oral and written consent of a final treatment plan should be achieved for legal purposes following discussion with patients, especially with those complex cases. If necessary, timely consultation and/or specialist referral should be undertaken (Glicksman 2001). After formulation of a treatment plan, treatment can be undertaken in four different phases (Salvi *et al* 2008). It usually starts with a systemic phase to eliminate or control the effects of systemic conditions or risk factors for the treatment outcome as well as to safe-guard the treatment process. Medical consultation or referral should be arranged as appropriate, especially for those with medically compromised health conditions such as uncontrolled diabetes. Subsequently, cause-related measures for arresting the disease progression by controlling pathogenic plaque biofilms and various risk factors should be undertaken. For the latter, the periodontal risk calculator has been developed and tested with promising results, and GDPs may consider using it in their practice (Page *et al* 2002, Page *et al* 2003). Following re-evaluation of treatment outcomes and control of active disease, corrective and reparative treatments are undertaken to create favourable conditions for maintenance of good plaque control and oral hygiene, to regenerate lost periodontal structures as appropriate, as well as to offer reconstruction work for comfort oral function desirably through a multidisciplinary approach. Long-term regular supportive care is of imperative importance for the prevention of disease recurrence and maintenance of periodontal health and implant stability.

The importance of 'periodontal clearance' in dental practice

As stated above, appropriate and adequate periodontal care must be undertaken, and periodontal stability should be achieved in periodontally compromised patients before proceeding with other definite corrective and reparative treatments, e.g. orthodontic, aesthetic, restorative, prosthodontic or implant treatments. The status of 'periodontal clearance' should be determined by establishment of a clinically healthy periodontal condition by the responsible dentists and/or specialists with the cooperation of the patient.

The outcome parameters, although they are arbitrary, debatable and classifiable, could desirably include

- reduction or resolution of gingivitis with full-mouth mean bleeding on probing (BOP) ≤25%
- reduction in probing depth (PD) and no residual pockets with PD >5 mm present
- elimination of open furcations in multi-rooted teeth and initial furcation involvement not exceed 3 mm
- absence of pain (Salvi *et al* 2008).

In the management of advanced periodontitis patients, in addition to reinforcement of the 'periodontal clearance' measures, various relevant factors relating to other dental disciplines need to be considered as well. Taking the periodontic-prosthodontic interface as an example, the following issues should be addressed:

- mucogingival considerations
- tooth preparation and crown margin placement
- biological width and consideration of crown lengthening procedures as appropriate
- crown contour and emergence profile
- prosthetic design
- the potential role of occlusion and occlusal

trauma in periodontal diseases and implant failure (Goldberg *et al* 2001).

In addition, appropriate evaluation should be undertaken when considering the use of periodontally involved teeth as abutments and critical concern may be given with the careful incorporation of shortened dental arch options. As mentioned above, the provisions of long-term regular supportive and maintenance care are of particular importance for periodontal patients.

Periodontal screening and recording in general dental practice

It is generally agreed that the first essential step in incorporation of the 'periodontal clearance' concept in general dental practice is to perform periodontal screening and recording for each new patient. Although no single screening system is currently universally accepted or routinely used by dental clinicians worldwide, two systems have been developed and recommended for use by dental professionals, including the Periodontal Screen and Recording (PSR) system (Covington *et al* 2003) and Basic Periodontal Examination (BPE) system (British Society of Periodontology 2001, Dowell and Chapple 2002). The former has been adopted by various professional organizations in North American, such as the American Dental Association, American Academy of Periodontology, Canadian Dental Association and Canadian Academy of Periodontology. The latter was developed by the British Society of Periodontology in 1986, and subsequently modified to screen all patients and to determine the level of examination needed by patients with differing disease levels (British Society of Periodontology 2001).

PSR is used for early detection of periodontal disease. It is virtually identical to the commonly used Community Periodontal

Index of Treatment Needs. It mainly assesses the three indicators of gingival bleeding, calculus and periodontal pockets (PD) using the PSR probe with a 0.5 mm ball tip and a black band between 3.5 and 5.5 mm. The oral cavity is screened systemically in six sextants consisting of teeth 18-14, 13-23 and 24-28 in the upper jaw, and teeth 38-34, 33-43 and 44-48 in the lower jaw. The coding system and interpretations are as follows:

Code 0 - Healthy
Code 1 - Bleeding observed after probing (PD <3.5 mm)
Code 2 - Calculus detected, all the black band visible (PD <3.5 mm)
Code 3 - PD 3.5 – 5.5 mm (gingival margin within the black band)
Code 4 - PD >5.5 mm (black band not visible)
Code X - Other abnormalities identified, e.g. furcation involvement, mobility, mucogingival problems and gingival recession >3.5 mm (Covington *et al* 2003).

As an alternative screening system, BPE identifies individuals who require a more detailed periodontal examination. The British Society of Periodontology recommends that it be carried out for all new patients who have not received a periodontal examination in the last year. Whilst it is not a monitoring tool for periodontal disease, BPE is undertaken by using a standardized periodontal probe with light pressure to examine periodontal tissue for bleeding on probing, plaque retentive factors and pocket depth. The coding system and interpretations are as follows:

Code 0 - No bleeding or pocketing detected
Code 1 - Bleeding on probing, no PD >3.5 mm
Code 2 - Plaque retentive factors present, no PD >3.5 mm
Code 3 - PD >3.5 mm but <5.5 mm
Code 4 - PD >5.5 mm
Code* - Loss of attachment (recession + PD >7 mm or furcation involvement.

The asterisk denotes that a full periodontal examination of the sextant is required regardless of the BPE score.

The patient examination method of BPE combined with appropriate radiographs forms the basis of a suitable screening examination for use in general dental practice. BPE is a useful tool for providing a guideline to where treatment should be undertaken and treatment complexity.

Professional guidelines on appropriate management of periodontal patients

Using the BPE the British Society of Periodontology (2001) has further established a referral policy and parameters of care (Dowell and Chapple 2002). According to the policy, after BPE is performed, the following care and referral could be considered accordingly:

- Complexity 1 cases: may be treated by GDPs
- Complexity 2 cases: either referred or treated by GDPs
- Complexity 3 cases: mostly referred to periodontal specialists.

It is believed that this policy and parameters of care could:

- provide useful guidance for practitioners, given the changing environment of primary and secondary care dentistry (e.g. specialists care in various mono-specialties)
- meet the dento-legal requirements and an increasing demand from the public for the provision of more advanced periodontal care
- promote periodontal therapy on both public and practitioner basis, and by promoting periodontology to the GDPs there is an inevitable knock-on effect to the patient base
- promote good referral practice, based upon the well-known tenets of BPE

- highlight the need for the practitioner to not only examine the periodontium but also to make a diagnosis and effect a treatment plan and defined therapeutic goals using evidence based procedures (Dowell and Chapple 2002).

It has also been shown that BPE may assist referring clinicians in identifying which patients to refer and encourage clinicians to perform periodontal screening (Snoad 2005).

Recently the American Academy of Periodontology (2006) published 'Guidelines for the management of patients with periodontal diseases', which provide useful guidance for GDPs in the co-management of various forms of periodontal patients together with periodontal specialists to achieve better treatment outcomes. In countries with emerging economics, there is a need to work out recommendations and subsequently develop professional guidelines for appropriate management of periodontal patients in general dental practice through an effective teamwork and co-management scheme.

Concluding remarks

It is evident that periodontal care is the foundation of general dental practice. Performance of periodontal screening/recording and assurance of appropriate periodontal clearance should be well incorporated in daily management of patients. This notion is fundamentally important for:

- more predictable and optimal treatment outcomes
- achieving greater patients' satisfaction and establishing life-long relationship with them
- more referral patients
- greater self-enhancement and professional satisfaction
- establishing a greater professional image in the community

- building up a more effective team and wider networking
- making a more successful and profitable practice.

Acknowledgements

References

American Academy of Periodontology. Guidelines for the management of patients with periodontal diseases. *J Periodontol* 2006;77:1607-1611.

Baehni P, Giovannoli JL. Patient profile and decision-making in periodontal practice. *Periodontol 2000* 2004;36:27-34.

British Society of Periodontology. Periodontology in general dental practice in the United Kingdom – A policy statement. http://www.bsperio.org.uk/members/policy.pdf Accessed 30 November 2009.

British Society of Periodontology. Referral policy and parameters of care. http://www.bsperio.org.uk/members/referral.htm Accessed 30 November 2009.

Buckley LA. A comparison of the attitudes of dentists and the public to periodontal health in Ireland. *Int Dent J* 1993;43(Suppl 1):161-166.

Covington LL, Breault LG, Hokett SD. The application of Periodontal Screening and Recording (PSR) in a military population. *J Contemp Dent Pract* 2003;4:36-51.

D'Aiuto F, Casas JP, Shah T, *et al*. C-reactive protein (+1444C > T) polymorphism influences CRP response following a moderate inflammatory stimulus. *Atherosclerosis* 2005a;179:413-417.

D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *J Dent Res* 2005b;84:269-273.

Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000* 1997;14:12-32.

Dowell P, Chapple IL, British Society of

Periodontology. The British Society of Periodontology referral policy and parameters of care. *Dent Update* 2002;29:352-353.

Gaunt F, Devine M, Pennington M, *et al*. The cost-effectiveness of supportive periodontal care for patients with chronic periodontitis. *J Clin Periodontol* 2008;35(8 Suppl):67-82.

Glicksman MA. Referral of the periodontal patient to the periodontist. *Periodontol 2000* 2001;25:110-113.

Goldberg PV, Higginbottom FL, Wilson TG. Periodontal considerations in restorative and implant therapy. *Periodontol 2000* 2001;25:100-109.

Gomez RS, Dutra WO, Moreira PR. Epigenetics and periodontal disease: Future perspectives. *Inflamm Res* 2009;58:625-629.

Hancock EB, Newell DH. Preventive strategies and supportive treatment. *Periodontol 2000* 2001;25:59-76.

Hardt CR, Gröndahl K, Lekholm U, Wennström JL. Outcome of implant therapy in relation to experienced loss of periodontal bone support: A retrospective 5 year study. *Clin Oral Implants Res* 2002;13:488-494.

Ide M, McPartlin D, Coward PY, *et al*. Effect of treatment of chronic periodontitis on levels of serum markers of acute-phase inflammation and vascular responses. *J Clin Periodontol* 2003;30:334-340.

Jin LJ. Periodontal infections, systemic inflammation and cardiovascular disease: Current evidence and perspectives. In: *A perspective of periodontal systemic relationships for the Asian Pacific region.* Bartold PM, Ishikawa I, Zhang J, eds. Asian Pacific Society of Periodontology. 2008a, p 114-121.

Jin LJ. An update on periodontal aetiopathogenesis and clinical implications. *Ann R Australas Coll Dent Surg* 2008b;19:96-101.

Jin LJ, Chiu GKC, Corbet EF. Are periodontal diseases risk factors for certain systemic disorders? What matters to medical practitioners? *Hong Kong Med J* 2003;9:31-37.

Jin LJ, Wang CY. An update on periodontal infections, systemic inflammatory biomarkers, and cardiovascular disease. *Chin J Dent Res*

2007;10:7-13.

Karoussis IK, Salvi GE, Heitz-Mayfield LJ, *et al*. Long-term implant prognosis in patients with and without a history of chronic periodontitis: A 10-year prospective cohort study of the ITI Dental Implant System. *Clin Oral Implants Res* 2003;14:329-339.

Kornman KS. Mapping the pathogenesis of periodontitis: A new look. *J Periodontol* 2008;79(8 Suppl):1560-1568.

Lee JH, Bennett DE, Richards PS, Inglehart MR. Periodontal referral patterns of general dentists: Lessons for dental education. *J Dent Ed* 2009;73:199-210.

Li X, Tse HF, Yiu KH, *et al*. Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. *J Clin Periodontol* 2009;36:933-939.

Mattila K, Vesanen M, Valtonen V, *et al*. Effect of treating periodontitis on C-reactive protein levels: A pilot study. *BMC Infect Dis* 2002;2:30.

Nunn ME. Understanding the etiology of periodontitis: An overview of periodontal risk factors. *Periodontol 2000* 2003;32:11-23.

Offenbacher S, Beck JD. A perspective on the potential cardioprotective benefits of periodontal therapy. *Am Heart J* 2005;149:950-954.

Ong G. Delivery of periodontal therapy by general practitioners in Singapore. *Singapore Dent J* 1990;15:13-15.

Ong CTT, Ivanovski S, Needleman IG, *et al*. Systematic review of implant outcomes in treated periodontitis subjects. *J Clin Periodontol* 2008;35:438-462.

Page RC, Krall EA, Martin J, *et al*. Validity and accuracy of a risk calculator in predicting periodontal disease. *JADA* 2002;133:569-576.

Page RC, Martin J, Krall EA, *et al*. Longitudinal validation of a risk calculator for periodontal disease. *J Clin Periodontol* 2003;30:819-827.

Page RC, Offenbacher S, Schroeder HE, *et al*. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. *Periodontol 2000* 1997;14:216-248.

Palmer RM, Cortellini P, Group B of European

Workshop on Periodontology. Periodontal tissue engineering and regeneration: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;35(8 Suppl):83-86.

Pihlstrom BL. Periodontology for the general practitioner: Introduction. *Periodontol 2000* 2001;25:7.

Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005;366:1809-1820.

Preshaw PM. Host response modulation in periodontics. *Periodontol 2000* 2008;48:92-110.

Salvi GE, Lindhe J, Lang NP. Treatment planning of patients with periodontal diseases. In: *Clinical periodontology and implant dentistry*. 5th ed, Vol. 2. Lang NP, Lindhe J editors. Blackwell Munksgaard. 2008, p 655-674.

Sanz M, Teughels W, Group A of European Workshop on Periodontology. Innovations in non-surgical periodontal therapy: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;35:3-7.

Seinost G, Wimmer G, Skerget M, *et al*. Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *Am Heart J* 2005;149:1050-1054.

Snoad R. Description of a system designed to assist primary dental care clinicians in decision-making with regard to specialist periodontal referrals and report of two clinical audits using the system. *Prim Dent Care* 2005;12:135-141.

Tonetti MS, D'Aiuto F, Nibali L, *et al*. Treatment of periodontitis and endothelial function. *N Engl J Med* 2007;356:911-920.

Van der Weijden GA, van Bemmel KM, Renvert S. Implant therapy in partially edentulous, periodontally compromised patients: A review. *J Clin Periodontol* 2005;32:506-511.

Zinman E. Dental and legal considerations in periodontal therapy. *Periodontol 2000* 2001;25:114-30.

Chapter 7

Periodontal systemic interrelationships: An overview of the evidence

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Introduction

Periodontitis is a multifactorial problem with three factors interplaying before disease becomes manifest: a susceptible host, environmental factors conducive to disease development and altered host responses leading to tissue destruction. A significant development in Periodontics in the past 20 years has been the development of the sub discipline of Periodontal Medicine.

In a landmark publication by the US Surgeon General titled "Oral Health in America" it was stated that "oral health and general health should not necessarily be dissociated as the have in the past. Further it stated that "in the interests of an holistic approach to patient care oral health must be considered as a critical issue for general well-being.

A central hypothesis of periodontal medicine states that periodontal infection presents a chronic inflammatory burden at the systemic level. Indeed if one were to take into account the total ulcerated periodontal tissue in an individual (28 teeth and periodontal pockets of around 5-6 mm) the total area of ulcerated tissue is about 75cm^2 (Page 1998). Such a large amount of inflamed tissue is likely to have systemic effects and influence inflammatory reactions elsewhere in the body.
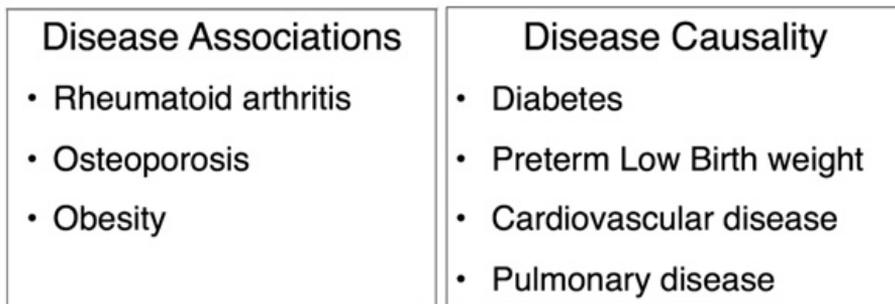
Of course, in many regards this concept is not new and the history of periodontal disease as a risk for systemic conditions has been chronicled by Williams (2008). In 1891 Miller published a paper detailing the focus of infection theory whereby microbes or their waste products obtain entrance to parts of the body adjacent to or remote from the mouth. In 1900 Hunter blamed oral sepsis for causing systemic diseases such as tonsillitis and emphysema and in the following years a number of authors advocated the prophylactic removal of teeth to avoid such complications (Billings 1912, Mayo 1922, Galloway 1931). However this concept was called into question by Cecil *et al* (1938) who noted that 156 rheumatoid arthritis patients who underwent full mouth extractions did not get better.

In more recent times the concepts of periodontitis affecting systemic health has been revisited and led to the suggestion of an "inverted paradigm" for the development of periodontitis (Page 1998). In this model systemic disease may modify the manifestation of periodontitis and conversely periodontitis may affect systemic health (Figure 1).

SUSCEPTIBLE + PRESENCE OF + ABSENCE OF + CONDUCIVE
HOST   PATHOGENS   BENEFICIAL   ENVIRONMENT

Neutrophils   **A.a**   **Actinomyces sp** **Temperature**
Imm. Resp   **B. forsythus**   **C. ochraces**   **Iron Conc.**
Diabetes   **F. nucleatum**   **S. mitis**   **Pocket dpth**
Smoking   **P. gingivalis** **S. oralis**   **Inflamm**
Medications   **P. intermedius** **V. parvula**
                  P. micros
                  Spirochetes
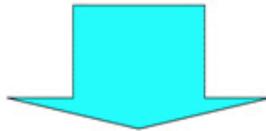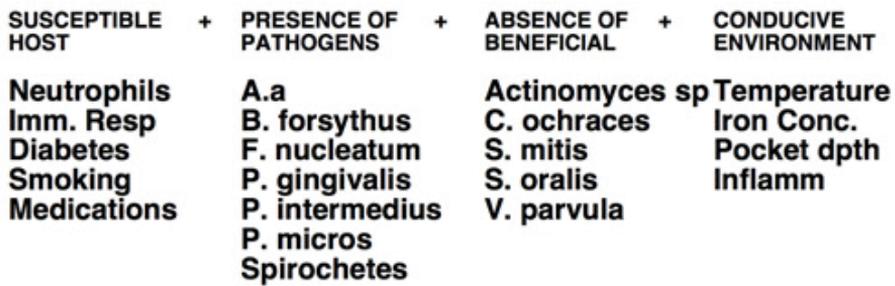
ACTIVE DISEASE
(Accepted paradigm)

Figure 1a. Accepted paradigm for development of periodontal disease in which a susceptible host provides a conducive environment for infection with pathogenic microbial subgingival flora

Disease Associations

- Rheumatoid arthritis
- Osteoporosis
- Obesity

Disease Causality

- Diabetes
- Preterm Low Birth weight
- Cardiovascular disease
- Pulmonary disease

PERIODONTITIS
(Inverted Paradigm)

Figure 1b. Inverted paradigm for periodontal disease in which acquisition of a periodontal infection leads to as association with a variety of systemic conditions

In a recent study the prevalence of systemic conditions in patients attending general dental or specialist periodontal practices was evaluated (Georgiou *et al* 2004). The results indicated that periodontitis patients had a high prevalence of systemic diseases. Periodontal patients had a higher prevalence of systemic diseases compared to the general practice population. Public patients had a greater prevalence of systemic diseases compared to patients in private practice for both general practice and periodontal patients. In patients with advanced periodontitis, bronchitis, hepatitis and rheumatoid arthritis were most prevalent. Patients with periodontitis also took more medications and were more likely to suffer from multiple conditions compared to the general dental population. It was concluded that patients with moderate or advanced periodontitis show an increase in the prevalence of some systemic diseases previously reported to be risk factors for periodontal disease.

What is the evidence for an association between systemic disease and periodontal disease?

Diabetes

The relationship between periodontal disease and diabetes mellitus has been recognized for many years with it being reported to be the sixth complication of diabetes (Loe 1993). Diabetes is an endocrine disorder demarcated by absolute or relative insulin deficiency and is characterized by insufficient secretion of insulin by the pancreatic beta-cells (Insulin-dependent diabetes mellitus Type-I) or sub responsiveness of tissues to circulating insulin (Non-insulin-dependent diabetes Type-II). The prevalence of diabetes is increasing at an alarming rate with many countries in the Asian Pacific region predicted to account for the

majority of cases by 2030 (Table 1).

A number of epidemiological and cross-sectional studies have reported a higher incidence and severity of periodontitis in patients with both type I and type II diabetes (Loe 1993, Oliver *et al* 1998, Albander 2000, Demmer *et al* 2008, Struch *et al* 2008). Moreover, individuals with untreated periodontitis have a higher risk of having diabetes (Emrich 1991). Landmark studies on Pima Indians during the 1990's showed that the prevalence of periodontal disease was 60% in subjects with diabetes compared with 36% in the non-diabetic subjects. 70% of the Pima Indians who had little or no evidence of periodontal disease at the initial examination, were followed for 2.6 years in order to evaluate the incidence of periodontal disease. The age and gender adjusted incidence rate of periodontal disease in diabetics was found to be 7%, whereas in subjects without diabetes the incidence rate was 2%. Therefore, it was shown that diabetes increases significantly the prevalence and incidence of periodontal disease (Nelson *et al* 1990, Emrich *et al* 1991, Taylor *et al* 1998).

One reason for the increased prevalence and severity of periodontal disease in diabetics is the impaired host defense system towards the microbial challenge. In particular, it has been shown that diabetics have decreased neutrophil chemotaxis (Manouchehr-Pour *et al* 1981) and impaired neutrophil phagocytosis and killing (Cutler *et al* 1991). Hyperglycemia results in the formation of advanced glycation end products which result in reduced solubility and reduced turnover of molecules such as collagen. As a result vascular basements become thickened, impeding oxygen diffusion, metabolic waste product elimination and immune defense. Through these processes wound healing is reduced and the capacity of the host defense system is decreased

Of interest in recent times has been the bidirectional relationship between diabetes

Ranking	Country	People w/ diabetes (mil) 2000	Country	People w/ diabetes (mil) 2003
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian Federation	4.6	Bangladesh	11.1
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	Bangladesh	3.2	Egypt	6.7

Table 1. Countries with highest number of estimated cases of diabetes for 2000 and 2003

and periodontitis. Well-managed diabetics appear to have better controlled periodontitis. On the converse infection for periodontal origin may contribute to poorer glycemic control and the risk of diabetic complication (Taylor and Borgnakke, 2008). Whether treated periodontitis is associated with improved glycemic control has been the subject of considerable debate although a recent meta-analysis of intervention studies do indicate that periodontal treatment led to a statistically and clinically significant reduction in HbA1c levels (Janket *et al* 2005).

Obesity

In the last decade many studies have investigated the relationship between obesity and periodontal disease. Obesity is defined as an excess amount of body fat in proportion to lean body mass, to the extent that health is impaired. Being overweight or obese is now recognized as important risk factors for a number of adult diseases (Figure 2). Adipose tissue is now considered as not being inert but rather it is a complex and metabolically active endocrine organ. Adipokines (which comprise more than 50 bioactive molecules) are released from adipose tissue and these have wide reaching effects (Figure 3). Some act locally, whereas others are released where they act as signaling molecules to the liver, muscle and endothelium. Given these significant relationships between adipokines and systemic health it is not surprising that obesity has emerged as one of the risk indicators for periodontal disease. In a study investigating various lifestyle factors in relation to periodontal disease, it was noted that there was a very clear association between obesity and periodontal disease, second only to smoking (Nishida *et al* 2005). This finding prompted
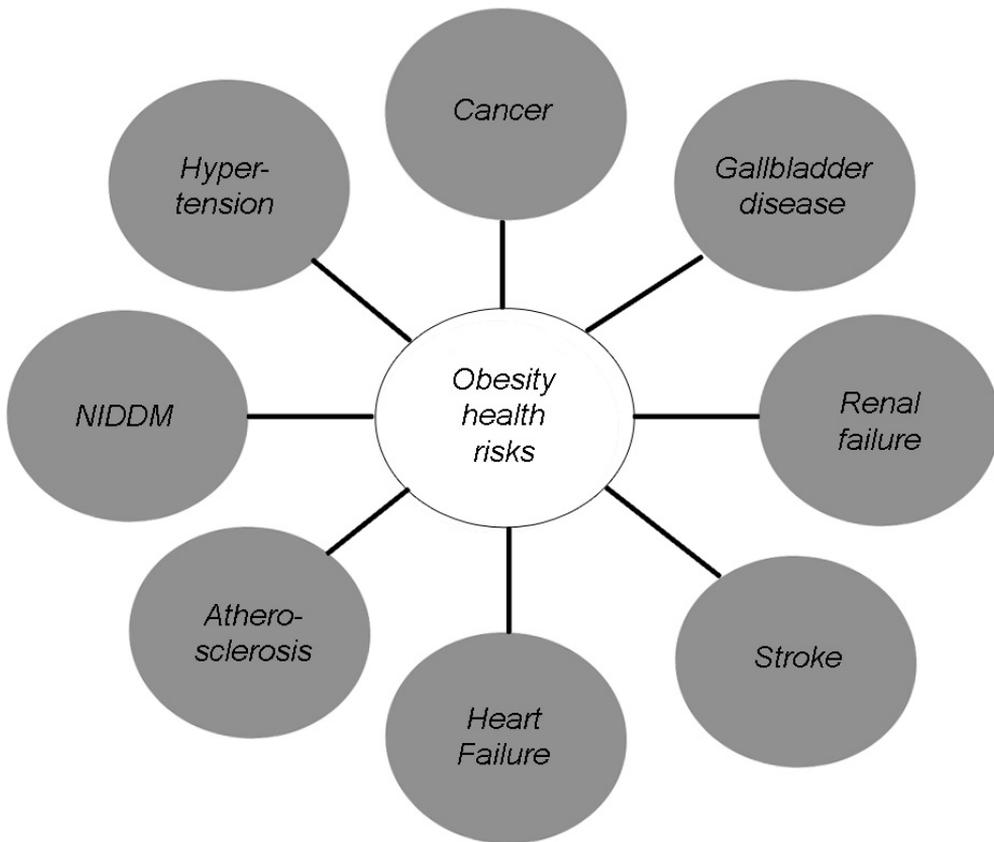
Figure 2. Health risks associated with obesity

studies to investigate how such a relationship could come about. One of the underlying features of this association appears to be a triumvirate between obesity, diabetes and periodontal disease (Saito and Shomazaki 2007). However, if obesity is a true risk factor for periodontal disease then the association between periodontal disease, obesity and diabetes is likely to be very complex since each is a confounding factor for the other. Initial studies in this area have indicated that the relationship between obesity indices and deep pockets is more significant than between the oral glucose tolerance test and deep pockets. Moreover, obesity is associated with periodontal disease independent of deteriorated glucose condition and diabetes (Nishimura 2000, Saito *et al* 2005). To date most studies in this area have been cross-sectional or case control studies. Prospective cohort studies and laboratory studies are now required to clarify whether obesity is one of the risk factors for periodontal disease or simply a risk indicator. In addition more studies on the causal relationships among diabetes, obesity and periodontal disease, with stringent consideration of confounders, are needed.

Preterm low birth weight infants

The classic studies of Löe and co-workers

in the early-sixties (Silness and Löe 1964, Löe and Silness 1966) revealed that 100% of pregnant women had signs of gingival inflammation in their second trimester compared to post partum women, and also that these signs of gingival inflammation were unrelated to the degree of plaque accumulation. Later, it was proposed that the increased gingival inflammation seen in pregnant women may be due to physiologic hormonal changes in the second trimester of pregnancy (Kornman and Loesche 1980).

More recently, research has focused not on the prevalence of periodontitis in pregnant women, but rather on the association between periodontitis and adverse birth outcomes. The rationale for such studies is based on observations that infectious processes elsewhere in the body may contribute to neonatal morbidity and mortality. Thus, periodontal disease may be one such infection. Early studies suggested that women who delivered preterm low birth weight infants had poorer periodontal health than mothers of normal birth weight infants (Offenbacher *et al* 1996). Indeed it was noted that pregnant women with periodontal disease had a 7.5 times increased risk of delivering an infant being preterm and with low birth weight (PLBW). This study indicated, for the first time, that periodontal disease represented a previously unrecognized and clinically significant risk factor for preterm low birth weight infants, after adjusting for all the other known risk factors such as tobacco use, alcohol consumption, level of prenatal care, parity, genitourinary infections and nutrition (Offenbacher *et al* 1996).

Since this study, numerous investigations have been carried out with mixed and conflicting results (Davenport *et al* 2002). With an expanding number of reports in the literature a meta-analysis was carried out (Khander and Ta'ani 2005). In this study 40 articles were identified but only 5 met the chosen inclusion criteria. Of the 5 studies included three were cohort studies and two were case control studies carried out between 1996 and 2002. The conclusions drawn were that there appeared to be a positive association between periodontitis and adverse pregnancy outcomes. Furthermore it was concluded that pregnant women with periodontal disease were at 4.28 times greater risk of having a preterm delivery than periodontally healthy women. More recent studies continue to explore this relationship but unfortunately mixed results continue to be reported and indicate that more rigorous studies are needed (Xiong *et al* 2006, Xiong *et al* 2007, Wimmer and Pihlstrom 2008). To date the studies appear to indicate that periodontal disease may be a risk factor for preterm low birth weight but additional longitudinal, epidemiologic and interventional studies are needed to validate this association and to determine whether it is causal. Indeed, at present it is not yet clear whether periodontal diseases play a causal role in adverse pregnancy outcomes. While treatment of periodontitis in pregnant women is safe it is interesting to note a very recent report indicating that periodontal therapy does not reduce the incidence of preterm delivery (Offenbacher *et al* 2009)

Cardiovascular Disease

Cardiovascular disease has been considered to be an inflammatory disease for many years (Ross 1999). In this model factors that induce or promote inflammation can have adverse effects on the vasculature. This is illustrated in Figure 3.

In recent times the connection between periodontitis and periodontitis has generated considerable interest (Karman 2009, Friedewald *et al* 2009). Evidence for an association between periodontitis and cardiovascular disease has come from studies demonstrating that periodontopathogens are
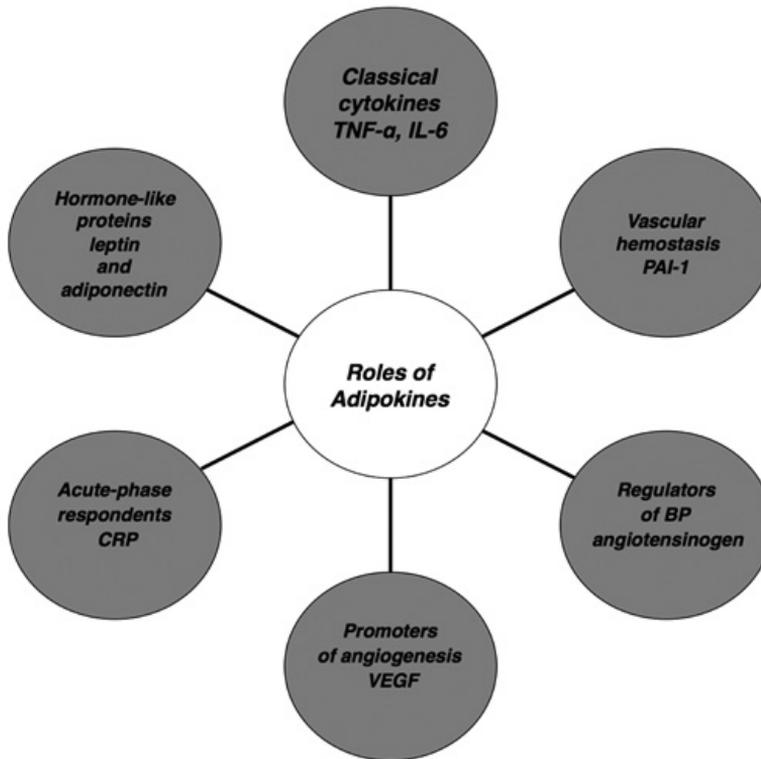
Figure 3. Biologic effects of adipokines

found in arterial plaques, elevated C-reactive protein levels in patients with periodontitis and that periodontopathogens may cause platelet aggregation. To date several studies have suggested that there is an elevated risk in periodontal patients for cardiovascular disease (De Stefano *et al* 1993, Howell *et al* 2001, Joshipura *et al* 1996, Matilla *et al* 1995, Wu *et al* 2000, Bahekar *et al* 2007).

However, one study has cast some doubt over the above findings (Hujoel *et al* 2000). In this study, the risk of chronic heart disease in 8032 people with periodontitis, gingivitis, and no periodontal disease was evaluated in a prospective cohort study. After adjusting for confounding factors they found that gingivitis and periodontitis were associated with a non-significant increased risk for chronic heart disease with a hazard ratio of 1.05 (95% CI 0.88-1.26) for gingivitis and a hazard ratio of 1.14 (95% CI 0.96-1.36) for periodontitis. In conclusion their study did not find convincing evidence of a causal association between periodontal disease and coronary heart disease. In a subsequent study evaluating the effect of the elimination of dental infection on the risk of developing chronic heart disease compared with the presence of dental infection (periodontitis) found that subjects with definite and long-term elimination of dental infections are not at lower risk of developing chronic heart disease than people who have dental infections (Hujoel *et al* 2001).

In conclusion, current evidence suggests a link and not a causal effect between periodontitis and cardiovascular disease. The risk for developing cardiovascular disease may increase 11.2 - 13.4 times in individuals with

periodontal infection. Further population based laboratory and clinical (molecular epidemiology) studies are required. Wile it is possible that treatment may provide some short term improvement in endothelial function (Tonetti *et al* 2007) However, in all the studies established risk factors for atherosclerosis have been adjusted indicating that the associations found are not confounded by these factors (Slavkin *et al* 1999). Despite the degree of association at this point in time the data cannot provide sufficient evidence of certain causality between oral infections (periodontitis) and cardiovascular disease (Chong *et al* 2000).

Periodontitis and respiratory disease

Pneumonia is an acute infection of the pulmonary parenchyma caused by a wide variety of infectious agents. Chronic obstructive pulmonary disease is defined as "a disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema" (Limeback 1998).

Microorganisms can contaminate the lower airways by four possible routes: aspiration of oropharyngeal contents, inhalation of infectious aerosols, spread of infection from contiguous sites, and hematogenous spread from extrapulmonary sites of infection. Aspiration of oropharyngeal contents is the most common route of infection (Scannapienco and Mylotte 1996).

With the above in mind it is no surprise that pulmonary infections and disease have been linked to oral and periodontal infections. Indeed, the oral cavity is now recognized as an important reservoir for bacterial pathogens which are capable of causing lung disease. The incidence of respiratory pathogen oropharyngeal colonization by respiratory pathogens is more common in dentate than edentulous patients. Poor oral hygiene and periodontal disease may foster respiratory

pathogen oropharyngeal colonization. Oral interventions which improve oral hygiene result in reduction in the rate of pneumonia in high risk patients. Dental plaque, especially from patients with periodontal disease, seems to be a logical source of anaerobic bacteria which can cause lung infections. It has been reported that plaque from patients with periodontal disease can harbor yeasts, enteric rods, pseudomonas, staphylococci and Candida, all which have been reported to be implicated in acute pneumonia (Slots *et al* 1988, Helovuo *et al* 1993, Morris and Sewell 1994). One study has suggested that after controlling for age, race, gender, and smoking, the presence of periodontal disease represented a 1.3 - 4.5 increased risk for development of chronic respiratory disease (Scannapieco *et al* 1998).

To date two systematic reviews have been carried out on this topic (Scannapieco *et al* 2003, Azarpazhooh and Leake 2006). Both reviews came to the conclusion that there is evidence of an association between oral health and chronic obstructive pulmonary disease and pneumonia. Nonetheless additional large scale randomized clinical trials are needed to provide further evidence to institute effective oral hygiene procedures in high risk patients to prevent nosocomial pneumonia.

Periodontitis and rheumatoid arthritis

Many studies have reported an association between RA and periodontitis. Analyses of self reported illnesses indicated the likely interrelationship between periodontitis and RA (Mercado *et al* 2000). Case/control studies have also reported a significantly higher incidence of tooth loss and alveolar bone loss in patients with RA (Mercado *et al* 2000, Kasser *et al* 1997). In addition, studies addressing cytokine profiles, HLA-DR shared epitopes and antibody titres to periodontopathic bacteria have strongly
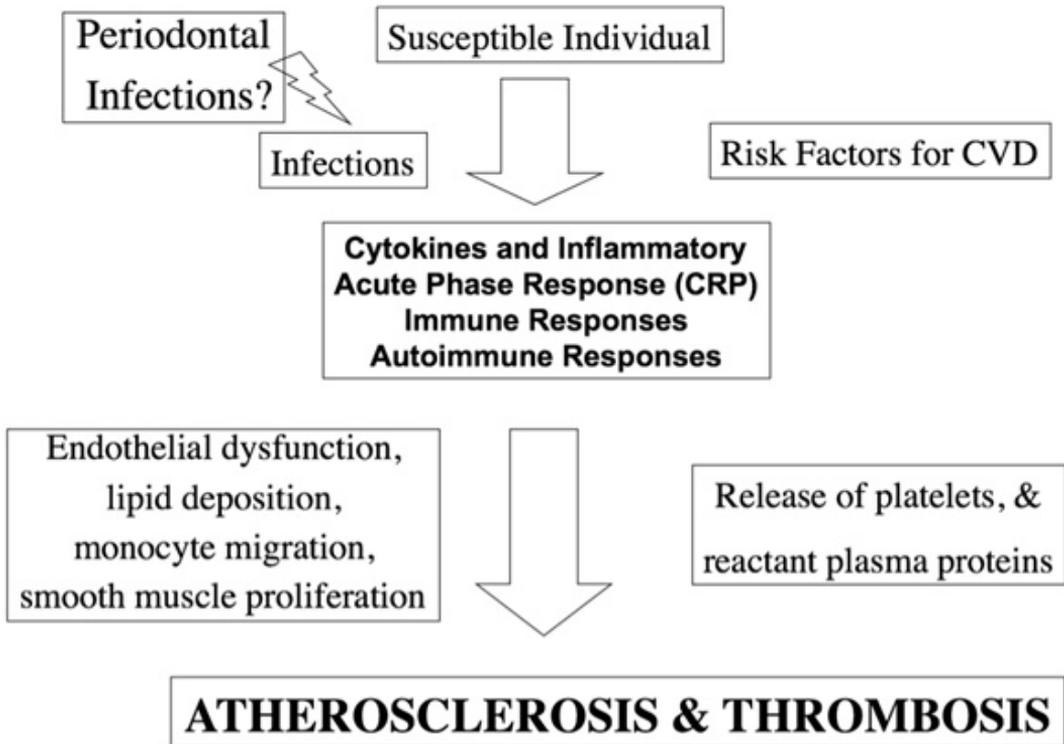
Figure 4. Relationship between periodontal infection and development of athersoclerosis

suggested an interrelationship between periodontitis and RA (Bozkurt *et al* 2000, Marrotte *et al* 2006). Of particular interest are studies reporting that periodontitis may serve as a risk factor or severity factor for RA and that periodontal treatment might even have a beneficial effect on RA (Mercado *et al* 2000, Riberio *et al* 2005, Riberio *et al* 2005, Al Katama *et al* 2007). A recent study reported that following induction of adjuvant experimental arthritis in rats, there was subsequent evidence of periodontal breakdown (Ramamurthy *et al* 2005). Most recently a report, using data from the NHANES III survey, demonstrated that RA is associated with tooth loss and periodontitis (de Pablo *et al* 2007).

One plausible explanation for the relationship between periodontitis and rheumatoid arthritis may be through a primed inflammatory response known as the "two-hit" model (Golub *et al* 2006). This model suggests that a primary "hit" of chronic inflammation (e.g. chronic periodontitis or other extra-synovial chronic inflammation) followed by an arthritogenic hit to induce rheumatoid arthritis could lead to an exacerbated response. It is also possible that the converse could occur and an initial hit of chronic inflammatory disease exacerbates the inflammatory response of developing periodontitis. If one were to take into account the large amount of ulcerated periodontal tissue in an individual with periodontitis, it is conceivable that such a large amount of inflamed tissue is likely to have systemic effects and influence inflammatory reactions elsewhere in the body.

To date, using standard clinical and laboratory parameters, rheumatoid arthritis patients appear to be more susceptible to

developing periodontitis. In addition there appears to be a subgroup of patients who have moderate to severe rheumatoid arthritis who are also susceptible to having severe periodontitis. Whether these relationships represent an underlying dysregulation of the inflammatory responses in these individuals remains to be established. Although some studies evidently show a strong association between RA and periodontal disease, and although the similarities in the pathogenic mechanisms of tissue destruction are obvious, more studies are needed in order to elucidate completely an understanding of the relationship between them.

Osteoporosis

Osteoporosis has long been suspected as a systemic risk factor for alveolar bone loss in periodontitis. However, surprisingly few studies have addressed this issue and mixed results have been reported (Famil *et al* 2005, Jeffcoat 2005). It is clear that additional well-controlled large-scale prospective studies are needed to clarify this issue.

Conclusion

Although there are many reports in the literature which link systemic conditions and periodontal disease, the precise mechanisms which generate such associations still remain to be elucidated. A number of conditions can be linked to periodontitis in either a causal, coexistent or contributory manner. However, in many cases, the literature is insufficient to make definite statements on links between systemic factors and periodontal disease. However with progression in understanding the pathogenic mechanisms of all chronic diseases, the time may come when clinicians will be fully informed regarding the systemic implications of periodontal diseases.

References

Albander JM. Global risk factors and risk indicators for periodontal diseases. *Periodontol 2000* 2002;29:177-206.

Al-Katma MK, Bissada NF, Bordeaux JM, *et al*. Control of periodontal infection reduces severity of active rheumatoid arthritis. *J Clin Rheumatol* 2007;13:134-137.

Azarpazhooh A, Leake JL. Systematic review of the association between respiratory diseases and oral health. *J Periodontol* 2006;77:1465-1482.

Bahekar AA, Singh S, Saha S, *et al*. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am Heart J* 2007;154:830-837.

Billings FA. Chronic focal infections and their etiologic relations to arthritis and nephritis. *Arch Int Med* 1912;9:484-498.

Bozkurt FY, Berker E, Akkus S, *et al*. Relationship between interleukin-6 levels in gingival crevicular fluid and periodontal status in patients with rheumatoid arthritis and adult periodontitis. *J Periodontol* 2000;71:1756-1760.

Cecil RL, Angevine DM. Clinical and experimental observations on focal infection, with an analysis of 200 cases of rheumatoid arthritis. *Ann Inter Med* 1938;12:5778-584.

Chong PH, Pharmd D, Kezele BS. Periodontal disease and atherosclerotic cardiovascular disease: Confounding effects or epiphenomenon? *Pharmachotherapy* 2000;20:805-818.

Cutler CW, Eke P, Arnold RR, Van Dyke TE. Defective Neutrophil function in an insulin-dependent diabetes mellitus patient. A Case Report. *J Periodontol* 1991;62:394-401.

Davenport ES, Williams CE, Sterne JA, *et al*. Maternal periodontal disease and preterm low birthweight: case-control study. *J Dent Res* 2002;81:313-318.

de Pablo P, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J Rheumatol* 2008;35:70-76.

Demmer RT, Jacobs DR, Desvarieux M. Periodontal disease and incident type 2 diabetes results from the First National Health and Nutrition

Examination Survey and its epidemiologic follow-up study. *Diabetes Care* 2008;31:1373-1379.

DeStefano F, Anda RF, Kahn HS, *et al*. Dental disease and risk of coronary heart disease and mortality. *Br Med J* 1993;306:688-691.

Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. *J Periodontol* 1991;62:123-31.

Famili P, Cauley J, Suzuki JB, Weyant R. Longitudinal study of periodontal disease and edentulism with rates of bone loss in older women. *J Periodontol* 2005;76:11-15.

Friedewald VE, Kornman KS, Beck JD, *et al*. Editors' consensus: Periodontitis and atherosclerotic cardiovascular disease. *J Periodontol* 2009;80:1021-1032.

Galloway CE. Focal Infection. *Am J Surgery* 1931;14:643-645.

Georgiou TO, Marshall RI, Bartold PM. Prevalence of systemic diseases in Brisbane general and periodontal practice patients. *Aust Dent J* 2004;49:177-184.

Helovuo H, Hakkarainen K, Paunio K. Changes in the prevalence of subgingival enteric rods, staphylococci and yeasts after treatment with penicillin and erythromycin. *Oral Microbiol Immunol* 1993;8:75-79.

Howell TH, Ridker PM, Ajani UA, *et al*. Periodontal disease and risk of subsequent cardiovascular disease in US male physicians. *J Am Coll Cardiol* 2001;37:445-450.

Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Examining the link between coronary heart disease and the elimination of chronic dental infections. *JADA* 2001;132:883-889.

Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Periodontal disease and coronary heart disease risk. *JAMA* 2000;248:1406-1414.

Hunter W. Oral sepsis as a cause of disease. *Br Med J* 215-216; 1900

Janket SJ, Wightman A, Baird AE, *et al*. Does periodontal treatment improve glycemic control in diabetic patients? A meta analysis of intervention studies. *J Dent Res* 2005;84:1154-1159.

Jeffcoat M. The association between osteoporosis and oral bone loss. *J Periodontol* 2005;76(11Suppl):2125-2132.

Joshipura KJ, Rimm EB, Douglass CW, *et al*. Poor oral health and coronary heart disease. *J Dent Res* 1996;75:1631-1636.

Käßer UR, Gleissner C, Dehne F, *et al*. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis Rheum* 1997;40:2248-2251.

Kornman KS. Editorial: a call to action. *J Periodontol* 2009;80:1019-1020.

Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. *J Periodont Res* 1980;15:111-122.

Limeback H. Implications of oral infections on systemic diseases on institutionalized elderly with a special focus on pneumonia. *Ann Periodontol* 1998, 3:262-275.

Löe H. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care* 1993;16:329-334.

Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1966, 21:533-549.

Manouchehr-Pour M, Spagnuglo PJ, Rodman HM, Bissada NF. Comparison of neutrophil chemotactic response in diabetic patients with mild and severe periodontal disease. *J Periodontol* 1981;52:410-415.

Marotte H, Farge P, Gaudin P, *et al*. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Ann Rheum Dis* 2006;65:905-909.

Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK. Dental infection and the risk of new coronary events: Prospective study of patients with documented coronary artery disease. *Clin Infect Dis* 1995;20:588-592.

Mayo C. Focal infection of dental origin. *Dent Cosmos* 1922;64:1206-1208.

Mercado F, Marshall RI, Klestov AC, Bartold PM. Is there a relationship between rheumatoid arthritis and periodontal disease? *J Clin Periodontol* 2000;27:267-272.

Miller WD. The human mouth as a focus of infection. *Dental Cosmos* 1891;33:13-23.

Morris JF. Sewell DL. Necrotizing pneumonia

caused by mixed infection with *Actinobacillus actinomycetemcomitans* and *Actinomyces israelli*: Case report and review. *Clin Infect Dis* 1994;18:450-452.

Nelson RG, Shlossman M, Budding LM. Periodontal disease and non-insulin-dependent diabetes mellitus in Pima Indians. *Diabetes Care* 1990;13:836-840.

Nishida N, Tanaka M, Hayashi N, *et al.* Determination of smoking and obesity as periodontitis risks using the classification and regression tree method. *J Periodontol* 2005;76:923-928.

Nishimura F, Kono T, Fujimoto C, *et al.* Negative effects of chronic inflammatory periodontal disease on diabetes. *J Int Acad Periodontol* 2000;2:49-55.

Offenbacher S, Beck JD, Jared HL, *et al.* Effects of periodontal therapy on rate of preterm delivery: A randomized controlled trial. *Obstet Gynecol* 2009;114:551-559.

Offenbacher S, Katz V, Fertik G, *et al.* Periodontal infection as a possible risk factor for pre term low birth weight. *J Periodontol* 1996;67:1103-1113.

Oliver RC, Brown LJ, Loe H. Periodontal disease in the United States population. *J Periodontol* 1998;69:269-278.

Page RC. The pathobiology of periodontal diseases may affect systemic diseases: Inversion of a paradigm. *Ann Periodontol* 1998;3:108-120.

Ramamurthy NS, Greenwald RA, Celiker MY, Shi EY. Experimental arthritis in rats induces biomarkers of periodontitis which are ameliorated by gene therapy with tissue inhibitor of matrix metalloproteinases. *J Periodontol* 2005;76:229-233.

Ribeiro J, Leao A, Novaes AB. Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol* 2005;32:412-416.

Ross R. Astherosclerosis – an inflammatory disease. *New Engl J Med* 1999;340:115-126.

Saito T, Shimazaki Y, Kiyohara Y, *et al.* Relationship between obesity, glucose tolerance, and periodontal disease in Japanese women: The Hisayama study. *J Periodontal Res* 2005;40:346-353.

Saito T, Shimazaki Y. Metabolic disorders related to obesity and periodontal disease. *Periodontol 2000* 2007;43:254-66.

Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for nosocomial bacterial pneumonia and chronic obstructive pulmonary disease. A systematic review. *Ann Periodontol* 2003;8:54-69.

Scannapienco FA. Mylotte JM. Relationship between periodontal disease and bacterial pneumonia. *J Periodontol* 1996;67:1114-1122.

Scannapienco FA, Papandonatos GD, Dunford RG. Association between oral conditions and respiratory disease in a national sample survey population *Ann Periodontol* 1998;3:252-256.

Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121-135.

Slots J, Rams TE, Listgarten MA. Yeasts, enteric rods and pseudomonads in the subgingival flora of severe adult periodontitis. *Oral Microbiol Immunol* 1988;3:47-52.

StruchF, Dau M, Schwahn C, *et al.* Interleukin-1 gene polymorphism, diabetes, and periodontitis: Results from the Study of Health in Pomerania (SHIP). *J Periodontol* 2008;79:501-507.

Taylor GW, Borgnakke WS. Periodontal disease: Associations with diabetes, glycemic control and complications. *Oral Dis* 2008;14:191-203.

Taylor GW, Burt BA, Becker MP, *et al.* Glycemic control and alveolar bone loss progression in type 2 diabetes. *J Clin Periodontol* 1998;24:505-510.

Tonetti MS, D'Aiuto F, Nibali L, *et al.* Treatment of periodontitis and endothelial function. *N Engl J Med* 2007;356:911-920.

Williams RC. Understanding and managing periodontal diseases: A notable past, a promising future. *J Periodontol* 2008;79(8 Suppl):1552-1559.

Wimmer G, Pihlstrom BL. A critical assessment of adverse pregnancy outcome and periodontal disease. J Clin Periodontol 2008;35(8 Suppl):380-397.

World Health Organization. World wide prevalence of diabetes. http://www.who.int/diabetes/facts/world_figures/en/index5.html Accessed 31 December 2009.

Wu T, Trevisan M, Genco RJ, *et al*. Periodontal disease and risk of cerebrovascular disease: The first national health and nutrition examination survey and its follow-up study. *Arch Intern Med* 2000;160:2749-2755.

Xiong X, Buekens P, Fraser WD, *et al*. Periodontal disease and adverse pregnancy outcomes: A systematic review. *Br J Obstet Gynecol* 2006;113:135-143.

Xiong X, Buekens P, Vastardis S, Yu SM. Periodontal disease and pregnancy outcomes: State-of-the-science. *Obstet Gynecol Surv* 2007;62:605-615.

Chapter 8

Management of inflammation in periodontal disease

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Introduction

Periodontitis is an inflammatory disease of the periodontium which is characterized by progressive destruction of the tissues supporting the teeth (Listgarten 1987). Its primary etiology is the biofilm that naturally forms on the teeth, which in disease comprises a series gram negative anaerobic bacteria, such as *Porphyromonas gingivalis* and *Tannerella forsythia* in the case of chronic periodontitis, and Aggregatibacter actinomycetemcomitans in the case of localized aggressive periodontitis (LAP) (Offenbacher 1996). While the etiology of periodontitis is bacterial, it is becoming clear that the pathogenesis of disease is mediated by the host response. A long-standing model for this concept comes from the study of Localized Aggressive Periodontitis (LAP, LJP). Until recently, defects associated with neutrophil functions were believed to predispose to infection. However, there is a growing body of evidence suggesting that neutrophil abnormalities in LAP are the result of a chronic hyperactivated state of the LAP neutrophil (Van Dyke and Serhan 2003, Hasturk e*t al* 2006). Hyperfunctional neutrophils release a barrage of noxious agents (i.e. reactive oxygen species and hydrolytic enzymes) that can inadvertently spill into surrounding tissues and cause tissue damage, amplifying local inflammatory sequelae. The available data strongly support the suggestion that neutrophil (or PMN) are responsible, at least in part, for the tissue destruction in chronic periodontal inflammation (Van Dyke and Serhan 2003). In addition, periodontal diseases are associated with increased levels of CRP, fibrinogen, and pro-inflammatory mediators such as PGE_2, IL-1β and $TNF\alpha$, which can stimulate many of the pathogenic events that may occur during periodontal disease (Offenbacher *et al* 1984, Shapira *et al* 1994, Kornman *et al* 1997, Loos *et al* 2000, Noack *et al* 2001a, Slade *et al* 2003, Graves and Cochran 2003, Graves 2008).

Despite the plethora of evidence supporting the inflammatory nature of periodontal diseases and activation of proinflammatory pathways, recent evidence has raised the possibility that this is only half the story. Active, host derived, pathways that regulate inflammation have now been described. However, there is still a gap in current knowledge regarding the cellular and molecular mechanisms associated with endogenous mediators that actively turn off inflammation and effectively rescue the host from tissue damage when functioning properly. This review will focus on the inflammatory nature of periodontal diseases with an emphasis on the importance of fatty

acids and how they regulate the balance between disease and health.

The changing paradigm of the role of fatty acids in health and disease: A look at periodontal disease

Fatty acids of endogenous or dietary origin have emerged as pivotal players mediating innate immunity and inflammation. The first observation linking essential long-chain polyunsaturated fatty acids (PUFA) and health was made by Burr and Burr in 1929. Burr and Burr fed weanling rats a fat-free diet throughout life. Despite gaining many times their original weight, they failed to grow at the rate of fat-fed rats, developed disease and died earlier. Their observations included:

"…the tip of the tail may become inflamed and swollen, and the whole tail soon is heavily scaled and ridged. Hemorrhagic spots may arise in the skin throughout the entire length of the tail. The swelling of the tip may gradually be replaced by a true necrosis, resulting in the loss of 1 to 3 cm of the tail. The hind feet become red and somewhat swollen at times, in some cases with large scales over the dorsal surfaces. The hair on the back of the body becomes filled with dandruff. There is a tendency to lose the hair, especially about the face, back, and throat. Sores often appear on the skin" (Burr and Burr 1929).

These landmark studies made it apparent to the scientific community that fatty acids play an essential role in the maintenance of health (Burr and Burr 1929). The discovery of essential fatty acids (i.e. those fats that must be acquired from the diet) provided the basis for current research of the function of polyunsaturated fatty acids.

Essential fatty acids, such as EPA and DHA are known for their protective actions in many diseases including, cancer, Alzheimer's disease, and cardiovascular diseases (Albert

et al 1998, Albert *et al* 2002, Harris and Von Schacky 2004, Larsson *et al* 2004, Lukiw and Bazan 2008). The cardioprotective actions of omega-3 EPA and DHA were first observed in the 1970's when researchers investigated the plasma lipid composition, diet and hemostasis of Greenlandic West Coast Eskimos, a population that had a remarkably low prevalence of cardiovascular diseases (Bang *et al* 1971, Bang *et al* 1976, Dyerberg and Bang 1979a, Dyerberg and Bang 1979b, Bang *et al* 1980). Collectively, these studies demonstrated that there were increased levels of EPA and DHA in the blood and their clotting time was increased compared to blood taken from people living in Denmark (Bang *et al* 1971, Dyerberg and Bang 1979a). The noticeable difference between the Greenland Eskimos and the Danish populations was the composition of fat in their diets; the Eskimos had a diet enriched in EPA and DHA whereas the Danish had a diet lacking omega-3 fatty acids (Bang *et al* 1980). Since these studies, numerous clinical investigations regarding the beneficial actions of omega-3 fatty acids have been reported (Albert *et al* 2002, Albert *et al* 1999, Harris and Von Schacky 2004).

The roles of essential fatty acids in disease, such as periodontitis, are also being investigated (Offenbacher *et al* 1984, Requirand *et al* 2000). Early studies indicate an increase of AA-derived PGE2 (Offenbacher *et al* 1984) and LTB4 in GCF of periodontal disease patients. Indeed, many of the early pathophysiologic events in periodontal diseases including chronicity can be attributed to lipid mediators (Offenbacher 1996). Leukotriene B4, produced mainly by activated leukocytes, initiates accumulation and superoxide generation by neutrophils within inflamed sites, stimulating the release of granule-associated enzymes and bone resorption (Majno and Joris 2004). PGE2 is a potent activator of osteoclast-mediated bone resorption (Offenbacher *et al* 1986) and, with

other eicosanoids, mediates inflammation and periodontal tissue destruction.

In addition, studies of serum PUFA levels in periodontal disease patients demonstrated that the level of fatty acids of the omega-6 (i.e. arachidonic acid) pathway was higher in patients with bone loss than in the control group, whereas the reverse was observed with fatty acids of the omega-3 (i.e. EPA, DHA) pathway (Requirand *et al* 2000). These investigations conclude that bone loss is linked to an imbalance between omega-6 and omega-3 fatty acids (Requirand *et al* 2000). Although omega-3 fatty acids seem to have protective roles in periodontal disease outcomes, the cellular and molecular mechanisms governing this protection remained of interest. Thus, it is of significance to understand the molecular and cellular basis of this protection.

Resolution of acute inflammation

Inflammation has been studied for centuries. Mesopotamian writings, Egyptian hieroglyphics and texts from ancient Greece and China all describe the flame of inflammation (Majno 1991), but only insofar as its observable signs, such as redness, heat, swelling and pain (initially noted by Celsus in 30 AD). These accounts, however, mistakenly concluded that inflammation is a disease unto itself (Majno 1991, Majno and Joris 2004). Centuries later in 1794, John Hunter, a Scottish surgeon who was noted for his contributions to the fields of anatomy and dentistry, posited that inflammation was actually a consequence of injury or disease (Majno 1991). Since this important clarification, there have been many investigations to elucidate the cellular and molecular mediators of inflammation.

Acute inflammation is a response that eliminates or neutralizes foreign organisms and is protective against injury or infection (Majno and Joris 2004). Under physiological conditions, catabasis (the return from disease state to normal) will occur, leaving little opportunity for the development of chronic inflammation (Kumar *et al* 2005). It is a popular misconception that once the inflammatory response has neutralized an injurious stimulus, inflammation somehow passively stops (Majno and Joris 2004). If the host cannot thwart the inflammatory insult or if there is a failure of endogenous resolution programs, inflammation persists and tissue damage is bound to occur (Serhan and Savill 2005).

Traditionally, it was thought that pro-inflammatory mediator catabolism was sufficient for inflammation to cease (Gilroy *et al* 2004). It is now known, however, that the resolution of acute inflammation is an active, highly coordinated process that is controlled by endogenous specialized pro-resolving lipid mediators (SPM) such as lipoxins, resolvins, protectins and maresins (Serhan 2007) (Figure 1).

Given the essential role of the innate immune system in regulating immunity, it is conceivable that dysfunction of the components of resolution can contribute to disease (Medzhitov and Janeway 2000). Prolonged inflammation can cease to be a beneficial event and contribute to the pathogenesis of many disease states. Periodontitis, a well-appreciated example of leukocyte-mediated inflammation followed by osteoclast-mediated bone loss, has pathogenic features similar to those observed in other inflammatory diseases such as arthritis (Weissmann *et al* 1980, Van Dyke and Serhan 2003, Hasturk *et al* 2006). Thus, it is important to achieve a complete understanding of the cellular and molecular events that govern the resolution of inflammation.

Most inflammatory processes are self-limiting, which implies the existence of endogenous anti-inflammation pathways (Majno and Joris 2004, Serhan *et al* 2004,
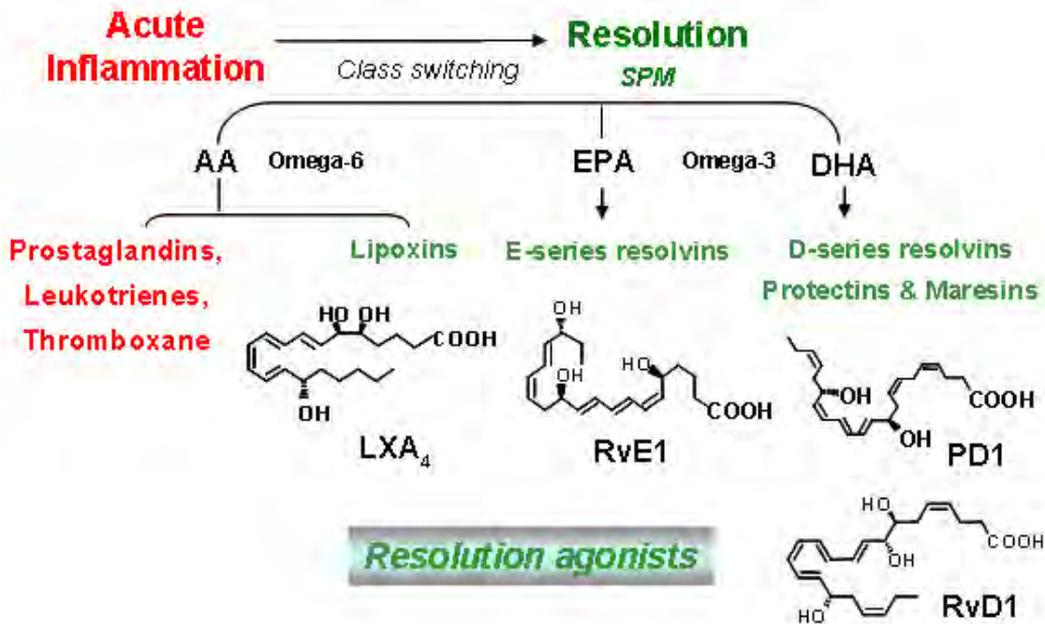
Figure 1. Specialized pro-resolving mediators in resolution of acute inflammation. The resolution of inflammation is actively regulated by pro-resolving lipid mediators. These local mediators are endogenously generated and include the lipoxins (LX) derived from arachidonic acid (AA) (Serhan *et al* 1984); resolvins derived from omega-3 PUFAs eicosapentaenoic acid (EPA; resolvins E series) (Serhan *et al* 2000); docosahexaenoic acid (DHA; resolvins D series (Serhan *et al* 2002, Serhan *et al* 2006, Sun *et al* 2007)); and protectins (Serhan *et al* 2002, Serhan *et al* 2006) and maresins (Serhan *et al* 2009) enzymatically derived from DHA.

Serhan and Savill 2005). Resolvins, first identified in resolving inflammatory exudates, are novel mediators generated from EPA and DHA that exhibit potent bioactions. The name Resolvin (resolution phase interaction product) was introduced for these active compounds that are endogenously biosynthesized via specific cell–cell interactions in murine inflammatory exudates in vivo, as well as during endothelial cell and leukocyte interactions in human vasculature (Serhan 2007). The autacoids that require enzymatic generation from omega-3 EPA are termed resolvins of the E series (i.e. RvE1) and those derived from DHA are members of the D series (i.e. RvD1). Other bioactive mediators from DHA that have a conjugated triene in their structure are known as protectins (Serhan 2007) (Figure 1).

Resolvin E1, as an example, has potent and protective actions in vivo by reducing inflammation and tissue destruction in peritonitis (Bannenberg *et al* 2005), 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, asthma, retinopathy, obesity and periodontitis (Arita *et al* 2005b, Hasturk *et al* 2006, Connor *et al* 2007, Hasturk *et al* 2007, Haworth *et al* 2008, Aoki *et al* 2008, Gonzalez-Periz *et al* 2009). In our rabbit model of experimental periodontitis, RvE1 treatment appeared to stimulate the resolution of inflammation by restoring *P. gingivalis* induced bone loss (Hasturk *et al* 2006, Hasturk *et al* 2007). RvE1 treatment resulted in a significant reduction in viable counts of oral microflora (Hasturk *et al* 2007). These results were not anticipated based on previous studies (Pouliot *et al* 2000) that failed to demonstrate

direct antibacterial action of RvE1 alone over vehicle (ethanol) treatment. The reasons for a reduction in the complexity of biofilm composition with RvE1 treatment remain unclear (Van Dyke 2008). One possibility is that resolvins promote the release of antimicrobial peptides, such as defensins and bactericidal/permeability-increasing protein, resulting in the destruction of select microorganisms (Levy *et al* 2003). A second possibility is that *P. gingivalis*, being an asaccharolytic organism that requires essential amino acids as its food source, survives on peptides formed by the degradation of collagen fragments, such as those produced during an inflammatory response (Van Dyke 2007). As such, an inflammatory state is a more hospitable environment for pathogenic bacteria, with formation of deep pockets favoring the survival of certain species. This explanation would suggest that the magnitude of inflammation generated by the host determines the composition of flora within the biofilm (Van Dyke 2008).

Recently, we also found that RvE1 topical treatment allowed for regeneration of hard and soft tissues of the periodontium lost to inflammatory disease (Hasturk *et al* 2007). Overall, RvE1 acts as a modulator of the inflammatory response by shifting the response to more rapid resolution effectively preventing the chronic phase. Elimination of inflammation in the healing lesion promotes tissue regeneration. These principles may be applicable to other inflammatory diseases including arthritis and cardiovascular disease due to the similarities between these diseases, such as the neutrophil induced panus formation in arthritis and the inflammatory damage to blood vessels stimulating atherogenesis (Abramson *et al* 1983, Libby *et al* 2002).

Periodontal and cardiovascular diseases

There is substantial epidemiological evidence for a link between periodontal diseases and cardiovascular diseases (Beck *et al* 1996, Dave *et al* 2004, Noack *et al* 2001b, Ridker and Silvertown 2008). The biological basis for the observed association is not described, although two hypotheses are presented in the literature. The first hypothesis holds that bacteremia results in direct action of bacteria on endothelial cells inducing injury followed by atheroma formation (Nassar *et al* 2002). This is an extension of observations in the cardiology literature suggesting that Chlamydia and Helicobacter infections were associated with cardiovascular events (Patel *et al* 1995, Pasceri *et al* 1998, Mayr *et al* 2000, Bloemenkamp *et al* 2002). However, intervention trials aimed at treating the infection and preventing cardiovascular events have all been negative (Zahn *et al* 2003, Cercek *et al* 2003, Muhlestein *et al* 2000). The other prevailing hypothesis is that periodontal diseases and cardiovascular disease associations are the result of common risk factors related to inflammation (Beck *et al* 1996, Ridker and Silvertown 2008). Recently, the landmark JUPITER Study provided convincing evidence that elevated systemic inflammation (elevated hsCRP, or high sensitivity C-reactive protein) is a major determinant in cardiovascular risk (Ridker 2003, Libby and Ridker 2004). Other independent observations have revealed that hsCRP is elevated in periodontal disease and that the elevation is reversible with treatment (Offenbacher *et al* 2009). Together, the data suggest that an elevated inflammatory phenotype, whether acquired or genetically determined, is a major risk factor for both diseases (Ridker and Silvertown 2008, Van Dyke 2008). The relationship of resolution
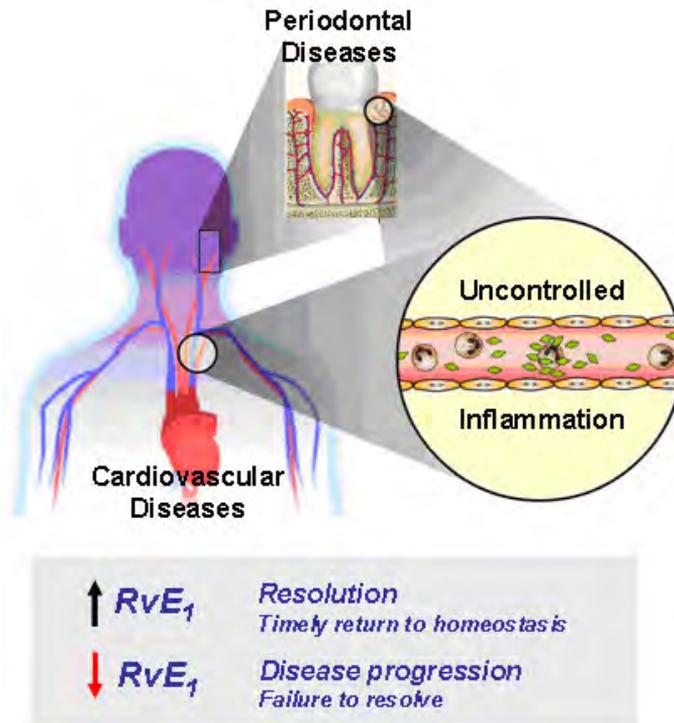
Figure 2. A unifying hypothesis for the common components of inflammation in the pathogenesis of inflammatory diseases. LAP or cardiovascular diseases have common components that are inflammatory in origin (Van Dyke and Serhan, 2003). Recent findings suggest that defective resolution mechanisms may underlie the inflammatory phenotypes that are believed to characterize many common human diseases (Serhan, 2008).   RvE1 (Serhan *et al* 2000), an E-series resolvin and member of the new genus of endogenous pro-resolving agonists, in known to have protective actions in animal models at periodontitis (Hasturk *et al* 2006, Hasturk *et al* 2007). In addition, RvE1 is generated via cell-cell interactions in the human vasculature (Arita *et al* 2005a) and may be an important regulatory component of the inflammatory process. As such, increased RvE1 (either endogenously and exogenously) may lead to a return to homeostasis, whereas a lack of RvE1 may result in chronicity. Thus, RvE1 and may be beneficial in the treatment of inflammatory diseases such as LAP and cardiovascular diseases.

pathways to the inflammatory phenotype in these diseases remains to be investigated.

Thus, we sought to investigate cause and effect in this association between cardiovascular diseases and periodontitis using animal models (Jain *et al* 2003). Twelve age and sex-matched New Zealand white rabbits were fed a 0.5% fat diet for 13 weeks, which is known to cause atheromatous changes in the large arteries of the animals. In our experiment, periodontitis was induced with a ligature and *Porphyromonas gingivalis*

in half the animals, while the other half maintained a normal periodontium. Using the area of the atheromatous lesion as the primary outcome, the animals that had periodontitis exhibited double the atheroma formation of the controls (Jain *et al* 2003). These data provide direct longitudinal evidence that suggests that periodontal inflammation can impact upon the progression of CVD. Further investigation was unable to isolate bacteria from the arterial lesions. These data suggest that the impact of infection is not at the level

of bacteremia and direct stimulation of arterial endothelium, but rather local inflammation inducing a systemic elevation of inflammation.

In order to determine the relationship between susceptibility to CVD and periodontitis and the innate inflammatory response, a 15-lipoxygenase (15-LO) transgenic rabbit was engineered (Serhan *et al* 2003). The overexpression of 15-LO leads to a rise in the product 15-HETE, which is rapidly metabolized by myeloid 5-LO into lipoxin A4. The resulting phenotype is a rabbit with increased circulating LXA4 that exhibits a lower innate inflammatory response. Interestingly, the lower response is not characterized by impaired innate immunity, but rather by reduced tissue damage (Serhan *et al* 2003). In addition, when fed with high fat diet accompanied by ligature-induced periodontitis, the transgenic animals were resistant to induction of CVD and were protected against development of periodontitis (Serhan *et al* 2003). From these data, we were able to conclude that inflammation plays a pivotal role in the pathogenesis of both periodontitis and cardiovascular disease and regulating the inflammatory response prevents both periodontitis and early vascular changes in the rabbit model.

Concluding remarks

Controlling the host response is an attractive hypothesis for interventions designed to reduce the inflammatory pathogenesis of periodontal disease. Since RvE1 was shown to rescue the primed phenotype of LAP PMN in isolation, it would be of great interest to investigate the systemic treatment of LAP with RvE1 (Hasturk *et al* 2007). There are no animal models for localised aggressive periodontitis, so human clinical trials will be needed to assess the systemic protective actions of RvE1. As the

role of inflammation in periodontal disease management is being unraveled, the concepts of actively resolving destructive host-mediated inflammatory pathways may offer a novel strategy toward prevention and treatment of periodontal diseases (Figure 2).

Acknowledgements

References

Abramson S, Edelson H, Kaplan H, *et al*. The neutrophil in rheumatoid arthritis: Its role and the inhibition of its activation by nonsteroidal anti-inflammatory drugs. *Sem Arth Rheum* 1983;13:148-153.

Albert CM, Campos H, Stampfer MJ, *et al*. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *New Eng J Med* 2002;346:1113-1138.

Albert CM, Hennekens CH, O'Donnell CJ, *et al*. Fish consumption and risk of sudden cardiac death. *JAMA* 1998;279:23-28.

Aoki H, Hisada T, Ishizuka T, *et al*. Resolvin E1 dampens airway inflammation and hyperresponsiveness in a murine model of asthma. *Biochemical and Biophysical Research Communications* 2008;367:509-515.

Arita M, Bianchini F, Aliberti J, *et al*. Stereochemical assignment, antiinflammatory properties and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med* 2005;201:713-722.

Arita M, Yoshida M, Hong S, *et al*. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc Nat Acad Sci USA* 2005;102:7671-7676.

Bang HO, Dyerberg J, Hjoorne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand* 1976;200:69-73.

Bang HO, Dyerberg J, Nielsen AB. Plasma lipid

and lipoprotein pattern in Greenlandic West-Coast Eskimos. *Lancet* 1971;1:1143-1145.

Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. *Am J Clin Nut* 1980;33:2657-2661.

Bannenberg GL, Chiang N, Ariel A, *et al*. Molecular circuits of resolution: Formation and actions of resolvins and protectins. *J Immunol* 2005;174:4345-4355.

Beck J, Garcia R, Heiss G, *et al*. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67:1123-1137.

Bloemenkamp DG, Mali WP, Tanis BC, *et al*. *Chlamydia pneumoniae*, *Helicobacter pylori* and cytomegalovirus infections and the risk of peripheral arterial disease in young women. *Atherosclerosis* 2002;163:149-156.

Burr GO, Burr MM. A new deficiency disease produced by the rigid exclusion of fat from the diet. *J Bio Chem* 1929;82:345-367.

Cercek B, Shah PK, Noc M, *et al*. Effect of short-term treatment with azithromycin on recurrent ischaemic events in patients with acute coronary syndrome in the Azithromycin in Acute Coronary Syndrome (AZACS) trial: A randomised controlled trial. *Lancet* 2003;361:809-813.

Connor KM, SanGiovanni JP, Lofqvist C, *et al*. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. *Nature Med* 2007;13:868-873.

Dave S, Batista EL Jr, Van Dyke TE. Cardiovascular disease and periodontal diseases: Commonality and causation. *Comp Cont Ed Dent* 2004;25:26-37.

Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet* 1979;2:433-435.

Dyerberg J, Bang HO. Lipid metabolism, atherogenesis and haemostasis in Eskimos: The role of the prostaglandin-3 family. *Haemostasis* 1979;8:227-233.

Gilroy DW, Lawrence T, Perretti M, Rossi AG. Inflammatory resolution: New opportunities for drug discovery. *Nature Reviews. Drug Discovery* 2004;3:401-416.

GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: Results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999;354:447-455.

Gonzalez-Periz A, Horrillo R, Ferre N, *et al*. Obesity-induced insulin resistance and hepatic steatosis are alleviated by Omega-3 fatty acids: A role for resolvins and protectins. *FASEB Journal* 2009;23:1946-1957.

Graves D. Cytokines that promote periodontal tissue destruction. *J Periodontol* 2008;79:1585-1591.

Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 200374:391-401.

Harris WS, Von Schacky C. The Omega-3 Index: A new risk factor for death from coronary heart disease? *Prevent Med* 2004;39:212-220.

Hasturk H, Kantarci A, Goguet-Surmenian E, *et al*. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis *in vivo*. *J Immunol* 2007;179:7021-7029.

Hasturk H, Kantarci A, Ohira T, *et al*. RvE1 pro ects from local inflammation and osteoclast-ediated bone destruction in periodo titis. *FASEB Journal* 2006;20:401-403.

Haworth O, Cernadas M, Yang R, *et al*. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nature Immunol* 2008;9:873-879.

Jain A, Batista EL Jr, Serhan C, *et al*. Role for periodontitis in the progression of lipid deposition in an animal model. *Infect Immun* 2003;71:6012-6018.

Kornman KS, Crane A, Wang H.Y, *et al*. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Perio* 1997;24:72-77.

Kumar V, Abbas AK, Fausto N, *et al*. Robbins and Cotran pathologic basis of disease. Elsevier/Saunders, Philadelphia. 2005.

Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: A review of potential

mechanisms. *Am J Clin Nutrit* 2004;79:935-945.

Levy O, Canny G, Serhan CN, Colgan SP. Expression of BPI (bactericidal/permeability-increasing protein) in human mucosal epithelia. *Biochem Soc Trans* 2003;31:795-800.

Libby P, Ridker PM. Inflammation and atherosclerosis: Role of C-reactive protein in risk assessment. *Am J Med* 2004;116(Suppl 6A):9S-16S.

Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-1143.

Listgarten MA. Nature of periodontal diseases: Pathogenic mechanisms. *J Perio Res* 1987;22:172-178.

Loos BG, Craandijk J, Hoek FJ, *et al*. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000;71:1528-34.

Lukiw WJ, Bazan NG. Docosahexaenoic acid and the aging brain. *J Nutrit* 2008;138:2510-2514.

Majno G. The healing hand: Man and wound in the ancient world. Harvard University Press, Cambridge, Mass. 1991.

Majno G, Joris I. Cells, tissues and disease: Principles of general pathology. Oxford University Press, New York. 2004.

Mayr M, Kiechl S, Willeit J, *et al*. Infections, immunity and atherosclerosis: associations of antibodies to *Chlamydia pneumoniae*, *Helicobacter pylori* and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. *Circulation* 2000;102:833-839.

Medzhitov R, Janeway C Jr. Innate immunity. *New Eng J Med* 2000;343:338-344.

Muhlestein JB, Anderson JL, Carlquist JF, *et al*. Randomized secondary prevention trial of azithromycin in patients with coronary artery disease: Primary clinical results of the ACADEMIC study. *Circulation* 2000;102:1755-1760.

Nassar H, Chou HH, Khlgatian M, *et al*. Role for fimbriae and lysine-specific cysteine proteinase gingipain K in expression of interleukin-8 and monocyte chemoattractant protein in *Porphyromonas gingivalis*-infected endothelial cells. *Infect Immun* 2002;70:268-276.

Noack B, Genco RJ, Trevisan M, *et al*. Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol* 2001;72:1221-1227.

Offenbacher S. Periodontal diseases: Pathogenesis. *Ann Periodontol* 1996;1:821-878.

Offenbacher S, Beck JD, Moss K, *et al*. Results from the Periodontitis and Vascular Events (PAVE) Study: A pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. *J Periodontol* 2009;80:190-201.

Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *J Perio Res* 1986;21:101-112.

Offenbacher S, Odle BM, Gray RC, Van Dyke TE. Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *J Perio Res* 1984;19:1-13.

Pasceri V, Cammarota G, Patti G, *et al*. Association of virulent *Helicobacter pylori* strains with ischemic heart disease. *Circulation* 1998;97:1675-1679.

Patel P, Mendall MA, Carrington D, *et al*. Association of *Helicobacter pylori* and *Chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk factors. *BMJ Clinical Research Ed* 1995;311:711-714.

Pouliot M, Clish CB, Petasis NA, *et al*. Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: A role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochem* 2000;39:4761-4768.

Requirand P, Gibert P, Tramini P, *et al*. Serum fatty acid imbalance in bone loss: Example with periodontal disease. *Clin Nutrit* 2000;19:271-276.

Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: Rationale and design of the JUPITER trial. *Circulation* 2003;108:2292-2297.

Ridker PM., Silvertown JD. Inflammation, C-

reactive protein and atherothrombosis. *J Periodontol* 2008;79:1544-1551.

Serhan CN. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Ann Rev Immunol* 2007;25:101-137.

Serhan CN. Controlling the resolution of acute inflammation: A new genus of dual anti-inflammatory and proresolving mediators. *J Periodontol* 2008;79:1520-1526.

Serhan CN, Savill J. Resolution of inflammation: The beginning programs the end. *Nat Immunol* 2005;6:1191-1197.

Serhan CN, Clish CB, Brannon J, *et al*. Novel functional sets of lipid-derived mediators with anti-inflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal anti-inflammatory drugs and transcellular processing. *J Exp Med* 2000;192:1197-1204.

Serhan CN, Gotlinger K, Hong S, Arita M. Resolvins, docosatrienes and neuroprotectins, novel omega-3-derived mediators and their aspirin-triggered endogenous epimers: An overview of their protective roles in catabasis. *Prost Other Lipid Med* 2004;73:155-172.

Serhan CN, Gotlinger K, Hong S, *et al*. Anti-inflammatory actions of neuroprotectin D1/ protectin D1 and its natural stereoisomers: Assignments of dihydroxy-containing docosatrienes. *J Immun* 2006;176:1848-1859.

Serhan CN, Hamberg M, Samuelsson B. Lipoxins: Novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Nat Acad Sci USA* 1984;81:5335-5339.

Serhan CN, Hong S, Gronert K, *et al*. Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 2002;196:1025-1037.

Serhan CN, Jain A, Marleau S, *et al*. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immun* 2003;171:6856-6865.

Serhan CN, Yang R, Martinod K, *et al*. Maresins: Novel macrophage mediators with potent anti-inflammatory and proresolving actions. *J Exp Med* 2009;206:15-23.

Shapira L, Warbington M, Van Dyke TE. TNF alpha and IL-1 beta in serum of LJP patients with normal and defective neutrophil chemotaxis. *J Perio Res* 1994;29:371-373.

Slade GD, Ghezzi EM, Heiss G, *et al*. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. *Arch Int Med* 2003;163:1172-1179.

Sun YP, Oh SF, Uddin J, *et al*. Resolvin D1 and its aspirin-triggered 17R epimer. Stereochemical assignments, anti-inflammatory properties and enzymatic inactivation. *J Bio Chem* 2007;282:9323-9334.

Van Dyke TE. Control of inflammation and periodontitis. *Periodontol 2000* 2007;45:158-166.

Van Dyke TE. The management of inflammation in periodontal disease. *J Periodont* 2008;79:1601-1608.

Van Dyke TE, Serhan CN. Resolution of inflammation: A new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003;82:82-90.

Weissmann G, Smolen JE, Korchak HM. Release of inflammatory mediators from stimulated neutrophils. *New Eng J Med* 1980;303:27-34.

Zahn R, Schneider S, Frilling B, *et al*. Antibiotic therapy after acute myocardial infarction: A prospective randomized study. *Circulation* 2003;107:1253-1259.

Chapter 9

Periodontic advances: Impact on practice

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Introduction

This article is designed to update the reader on recent advances in periodontics over the past five years. The article addresses the etiology and pathogenesis of periodontal disease, various therapeutic modalities including host modulation, topical antimicrobials, locally delivered agents, systemic antimicrobials, agents for repair and regeneration of the periodontium, current concepts in peri-implant mucositis and the relationship between oral health and general health.

Recent advances in periodontics have had a strong impact on the clinical practice of periodontics. These advances have emanated from research funded by government, industry and foundations. Working together these groups have made us realize that the research of today is the promise for tomorrow's therapy. This article will review the various scientific advances that have had a significant impact on clinical periodontics.

Etiology and pathogenesis

Both animal and human studies have established that bacteria are important in the etiology and progression of periodontal diseases (Löe 1965, Theilade 1966, Saxe 1967, Lindhe 1975). The following bacteria appear to be most often associated with periodontal disease incidence, severity and progression: *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Campylobacter rectus, Streptococcus intermedius, Eubacterium nodatum, Aggregatibacter actinomycetemocmitans,* and *Prevotella intermedia.* The realization that these bacteria form highly organized biofilms which are complex polymicrobial communities resistant to externally applied antimicrobial agents has led investigators to look not for just the ability of antimicrobials to kill bacteria in test tube but, more importantly, those in biofilms (Kolenbrander 2006, Socransky 2002). Recent studies with mouthrinses containing essential oils and chlorhexidine have demonstrated that these products can effectively penetrate biofilms and kill the bacteria within them (Pan 2000). This has also been demonstrated with dentifrices containing triclosan/copolymer (Amornchat 2004).

Host modulation

It is also clear that these bacterial biofilms can initiate destructive inflammatory responses by the host and that the inflammatory process can mediate the

destruction of connective tissue and bone. Therefore the concept of host modulation now must be considered as an important part of therapy for periodontal and peri-implant tissues. Strong evidence supports the use of low dose doxycycline (20 mg twice daily) as a host modulating agent and its benefit to scaling and root planing in the treatment of chronic periodontitis (Reddy 2003). The mechanism of action is by suppression of the activity of collagenase, particularly that produced by polymorphonuclear leukocytes (PMNs). However, in the antibiotic family, doxycycline hyclate does not produce antibacterial effects because the dose of 20 mg bid is too low to affect bacteria. As a result, resistance to this medication has not been seen. Four double-blind, multicenter clinical studies of more than 650 patients have demonstrated that doxycycline hyclate improves the effectiveness of professional periodontal care and slows the progression of the disease process. The results of the first three studies showed that doxycycline hyclate resulted in approximately a 50% improvement in clinical attachment levels in pockets with probing depths of 4 to 6 mm and a 34% improvement in pockets with probing depths of 7 mm or greater. Attachment loss was prevented in sites with normal probing depths (0-3 mm), whereas the placebo groups lost 0.13 mm at 12 months (p = 0.05) (Caton et al 1997).

Caton et al (2000) showed statistically significant reductions in probing depths and increases in clinical attachment levels with adjunctive doxycycline hyclate in conjunction with root planing at 3, 6, and 9 month evaluations compared with placebo groups undergoing root planing alone. Although statistically significant, the net changes were considered limited alterations in patients with moderate to severe chronic periodontitis (American Academy of Periodontology 2004).

Results of safety studies have shown that doxycycline hyclate (20 mg bid) with or without mechanical therapy (scaling and root planing) did not exert an anti-infective effect on the periodontal microflora and did not result in a detrimental shift in the normal flora. The colonization or overgrowth of the periodontal pocket by bacteria resistant to doxycycline, tetracycline, minocycline, amoxicillin, erythromycin, or clindamycin has not been observed. In addition, no evidence of a tendency toward the acquisition of multiantibiotic resistance was found (Walker et al 1981). Periostat (Collagenex Pharmaceutical Inc) and generic forms are currently available in a dose of 20 mg of doxycycline.

Nonsteroidal anti-inflammatory drugs (NSAIDs) may be of therapeutic value in treating periodontal disease because of their ability to interfere with arachidonic acid metabolism and thereby inhibit the inflammatory process. This theory has been validated in studies in both animals and humans (Feldmen et al 1983, Offenbacher et al 1987, Waite et al 1981, Williams et al 1987, Williams et al 1985). Some NSAIDs have been shown to affect the response of PMNs to inflammation not related to prostaglandin inhibition (Feres et al 2002, Kaplan 1984). Beneficial effects of NSAIDs have also been found after topical application (Heasom et al 1993, Vogel 1986, Williams et al 1988). Drugs such as flurbiprofen, ibuprofen, mefenamic acid, and naproxen have been studied (Williams et al 1989).

Flurbiprofen appears to be an NSAID worthy of further investigation. It inhibits PMN migration, reduces vascular permeability, and inhibits platelet aggregation by inhibiting cyclooxygenase (Heasom et al 1993). In a 3 year study, Williams et al (1988) reported that flurbiprofen significantly inhibited radiographic alveolar bone loss when compared with placebo. Unfortunately, by 24 months, the difference in the rate of bone loss had disappeared. This group also reported a

return to baseline in the rate of bone loss after treatment with flurbiprofen was discontinued (Williams *et al* 1985).

A recent study suggests that concomitant administration of doxycycline and flurbiprofen may result in enhancement of the anticollagenase effects of doxycycline and deserves further investigation (Lee *et al* 2004).

Regeneration of periodontal tissues

A number of controlled studies have evaluated the effects of periodontal flap procedures alone compared to flap procedures plus insertion of various bone-replacement graft materials. A systematic review and meta-analysis of the studies found that use of bone replacement grafts as part of a flap procedure resulted in statistically significant increased bone and clinical attachment levels and reduced probing depth compared to flap alone procedures (Reynolds *et al* 2003). Over the years there have been a number of studies on the clinical effectiveness of Guided Tissue Regeneration (GTR). These studies have also been evaluated by a meta-analysis with the findings that GTR procedures can promote the gain of clinical attachment levels and reduction in probing depths (Murphy *et al* 2003). Growth factors have also been recently introduced. They are naturally occurring mediators produced by a variety of cells that affect the process of wound healing. Growth factors can regulate the timing of cell division, cell differentiation, synthesis of extracellular matrix and recruitment of progenitor cells. Natural growth factors have been isolated and recombinant forms have been developed. Initial studies of these agents have suggested that they are capable of supporting periodontal regeneration (Giannobile *et al* 2003). Use of these agents as well as stem cell research offer promise for even more predictable periodontal regeneration (Slavkin and Bartold 2006).

Peri-implant mucositis/implantitis

Since there are a number of similarities between the pathogens involved in periodontitis and peri-implantitis, it is logical that similar clinical approaches should be implemented for peri-implant mucositis/implantitis. An early study by Mombelli *et al* (2001), utilizing tetracycline containing fibers (which are no longer available), demonstrated that, in 25 patients with peri-implantitis, there was a significant reduction of four periopathogenic species, but other species, such as *P. gingivalis* and *A. actinomycetemcomintans*, had very low baseline values and were not significantly affected. There was a slight, but not significant, improvement in the probing depth at 12 months.

In another study, investigators applied the controlled release of doxycycline (Atridox™) into peri-implant pockets and noted differences from scaling and root planning alone. This study also showed the efficacy of such devices in treating peri-implant infections. Patients who received the doxycyline treatment showed significantly greater probing attachment levels and lesser pocket probing depth and bleeding index (p < 0.05), than those who received scaling/ root planning alone (Bueno *et al* 1988)

A study by Salvi *et al* (2002) evaluated 31 implants with peri-implantitis in 25 patients and monitored clinical and radiographic changes over 12 months following adjunctive placement of minocycline microspheres (Arestin). Minocycline microspheres were locally delivered to each implant site with bone loss and a probing pocket depth (PPD) equal to or greater than 5 mm. Rescue therapy with Arestin was allowed at days 180 and 270 at any site exhibiting an increase in PPD equal or greater than 2 mm from the previous visit. Six implants in six subjects were either

rescued or exited because of persisting active peri-implantitis. Successful implants showed a statistically significant reduction in both PPD and percentage of sites with BOP between baseline and Day 360 (P<0.05). At mesial implant sites, the mean PPD reduction amounted to 1.6 mm (95% CI 0.9 – 2.2 mm, P<0.001) and was accompanied by a statistically significant reduction of the BOP value (P<001).

Although studies evaluating the use of the Perio-chip (chlorhexidine) for peri-implant infections were not identified, one 12 month study compared the clinical and microbiological results after application of minocycline microspheres as an adjunct to mechanical treatment of incipient peri-implant infections compared with an adjunctive treatment using application of 1 ml of a 1% chlorhexidine gel (Renvert 2006). Sixteen patients in the minocycline group and fourteen in the chlorhexidine group completed the study. The findings of the study were that the adjunctive use of minocycline microspheres resulted in improvements of probing depths and bleeding scores, whereas the adjunctive use of chlorhexidine only resulted in limited reduction of bleeding scores. For the deepest sites of the treated implants in the minocycline group, the mean probing depth was reduced from 5.0 to 4.4 mm at 12 months. This study could not show any significant difference in the levels of bacterial species or groups at any time point between the two antimicrobial agents tested.

In the author's practice, local delivery agents have been utilised to treat peri-implantitis and have been supplemented with systemic administration of low dose doxycycline (20 mg twice daily) as well as clindamycin administered in a dose of 150-300 mg three times daily for ten days prior to administration of low dose doxyclcine. This form of therapy has arrested the disease process for up to 6 months.

Topical antimicrobial agents

Topical antimicrobials in the form of mouthrinses, pastes and gels have been shown to be effective for both the prevention and reduction of gingivitis, and, in the case of triclosan, to also arrest the progression of periodontitis (Rosling *et al* 1997, Rosling *et al* 1997, Ellwood *et al* 1998, Furuichi *et al* 1999).

A 2007 statement by the American Dental Association (ADA) advised consumers that "Use of an ADA Accepted antimicrobial mouthrinse or toothpaste helps prevent and reduce plaque and gingivitis." (www.ada.org). Products currently accepted by the ADA for the reduction of plaque and gingivitis are Listerine antiseptic mouthrinse, chlorhexidine mouthrinses, Colgate Total toothpaste and Crest Pro-Health toothpaste.

The rationale for incorporating antimicrobial mouthrinses into a daily oral hygiene regimen is based on the fact that most patients have difficulty with mechanical methods of home care (Barnett 2006, Morris 2001, Hugosan 1998).

In view of the problems associated with mechanical plaque control, worldwide studies in developed countries have shown that people who claim to use dental floss or some other interdental cleaning device daily are between 11 and 51% with the average usage being approximately 20% (Morris 2001, Christensen 2003, Segelnick 2004, American Dental Association 2004, Bakdash 1995, Albandar 1999). Gingivitis is present in over 50% of the population.

Although brushing and interdental cleaning remove some bacterial biofilm from tooth surfaces, tooth surfaces only comprise 25% of oral surface areas exposed to bacteria. The other 75% is found on oral mucosal surfaces. Studies have shown that oral soft tissues serve as reservoirs for bacteria and can be the source of pathogens that re-colonise

Product	# of studies	Significant Plaque Reduction	Significant Gingivitis Reduction	ADA Accepted
Chlorhexedine 0.12%	6	22-61	18-42	Yes
Essential Oils	25 (4 had 2 active arms)	12-56	14-36	Yes
Cetylpyridinium Chloride 0.045-0.05%	5	7-16	15-20	No
Cetylpyridinium Chloride 0.07%	2	7-16	15-20	No

Table 1. Six month studies on the effectiveness of mouthrinses

teeth after a dental prophylaxis or periodontal therapy (Danser 1996, Somer 1996, Eger *et al* 1996, Tanner *et al* 2006, Ximenez 2000). A recent article states "The finding that the oral mucosa serve as reservoirs of pathogenic bacteria that can be transferred to the tooth surface provides a further rationale for supplementing mechanical plaque control methods with effective antimicrobial mouthrinses" (Barnett 2006).

The only systematic review (meta-analysis) of six-month clinical trials of mouthrinses with agents to control plaque and gingivitis was published in the *Journal of the American Dental Association* in 2006 (Gunsolley 2006). In this systematic review, relative to dentifrices, 17 studies supported the antiplaque, antigingivitis effects of dentifrices containing 0.30% triclosan, 2.0% Gantrez copolymer. There was no evidence of efficacy for triclosan products containing either soluble pyrophosphate or zinc citrate. Dentifrices with stannous fluoride had statistically significant, but marginally clinically significant evidence of an antiplaque effect; however, there was both a statistically and clinically significant antigingivitis effect. The largest body of studies (21 studies) supported the efficacy of mouthrinses with essential oils. A smaller body of 7 studies supported a strong antiplaque,

antigingivitis effect of mouthrinses with 0.12% chlorhexidine. Results for mouthrinses with cetylpyridinium chloride varied and depended on the product's formula (Table 1).

It should be noted that, in addition to the efficacy of various mouthrinse ingredients against bacteria associated with the etiology of gingivitis, chlorhexidine containing and essential oil containing mouthrinses have good anti-candida effects and can be used to treat oral candidiasis.

The data presented in this table clearly show that the two most often studied mouthrinse ingredients are chlorhexidine and essential oils, with mouthrinses containing essential oils being the most often studied. These studies have established both the safety and efficacy of these mouthrinses.

Alcohol in mouthrinses

Over the years concern has been expressed about the adverse effects of alcohol-containing mouthrinses (Silverman 2006). However, numerous published studies have demonstrated the safety of alcohol-containing mouthrinses, and they have not found a relationship between the use of these products and safety concerns. In reviewing the various studies that evaluated the potential for alcohol

in antimicrobial mouthrinses to cause oral cancer, a review in *Biological Therapies in Dentistry* noted that the studies suggesting a relationship between oral cancer and use of alcohol containing mouthrinses had a number of deficiencies in design and analysis and that these deficiencies had been identified by the American Dental Association, the United States National Cancer Society and the United States Food and Drug Association (Ciancio 1993). All three organizations stated that studies have not established an association between use of alcohol containing mouthrinses and oral cancer.

In April 2009 the International Academy of Periodontology issued the following statement:

"The International Academy of Periodontology (IAP) is concerned about a recent article which concludes there is now "sufficient evidence" to suggest that alcohol containing mouthwashes are a contributing factor to oral cancer (McCullough and Farah 2008). However, it should be noted that this issue was evaluated by the United States Food and Drug Administration, the American Dental Association and the National Cancer institute over 10 years ago with the conclusion that alcohol in mouthrinses does not cause cancer" (Ciancio 1993).

More recently a review article in Great Britain reached the same conclusion (Lewis 2006) as do three thorough review papers: one in Australia, one in Brazil and one in Italy. These papers all agreed that, relative to an association between alcohol in mouthrinses and oral cancer risk, there is no "relevant association between alcohol containing mouthrinses and oral cancer risk" The Brazilian paper stated "The alleged correlation between oral cancer and alcohol-based mouthrinses presents little, weak, inconsistent and even contradictory evidence in the literature that any kind of risk-warning to patients would be uncalled for (La Vecchia

2009, Lemos 2008, Walsh 2008).

In view of the current trend towards evidenced based decisions in medicine and dentistry, the International Academy of Periodontology encourages a well designed systematic review of this topic when adequate studies are available.

The International Academy of Periodontology is in agreement with the following positions of the British Dental Health Foundation and the British Dental Association.

British Dental Health Foundation Statement

"A recent, and more thorough review of all available evidence carried out by leading experts on behalf of the foundation concluded there were no proven links between alcohol-containing mouthwashes and increased incidence of mouth cancer. The public should not worry."

British Dental Association Statement

"Excessive consumption of alcohol and tobacco are well recognized in the UK as risk factors for developing oral cancers. This paper raises interesting issues but the evidence showing any link between the prolonged use of mouthwashes containing alcohol and oral cancer is not conclusive. Further research is required to establish if there is a genuine connection. Where patients are in any doubt about using mouthwash, they should consult their dentist."

Other positions

Other dental professional organisations have also stated positions similar to those of the International Academy of Periodontology. Two recent reviews confirmed that the findings in the literature are inconsistent and

contradictory and do not fulfill the basic pharmacologic requirement of a dose response to establish a causal relationship between alcohol-containing rinses and oral cancer (Lewis 2006, La Vecchia 2009). Lewis and Murray (2006) concluded that the main concern regarding alcohol in mouthrinses involves people who are recovering from alcohol abuse, since the taste of the alcohol in the mouthrinse may put them at risk of experiencing a relapse to their alcohol consumption habit.

Locally delivered antimicrobial agents

Locally delivered antimicrobial agents are available as adjuncts to scaling and root planing. When placed into periodontal pockets, they reduce the subgingival microflora, probing depths, and clinical signs of inflammation. A recent systematic review concluded "Adjunctive local therapy generally reduced probing depth levels. Differences between treatment and SRP-only groups in the baseline to followup period typically favored treatment groups but usually only modestly (e.g. from about 0.1 mm to nearly 0.5 mm. Effects for clinical attachment level gains were smaller and statistical significance less common" (Bonito *et al* 2005). A report on locally delivered agents prepared by the American Academy of Periodontology stated "The clinician's decision to use locally delivered agents (LDA) should be based upon a consideration of clinical findings, the patient's dental and medical history, scientific evidence, patient preferences and advantages and disadvantages of alternative therapies". The report also stated that use of LDAs may be of value when probing depths greater than 5 mm with inflammation are still present after conventional therapy. However if multiple sites are present in the same quadrant, therapy other than LDAs should be considered (Baker *et al* 1985).

Available agents for local delivery include minocycline (Arestin), doxycycline (Atridox), chlorhexidine (Perio Chip) and metronidazole. There are extensive reviews of the local delivery agents available for periodontitis (Killoy 1998, Killoy *et al* 1998, Hill *et al* 2004, Hanes *et al* 2003).

Comparative studies

Few researchers have attempted to compare these devices side-by-side, but in one study, doxycycline polymer, metronidazole gel, and Perio Chip were compared in 47 periodontitis patients. The study found that all controlled release polymer devices increased gingival attachment levels, but that there was a slightly greater improvement in patients treated with the doxycycline polymer (Salvi *et al* 2002).

Systemic administration of antimicrobials

A number of antibiotics have been evaluated as adjuncts to periodontal therapy. A recent meta-analysis shows that systemic antibiotics combined with mechanical plaque removal by scaling and root planning has beneficial adjunctive effects, including gains in clinical attachment and reduction of pocket probing depths (Haffajee 2003). Because periodontal infections may contain a wide diversity of bacteria, no single antibiotic is effective against all putative pathogens. Indeed, differences exist in the microbial flora associated with the various periodontal disease syndromes (Walker *et al* 1993). These "mixed" infections can include a variety of aerobic, microaerophilic, and anaerobic bacteria, both gram negative and gram positive. In these cases it may be necessary to use more than one antibiotic, either serially or in combination (Rams and Slots 1992). Before combinations of antibiotics are used,

Antibiotic	Regimen	Dosage Duration
Amoxicillin	500 mg	Three times daily for 10 days
Clindamycin	150-300 mg	Three times daily for 10 days
Doxycycline/minocycline	100 mg	One bid for day 1, 1 daily for 14-21 days
Metronidazole	500 mg	Three times daily for 10 days
Metronidazole + amoxycillin	250 mg of each	Three times daily for 14-21 days
Metronidazole + ciprofloxacin	500 mg of each	Twice daily for 8 days

Table 2. Common antibiotic regimens used to treat periodontal diseases

where possible the periodontal pathogen(s) being treated must be identified and antibiotic-susceptibility testing performed.

Antibiotics that are bacteriostatic (e.g. tetracycline) generally require rapidly dividing microorganisms to be effective. They do not function well if a bactericidal antibiotic (e.g. amoxicillin) is given concurrently. When both types of drugs are required, they are best given serially, not in combination.

Rams *et al* (1992) reviewed combination therapy using systemic metronidazole along with amoxicillin, amoxicillin-clavulanate (Augmentin), or ciprofloxacin. The metronidazole-amoxicillin and metronidazole-augmentin combinations provided excellent elimination of many organisms in adult and localized aggressive periodontitis that had been treated unsuccessfully with tetracyclines and mechanical debridement. These drugs have an additive effect regarding suppression of *A. actinomycetemcomitans*. Tinoco *et al* (1998) found metronidazole and amoxicillin to be clinically effective in treating LAP, although 50% of patients harbored *A. actinomycetemcomitans* 1 year later. Metronidazole-ciprofloxacin combination is effective against *A. actinomycetemcomitans* as metronidazole targets obligate anaerobes and ciprofloxacin targets facultative anaerobes. Studies of this drug combination

in the treatment of refractory periodontitis have documented marked clinical improvement (Rams *et al* 1992).

Systemic antibiotic therapy combined with mechanical therapy appears valuable in the treatment of recalcitrant periodontal infections and LAP infections involving *A. actinomycetemcomitans*. Antibiotic treatment should be reserved for specific subsets of periodontal patients who do not respond to conventional therapy. Selection of specific agents should be guided by the results of cultures and sensitivity tests for subgingival plaque microorganisms. Some of the more commonly prescribed antibiotics are presented in Table 2.

Relationship between oral health and general health

Although the major concern about periodontal disease is loss of teeth, evidence is also accumulating to suggest that the presence of periodontal disease does not just have negative implications for oral health, but can also adversely affect certain other, potentially fatal co-morbid systemic conditions.

More specifically, a number of studies are ongoing to determine whether there is a cause-and-effect relationship between periodontal

disease and:

- an increased risk of atherosclerosis, heart attack and stroke (Southerland 2006, Paquette 2007)
- an increased risk of preterm, low birth weight babies (Wimmer 2008)
- increased severity of diabetes (Mealy 2003)
- an increased risk for pneumonia in institutionalized subjects (Ragavendran 2007).

At the time of writing, data are emerging that periodontal disease is linked to a number of other systemic diseases. These include:

- osteoporosis (Reinhardt *et al* 1999)
- Alzheimer's disease (Kornman 2006, New York University 2006)
- rheumatoid arthritis (Mercado *et al* 2001)
- oral cancer (Tezel 2005)
- androgen deprivation therapy (Famili 2007).

Although it is generally accepted that these complex diseases have multi-faceted etiologies, the growing body of evidence linking these oral and systemic complications appears to have great merit.

References

Albandar JM, Kingman A. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 1999;70:30-43.

Allen DR, Davies R, Bradshaw B, *et al*. Efficacy of a mouthrinse containing 0.05% cetylpyridinium chloride for the control of plaque and gingivitis: a 6-month clinical study in adults. *Compend Cont Educ Dent* 1998;19(Supp2):20-26.

American Academy of Periodontology, Research, Science and Therapy Committee: Systemic antibiotics in periodontics. *J Periodontol* 2004;75:1553.

American Academy of Periodontology. Statement on local delivery of sustained or controlled release antimicrobials as adjunctive therapy in the treatment of periodontitis. *J Periodontol* 2006;77:1458.

American Dental Association. 2003 public opinion survey: Oral health of the US population. Chicago, American Dental Association. 2004.

Amornchat C, Kraivaphan P, Triratana T. Investigation of a triclosan/copolymer dentifrice on plaque viability and triclosan retention in dental plaque in a Thai population. *Mahidol Dent J* 2004:103-111.

Bakdash B. Current patterns of oral hygiene product use and practices. *Periodontol 2000* 1995;8:11-114.

Baker PJ, Evans RT, Slots J, *et al*. Susceptibility of human oral anaerobic bacteria to antibiotics suitable for topical use. *J Clin Periodontol* 1985;12:201-208.

Barnett ML. The rationale for the daily use of an antimicrobial mouthrinse. *J Am Dent Assoc* 2006;137:16S–21S.

Bauroth K, Charles CH, Mankodi SM, *et al*. The efficacy of an essential oil antiseptic mouthrinse vs. dental floss in controlling interproximal gingivitis: a comparative study JADA 2003;134:359-365 (published correction appears in JADA 2003;134:558).

Bauroth K, Goyal, Charles CH. Efficacy of FreshBurst mouthrinse and Listerine antiseptic compared to a hydroalcohol control in inhibiting the development of supragingival dental plaque and gingivitis. Morris Plains, NJK: Pfizer, 2004. Research report 6361003:1-25.

Bonito, A, Lus L, Lohr KN. Impact of local adjuncts to scaling and root planning in periodontal disease therapy: A systematic review. *J Periodontal* 2005;76:1227-1236.

Bueno L, Walker C, van Ness W, *et al*. Effect of augmentin on microbiota associated with refractory periodontitis. *J Dent Res* 1988;67:246.

Caton J, Bleiden T, Adams D, *et al*. Subantimicrobial doxycycline therapy for periodontitis. *J Dent Res* 1997;76:1307.

Caton JG, Ciancio SG, Blieden TM, *et al*. Treatment with subantimicrobial dose doxycycline improves the efcacy of scaling and root planing in patients with adult periodontitis, *J Periodontol* 2000;71:521-532.

Charles CH, Mostler KM, Bartela LL, Mankodi SM.

Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. *J Clin Periodontol* 2004;31:878-884.

Charles CH, Sharma NC, Galustians HJ, *et al*. Comparative efficacy of an antiseptic mouthrinse and an antiplaque/antigingivitis dentifrice: a six-month clinical trial. *JADA* 2001;132:670-675.

Christensen LB, Petersen PE, Krustrup U, Kjoller M. Self-reported oral hygiene practices among adults in Denmark. *Commun Dent Health* 2003;20:229-235.

Ciancio SG. Alcohol in mouthrinse: lack of association with cancer. *Biol Ther Dent* 1993;9:1-2.

Ciancio SG. Effect of Cepacol on gingivitis and supragingival plaque. Cincinnati: Merrell Dow Pharmaceuticals. Submitted to U.S. Food and Drug Administration Plaque Subcommittee; 1979:548-557.

Danser MM, Timmerman MF, Van Winklehoff AJ, Van Der Velden U. The effect of periodontal treatment on periodontal bacteria on the oral mucous membranes. *J Periodontol* 1996;67:478-485.

DePaola LG, Overholser CD, Meiller TF, Minah GE, Niehaus C. Chemotherpeutic inhibition of supragingival dental plaque and gingivitis development. *J Clin Periodontol* 1989;16:311-315.

Eger T, Zoller L, Muller HP, *et al*. Potential diagnostic value of sampling oral mucosal surfaces for *Actinobacillus actinomycetemcomitans* in young adults. *Eur J Oral Sci* 1996;104:112-117.

Ellwood RP, Worthington HV, Blinkhorn AS, *et al*. Effect of a triclosan/copolymer dentifrice on the incidence of periodontal attachment loss in adolescents. *J Clin Periodontol* 1998;25:363-367.

Famili P, Cauley JA, Greenspan SL. The effect of androgen deprivation therapy on periodontal disease in men with prostrate cancer. *J Urol* 2007;177:921-924.

Feldman R, Szeto B, Chauncey H, *et al*. Nonsteroidal anti-inflammatory drugs in the reduction of human alveolar bone loss, *J Clin Periodontol* 1983;10:131.

Feres M, Haffajee AD, Allard K, *et al*. Antibiotic resistance of subgingival species during and after periodontal therapy. *J Clin Periodontol* 2002;29:724-735.

Flemmig TF, Newman MG, Doherty FM, *et al*. Supragingival irrigation with 0.06% chlorhexidine in naturally occurring gingivitis, part I: six month clinical observations. *J Periodontol* 1990:61:112-117.

Furuichi Y, Rosling B, Volpe AR, Lindhe J. The effect of a triclosan/copolymer dentifrice on healing after no-nsurgical treatment of recurrent periodontitis. *J Clin Periodontol* 1999;26:63-66.

Giannobile WV, Somerman MJ. Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Ann Periodontol* 2003;8:193-204.

Gordon JM, Lamster IB, Seiger MC. Efficacy of Listerine antiseptic in inhibiting the development of plaque and gingivitis. *J Clin Periodontol* 1985;12:697-704.

Grossman E, Meckel AH, Issacs Rl, *et al*. A clinical comparison of antibacterial mouthrinses: Effects of chlorhexidine, phonolics, and sanguinarine on dental plaque and gingivitis. *J Periodontol* 1989:60:435-440.

Grossman E, Rijn G, Sturzenberger OP, *et al*. Six-month study of the effects of a chlorhexidine mouthrinse on gingivitis in adults. J Periodont Res 1986;(Supp):33-43.

Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *JADA* 2006;137:1649-1657.

Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann Periodontol* 2003;8:115-181.

Hanes PJ, Purvis JP. Local anti-infective therapy pharmacological agents. A systematic review. *Ann Periodontol* 2003;79-98.

Heasam PA, Benn DK, Kelly PJ, *et al*. The use of topical flurbiprofen as an adjunct to non-surgical management of periodontal disease, *J Clin Periodontol* 1993;20:457.

Hill M, Moore R. Locally acting oral chemotherapeutic agents. In: *Periodontics:*

Medicine, Surgery, and Implants. Rose LF, Mealey BL, Genco RJ, Cohen DW, ed. St. Louis, Elsevier. 2004; pp 277–286.

Hugosan A, Norderyd O, Slotte C, Thorstensson H. Oral hygiene and gingivitis in a Swedish adult population 1973, 1983 and 1993. *J Clin Periodontol* 1998;25:807–812.

Kaplan H, Edelson H, Korchak H, *et al.* Effects of nonsteroidal anti-inflammatory agents on neutrophil functions in vitro and in vivo. *Biochem Pharmacol* 1984;33:371.

Killoy WJ. Chemical treatment of periodontitis: Local delivery of antimicrobials. *In Dent J* 1998;48(Suppl 1):305-315.

Killory WJ, Poison AM, Controlled local delivery of antimicrobials in the treatment of periodontitis. *Dent Clin North Am* 1998;42;263-283.

Kolenbrander PE, Palmer RJ Jr, Rickard AH, *et al.* Bacterial interactions and successions during plaque development. *Periodontology 2000* 2006;42:47-49.

Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr* 2006;83;47S5S-483S.

Kumar LD. Comparative effectiveness of an essential oil mouthrinse and dental floss in controlling interproximal gingivitis and plaque. *Am J Dent* 2002;15:351-355.

Lamster IB. The effect of Listerine antiseptic on reduction of existing plaque and gingivitis. *Clin Prev Dent* 1983;5:12-16.

La Vecchia, C. Alcohol containing mouthwash and oral cancer risk: An update. *Oral Oncol* 2009;45:198-200.

Lee HM, Ciancio SG, Tuter G, *et al.* Subantimicrobial dose doxycycline efcacy as a matrix metalloproteinase inhibitor in chronic periodontitis is enhanced when combined with a non-steroidal anti-inflammatory drug, *J Periodontol* 2004;75:453.

Lemos-Junior CA and Villoria GEM. Reviewed evidence about the safety of alcohol based mouthrinses. *Braz Oral Res* 2008;22(Spec Iss 1):24-31.

Lewis MAO, Murray S. Safety of alcohol containing mouthwashes. A review of the evidence. *Dental Health* 2005;45.

Lindhe J, Hamp DS-E, Löe H. Plaque induced periodontal disease in beagle dogs. A 4-year clinical, roentgenographical and histometrical study. *J Peridont Res* 1975;10:243-255.

Lobene RR. Effect of Cepacol on gingivitis and supragingival plaque. Merrell Dow Pharmaceuticals. Submitted to U.S. Food and Drug Administration Plaque Subcommittee; 1977:519-527.

Löe H, Theilade E, Jensen SB, Experimental gingivitis in man. *J Periodontol* 1965:36:177-187.

Mankodi S, Bauroth K, Witt JJ, *et al.* A 6-month clinical trial to study the effects of a cetylpyridinium chloride mouthrinse on gingivitis and plaque. *Am J Dent* 2005;19(special number):9A-14A.

Mankodi S, Besselaar, Vincent JW, Hovliares CA. Comparative efficacies of Listerine minus methyl salcylate, Listerine antiseptic and a vehicle control in inhibiting the development of supragingival dental plaque and gingivitis (research report on file). Morris Plains, NJ: Warner-Lambert (Pfizer); 1993. Research report 931-0902, study number 931-939:1-30.

Mankodi S, Hovliaras CA, Kohut B. Efficacy of Cool Mint Listerine and Listerine antiseptic compared to a hydralcohol control in inhibiting the development of supragingival dental plaque and gingivitis (research report n file). Morris Plains, NJ: Warner-Lambert (Pfizer); 1991. Research report 931-0866, study number 931-838:1-20.

Mankodi S, Ross NM, Charles CH. Efficacy of Listerine and Listerine Plus Mint in inhibiting the development of dental plaque and gingivitis (research report on file). Morris Plains, NJ: Warner-Lambert (Pfizer); 1989. Research report 931-0780.

McCullough MJ, Farah CS. The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouthwashes. *Aust Dent J* 2008;53:302-305.

Mealy DL, Rethman MP. Peridontal disease and diabetes mellitus. Bidirectional relationship. *Dent Today* 2003;22:107-113.

Menaker L, Ross NM. Efficacy of Listerine

antiseptic and control for inhibiting the development of dental plaque and gingivitis (research report on file). Morris Plains, NJ: Warner Lambert (Pfizer):1981. Research report 9310135, study number 931-014:1-17.

Menaker L, Ross NM, Olshan AM. Efficacy of Listerine antiseptic compared to Scope and a hydroalcohol control (research report on file). Morris Plains, NJ: Warner-Lambert (Pfizer): 1986. Research report 931-0649, study number 931-453:1-15.

Mercado FB, Marshall RI, Klestov AC, *et al.* Relationship between rheumatoid arthritis and periodontitis. *J Periodontol* 2001;72;779-787.

Mombelli A, Feloutzis A, Brägger U, Land NP. Treatment of peri-implantitis by local delivery of tetracycline. *Clinical Oral Implants Research* 2001;12:287-294.

Morris AJ, Steele J, White DA. The oral cleanliness and periodontal health of UK adults in 1998. *Br Dent J* 2001;191:186-192.

Mosby's GenRx 10th Ed. Mosby, St Louis. 2001.

Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A Systematic review. *Ann Periodontol* 2003;8:266-302.

New York University, NYU Dental College, Medical School Partner to study Possible Periodontal Disease/Alzheimer's Link. September 2006. http://www.nyu.edu/public.affairs/releases/detail/1192. Accessed on September 21, 2007.

Offenbacher S, Braswell L, Loos A, *et al.* Effects of flurbiprophen on the progression of periodontitis in Macaca mulatta. *J Periodontal Res* 1987;22:473-481.

Overholser CD, Charles CH, Vincent JW. Efficacy of Listerine minus methyl salicylate and Listerine antiseptic compared to a vehicle control in inhibiting the development of supragingival dental plaque and gingivitis (research report on file). Morris Plains, NJ:Warner-Lambert (Pfizer); 1992. Research report 931-0899, study number 931-938;1-26.

Overholser CD, Meiller TF, DePaola LG, Minah GE, Niehaus C. Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. *J Clin Periodontol* 1990:17:575-579.

Pan P, Barnett ML, Coelho J, Brogdon C, Finnegan MB, Determination of the in situ bactericidal activity of an essential oil mouthrinse using a vital stain method. *J Clin Periodontol* 2000;27:256-261.

Paquette DW, Brodala N, Nichols TC. Cardiovascular disease, inflammation, and periodontal infection. *Periodontology 2000* 2007;44:113-126.

Ragavendran K, Mylotte JM, Scannapieco FA. Nursing home associated pneumonia, hospital acquired pneumonia and ventilator associated pneumonia: The contribution of dental biofilms and periodontal inflammation. *Periodontology 2000* 2007;44:164-177.

Rams TE, Slots J. Antibiotics in periodontal therapy: An update. *Compend Contin Edu Dent* 1992;13:1130.

Rams TE, Feik D, Slots J. Ciprofloxacin/metronidazole treatment of recurrent adult periodontitis (abstract). *J Dent Res* 1992;71:319.

Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann Periodonitol* 2003;8:12-34.

Reinhardt RA, Payne JB, Maze CA, *et al.* Influence of estrogen and osteopenia/osteoporosis on clinical periodontitis in postmenopausal women. *J Periodontol* 1999;70:923-828.

Renvert S, Lessem J, Dahlén G, Lindahl C, Svensson M. Topical minocycline microspheres versus topical chlorhexidine gel as an adjunct to mechanical debridement of incipient peri-implant infections: A randomized clinical trial. *J Clin Periodontol* 2006;33:362-369.

Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Ann Periodontol* 2003;8:227-265.

Rosling B, Dahlén G, Volpe A, *et al.* Effect of triclosan on the subgingival microbiota of periodontitis-susceptible subjects. *J Clin Periodontol* 1997 Dec;24:881-887.

Rosling B, Wannfors B, Volpe AR, *et al.* The use of a triclosan/copolymer dentifrice may retard the

progression of periodontitis. *J Clin Periodontol* 1997;24:873-80.

Salvi GE, Lawrence HP, Offenbacher S, Beck JD. Influence of risk factors on the pathogenesis of periodontitis. *Periodontol 2000* 1998;14:173-201.

Salvi GE, Mombelli A, Mayfield L, *et al*. Local antimicrobial therapy after initial periodontal treatment. *J Clin Periodontol*. 2002;29:540-550.

Saxe SR, Greene JC, Bohannan HM, Vermillion JR. Oral debris, calculus, and periodontal disease in the beagle dog. *Periodontics* 1967;5:217:225.

Segelnick SL. A survey of floss frequency, habit and technique in a hospital dental clinic and private periodontal practice. *N Y State Dent J* 2004;70:28-33.

Segreto V, Collins E. A clinical investigation to assess the effects on plaque, gingivitis, and staining potential of an experimental mouthrinse (research report on file). Cincinnati: Procter & Gamble; 1993. Study number 002392:1-27.

Sharma N, Charles CH, Lynch MC, *et al*. Adjunctive benefit of an essential oil-containing mouthrinse in reducing plaque and gingivitis in patients who brush and floss regularly:a six-month study. *JADA* 204;135:496-504.

Sharma N, Charles CH, Vincent JW. Efficacy of a reduced alcohol Listerine mouthrinse in inhibiting the development of supragingival dental plaque and gingivitis when used as an adjunct to usual oral hygiene for 6 months (research report on file). Morris Plains, NJ: Warner-Lambert (Pfizer): 1997. Research report 931-1054, study number 931-1176:1-14.

Silverman S Jr, Wilder R. Antimicrobial mouthrinse as a part of a comprehensive oral care regimen: Safety and compliance factors. *J Am Dent Assoc* 2006;137:22S-26S.

Slavkin HC, Bartold PM. Challenges and potential in tissue engineering. *Periodontology 2000* 2006:41:9-15.

Slots J, Rams TE. Antibiotics and periodontal therapy: Advantages and disadvantages. *J Clin Periodontol* 1990;17:479-493.

Slots J, Rosling BG. Suppression of periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. *J Clin Periodontol* 1983;10:465-486.

Slots J, McDonald ES, Nowzari H. Infectious aspects of periodontal regeneration. *Periodontol 2000* 1999;19:164-172.

Socransky SS, Haffajee AD. Dental biofilms:difficult therapeutic targets. *Periodontology 2000* 2002;28:12-55.

Somer H, Asikainen S. Source of suspected periodontal pathogens re-emerging after periodontal treatment. *J Clin Periodontol* 1996;23:601-607.

Southerland JH, Taylor GW, Moss K, *et al*. Commonality in chronic inflammatory diseases: Periodontitis, diabetes and coronary artery disease. *Periodontology 2000* 2006;40:130-143.

Stookey GK, Beiswanger B, Mau M, *et al*. A 6-month clinical study assessing the safety and efficacy of two cetylpyridinium chloride mouthrinses. *Am J Dent* 2005;18:24A-28A.

Tanner AC, Paster BJ, Lu SC, *et al*. Subgingival and tongue microbiota during early periodontitis. *J Dent Res* 2006;85:318–323.

Tezal M, Gross SG, Genco RJ. Is periodontitis associated with oral neoplasms? *J Periodontol* 2005;76:406-410.

Theilade E, Wright WH, Jensen SB, Löe H. Experimental gingivitis in man. II longitudinal clinical and bacteriological investigation. *J Periodont Res* 1966;1:1-13.

Tinoco EM, Beldi M, Campedelli F, *et al*. Clinical and microbiologic effects of adjunctive antibiotics in treatment of localized aggressive periodontitis: A controlled clinical study. *J Periodontol* 1998;69:1355.

Vogel R, Schneider L, Goteinter D. The effects of a topical nonsteroidal anti-inflammatory drug on ligature induced periodontal disease in the squirrel monkey. *J Clin Periodontol* 1986;12:139.

Waite I, Saxon C, Young A, *et al*. The periodontal status of subjects receiving nonsteroidal anti-inflammatory drugs. *J Periodontal Res* 1981;16:100.

Walker CB, Gordon JM, Magnusson I, *et al*. A role for antibiotics in the treatment of refractory periodontitis. J Periodontol 1993;64:772.

Walker CB, Gordon JM, McQuilkin SJ, *et al*. Tetracycline: Levels achievable in gingival

crevice fluid and in vitro effect on subgingival organisms. Part II. Susceptibilities of periodontal bacteria. *J Periodontol* 1981;52:613.

Walsh LJ. Are alcohol containing dental mouthrinses safe? A critical look at the evidence. *Australian Dental Practice* 2008;64-68.

Williams RC, Jeffcoat MK, Howell T, *et al.* Altering the progression of human alveolar bone loss with the non-steroidal anti-inflammatory drug flurbiprofen. *J Periodontol* 1989;60:485.

Williams RC, Jeffcoat MK, Howell T, *et al.* Ibuprofen: An inhibitor of alveolar bone resorption in beagles. *J Periodontal Res* 1988;23:225.

Williams RC, Jeffcoat MK, Howell T, *et al.* Indomethacin or flurbiprofen treatment of periodontitis in beagles: comparison of effect on bone loss. *J Periodontal Res* 1987;22:403.

Williams RC, Jeffcoat MK, Howell T, *et al.* Three year trial of flurbiprofen treatment in humans: post-treatment period (abstract). *J Dent Res* 1991;70:448.

Williams RC, Jeffcoat MK, Wechter WJ, *et al.* Flurbiprofen: A potent inhibitor of alveolar bone resorption in beagles. *Science* 1985;27:640.

Wimmer G, Pihlstrom BL. A critical assessment of adverse pregnancy outcome and periodontal disease. *J Clin Periodontol* 2008;35(Suppl 8):380-397.

Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. *J Clin Periodontol* 2000;27:722–732.

Chapter 10

Periodontal regeneration based on cell sheet engineering: The future of periodontal therapy

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Introduction

A major goal in the treatment of periodontal defects is the simultaneous regeneration of cementum, periodontal ligament and alveolar bone structures in the face of the microbial assault and altered host immune response. Current periodontal therapies, such as tissue-banked bone allografts, cell-occlusive barriers, or enamel matrix proteins, have contributed to the development of periodontal regeneration. However, complete regeneration using these procedures is still limited. Recently, cell-based therapies have been developed as a foundation for regenerative medicine. General approaches for cell delivery have involved direct application of single cell suspensions into the target tissues. Tissue engineering with the purpose of seeding cells into biodegradable scaffolds has also evolved as a method for the reconstruction of various tissues and organs. With success in clinical trials, regenerative therapies using these approaches have therefore garnered significant interest and attention. As a novel alternative, we have utilized nanotechnology for regenerative medicine to develop temperature-responsive culture dishes, on which a temperature-responsive polymer is covalently grafted with precise control of density and thickness in a nanometer scale. Confluent cells cultured on the dishes are harvested as a single contiguous cell sheet by reducing the temperature below 32ºC and harvested cell sheets used for tissue reconstruction in a process called cell sheet engineering. Using this approach, cell sheets can be directly transplanted into host tissues without the use of scaffold or carrier materials, or can be utilized to create *in vitro* tissue constructs via the layering of individual cell sheets. In addition to simple transplantation, cell sheet engineered constructs have been applied in alternative therapies such as endoscopic transplantation, combinatorial tissue reconstruction and polysurgery to overcome the limitations of regenerative therapies and cell delivery using conventional approaches. In this manuscript, we introduce our novel tissue regeneration method based on cell sheet engineering and overview available ongoing cell therapies.

Development of cell sheet engineering

The tissue engineering concept proposed by Langer and Vacanti (2003), is the use of a combination of cells, signaling molecules and scaffolds to improve or replace biological function. In addition, recent studies have indicated that neoangiogenesis is also needed to supply the nutrition required to maintain
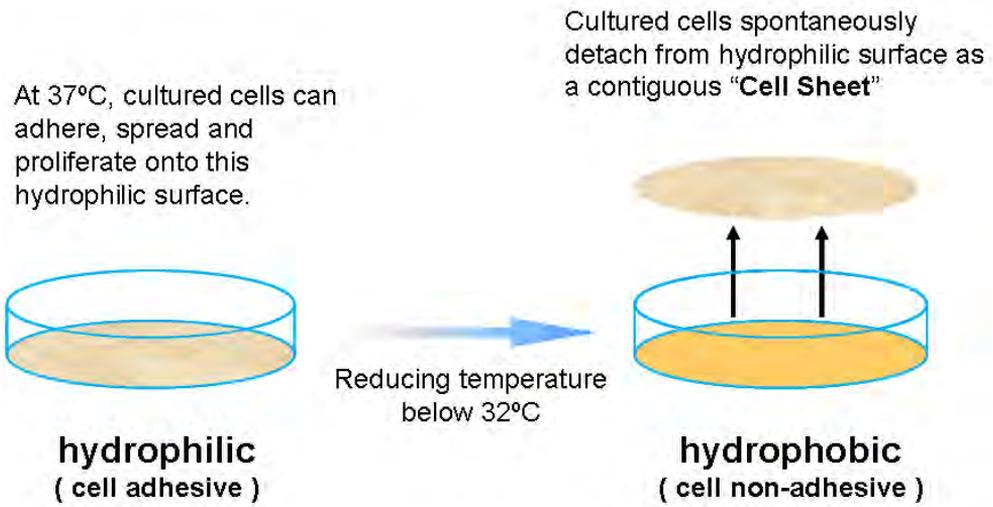
At 37ºC, cultured cells can adhere, spread and proliferate onto this hydrophilic surface.

Cultured cells spontaneously detach from hydrophilic surface as a contiguous "**Cell Sheet**"

Reducing temperature below 32ºC

hydrophilic
(cell adhesive)

hydrophobic
(cell non-adhesive)

Figure 1. The mechanism of cell sheet harvesting using a temperature responsive dish

the transplanted cells, especially for mesenchymal tissues (Shimizu 2001). Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell and developmental biology, as well as materials science and bioinformatics. Most tissue engineering experiments have been focused on creating target tissues in a laboratory by culturing and proliferating cells together with scaffold material before their transplantation into the body. Generally cultured cells were detached from the substratum by treatment with proteolytic enzymes such as trypsin and dispase. It has been noted however that detachment with the enzymes induced the degradation of deposited extracellular matrix and cell junctions. The enzymatic treatment inflicts damage to cell membranes by hydrolyzing various membrane-associated proteins, resulting in impairment of cell function. New techniques to detach the cultured cells without using of enzymes were required. Okano *et al* (1990) developed a new method to control cell surface adhesion by exploiting cell culture temperature and the use of a surface-grafted temperature-responsive polymer *Poly N-isopropylacylamide* (PIPAAm). PIPAAm is fully hydrated with an extended chain configuration below 32ºC and is extensively dehydrated and compacted above 32ºC (Figure 1). A characteristic of the cells is that they adhere onto hydrophobic surfaces but not onto highly hydrated hydrophilic surfaces. By applying this characteristic to the PIPAAm, temperature-responsive culture dishes were developed by grafting PIPAAm onto tissue culture grade polystyrene dishes using irradiation with an electron beam (Yamada *et al* 1990). These dishes made it possible to harvest cultured cells through temperature change, without using enzymes. These sheets showed intact surfaces and maintained normal cell junctions (Kwon *et al* 2000). These temperature-responsive dishes allowed cells to maintain substrate adhesiveness, growth and secretion activities although the culture temperature was changed. Thus, cell sheet

engineering by use of thermo-responsive dishes provides a novel strategy to produce tissues without a specified scaffold.

The obtained cell sheets hold their original extracellular matrix and cell-cell contact due to non-usage of proteolytic enzymes. The cell sheets can be multilayered to strengthen the sheets and to intensify the number of cells. The cell sheets can be manipulated easily with tweezers, thus transplantable to the body without special equipment. Transplanted cell sheets can be placed and retained on recipient tissues without suturing. These findings indicate that cell sheet engineering may be useful for to the regeneration of tissues and organs. These temperature responsive dishes are now commercially available under the name of UpCell ™ (CellSeed Inc, Tokyo, Japan).

Regeneration of various tissues with cell sheet engineering

The use of temperature-responsive culture systems created new strategies to manufacture transplantable sheets composed only of living cells and this procedure is well suited for the regeneration of a wide variety of tissues and organs. Numerous cell types including epidermal keratinocytes, vascular endothelial cells, renal epithelial cells and cardiomyocytes can be created as cell sheets according to this method and the cell sheets maintain differentiated functions.

Nishida *et al* (2004) developed a method for transplanting autologous oral mucosal epithelial cell sheets onto a denuded corneal surface without sutures. Corneal transparency was restored and postoperative visual acuity improved remarkably.

With the recent development of endoscopic submucosal dissection (ESD), a wide range early esophageal cancers limited to the mucosal layer could be removed, but postoperative inflammation and stenosis were

often induced. Ohki *et al* (2006) developed a method combining ESD and the endoscopic transplantation of tissue-engineered cell sheets produced by using autologous oral mucosal epithelial cells in a dog model. The transplanted cell sheets effectively enhanced wound healing and possibly prevented postoperative oesophageal stenosis compared with control sites treated only by ESD.

Shimizu *et al* (2001) produced cardiac myocyte sheets without scaffolds. The cell sheets retained cell morphology and pulsation and the cardiac myocyte sheets could create 3-dimensional myocardial tissues by layering the cell sheets without any scaffolds. The tissues retained the function of electrically communicative pulsation (Shimizu *et al* 2002). It was also reported that cell sheets transplanted onto damaged hearts improved heart function in several animal models (Hata *et al* 2006, Kondoh *et al* 2006, Shimizu *et al* 2006).

Ohashi *et al* (2007) developed a method to transplant uniformly continuous hepatocytes sheets constructed by primary hepatocytes into a subcutaneous space in a mouse model. The engrafted hepatic tissues connected to the surrounding cells and regenerated two-dimensional hepatic tissues with several liver-specific functionalities. Furthermore, three-dimensional miniature liver systems were developed by layering the hepatic tissue sheets in vivo. The engineered livers were stable and survived well.

Periodontal application of cell sheet engineering

We investigated the application of cell sheet engineering for periodontal therapy in collaboration with Professor Okano's laboratory and showed that periodontal ligament cells can be harvested from temperature-responsive culture dishes as a contiguous sheet at low-temperature (Figure
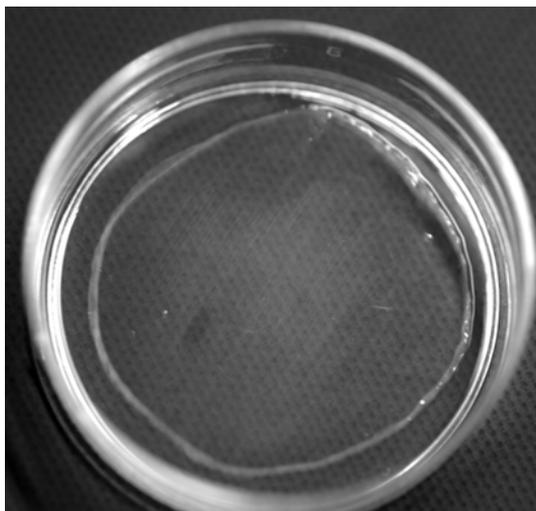
Figure 2. Transplantable human periodontal ligament cell sheet

2).

Hasegawa *et al* (2005) investigated the characteristics of human periodontal ligament cell sheets obtained from temperature-responsive culture dishes and then examined whether these cell sheets could regenerate periodontal tissues. The human periodontal ligament cell sheets were transplanted into a mesial dehiscence model in immunodeficient rats. The periodontal ligament cells were recovered from the culture dishes as a contiguous sheet accompanied with abundant extracellular matrix including type 1 collagen, integrin and fibronectin. Histology of the transplanted cell sheet of human periodontal ligament indicated uneventful healing during the experimental period. Newly formed immature fibers were obliquely anchored to dentin surfaces in the experiment site. Fibre formation was not observed in the control site at 4 weeks (Figure 3). These results suggest that this approach based on the cell sheet engineering can be useful for periodontal regeneration.

Akizuki *et al* (2005) investigated periodontal healing after application of periodontal ligament cell sheet in beagle dogs. Following primary culture the periodontal

ligament cell sheet was prepared using temperature responsive cell culture dishes. When cells reached confluence the medium was supplemented with 50µg/ml of ascorbic acid to increase production of type 1 collagen to strengthen the cell sheet. The PDL cells were then incubated for another 2 weeks. At the time of transplantation, hyaluronic acid sheets (Seikagaku Kogyo, Tokyo, Japan) were placed on the culture dishes as a reinforced carrier. Defects of 5 mm x 5 mm were surgically prepared on the buccal root surfaces of mandibular first molars. Combined cell sheets were transplanted to the deficient defect. Periodontal tissue healing with bone, cementum and periodontal ligament formation was found in 60% of experimental defects. In most of the experimental defects, collagen fibers were inserted into the newly formed bone and cementum (Figure 4). In the control group (without periodontal ligament cell sheet) neither bone nor cementum was found in the defects. Histomorphometric analysis indicated that the formation of new cementum in the experimental group was significantly higher than that in the control group.

Flores *et al* (2008) explored the possibility of cementum-periodontal ligament complex
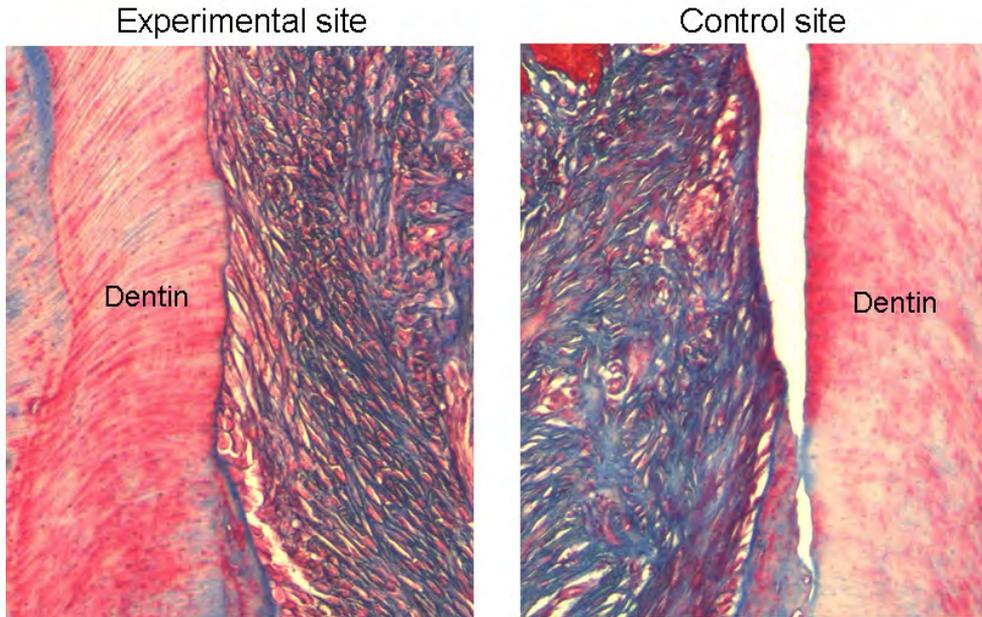
Figure 3. Histological sections (Azan staining) at 4weeks post-transplantation of human periodontal ligament cell sheets to athymic rats
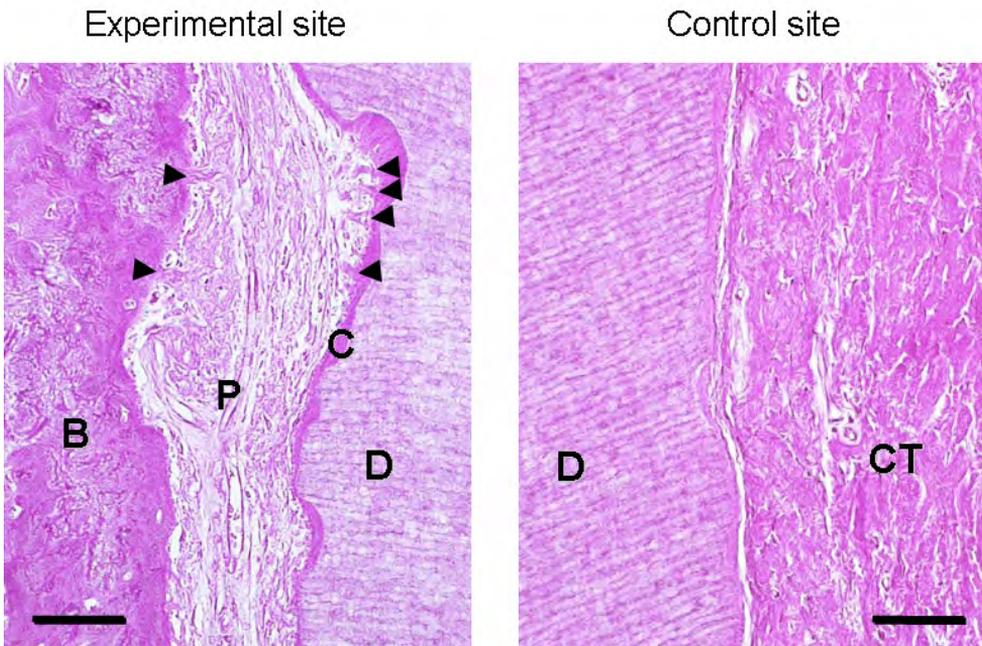

Figure 4. Periodontal regeneration at 8 weeks post-transplantation using autologous periodontal ligament cell sheets in Dogs (HE staining)
B: New bone, **P:** New PDL, **C:** New cementum, **D:** Dentin, **CT:** Connective tissue, bar: 50ìm. Arrow heads show areas that collagen fibers were inserted perpendicularly into cementum-like tissue.
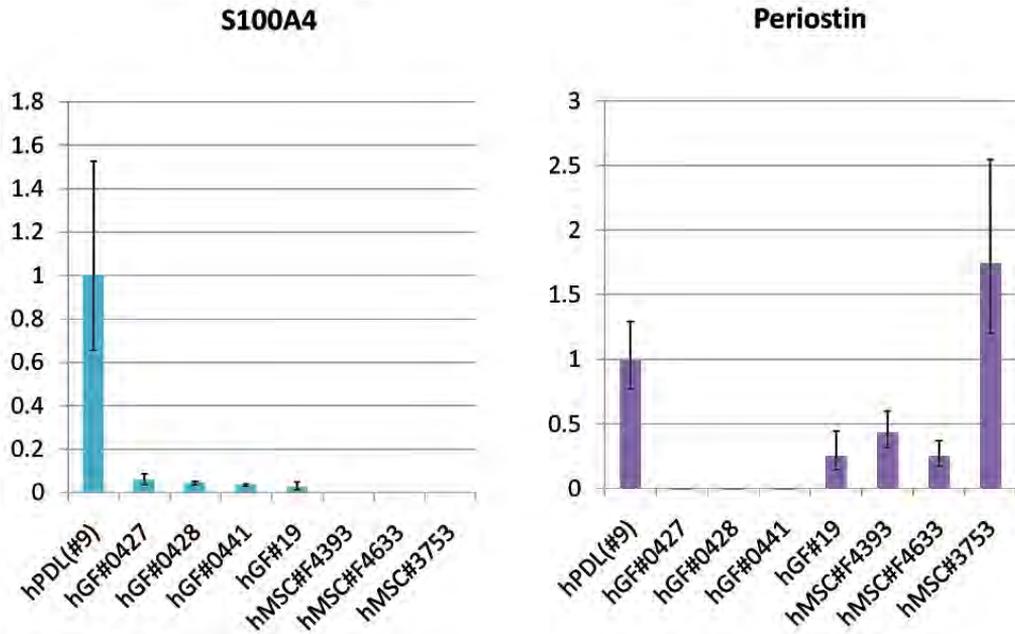
S100A4

Periostin

Figure 5. Expression levels of periodontal maker genes (S100A4 and periostin) in human periodontal ligament, human gingival fibroblasts, and human mesenchymal stem cells

regeneration by improving two processes of the periodontal cell sheet engineering. First, the experimental group was cultured in supplemented minimum essential medium with 50 µg/ml of ascorbic acid, 10 nM dexamethasone and 10 nM β-glycerophosphate as osteogenic differentiation medium. Dexamethasone has been shown to induce osteoblast differentiation of human bone marrow cells (Cheng *et al* 1994).

Nagatomo *et al* (2006) reported that about 30% of periodontal ligament cells showed calcifying differentiation in osteogenic differentiation medium. Secondly, the cell sheets were multilayered using fibrin gel. Multilayered sheet constructs were then placed onto the dentin blocks and transplanted subcutaneously in the dorsa of immunodeficient rats. The multilayered constructs were left in situ for 6 weeks and were then excised for histological investigation. Human periodontal ligament cells cultured with an osteodifferentiation

medium showed a marked increase of alkaline phosphatase and calcium deposition at 3 weeks. Newly regenerated cementum-like hard tissue on the dentin surfaces were observed in more than 60% of histological section samples of the periodontal ligament cell-dentin block constructs. Many collagen fibers were inserted perpendicularly into the newly formed cementum-like tissue which closely resembled native periodontal and Sharpey's fibers.

Flores *et al* (2008) transplanted the cell sheet onto the root surface of a defect created in the mandible of an immunodeficient rat prepared following King's method (King *et al* 1997, King *et al* 2001). This method makes it possible to observe the regenerated cementum and periodontal ligament. Results indicated that the majority of the experimental group exhibited a new layer of cementum and new attachment of periodontal ligament fibers to the layer. The control group cultured without osteodifferentiation medium revealed

no clear cementum layer. Thus, periodontal ligament cells cultured in osteogenic differentiation medium could regenerate both cementum and periodontal ligament. It was recently reported that periodontal ligament cells cultured with IL-11 instead of dexamethasone enhanced alkaline phosphatase activity (Leon *et al* 2007). This may be a possible novel method to regenerate the periodontium.

Iwata *et al* (2009) performed periodontal regeneration with multi-layered periodontal ligament cell sheets in a canine model. Periodontal marker genes were found in the cultured cells and S-100 calcium binding protein A and periostin were identified as the markers (Figure 5). Three layered periodontal ligament cell sheets supported with woven polyglycolic acid were transplanted onto root surfaces with 3 wall bony defects. Bone defects were also filled with porous ß-tricalcium phosphate. The cell sheet transplantation experimental sites exhibited regeneration of both new bone and cementum together with periodontal ligament fibers while control sites showed only limited regeneration. Thus, it has been shown that periodontal ligament cells have multi-differentiation properties to regenerate all the components of the periodontium.

Conclusion

The aim of our studies has been to evaluate cementum and periodontal regeneration using periodontal cell sheet engineering without scaffolds. We used periodontal ligament cells from extracted teeth, which retain healthy periodontal ligament tissue, as the cell source. We consider that these cells are important cell sources for regenerative medicine. To date periodontal ligament cells and dental pulp cells have been examined for their stem cell properties in dog and pig models and were found to be effective and convenient cell sources in the regenerative studies. Additionally, previous reports indicated that these periodontal ligament cells had the ability to express osteoblast-like phenotypes, such as a capacity to form mineralized nodules and expression of several bone-associated proteins when the periodontal ligament cells were cultured in the presence of dexamethasone, ß-glycerophosphate and ascorbic acid. In our experiments, we have confirmed that transplanted periodontal ligament cells when cultured with osteogenic differentiation medium induce cementum-like tissue and periodontal ligament together with Sharpey's fibers (Flores *et al* 2008a, Flores *et al* 2008b, Iwata *et al* 1009).

We also investigated distinguishable marker genes for human periodontal ligament cells which have been reported by Han *et al* (2002), Fujita *et al* (2007) and Pi *et al* (2007). We examined five genes among known periodontal ligament cell markers using available PCR primers. We showed that both S100A4 and periostin were consistently expressed in the examined periodontal ligament cells.

Immunohistochemical studies also confirmed that both S100A4 and periostin are specific signals in human periodontal ligament cells indicating these two molecules are useful markers to distinguish them from gingival fibroblast and other cells (Duarte *et al* 1998, Horiuchi *et al* 1999, Iwata *et al* 2009). Clinical applications of the periodontal ligament cell sheet engineering using cell sources from human wisdom teeth and application of the cell sheet in periodontal defects in the same patients with periodontal disease has been approved. We are now preparing the cell sheet at an authorized cell-processing center. These processes should meet the government guidelines of stem cell application and will contribute to predictable development of periodontal regeneration.

References

Akizuki T, Oda S, Komaki M, *et al*. Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. *J Periodontal Res* 2005;40:245-251.

Cheng SL, Yang JW, Rifas L, *et al*. Differentiation of human bone marrow osteogenic stromal cells in vitro: induction of the osteoblast phenotype by dexamethasone. *Endocrinology* 1994;134:277-286.

Duarte WR, Kasugai S, Iimura T, *et al*. cDNA cloning of S100 calcium-binding proteins from bovine periodontal ligament and their expression in oral tissues. *J Dent Res* 1998;77:1694-1699.

Flores MG, Hasegawa M, Yamato M, *et al*. Cementum-periodontal ligament complex regeneration using the cell sheet technique. *J Periodontal Res* 2008;43:364-371.

Flores MG, Yashiro R, Washio K, *et al*. Periodontal ligament cell sheet promotes periodontal regeneration in athymic rats. *J Clin Periodontol* 2008;35:1066-1072.

Fujita T, Iwata T, Shiba H, *et al*. Identification of marker genes distinguishing human periodontal ligament cells from human mesenchymal stem cells and human gingival fibroblasts. *J Periodontal Res* 2007;42:283-286.

Han X, Amar S. Identification of genes differentially expressed in cultured human periodontal ligament fibroblasts vs. human gingival fibroblasts by DNA microarray analysis. *J Dent Res* 2002;81:399-405.

Hasegawa M, Yamato M, Kikuchi A, *et al*. Human periodontal ligament cell sheets can regenerate periodontal ligament tissue in an athymic rat model. *Tissue Eng* 2005;11:469-478.

Hata H, Matsumiya G, Miyagawa S, *et al*. Grafted skeletal myoblast sheets attenuate myocardial remodeling in pacing-induced canine heart failure model. *J Thorac Cardiovasc Surg* 2006;132:918-924.

Horiuchi K, Amizuka N, Takeshita S, *et al*. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res* 1999;14:1239-1249.

Iwata T, Yamato M, Tsuchioka H, *et al*. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials* 2009: 30: 2716-2723.

King GN, Hughes FJ. Bone morphogenetic protein-2 stimulates cell recruitment and cementogenesis during early wound healing. *J Clin Periodontol* 2001;28:465-475.

King GN, King N, Cruchley AT, *et al*. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. *J Dent Res* 1997;76:1460-1470.

Kondoh H, Sawa Y, Miyagawa S, *et al*. Longer preservation of cardiac performance by sheet-shaped myoblast implantation in dilated cardiomyopathic hamsters. *Cardiovasc Res* 2006;69:466-475.

Kwon OH, Kikuchi A, Yamato M, *et al*. Rapid cell sheet detachment from poly(N-isopropylacrylamide)-grafted porous cell culture membranes. *J Biomed Mater Res* 2000;50:82-89.

Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920-926.

Leon ER, Iwasaki K, Komaki M, *et al*. Osteogenic effect of interleukin-11 and synergism with ascorbic acid in human periodontal ligament cells. *J Periodontal Res* 2007;42:527-535.

Nagatomo K, Komaki M, Sekiya I, *et al*. Stem cell properties of human periodontal ligament cells. *J Periodontal Res* 2006;41:303-310.

Nishida K, Yamato M, Hayashida Y, *et al*. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 2004;351:1187-1196.

Ohashi K, Yokoyama T, Yamato M, *et al*. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. *Nat Med* 2007;13:880-885.

Ohki T, Yamato M, Murakami D, *et al*. Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. *Gut* 2006;55:1704-1710.

Okano T, Bae YH, Jacobs H, Kim SW. Thermally on-off switching polymers for drug permeation and release. *J Controlled Release* 1990;11:255-

265.

Pi SH, Lee SK, Hwang YS, *et al*. Differential expression of periodontal ligament-specific markers and osteogenic differentiation in human papilloma virus 16-immortalized human gingival fibroblasts and periodontal ligament cells. *J Periodontal Res* 2007;42:104-113.

Shimizu T, Sekine H, Isoi Y, *et al*. Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. *Tissue Eng* 2006;12:499-507.

Shimizu T, Yamato M, Isoi Y, *et al*. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res* 2002;90:e40.

Shimizu T, Yamato M, Kikuchi A, Okano T. Two-dimensional manipulation of cardiac myocyte sheets utilizing temperature-responsive culture dishes augments the pulsatile amplitude. *Tissue Eng* 2001;7:141-151.

Yamada N, Okano T, Sakai H, *et al*. Thermo-responsive polymeric surfaces; control of attachment and detachment of cultured cells. *Makromol Chem Rapid Commun* 1990;11:571-576.

Chapter 11

How do patient data affect treatment planning and prognosis?

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Introduction

A clinician needs to make treatment decisions based on patient data. The following basic steps need to be performed as a routine when presented with a new patient: The patient first needs to be screened. A diagnosis needs to be made and a treatment plan is then laid out. Treatment mostly involves initial therapy, re-evaluation, and any other subsequent corrective therapy like regeneration. A risk assessment is then done on the treated patient and the patient is placed on maintenance therapy tailored to fit the patient's risk profile. However, what do we consider quality management of the patient? State of the science treatment planning involves well-defined criteria and parameters to define treatment outcomes.

Treatment goals

In every patient diagnosed with periodontitis, a treatment strategy must define the clinical outcome parameters to be reached through therapy.
Optimal clinical parameters include:
- no probing depth >4 mm
- minimal bleeding on probing (<10%)
- no visible hard or soft deposits
- aesthetically satisfactory conditions of all periodontal sites
- no pain
- individually optimal function.

Satisfactory clinical outcomes include:
- no residual pockets >5 mm
- no suppuration
- occasional bleeding on probing ($\leq$25%)
- low plaque accumulation ($\leq$30%)
- minor impairment of aesthetics
- no pain
- satisfactory function.

Initially these treatment goals were defined by a panel of experts, but a recent retrospective 11 year study by Matuliene *et al* (2008) had the following conclusions.
- residual PPD $\geq$6 mm after active periodontal therapy represented a risk factor for both progression of periodontitis and tooth loss during the SPT on a patient, tooth and site level.
- multiple sites ($\geq$9) with residual PPD $\geq$5 mm represented a risk factor for further progression of periodontitis on a patient level.
- bleeding on probing (BOP) on a site and tooth level increased the probability of tooth loss with OR 2.0 and 1.9, respectively.
- on a patient level, FMBS $\geq$30% represented a strong risk factor for tooth loss.

This study demonstrates the increasing evidence for dentists to strive to achieve the treatment goals outlined above.

Screening

A patient seeking periodontal care is first screened for periodontal conditions to facilitate treatment planning. Screening is carried out using a Basic Periodontal Examination (BPE). A thin periodontal probe is used to probe at least 2 sites per tooth using a light force. Each dentate sextant is given a BPE score, whereby the highest individual site score is used.

BPE system code:

0: Periodontal heath
1: Bleeding on probing (BOP), gingivitis
2: BOP, supra-and/or subgingival calculus, ill fitting restoration, gingivitis
3: Probing pocket depth (PPD) >3 mm - ≤5 mm, gingivitis, mild periodontitis
4: PPD ≥6 mm, periodontitis
*: Furcation involvement, tooth mobility >2, mucogingival problems, esthetically disturbing buccal recessions

Patients with sextants given codes 0,1 or 2 belong to the relatively periodontally healthy category. Prophylaxis and maintenance care is carried out for these patients. A patient exhibiting a sextant with codes of 3 or 4 must undergo a more comprehensive periodontal examination while patients with code * should be referred to a specialist for treatment.

Diagnosis and treatment plan

The basis for treatment planning is established by the clinical data collected from the patient's examination. Clinical data that need to be collected include recession, probing depths, bleeding on probing, furcation involvement, mobility, and radiographs. This will enable us to arrive at a diagnosis and the possible need for referral to a specialist as part of the treatment plan.

Initial therapy and re-evaluation

At this stage in the management of the patient, it is almost impossible to make definite decisions regarding the treatment sequence as the degree of success of initial therapy and the patient's "subjective" need for additional therapy is unknown. As a rule of thumb though, "teeth irrational to treat" are first extracted and the patient undergoes motivational oral hygiene instructions where they are taught good oral home care. The mouth is then disinfected with thorough scaling and root planing where the biofilm is disrupted and calculus removed. The patient is then re evaluated 1 to 2 months later where clinical data such as the plaque index, probing depths, bleeding on probing and furcation involvement is collected to make a treatment decision on the next treatment strategy.

Additional treatment and re-evaluation

If the treatment goals of the initial therapy outlined above are met, a periodontal risk assessment is done and the patient is placed on a maintenance program. If the treatment goals are not met, a referral to a specialist at this point might be necessary and a microbiological and systemic check performed. Local antibiotic therapy, systemic antibiotic therapy, periodontal surgery and furcation therapy are some of the additional therapeutic procedures needed.

As a basic rule, if the patient still has poor oral hygiene, periodontal surgery is not recommended. The patient will have to undergo additional rounds of motivation and instruction to improve on the oral hygiene.

If the goals of the hygienic phase are met but probing depths of ≥6 mm still remain, the specialist might indicate additional surgical corrective therapy such as regenerative

therapy. Regenerative therapy shows increasing predictability if it is a 3 wall, narrow and deep defect. A meta analysis showed greatest clinical attachment level gain with guided tissue regeneration, followed by Emdogain®, demineralized freeze dried bone allograft then access flap surgery (Cortellini and Tonetti 2000). This study also concluded that a good treatment outcome with respect to clinical attachment level is expected for a site with a narrow, angular defect with an osseous component of at least 3 mm and a periodontal probing depth of at least 6 mm, in a non-smoker with a high standard of oral hygiene.

Re-evaluation after the corrective phase again involves collecting patient data such as probing depth, bleeding on probing, furcation involvement, and the plaque index.

Risk assessment and maintenance care

The results of the re-evaluation form the basis for the assessment of the residual periodontal risk. The outcomes of the periodontal risk assessment, in turn, will determine the recall frequency of the patient during the maintenance phase.

Conclusion

Care for the periodontal patient involves careful examination of patient data and arriving at individually tailored treatment strategies for the patient based on the diagnosis. Ultimately, the treatment goals outlined above should be used as the endpoints of periodontal therapy.

References

Cortellini P, Tonetti MS. Focus on intrabony defects: guided tissue regeneration. *Perio 2000* 2000;22:104-132.

Matuliene G, Pjetursson BE, Salvi GE, *et al*. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. *J Clin Perio* 2008;35:685-695.

Chapter 12

Periodontology beyond the pocket: Now totally lost

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Introduction

The theme for the eighth meeting of the Asian Pacific Society of Periodontology was "Beyond the Pocket". This theme implies that periodontology has now moved beyond the management of periodontal pockets, even though periodontal pockets still remain as very important pathological entities in determining periodontal disease behaviour (Matuliene *et al* 2008). The direction in which periodontology has drifted in moving "beyond the pocket" is exemplified in the titles given to top selling textbooks in the field e.g. Clinical Periodontology and Implant Dentistry (Lindhe *et al* 2009) or Periodontics Medicine, Surgery and Implants (Rose *et al* 2004).

A very insightful guest editorial by Yvonne de Paiva Buischi in Volume 28, Number 5 of *The International Journal of Periodontics and Restorative Dentistry* (2008) articulated issues which trouble many about periodontology going "Beyond the Pocket". She asked two questions: (1) Are we moving away from our primary professional competencies to become implantologists? and (2) Is this a positive change?

Periodontology and implant dentistry

Periodontology developed as in response to humankind being afflicted by periodontal diseases. Basically evidence exists that periodontal disease in adults can be controlled by the measures currently available and employed (Axelsson *et al* 2004). Periodontists as health care professionals should therefore have as their primary professional responsibilities the contribution to the prevention, diagnosis and management of periodontal diseases.

Dental implants in general can be considered to be successful means to replace missing teeth, and at present there is no evidence to show that any particular implant type has superior long-term success over any other (Esposito *et al* 2007). Indeed it has been claimed that dental implants have the highest survival rates in the human body subsequent to implantation for all implantable medical devices (Ratner 2001). Although it must be recognised that the success of dental implants in term of patient-based assessments is to do with aesthetics and satisfaction is not well documented (Den Hertog *et al* 2008).

Dental specialties and implant dentistry

Periodontists are recognised by their various specialty bodies as being appropriate health care professionals for the placement of dental implants and for the delivery of supportive care for dental implants. Periodontology is far from being the only specialty to recognise dental implant placement as part of its professional responsibilities. Obviously oral surgery, under whatever guise, includes dental implant surgery. Following the recognition in 2004 that implant placement was being taught in American Prosthodontics programmes (Sukotjo and Arbree 2008), the American Academy of Prosthodontics added surgical implant placement to the Accreditation Standards for Advanced Specialty Education Programmes in Prosthodontics (Commission on Dental Accreditation). The Commission on Accreditation Standards for Advanced Specialty Education Programmes in Endodontics currently allows for clinical training to the level of exposure in implant dentistry. A recent report (Potter *et al* 2009) shows that the majority of American endodontists believe that dental implant placement is within the scope of endodontic practice. If the same pattern holds as seen for prosthodontics, implant placement will come to be taught as part of the practice of endodontics. A 2004 survey of Deans of North American dental schools revealed that while most schools included restoration of single tooth implants and implant over-dentures in the predoctoral education and training there was no predoctoral competency requirement in implant placement in all schools surveyed (Petropoulos *et al* 2006). In Europe the Proceedings of the First European Consensus Workshop on Implant Dentistry University Education deals with the rationale for the introduction of implant dentistry into the dental curriculum (Lang and De Bryun 2009) and the assessment of competencies related to implant dentistry in undergraduate university education (Mattheos *et al* 2009). So it can be envisaged that not only will the specialties of Oral Surgery, periodontology, prosthodontics and endodontics consider implant placement to be part of the essential practice of their respective specialties, general dentists will come to have surgical implant placement as a component of the dental practice for which they have been educated and trained.

Influence of implant dentistry on periodontology

For the time being, periodontology behaves as if implant dentistry belongs to periodontology. There is a very close relationship between dental implant companies and organised periodontology.

EuroPerio6 (6th Congress of the European Federation of Periodontology, June 2009) recently held in Stockholm, Sweden can be used as an example of this close relationship. Two of the three diamond sponsors were implant companies, four of the eight platinum sponsors were implant companies, and three of the gold sponsors were implant companies. In the trade exhibition the majority of the booths were either implant companies of companies dealing in implant related materials or instrumentation. These companies can hardly have their economic interests best served by a dental specialty devoted to preventing tooth loss through the prevention, diagnosis, treatment and supportive care of periodontal diseases. The close relationship which has developed between periodontal organisations and dental implant companies can certainly be observed to have influenced the content of professional congresses and symposia, even if only through the inclusion of so many sessions with direct sponsorship

from industry with their own selection of speakers and topics.

It can be conceded that periodontology is the only specialty taking an interest in the prevention and treatment of peri-implant biological complications such as peri-implantitis. Periodontal biological complications, such as periodontitis, are uncommon in the first three decades (Hugoson *et al* 1998), whereas follow-up of dental implants has shown that peri-implant disease is common relatively much earlier around implants in the absence of regular and devoted supportive care (Roos-Jansåker 2006a, 2006b, 2006c). The close cooperation between dental implant companies and periodontal organisations does not seem at the moment to be predicated on the maintenance of peri-implant health; perhaps that will come later (Berglundh *et al* 2002). It must be admitted that at this stage the evidence base for the prevention and management of peri-implant biological complications is not strong (Grusovin *et al* 2007). If the special relationship between periodontology and implant dentistry develops to be that of maintaining peri-implant health and managing peri-implant complications, periodontology will find itself often dealing with mistakes of others. The outcomes in terms of patient satisfaction will often be only marginally less unhappy patients. Implant placement errors account for many examples of implant component exposures. Cleansability of implant bone restorations is key to long term peri-implant health (Serino and Strom 2009).

So keen are they in Europe for periodontology to be recognised as one of the, if not the, dental specialty which includes special knowledge and skills in all aspects of implant dentistry that the Quality Standards for Graduate Programmes in Periodontology of the European Federation of Periodontology states that postgraduate specialists in periodontology should be proficient in surgical implant therapy including fully edentulous patients. Periodontology has the base of "peri" around and "odont" a tooth. If there is no tooth, as in the fully edentulous, it really can be questioned whether periodontology should have a role. Perhaps the role in the fully edentulous should be restricted to the maintenance of peri-implant health and peri-implant mucosal stability when fully edentulous patients have had dental implant therapy delivered by others.

"Is endodontic therapy passé?"

Within endodontics, the success of endodontic therapy is being compared to the success of dental implant therapy with claims of equivalent outcomes (Iqbal and Kim 2007, Salinas and Eckert 2007, Torabinejad *et al* 2007, Hannahan and Eleazer 2008, Blicher *et al* 2008, Torabinejad *et al* 2008, Zitzmamn *et al* 2009). It has even been questioned whether endodontics should be dropped in favour of dental implant therapy, given that both are successful. Whether endodontic treatment is passé has been discussed in an Editorial (Spångberg 2008) which very forcefully concluded that endodontic treatment, orthograde or retrograde, should always be first treatment of choice for a tooth having disease endodontic in origin. This editorial was applauded by the President of the American Association of Endodontics in a letter to the editor (Rossman 2009). In that letter it was highlighted, that while both endodontic treatment and dental implant tooth replacement may both be very successfully applied, at least one study (Hannahan and Eleazer 2008) noted that dental implant replacement required more extensive and time-consuming follow up compared to endodontic therapy and tooth restoration. Within the specialty of endodontics there is however no evidence that a tooth lost because of endodontic disease is less successfully

Authors	Years	Association of periodontitis with implant problems
Van de Weijden *et al*	2005	Yes
Schou *et al*	2006	Yes
Karoussis *et al*	2007	Yes
Quirynen *et al*	2007	Yes
Ong *et al*	2008	Yes
Heitz-Mayfield	2008	Yes
Safii *et al*	2009	Yes

Table 1. Reviews dealing with dental implants and periodontitis

replaced by a dental implant than if the tooth has been lost for other reasons. Hence the consideration of whether endodontics is passé has some legitimate basis. This situation does not hold in periodontology.

Dental implants in the periodontally susceptible

There have been seven reviews of clinical trials of dental implants placed in periodontally susceptible patients (Table 1). If periodontally involved teeth are to be extracted to be replaced by dental implants, it is imperative that it be known if dental implants behave the same way in periodontally susceptible patients as they do, say, in patients whose teeth to be replaced by dental implants have been lost for endodontic reasons. All reviews approached the topic from somewhat different view points. Van der Weijden (2005) considered dental implant therapy in partially edentulous periodontally compromised patients. Schou *et al* (2006) considered the outcome of dental implant therapy in patients with previous tooth loss due to periodontitis. Karoussis *et al* (2007) was a comprehensive and critical review of dental implant prognosis in periodontally compromised partially edentulous patients. Quirynen *et al* (2007) considered two aspects - (i) the impact of supportive periodontal therapy, and (ii) affects of implant surface roughness - on the therapeutic outcomes of dental implants in patients with a history of periodontitis. Ong *et al* (2008) focused on dental implant treatment outcomes in treated periodontitis patients. Another view point was to highlight the risk indicators for peri-implant diseases, which was covered by the sixth review (Heitz-Mayfield 2008). The most recent review concentrated on dental implant failure and marginal bone loss in patients with a history of periodontitis (Safii *et al* 2009). All of these have confirmed that the outcome of dental implant therapy is adversely affected by periodontitis. This may be noted in terms of lower survival in the long term, greater frequency of peri-implant biological complications and greater peri-implant marginal bone loss. This is constant in the periodontally susceptible, periodontally compromised, periodontally treated and those losing teeth due to periodontal destruction. Even immediately placed and restored dental implants do less well in periodontally

Figure 1. Upper arch reconstruction in a patient with history of periodontitis

susceptible patients (Horwitz *et al* 2008). Lindhe and Meyle (2008) writing on behalf of Group D of the Sixth European Workshop on Periodontology having considered the review of Heitz-Mayfield (2008), which did not cover Ong *et al* (2008) and Safii *et al* (2009), concluded that a history of periodontitis is a risk for peri-implant disease. The Group D also concluded that there is evidence that poor oral hygiene and cigarette smoking are established risks for peri-implant disease.

Hence, it should be clearly noted that peri-implant disease and periodontal disease share key risk factors, namely poor oral hygiene and tobacco smoking. This has very practical implications. If it becomes impossible to treat and retain teeth in a periodontally susceptible individual because of deficiencies in plaque control, replacement of the missing teeth by means of dental implants will leave the dental implants at considerable risk of developing peri-implant disease. The reasons are two-fold; firstly the patient has a history of periodontitis, secondly deficiencies in plaque control are not themselves corrected by replacing teeth with dental implants. There is no evidence to date that patients with dental implants clean the implants and implant borne restorations better than they cleaned the teeth which preceded the dental implants. The direct association between inadequate plaque control and peri-implantitis has recently been demonstrated by Serino and Strom (2009). Figure 1 shows the upper arch reconstruction of a 62 year-old patient with a history of periodontitis whose tooth loss was occasioned by periodontal attachment and alveolar bone loss. The reconstruction is barely cleansable by a dentist with the aid of dental surgery (chairside) assistance, the provision of suction and the use of illumination and magnification. Maintaining adequate plaque control on the part of the patient on a day-to-day basis is a major, if not insurmountable, challenge. Yet this day-to-day plaque control required if this patient, with her history of periodontitis, is to manage to avoid peri-implant disease. For all patients in whom dental implant borne restorations are delivered, and especially so for those with a history of periodontitis, it is essential that the restorations are amenable to the maintenance of high standards of plaque control.

To 'save or extract' periodontally compromised teeth?

A prerequisite for treatment planning in implant dentistry, when the issue arises as to whether to treat and retain teeth or to extract and replace using dental implants, is the "periodontal prognostication of comprised teeth" (Greenstein *et al* 2007). Yet establishing periodontal prognoses for periodontally compromised teeth is not an aspect of periodontology which has received sufficient research attention. Indeed, standard textbooks such as "Clinical Periodontology and Implant Dentistry, 5th Edition" (Linde *et al* 2009), which runs to over 1300 pages, deals with prognostication to only a very small extent.

Prognosis of periodontally compromised teeth after periodontal therapy

A recent pair of papers from Germany sheds some light on patient-related risk factors for prognosis, treatment outcomes and tooth loss after active periodontal therapy (Eickholz *et al* 2008) and tooth-related risk factors for tooth loss after active periodontal therapy (Pretzl *et al* 2008). The most important patient related risk factors identified in this dental hospital patient group were inadequate and ineffective oral hygiene and irregular attendance for supportive periodontal care, furthermore smoking, initial diagnosis, age and gender all also influenced treatment outcomes, prognosis and tooth loss (Eickholz *et al* 2008). At the tooth level, advanced baseline bone loss, furcation involvement (for multi-rooted teeth) and if the tooth functioned as an abutment tooth all negatively influenced the prognosis and were risks for tooth loss after active therapy (Pretzl 2008). The importance of the persistence of deep pockets after therapy on influencing disease progression and being risks for tooth loss was recently further

emphasised (Matuliene *et al* 2008). Decision making on whether to retain a periodontally involved or compromised tooth or to extract it requires a better evidence-base. A recent decision making schema developed on the basis of reviewing available literature has been proposed (Avila *et al* 2009). This is a very promising start, although assumptions have been built into the schema which would not find universal favour.

Decisions on when to save and when to extract are contentious (Greenstein *et al* 2008) and further evidenced by heated discussions, ever among periodontists, on their professional societies' webpages. The discussion comes perhaps from firm opinions being formed in the absence of a sufficient evidence-base on which to ground decisions.

Dental implants do not seem to compromise adjacent teeth during a 10-year period (Misch *et al* 2008) whereas removable partial dentures in periodontal patients responsible for their own supportive care arrangements were associated with greater attachment loss in the denture bearing arch (Leung *et al* 2006).

If a decision is made to extract periodontally involved teeth because the patient or the teeth appear to be refractory to periodontal treatment and to replace these teeth by means of dental implants, it has been shown that dental implants in such patients also show high experience of peri-implant biological complications and implant loss (Fardal and Linden 2008).

Supportive periodontal care and supportive care for dental implants

Periodontal susceptible patients both with teeth or teeth and implants are in need of supportive periodontal (and peri-implant) care. Inadequate oral hygiene is a risk for both periodontal disease and peri-implant disease and irregular supportive care is a risk for

Authors	Year	Country	Compliant
Anneken *et al*	2001	Germany	53%
Checchi *et al*	1994	Italy	30%
Demetriou *et al*	1995	Greece	27%
Demirel & Efeodlu	1995	Japan	37%
Fardal *et al*	2003	Norway	87%
König *et al*	2001	Germany	62%
Mendoza *et al*	1991	Australia	36%
Matthews *et al*	2001	Canada	85%
Novaes *et al*	1996	Brazil	30%
Novaes *et al*	1999	South America	26%
Novaes & Novaes	2001	Brazil	46%
Ojima *et al*	2001	Japan	72%
Soolari & Rokn	2003	Iran	35%
Wilson *et al*	1984	USA	16%
Wilson *et al*	1993	USA	32%

Table 2. Studies on compliance with supportive periodontal care

periodontal disease progression and tooth loss (Eickholz *et al* 2008). Periodontal supportive care delivered in a university setting has been reported to stabilise successfully periodontal conditions after active periodontal therapy (Lorentz *et al* 2009). It must be acknowledged, however, that the evidence base for maintaining soft tissue health around dental implants is not yet secure (Grusovin *et al* 2007).

Generally, supportive periodontal for periodontitis patients care can limit periodontal disease progression at least to that experienced by non-periodontally susceptible persons of the same age in the community (Jansson and Lagervall 2008). In comparison, selected periodontally healthy individuals enrolled in a preventive regime periodontitis patients undergoing regular supportive periodontal care display more periodontal attachment loss (Teles *et al* 2008). Given current practice of extracting periodontally involved teeth and replacing these teeth with treatments involving dental implants, compliance with supportive periodontal regimes may, while achieving low plaque levels, result in greater tooth loss (Miyamoto *et al* 2006), a finding which was noted with comment (Hujoel *et al* 2006).

Periodontal disease	Peri-implant disease
Approaches evidence based	Approaches not yet evidence based
If treatment fails, it fails because of risks: oral hygiene, smoking, undisturbed biofilm	If treatment fails, it fails because of risks: oral hygiene, smoking, undisturbed biofilm
Gingival recession (because of disease, prevention or treatment) leaves roots showing	Peri-implant mucosal "recession" (because of disease, prevention, or treatment) leaves implant connections or surfaces exposed
Management of gingival recession is evidence based	Management of peri-implant mucosal recession is "trial and error"
Malpositioned tooth/teeth can be removed orthodontically	Malpositioned implants difficult to move

Table 3. Considerations in preventing and treating peri-implant disease

Compliance with supportive care arrangements

Good compliance with supportive periodontal care is usually associated with overall positive oral health behaviours, including toothbrushing and interdental cleaning, and poor compliance is associated with unfavourable oral health care practices and attitudes (Ojima *et al* 2005). Hence, compliance with supportive periodontal care can be expected to assist in the maintenance not only of periodontal attachment levels but also peri-implant health and maintenance of marginal bone levels.

Those studies on compliance with supportive periodontal care regimens which report on or allow for the calculation of rates of compliance are summarised in Table 2. It can be noted from Table 2 that these studies were conducted in many countries across five continents. A range of oral health delivery settings (private practices, hospitals and universities) collectively contributed to these studies. An analysis of all subjects included in these studies reveals that world-wide of the treated periodontal patients entered into supportive periodontal care, less than 40%

were found to be compliant with supportive periodontal care arrangements. The Novaes and Novaes (1999) report was excluded from calculation of the rate of compliance. Hence it can be expected, unless patients with dental implants have a greater level of compliance with supportive periodontal care arrangements than those captured in these studies in Table 3, that the majority of periodontally susceptible patients will not be fully complaint with supportive periodontal (and peri-implant) care arrangements. The series of reports from Roos-Jansåker and colleagues (2006a, 2006b, 2006c) reports on peri-implant disease in those dental implant recipients with a history of periodontitis who did not attend for supportive care. Peri-implant biological complications appear 10 to 14 years after dental implant reconstruction, as far as is known.

Treatment of peri-implant diseases

It has been concluded that there is little evidence to determine which, of any, of the currently employed treatment approaches is the most effective way to treat peri-implant diseases (Esposito *et al* 2008). This however does not imply that peri-implant diseases

cannot be treated. Non-surgical treatment of peri-implant diseases has been recently reviewed (Renvert *et al* 2008). Surgical treatment of peri-implantitis has also been subjected to review (Claffey *et al* 2008). In considering these two reviews and reporting on behalf of Group D, Lindhe and Meyle (2008) concluded that the outcomes of non-surgical therapy of peri-implant disease is unpredictable, and that while several studies have reported on the long term success of treatment of periodontitis, the same cannot be said for peri-implantitis (Lindhe and Meyle 2008). It was further concluded that there is no evidence that so called peri-implant "regenerative procedures" have additional beneficial effects on peri-implant disease treatment outcomes.

Table 3 presents a 'compare and contrast' of the prevention and treatment of periodontal and peri-implant disease. Given the uncertainties in the column under peri-implantitis, it can be strongly argued that inflammatory diseases around teeth are more manageable than around implants. If periodontal disease cannot be managed in a patient, this is often (always) due to ineffective control of the causative agent plaque, and failure to modify modifiable risk factors, e.g. smoking.

Peri-implant diseases, in such periodontally susceptible patients, are brought on by what caused periodontal therapy to fail. Thus dental implants seem to have enhanced likelihood of greater failures and biological complications in those patients whose periodontal diseases have proved refractory to treatment (Fardal and Linden 2008).

Conclusions

This author's sad conclusion is that by moving 'beyond the pocket' and away from its mission in preventing and treating periodontal diseases periodontology has

become totally lost. Periodontology has been too keen to act as if implant dentistry somehow belongs to periodontology, when in fact many specialties have the same claim. Certainly there is scientific interest in the behaviour of the tissues which surround dental implants, and this interest belongs in part to periodontology. Now there comes a move to have dental undergraduates become competent in dental implant therapy, so dental implants will become even less within the purview of any one of the specialties.

Periodontology has become too intertwined with dental implant companies, which because of their relative economic strength are in a position to dictate agendas and directions. It is unlikely that dental implant companies fully support the treatment and retention of periodontally compromised teeth, when their coffers are swelled by the loss of such teeth and the use of dental implants in their replacement.

Periodontology has failed to pay sufficient attention to "prognostication" of periodontally compromised teeth. Periodontology has become too keen on extracting periodontally compromised teeth and on using dental implants in the replacement of teeth in periodontally susceptible patients. This is despite a substantial body of evidence showing that dental implants perform less well in terms of survival and freedom from peri-implant biological complications.

Further, periodontology has ignored the evidence that periodontitis and peri-implant disease share common risk factors, a major one being inadequacies in plaque control. If periodontitis proves itself refractory to therapy, periodontology has in the past two decades collectively taken recourse to dental implant therapy. Yet refractory periodontitis patients may experience more dental implant loss and peri-implant biological complications.

Periodontology has collectively failed to recognise that dental implants require just as

assiduous plaque control over the long term as in the management of periodontitis.

Since many dental specialties and general dental practitioners are actively involved in dental implant therapy there is a real danger that periodontology will become the specialty for performing peri-implant mucosal plastic procedures to try to cover unaesthetic exposures of margins, abutments and implants, and for attempting to manage peri-implant disease. These therapeutic endeavours will not curry favour with dental implant companies and periodontology may well find the supportive relationships fostered over recent years to be ephemeral.

References

Anneken C, Treinen J, Willershausen B. Patients compliance in periodontal therapy: A retrospective investigation on the basis of a clinical group. *Eur J Med Res* 2001;28:75-82.

Avila G, Galindo-Moreno P, Soehren S, *et al*. A novel decision-making process for tooth retention or extraction. *J Periodontol* 2009;80:476-491.

Axelsson P, Nyström B, Lindhe J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *J Clin Periodontol* 2004;31:749-757.

Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. *J Clin Periodontol* 2002;29(Suppl 3):197-212.

Blicher B, Baker D, Lin J. Endosseous implants versus nonsurgical root canal therapy: A systemic review of the literature. *Gen Dent* 2008;56:576-580.

Checchi L, Pelliccioni GA, Gatto MR, Kelescian L. Patient compliance with maintenance therapy in an Italian periodontal practice. *J Clin Periodontol* 1994;21:309-312.

Claffey N, Clarke E, Polyzois I, Renvert S. Surgical treatment of peri-implantitis. *J Clin Periodontol* 2008;35(Suppl 8):316-332.

De Paiva Buischi Y. Where is Periodontology heading? *Int J Periodontics Restorative Dent* 2008;5:428-439.

Demetriou N, Tsami-Pandi A, Parashis A. Compliance with supportive periodontal treatment in private periodontal practice. A 14-year retrospective study. *J Periodontol* 1995;66:145-149.

Demirel K, Efeodlu A. Retrospective evaluation of patient compliance with supportive periodontal treatment. *J Niho Univ Sch Dent* 1995;37:131-137.

Den Hartog L, Slater JJ, Vissink A, *et al*. Treatment outcome of immediate, early and conventional single-tooth implants in the aesthetic zone: A systemic review to survival, bone level, soft-tissue, aesthetics and patient satisfaction. *J Clin Periodontol* 2008;35:1073-1086.

Eickholz P, Kaltschmitt J, Berbig J, *et al*. Tooth loss after active periodontal therapy. Patient-related factors for risk, prognosis, and quality of outcome. *J Clin Periodontol* 2008;35:165-174.

Esposito M, Grusovin MG, Kakisis I, *et al*. Interventions for replacing missing teeth: Treatment of peri-implantitis. *Cochrane Database of Systematic Reviews* 2008;CD004970.

Esposito MM, Murray-Curtis L, Grusovin MG, *et al*. Interventions for replacing missing teeth: Different types of dental implants. *Cochrane Database of Systematic Reviews* 2007;CD003815.

Fardal Ø, Johannessen AC, Linden GJ. Compliance in a Norwegian periodontal practice. *Oral Health Prev Dent* 2003;1:93-98.

Fardal Ø, Linden GJ. Tooth loss and implant outcomes in patients refractory to treatment in a periodontal practice. *J Clin Periodontol* 2008;35:733-738.

Greenstein G, Cavallaro J, Tarnow D. When to save or extract a tooth in the esthetic zone: A commentary. *Compend Contin Educ Dent* 2008;29:136-145.

Greenstein G, Greenstein B, Cavallaro J. Prerequisite for treatment planning implant dentistry: Periodontal prognostication of

compromised teeth. *Compend Contin Educ Dent* 2007;28:436-446.

Grusovin MG, Coulthard P, Jourabchian E, *et al*. Interventions for replacing missing teeth: Maintaining and recovering soft tissue health around dental implants. *Cochrane Database of Systematic Reviews* 2007;CD003069.

Hannahan JP, Eleazer PD. Comparison of success of implants versus endodontically treated teeth. *J Endod* 2008;34:1302-1305.

Heitz-Mayfield LJA. Peri-implant diseases: Diagnosis and risk indicators. *J Clin Periodontol* 2008;35(Suppl 8):292-304.

Horwitz J, Zuabi O, Machtei E. Radiographic changes around immediately restored dental implants in periodontally susceptible patients: 1-year results. *Int J Oral Maxillofac Implants* 2008;23:531-538.

Hugoson A, Norderyd O, Slatte C, Thorstensson H. Distribution of periodontal disease in a Swedish adult population 1973, 1983 and 1993. *J Clin Periodontol* 1998;25:542-548.

Hujoel P, Selipsky H, Cunha-Cruz J. Compliance with periodontal maintenance may be associated with increased tooth loss. *J Evid Based Dent Pract* 2006;6:262-264.

Iqbal MK, Kim S. For teeth requiring endodontic treatment, what are the differences in outcomes of restored endodontically treated teeth compared to implant-supported restorations? *Int J Oral Maxillofac Implants* 2007;22(Suppl):96-116.

Jansson L, Lagervall M. Periodontitis progression in patients subjected to supportive maintenance care. *Swed Dent J* 2008;32:105-114.

Karoussis IK, Kotsovills S, Fourmousis I. A comprehensive and clinical review of dental implant prognosis in periodontally compromised partially edentulous patients. *Clin Oral Impl Res* 2007;18:669-679.

König J, Plagmann HC, Langenfeld N, Kocher T. Retrospective comparison of clinical variables between compliant and non-compliant patients. *J Clin Periodontol* 2001;28:227-232.

Lang NP, De Bruyn H. The rationale for the introduction of implant dentistry into the dental curriculum. *Eur J Dent Educ* 2009;13(Suppl 1):19-23.

Leung WK, Ng DK, Jin L, Corbet EF. Tooth loss in treated periodontitis patients responsible for their supportive care arrangements. *J Clin Periodontol* 2006;33:265-275.

Lindhe J, Lang NP, Karring T. Clinical Periodontology and Implant Dentistry, 5th Edition. Wiley-Blackwell. 2009.

Lindhe J, Meyle J. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;35(Suppl 8):282-285.

Lorentz TC, Cota LO, Cortelli JR, *et al*. Prospective study of compiler individuals under periodontal maintenance therapy: Analysis of clinical periodontal parameters, risk predictors and the progression of periodontitis. *J Clin Periodontol* 2009;36:58-67.

Mattheos N, Ucer C, Van de Velde T, Nattestad A. Assessment of knowledge and competencies related to implant dentistry in undergraduate and postgraduate university education. *Eur J Dent Educ* 2009;13(Suppl 1):56-65.

Matthews DC, Smith CG, Hanscom SL. Tooth loss in periodontal patients. *J Can Dent Assoc* 2001;67:207-210.

Matuliene G, Pjetursson BE, Salvi GE, *et al*. Influence of residual pockets on progression of periodontitis and tooth loss: Results after 11 years of maintenance. *J Clin Periodontol* 2008;35:685-695.

Mendoza AR, Newcomb GM, Nixon KC. Compliance with supportive periodontal therapy. *J Periodontol* 1991;62:731-736.

Misch CE, Misch-Dietsh F, Silc J, *et al*. Posterior implant single-tooth replacement and status of adjacent teeth during a 10-year period: A retrospective report. *J Periodontol* 2008;79:2378-2382.

Miyamoto T, Kumagai T, Jones JA, *et al*. Compliance as a prognostic indicator: Retrospective study of 505 patients treated and maintained for 15 years. *J Periodontol* 2006;77:223-232.

Novaes AB Jr, Novaes AB, Bustamanti A, *et al*. Supportive periodontal therapy in South America. A retrospective multi-practice study on compliance. *J Periodontol* 1999;70:301-306.

Novaes AB Jr, Novaes AB. Compliance with

supportive periodontal therapy. Part I. Risk of non-compliance in the first 5-year period. *J Periodontol* 1999;70:679-682.

Novaes AB Jr, Novaes AB. Compliance with supportive periodontal therapy. Part II: Risk of non-compliance in a 10-year period. *Braz Dent J* 2001;12:47-50.

Novaes AB, Novaes AB Jr, Moraes N, *et al*. Compliance with supportive periodontal therapy. *J Periodontol* 1996;67:213-216.

Ojima M, Hanioka T, Shizukuishi S. Survival analysis for degree of compliance with supportive periodontal therapy. *J Clin Periodontol* 2001;28:1091-1095.

Ojima M, Kanagawa H, Nishida N, *et al*. Relationship between attitudes toward oral health at initial office visit and compliance with supportive periodontal treatment. *J Clin Periodontol* 2005;32:364-368.

Ong CT, Ivanovski S, Needleman IG, *et al*. Systemic review of implant outcomes in treated periodontitis subjects. *J Clin Periodontol* 2008;35:438-462.

Petropoulos VC, Arbree NS, Tarnow D, et al. Teaching implant dentistry in the predoctoral curriculum: A report from the ADEA implant workshop's survey of Deans. *J Dent Educ* 2006;70:580-588.

Potter KS, McQuistan MR, Williamson AE, *et al*. Should endodontists place implants? A survey of US endodontists. *J Endod* 2009;35:966-970.

Pretzl B, Kaltschmitt J, Kim TS, *et al*. Tooth loss after active periodontal therapy. Tooth-related factors. *J Clin Periodontol* 2008;35:175-182.

Quirynen M, Abarca M, Van Assche N, *et al*. Impact of supportive periodontal therapy and implant surface roughness on implant outcome in patients with a history of periodontitis. *J Clin Periodontol* 2007;34:805-815.

Ratner BD. Replacing and renewing: Synthetic materials, biomimetics and tissue engineering in implant dentistry. *J Dent Educ* 2001;65:1340-1347.

Renvert S, Roos-Jansåker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: A literature review. *J Clin Periodotol* 2008;35(Suppl. 8):305-315.

Roos-Jansåker AM, Lindahl C, Renvert H, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part I: Implant loss and associations to various factors. *J Clin Periodontol* 2006a;33:283-289.

Roos-Jansåker AM, Lindahl C, Renvert H, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part II: Presence of peri-implant lesions. *J Clin Periodontol* 2006b;33:290-295.

Roos-Jansåker AM, Renvert H, Lindahl C, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part III: Factors associated with peri-implant lesions. *J Clin Periodontol* 2006c;33:296-301.

Rose LF, Mealey BL, Genco RJ, Cohen W. Periodontics - Medicine, Surgery and Implants. Mosby, United States. 2004.

Rossman LE. Letter to the Editor. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:450-451.

Safii SH, Palmer RM, Wilson RF. Risk of implant failure and marginal bone loss in subjects with a history of periodontitis: A systematic review and meta-analysis. *Clin Implant Dent Relat Res* 2009 May 7. [Epub ahead of print]

Salinas TJ, Eckert SE. In patients requiring single-tooth replacement, what are the outcomes of implant- as compared to tooth-supported restorations? *Int J Oral Maxillofac Implants* 2007;22(Suppl):71-95.

Schou S, Holmstrup P, Worthington HV, Exposito M. Outcome of implant therapy in patients with previous tooth loss due to periodontitis. *Clin Oral Impl Res* 2006;17(Suppl 2):104-123.

Serino G, Strom C. Peri-implantitis in partially edentulous patients: Association with inadequate plaque control. *Clin Oral Impl Res* 2009;20:169-174.

Soolari A, Rokn AR. Adherence to periodontal maintenance in Tehran, Iran. A 7-year retrospective study. *Quintessence Int* 2003;34:215-219.

Spångberg LSW. Is endodontic treatment passé? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;106:465-466.

Sukotjo C, Arbree NS. Prosthodontic program directors' perceptions regarding implant placement by prosthodontic residents: A 2004 survey conducted by the Educational Policy

Subcommittee of the American College of Prosthodontists. *J Prosthodont* 2008;17:662-668.

Teles RP, Patel M, Socransky SS, Haffajee AD. Disease progression in periodontally healthy and maintenance subjects. *J Periodontol* 2008;79:784-794.

Torabinejad M, Anderson P, Bader J, *et al*. Outcomes of root canal treatment and restoration, implant-supported single crowns, fixed partial dentures, and extraction without replacement: A systematic review. *J Prosthet Dent* 2007;98:285-311.

Torabinejad M, Lozada J, Puterman I, White SN. Endodontic therapy or single tooth implant? A systematic review. *J Calif Dent Assoc* 2008;36:429-437.

Van der Weijden G, van Benmei K, Revert S. Implant therapy in periodontally edentulous, periodontally compromised patients: A review. *J Clin Periodontol* 2005;32:506-511.

Wilson TG Jr, Glover ME, Schoen J, *et al*. Compliance with maintenance therapy in a private periodontal practice. *J Periodontol* 1984;55:468-473.

Wilson TG Jr, Hale S, Temple R. The results of efforts to improve compliance with supportive periodontal treatment in a private practice. *J Periodontol* 1993;64:311-314.

Zitzmann NU, Krasti G, Hecker H, *et al*. Endodontics or implants? A review of decisive criteria and guidelines for single tooth restorations and full arch reconstructions. *Int Endod J* 2009;42:757-774.

Chapter 13

Evaluation of root coverage with autogenous connective tissue and acellular dermal matrix graft

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Introduction

Definitions and classifications of gingival recession

Gingival recession is commonly defined as a condition in which the location of the gingival margin is apical to the cemento-enamel junction (AAP 2001). Sullivan and Atkins (1968a) initially defined gingival recession as exposure of the root surface by an apical shift in the position of the marginal tissue. A recent definition was introduced by Miller (1985a) related to the height of the interproximal bone and the relation of gingival recession to mucogingival junction (MGJ):

- Class I: marginal tissue recession which does not extend to the MGJ. There is no periodontal loss (bone or soft tissue) in the interdental area, and 100% root coverage can be anticipated
- Class II: marginal tissue recession which extends to or beyond the mucogingival junction. There is no periodontal loss (bone or soft tissue) in the interdental area, and complete root coverage can be achieved
- Class III: marginal tissue recession which extends to or beyond the MGJ. Bone or tissue loss is present in the interdental area

or there is malpositioning of the teeth which prevents the attempt for 100% root coverage. Partial root coverage can be anticipated
- Class IV: marginal tissue recession which extends to or beyond the MGJ. The bone of soft tissue loss in the interdental area and/or malpositioning of teeth is so severe that no root coverage is feasible.

Prevalence

There are a number of studies reporting the prevalence of gingival recession in various populations. It has been shown that there is a more generalized pattern of prevalence and severity of recession as age increases (Yoneyama *et al* 1988, Loe *et al* 1992, Serino *et al* 1994). Individuals of all age groups had varying degrees of gingival recession which ranged from 44-54.5% in individuals under 30 years old to 100% in individuals over 40 years old (Gorman 1967, Serino *et al* 1994). Löe *et al* (1992) reported that gingival recession was already prevalent in both good (60% in Norwegians) and poor (80% in Sri Lankans) oral hygiene populations aged in their 20s. By age 50, >90% of all Norwegian exhibited some gingival recession whereas all

the Sri Lankans participants had gingival recession. Gingival recession was predominantly found on the buccal surfaces of the teeth (Yoneyama *et al* 1988, Löe *et al* 1992, Joshipura *et al* 1994, Serino *et al* 1994, Cecchi *et al* 1999).

Etiology

Recession defects may exist in the presence of normal sulci and undiseased crestal bone levels or they may occur as part of the pathogenesis of periodontal disease with the loss of alveolar bone. However, recession frequently results from a combination of predisposing factors. Factors identified are mechanical trauma via tooth brushing trauma, impaction of foreign bodies or self-inflicted injury such as fingernail pricking habits, presence of debri and calculus which causes attachment loss in periodontal disease, malposition of the teeth erupting close to mucogingival junction with narrow/ nonexistent keratinized tissue, orthodontic tooth movement out of the cortical plate such as excessive proclination of teeth and arch expansion, anatomical defects where a thin buccolingual gingival dimension is present at sites with alveolar bone dehiscences and presence of frenal pull (Trott & Love 1966, Gorman 1967, Sangnes & Gjermo 1976, Modéer and Odenrick 1980, Steiner *et al* 1981, Löst 1984, Wennström *et al* 1987, Löe *et al* 1992, Källestål and Uhlin 1992, Beck & Koch 1994, Joshipura *et al* 1994, Cecchi *et al* 1999, Tugnait & Clerehugh 2001). Any iatrogenic factors related to restorative (subgingival margins) and periodontal treatment (tissue trauma induced by root planing and conformation of healed gingival to underlying bone after surgical modalities) could contribute to the development of gingival recession (Lindhe and Nyman 1980, Lindhe *et al* 1987).

Consequences of gingival recession and indications for treatment

A common symptom associated with recession is cervical dentine hypersensitivity and this is often the patient's primary concern (Tugnait and Clerehugh 2001, Oates *et al* 2003, Zaher *et al* 2005). Other indications for treatment include esthetic demands, elimination of recession sites as plaque retentive sites and to facilitate adequate oral hygiene control, prevention of further progression of the defect and root caries and fear of losing the tooth (Zaher *et al* 2005, Tugnait and Clerehugh 2001).

Treatment modalities

Patients should be provided with a clear explanation of the factors that may have caused the gingival recession and how further progression may be prevented. The patient should also be advised on the long-term prognosis of the tooth and reassured as appropriate.

Management of etiological factors associated with recession

Gingival recession can be avoided with the utilization of atraumatic brushing techniques, particularly at sites that are plaque free but have been subjected to a vigorous and destructive brushing technique (Addy *et al* 1987a). Wennström *et al* (2008) advised that these patients should use a brushing technique with soft bristled brushes creating as little apically directed pressure on soft tissue margin as possible, instead. Plaque control is extremely important in preventing the occurrence of recession or its progression. A tooth that is positioned facially within the alveolar process may show an alveolar dehiscence with a thin covering of soft tissue. When such a tooth is moved lingually during

orthodontic treatment, the gingival dimensions on the labial aspects will increase in thickness and height (Zachrisson 2008). Gingival recession which is induced by habits, such as gingival trauma with fingernail or pencil, should be identified and discussed with the patient.

Management of the sign and symptoms of recession

In addressing dentine hypersensitivity, a full diet history should be obtained to assist in identifying any potential contribution from dietary acids and appropriate dietary advice can then be provided (Davis *et al* 1980, Addy *et al* 1987b). There are a large number of products available for the treatment of dentine hypersensitivity. The mechanism of action aimed by most agents is to block the dentinal tubules reducing pain transmission. An alternative approach is to use NdYAG lasers, reported to be effective in treating dentine hypersensitivity (Renton-Harper and Midda 1992).

Restorations using glass ionomer, composite, and compomer can be placed in cases where there is marked loss of tooth substance in association with cervical dentine hypersensitivity. In extreme situations, the tooth can be devitalized and endodontically treated. Recession in 'black triangles' is not amenable for surgical correction due to the degree of soft and hard tissue loss. Thus, approaches to improve aesthetics include construction of a silicone flexible gingival mask or gingival veneers, fabrication of crowns and veneers with extended clinical crown length and placement of pink coloured composite on the cervical part of tooth (Greene 1998, Tugnait and Clerehugh 2001, Zalkind and Hochman 1997).

There are a variety of surgical procedures that have been developed to increase the width of keratinized tissue and to cover root surfaces.

- Frenectomy may be advocated in localized gingival recession that has resulted from a frenal pull. It is also required in cases of high frenal attachment whereby mechanical oral hygiene procedures are impeded.
- Mucogingival surgical intervention, recently termed as periodontal plastic surgery, include manipulation of the patient's tissues to augment the soft tissues and cover the exposed root surface. The following procedures have been described:
- Pedicle grafts which can be divided, depending on the direction of transfer, into rotational flaps (e.g. laterally sliding flap, papilla flap, double papilla flap) and advanced flaps without rotation or lateral movement (e.g. coronally positioned flap).
- Guided tissue regeneration (GTR) which involves the use of resorbable and non-resorbable membrane barriers to re-establish soft tissue dimensions over areas of recessions combined with the pedicle soft tissue graft procedures (Pini Prato *et al* 1992, Tinti *et al* 1992).
- Free soft tissue grafts can be performed as an epithelialized soft tissue graft or a subepithelial connective tissue graft.

Epithelialized free gingival grafts

To achieve root coverage, a 2-stage procedure was advocated whereby the graft was initially placed apical to the recession defect. In second surgical procedure, the healed graft was coronally positioned over the exposed root surface (Bernimoulin *et al* 1975, Maynard 1977) which was found to be more successful and predictable with respect to root coverage (Miller 1985b, Holbrook and Ochsenbein 1983).

Subepithelial connective tissue grafts

Subepithelial connective tissue grafts (sCTG) were introduced for root coverage in 1985 (Langer and Langer 1985, Raetzke 1985). It involved the use of a piece of tissue consisting of connective tissue as a donor source, usually from the hard palate and edentulous alveolar ridge. This donor tissue is usually harvested by the use of a trap door approach. The graft can then be placed directly on the exposed root and covered with a coronally or laterally repositioned mucosal flap (Langer and Langer 1985, Nelson 1987, Harris 1992) or placed within an "envelope" prepared by an undermining partial thickness incision from the soft tissue margin (Raetzke 1985).

The success of these grafts is due to the double blood supply at the recipient site from the underlying connective tissue base and the overlying recipient flap. The donor site is a closed wound ensuring less postoperative discomfort than conventional free gingival grafts. Langer and Langer (1985) found that an increase of 2-6 mm of root coverage was achieved.

Acellular dermal matrix (ADM)

Recently, acellular dermal matrix allograft (AlloDerm[TM], LifeCell, USA) has become increasingly popular as a donor tissue alternative to the autogenous palatal mucosal graft. AlloDerm[TM], a processed dermal matrix that is obtained from human donors under sterile operating conditions was originally developed for plastic surgery to cover full-thickness burn wounds. AlloDerm[TM] is derived through a process of removing the epidermal layer and all cells within the dermis. Because all cells are removed during tissue processing, viruses cannot be transmitted in AlloDerm[TM]. A second antiviral step is the addition of an antiviral agent, which inactivates HIV. In addition, removal of cells leaves no antigenic components to cause the recipient to reject the graft. Finally, the tissue is further processed through freeze-drying. The resultant allograft is described as an acellular dermal matrix with normal and undamaged collagen bundling and organization, as well as elastin matrices that function as a scaffold to allow ingrowth by host tissues. Furthermore, the basement membrane complex is intact. Due to its nonvital structure, it is dependant on cells and blood vessels from the recipient site to achieve reorganization.

With its advantages of being incorporated into host tissues, eliminating donor site morbidity, a reduction in postoperative discomfort, and lessened complications, AlloDerm[TM] grafts are being used in dentistry as an alternative to autogenous free gingival grafts to increase the zone of keratinized gingiva around teeth and implants, root coverage, and ridge preservation procedures (Callan and Silverstein 1998, Harris 1998). It does not have to be removed and can be left exposed to the oral cavity while increasing keratinized tissue. AlloDerm[TM] was consistently integrated into host tissue, maintaining structural integrity of the tissue and revascularization via preserved vascular channels (Harris 1998).

Comparative studies of AlloDerm[TM] (ADM) and Connective Tissue Grafts (CTG)

There have been few studies comparing ADM with CTG for the treatment of gingival recession. Harris (2000) treated 107 recession defects and found no statistically significant difference in the mean root coverage obtained (96.2% in CTG and 95.8% in ADM, respectively). There was a statistically significant reduction in probing depth (1.2 mm in CTG and 0.7 mm in ADM) and an increase in keratinized tissue (2.0 mm in CTG and 1.2

mm in ADM) in both groups. It was observed that the connective tissue graft produced a greater mean probing reduction and mean keratinized tissue increase in acellular dermal matrix but did not appear to be clinically significant. it was also reported that both procedures were aesthetically acceptable to the patients and clinically acceptable in all cases.

Novaes *et al* (2001) randomly assigned a total of 30 recession treated to CTG and ADM. The authors found that there were no statistically significant differences between the two groups in terms of recession reduction, clinical attachment gain, and reduction in probing depth. The control group (CTG) had a statistically significant increased area of keratinized tissue after 3 months compared to the test group. However, both procedures resulted in an increase in keratinized tissue after 6 months, with no statistical difference. In another study involving 30 recession defects done by Paolantonio *et al* (2002), a similar conclusion was obtained. However, the autogenous CTG showed a significantly greater increase in the width of keratinized tissue, and a shorter complete healing period. Tal *et al* (2002) who treated 14 recessions had concluded that recession defects may be covered using ADM or CTG with no practical difference. It was found that CTG resulted in a significantly greater gain of keratinized gingiva.

The efficacies of ADM and CTG with time have been evaluated by Harris (2004). It was reported that in the short term (mean 12.3 to 13.2 weeks) the mean root coverage for ADM was 93.4% and subepithelial CTG was 96.6%. The long term (48.1 to 49.2 months) results for CTG was 97.0% and for ADM was 65.8% only. The comparison of these results was statistically significant. Thus the author concluded that the mean results with CTG were better than those achieved with ADM. However, 32% of ADM cases improved or remained stable with time. Hirsch *et al* (2005)

compared the effectiveness of CTG and ADM in achieving root coverage two years postoperatively. The study involved 101 patients who were treated with dermal matrix allograft and 65 patients with connective tissue graft. Their results indicated that the root coverage by subpedicle ADM or CTG was a very predictable procedure which is stable for two years postoperatively. However CTG demonstrated significant increases in defect coverage, keratinized gingiva, and residual probing depth.

In a recent report by Rahmani and Lades (2006), a total of 20 gingival recessions of Miller Class I and II were selected and randomized into subepithelial CTG and ADM. The percentage of root coverage for the subepithelial CTG and ADM groups were 70.12% ±22.81% and 72.08% ±14.12%, respectively. The changes from baseline to the 6 month visit were significant for both groups in terms of all parameters but probing depth. However, there was no significant difference between ADM and CTG in terms of mean changes in any parameters 6 months postoperatively.

Based on the findings in the literature, ADM and CTG appear to be successful in treating recession. Most studies have shown insignificant difference comparing the two approaches. Despite the slightly more satisfactory results, CTG is however a two-sites surgical procedure leaving a wound of considerable size in the palatal donor area, and the supply of the donor tissue is limited. Hence, the use of ADM is more convenient as only one operating site is involved and a greater patient compliance can be expected. ADM is commercially available in a variety of sizes and hence could be a suitable substitution in treating both single and multiple recession defects in one surgical procedure. These studies have been carried out in Caucasian groups which generally have thicker biotype whereas Asian populations

generally have a thinner gingival biotype. Since there is a dearth of literature in the latter population to investigate the effect of these two tissue grafts, the current investigation was being undertaken.

Objectives of the study

To assess and compare the clinical success of subepithelial connective tissue graft (sCTG) and acellular dermal matrix (ADM) allograft (AlloDerm™) in the treatment of gingival recessions in relation to recession height and width in Malaysian population. In addition, reduction of probing pocket depth, increase in probing clinical attachment level and increase in width of keratinized gingiva were assessed.

Materials and methods

This is a prospective observational study of a case series and was approved by the Ethics Committee, Faculty of Dentistry, University of Malaya, Kuala Lumpur. Details of the study were provided and written consent was obtained. Prior to the study, a reproducibility study was done to validate intra-examiner reproducibility.

Study population

Both males and females between 20-60 years of age who presented with Miller's Class I and II recession defects were considered from a pool of patients seeking treatment at the Dental Clinic, Faculty of Dentistry, University of Malaya, Kuala Lumpur, and all relevant clinical measurements were performed by the co-author. Six patients (3 males and 3 females), ranging from 23 to 58 years of age (mean = 37.8 years) with eight sites of gingival recessions participated. The patients met the following inclusion criteria: non-compromised systemic health and no

contraindications for periodontal surgery, with gingival recession ≥ 3 mm in the apico-coronal dimension on the facial aspects of incisors, canine or premolars, no pathological periodontal pockets in the defect area, no restorations in area to be treated, were non-smokers and not pregnant.

Pre-surgical treatment

All patients initially completed a plaque control program and were instructed to avoid brushing techniques which could cause further damage to the marginal tissue and to avoid using a toothbrush with hard bristles or abrasive toothpaste. After two weeks of initial therapy, the patients were re-evaluated for oral hygiene and gingival inflammation. Surgery was scheduled if the patient had an optimal plaque index of $\leq 20\%$.

Clinical measurements and records

Baseline measurements of the parameters and clinical photographs were taken on the day of the surgery. Full mouth plaque scores and full mouth bleeding scores were recorded as the percentage (%) of tooth surfaces with plaque and bleeding after probing. The plaque scoring was done on four surfaces of each tooth present in the mouth whereas bleeding score was recorded on six sites. Measurements were made at mid-facial/buccal aspects of each tooth along the defect for recession height (RH) which was measured from cemento-enamel junction (CEJ) to the free gingival margin, recession width (RW) was measured at the widest point mesio-distally, probing pocket depths (PPD) and clinical attachment loss (CAL) were measured as the distance from the base of the pocket to the gingival margin and CEJ, respectively and keratinized gingiva was measured from the gingival margin to mucogingival junction (MGJ). These measurements were made to the nearest

half millimeter using a calibrated straight periodontal probe (UNC15 Hu Friedy, Chicago, IL, USA). All clinical data were recorded at three intervals by the author: at baseline, 3 months and 6 months post-surgically. Acrylic stents was used as reference points to determine the exact sites of measurements, ensuring reproducibility during re-evaluation assessments for all the patients. Recession sites were randomly assigned to the test group or control group. Randomization was done by a SPSS statistical package. In the control group, the exposed root surfaces were treated with a connective tissue graft (sCTG) combined with a coronally repositioned flap. An acellular dermal matrix (ADM) allograft was used, as a substitute for palatal donor tissue, in the test group.

Surgical procedures

All patients were treated by the co-author, closely supervised by the principle author. Patients rinsed with 0.12% Chlorhexidine gluconate solution prior to the surgery.

Recipient bed preparations

Local anaesthesia was administered using 2% lignocaine HCL with 1:100,000 epinephrine. Intrasulcular incisions were placed on the facial / buccal aspect of the experimental tooth and connected to horizontal incisions interdentally (Figure 1). These small horizontal incisions were made at CEJ level. The upper part of the papillae was de-epithelialized. At the end of these horizontal incisions, two vertical releasing incisions were placed which were extended apically beyond MGJ. The design of the flaps was trapezoidal to provide optimal vascular circulation. A partial thickness flap was reflected (Figure 2) using double-sided microsurgical blade. To allow passive repositioning of the flap, sharp dissection of

the periosteal fibers at the apical extent of the flap was done. The exposed root surfaces were cleaned and planed with ultrasonic scaler and Gracey's hand curettes to remove calculus, caries, and softened tooth structures. A fine fissured-shaped diamond bur was used to smooth any obvious irregularities or sharp edges. Root surfaces were then conditioned with tetracycline solution (125 mg/ml saline) (Figure 3) and subsequently thoroughly flushed with sterile normal saline.

Test group

Recession sites in this group received AlloDerm™ (Figure 4) which had been rehydrated in sterile saline according to the manufacturer's instructions. The rehydrated graft was soft and pliable and it was ready for application to the recipient bed. AlloDerm™ has a distinct basement membrane (upper) and dermal surface (lower). To determine its orientation once the graft had been rehydrated, a drop of patient's blood was added to both sides. The dermal side had a bloody appearance, whereas the basement membrane side appeared pinkish (Figure 5). The allograft was trimmed to the desired dimension (Figure 6). In this study the ADM allograft was oriented so that the connective tissue (dermal side) was placed against the denuded root surface, with the basement membrane facing up, as recommended by the manufacturer.

Control group

The recipient beds received sCTG, harvested from same side of the hard palate, in the region of the second premolar to first molar. The desired size of the graft was estimated using a sterilized tinfoil template, wide enough to cover the recipient bed mesiodistally and coronoapically. The sCTG procedure carried out in this study was a modification of the method described by

Figure 1. Outline of the incision on tooth 24

Figure 2. A partial thickness flap was reflected and tip of the interdental papillae were de-epithelialized

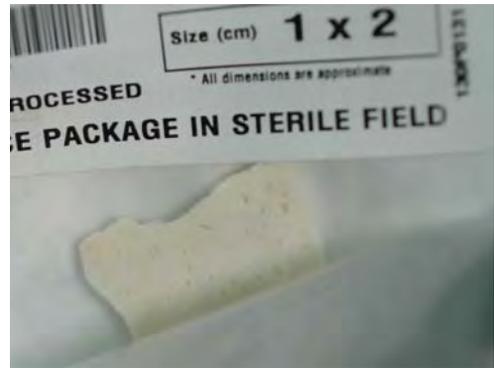**Figure 3.** Root conditioning done with tetracycline solution (125 mg/ml)

Figure 4. Acellular dermal matrix allograft (AlloDerm™) in the inner pouch of the packaging

Figure 5. A fully rehydrated AlloDerm™ in sterile dish after adding drops of patient's blood on its surface

Figure 6. AlloDerm™ was trimmed to get the desired size and dimension

Figure 7. Sounding of bone with a periodontal probe

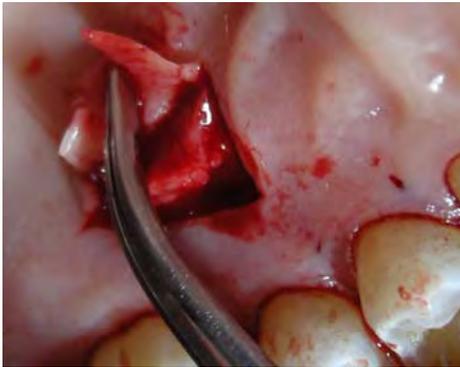**Figure 8.** The outline incision on the palatal donor site

Figure 9. The 'trap door' was established

Figure 10. The harvested donor tissue corresponds to the size of tin foil template

Figure 11. The palatal site was sutured

Figure 12. AlloDerm™ was adapted and secured on the recipient bed of 34, tooth 33 with a 2 mm recession was included

Figure 13. Subepithelial connective tissue graft was adapted and secured on tooth 24

Figure 14. A coronally positioned flap was done in an attempt to fully cover the AlloDerm™

Figure 15. A coronally positioned flap was done to cover the entire autogenous graft

Langer and Langer (1985). Sounding with a periodontal probe was done (Figure 7) to estimate harvested tissue thickness. An initial horizontal incision parallel to the gingival margin was made with a vertical (perpendicular) incision on mesial end of this initial incision creating an 'L-shaped' trapped door (Figure 8). A partial thickness flap was reflected and a connective tissue graft of 1.5-2 mm thickness was harvested (Figures 9 and 10). The palatal flap was repositioned sutured with Vicryl 5/0 sutures (Figure 11).

Adaptation of grafts to the recipient beds

The donor grafts were then secured to the

periosteum and adjacent connective tissue by placing resorbable sutures (Figures 12 and 13). The overlying partial thickness gingival flaps were coronally advanced and positioned in an attempt to cover the entire ADM/sCTG (Figures 14 and 15). The flaps were sutured without tension. To reduce the incidence of hematoma formation, direct pressure on the surgical sites was applied postoperatively for a few minutes to ensure close adaptation of the mucoperiosteum and the grafts to the bone and root surfaces.

Post operative instructions

All patients underwent similar postsurgical management, consisting of the administration of analgesic 400 mg ibuprofen tds for 3-5 days, 0.12% Chlorhexidine gluconate mouthrinse 2 times daily for 8 weeks, Gengigel spray 3 times daily over the wound was advocated. The patients were instructed in appropriate postoperative home care including soft diet, discontinuation of tooth brushing and to avoid trauma or pressure at the surgical site. Sutures were removed after 15 days post-surgically, in most cases. After 8-10 weeks, patients were allowed to resume mechanical tooth cleaning of the treated areas using a soft postsurgical toothbrush. Flossing was not resumed in the area until 3 months postoperatively. Patients

were reviewed weekly for the first month, then fortnightly for 12 weeks (3 months), and then monthly until 6 months post-operatively. All clinical measurements were obtained at 3 and 6 months postoperatively.

Results

Statistical analysis

Microsoft Excel was used to perform the data analysis. The descriptive statistics are expressed as means ± standard deviations (SD). As the sample size was small, effect size was reported to assess the strength of the relationship of the treatment rendered and to determine whether a statistically significant difference is a difference of practical concern. To interpret the resulting magnitude, a general guide developed by Cohen (1988) was used: an effect size of ~0.20 is a small effect size, 0.50 is a moderate effect size and ≥0.8 is a large effect size. Analysis of the clinical data between the experimental groups was made by comparing the mean values at baseline, 3 months and 6 months and mean changes throughout the study period.

Baseline data

Four recession sites with similar sized initial defects were treated with ADM and four sites were treated with sCTG. There was no statistically significant difference seen in all parameters between test and control groups (Table 1).

Root coverage

Root coverage obtained ranged from 17-100% in the eight sites, with mean range of 54.2% to 71.75% (Table 2). If site 8, which appeared to be an outlier, was to be excluded higher root coverage was obtained, ranging from 50% to 100%. Root coverage percentage

was higher in the ADM group (71.75 ± 19.12) at both the 3 and 6 month intervals in comparison to sCTG group (58.25 ± 21.56, 54.25 ±34.24, respectively). There was no statistically significant difference between the outcomes of the two groups.

Changes in clinical parameters within the groups

Recession height

Both the ADM and sCTG groups showed statistically significant reduction in *recession height* from the baseline to both 3 months and to 6 months within groups (p<0.05) (Tables 3 and 4). In sites treated with ADM grafts, mean reductions in recession height at 3 and 6 months were greater as compared to sCTG. Effect size indicated that these reductions in *recession height* were large within both the ADM and sCTG group. The ADM group (ES = 5.50) showed effect size of approximately twice that of sCTG (ES = 2.71) at 3 months and 3.5 times reduction in recession height from baseline to 6 months (ES of ADM = 5.5 and sCTG = 1.58) (Tables 3 and 4).

Recession width

There were no statistical differences (p>0.05) observed in RW within each group from baseline to 3 months and to 6 months (Tables 3 and 4). Despite the absence of significant differences, the ADM group demonstrated moderate clinical changes in recession width from baseline to 3 months and to 6 months (ES = 0.71). On the other hand, the sCTG group showed relatively large clinical changes from baseline to 3 months and to 6 months (ES = 1.22 and 0.87, respectively).

Probing pocket depth

There were no statistically significant

Clinical parameters	Test group (ADM)	Control group (sCTG)	p value
Recession height	4.00 ± 1.15 mm	3.00 ± 0.00 mm	0.134 (NS)
Recession width	3.00 ± 0.82 mm	3.72 ± 0.50 mm	0.310 (NS)
Probing pocket depth	1.00 ± 0.00 mm	1.00 ± 0.00 mm	- (NS)
Keratinized gingiva	1.25 ± 1.50 mm	3.50 ± 1.29 mm	0.159 (NS)
Clinical attachment loss	5.00 ± 1.15 mm	4.00 ± 0.00 mm	0.267 (NS)
Plaque score	16.00 ± 3.80 %	13.98 ± 2.86 %	0.569 (NS)
Bleeding scores	17.05 ± 3.02 %	11.65 ± 4.82 %	0.228 (NS)

Table 1. Comparison of the means of the pre-operative measurements in control and test groups (α = 0.05, NS = not significant)

Site	Tooth	Treatment approach	Recession defect (mm)	Recession coverage 3 months mm (%)	6 months mm (%)
1	22	sCTG	3.0	2.5 (83)	3.0 (100)
2	41	ADM	5.0	3.0 (60)	3.0 (60)
3	31	ADM	5.0	3.0 (60)	3.0 (60)
4	13	ADM	3.0	3.0 (100)	3.0 (100)
5	14	sCTG	3.0	1.5 (50)	1.5 (50)
6	24	sCTG	3.0	2.0 (67)	1.5 (50)
7	34	ADM	3.0	2.0 (67)	2.0 (67)
8	31	sCTG	3.0	1.0 (33)	0.5 (17)
	Mean	sCTG	3.00±0.00	1.75±0.65 (58.25±21.56)	1.63±1.03 (54.25±34.24)
		ADM	4.00±1.15	2.75±0.50 (71.75±19.12)	2.75±0.50 (71.75±19.12)
	p		0.134	0.532	0.551
	Effect size (ES)		1.23	0.468	0.446

Table 2. Root coverage (measurements and proportions) obtained 3 months and 6 months post-operatively at the eight sites treated by ADM and sCTG

Clinical Parameter	Baseline	3 mth postop	Change	p value	Effect size(ES)
Recession Height					
ADM	4.00+1.15	1.25+0.96	2.75+0.50	0.002*	5.50
sCTG	3.00+0.00	1.25+0.65	1.75+0.65	0.012*	2.71
Recession Width					
ADM	3.00+0.82	2.00+1.41	1.00+1.41	0.252	0.71
sCTG	3.75+0.50	2.75+0.96	1.00+0.82	0.092	1.22
Probing Pocket Depth					
ADM	1.00+0.00	1.25+0.50	0.25+0.50	0.391	0.50
sCTG	1.00+0.00	1.25+0.50	0.25+0.50	0.391	0.50
Clinical Attachment Loss					
ADM	5.00+1.15	2.50+1.00	2.50+1.00	0.015*	2.50
sCTG	4.00+0.00	2.50+0.71	1.50+0.71	0.024*	2.12
Keratinized Gingiva					
ADM	1.25+1.50	4.25+0.50	3.00+1.41	0.024*	2.12
sCTG	3.50+1.29	5.50+1.91	2.00+2.16	0.161	0.93
Plaque Score					
ADM	16.00+3.80	15.55+3.55	0.45+0.52	0.182	0.87
sCTG	13.98+2.86	13.98+2.29	0.00+0.65	1.00	0.00
Bleeding Score					
ADM	17.05+3.02	16.15+2.01	0.90+1.04	0.182	0.87
sCTG	11.65+4.82	8.85+5.90	2.80+3.33	0.191	0.84

Table 3. Changes in clinical parameters from baseline to 3 months post-operative within groups

differences in probing pocket depth within both groups at all intervals (p> 0.05, Tables 3 - 5). Mean changes in probing pocket depth increased at the 3 month interval in both groups and then decreased from 3 to 6 months in both groups.

Clinical attachment gain

As shown in Tables 3 and 4, there were statistically significant differences (p<0.05) within each group from baseline to 3 and to 6 months in clinical attachment gain. The effect size values indicated large changes from baseline to 3 months in both groups (ES = 2.50 in ADM, ES = 2.12 in sCTG) (Table 3). From baseline to 6 months (Table 4), there were greater gains of clinical attachment in ADM (ES = 5.50) as compared to sCTG (ES = 1.58).

Keratinized gingiva

Width of keratinized gingiva from baseline of the study to 3 months and to 6 months demonstrated statistically significant mean increase (p<0.05) in the ADM group, whereas the sCTG group did not show any statistically significant mean change. The effect size values in both the ADM (ES = 2.12) and sCTG (ES = 0.92) groups indicated that large changes had taken place clinically from baseline to 3 months and to 6 months (Tables 3 and 4). The width of keratinized gingiva in ADM group remained constant after 3 months

Clinical Parameter	Baseline	6 mth postop	Change	p value	Effect size(ES)
Recession Height					
ADM	4.00+1.15	1.25+0.96	2.75+0.50	0.002*	5.50
sCTG	3.00+0.00	1.38+1.03	1.63+1.03	0.051*	1.58
Recession Width					
ADM	3.00+0.82	2.00+1.41	1.00+1.41	0.252	0.71
sCTG	3.75+0.50	2.25+1.50	1.50+1.73	0.182	0.87
Probing Pocket Depth					
ADM	1.00+0.00	1.00+0.00	-	-	-
sCTG	1.00+0.00	1.00+0.00	-	-	-
Clinical Attachment Loss					
ADM	5.00+1.15	2.25+0.96	2.75+0.50	0.002*	5.50
sCTG	4.00+0.00	2.38+1.03	1.63+1.03	0.051*	1.58
Keratinized Gingiva					
ADM	1.25+1.50	4.25+0.50	3.00+1.41	0.024*	2.12
sCTG	3.50+1.29	5.50+1.50	1.75+1.89	0.162	0.92
Plaque Score					
ADM	16.00+3.80	12.55+2.83	3.45+3.23	0.122	1.07
sCTG	13.98+2.86	11.25+1.70	2.73+2.17	0.087	1.25
Bleeding Score					
ADM	17.05+3.02	14.10+3.49	2.95+1.98	0.059	1.49
sCTG	11.65+4.82	7.50+5.10	4.15+2.99	0.069	1.39

Table 4. Changes in clinical parameters from baseline to 6 months post-operative within groups

(Table 5).

Full mouth plaque score and bleeding scores

There were no statistically significant differences (p>0.05) seen in plaque score and bleeding scores within each group throughout the study period (Tables 3-5). Both study groups demonstrated low percentage of plaque score and bleeding scores (≤20%) throughout the study.

Changes in clinical parameters between the groups

Recession height

The difference in mean reductions in recession height between the two groups was statistically significant (p = 0.049) from baseline to 3 months. However this difference was not significant from baseline to 6 months. Effect size values indicated that the differences in the reductions were considerably large at both time intervals (ES = 1.73 at 3 months; ES = 1.39 at 6 months).

Clinical Parameter	3 mth postop	6 mth postop	Change	p value	Effect size(ES)
Recession Height					
ADM	1.25±0.96	1.25±0.96	-	-	-
sCTG	1.25±0.65	1.38±1.03	-0.13±0.48	0.638	0.26
Recession Width					
ADM	2.00±1.41	2.00±1.41	-	-	-
sCTG	2.75±0.96	2.25±1.50	0.50±2.38	0.703	0.21
Probing Pocket Depth					
ADM	1.25±0.50	1.00±0.00	0.25±0.50	0.391	0.50
sCTG	1.25±0.50	1.00±0.00	0.25±0.50	0.391	0.50
Clinical Attachment Loss					
ADM	2.50±1.00	2.25±0.96	0.25±0.50	0.391	0.50
sCTG	2.50±0.71	2.38±1.03	0.13±0.48	0.638	0.26
Keratinized Gingiva					
ADM	4.25±0.50	4.25±0.50	-	-	-
sCTG	5.50±1.91	5.50±1.50	0.25±0.50	0.391	0.50
Plaque Score					
ADM	15.55±3.55	12.55±2.83	3.00±2.72	0.114	1.10
sCTG	13.98±2.29	11.25±1.70	3.00±2.72	0.114	1.10
Bleeding Score					
ADM	16.15±2.01	14.10±3.49	2.05±2.37	0.182	0.87
sCTG	8.85±5.90	7.50±5.10	1.35±1.57	0.183	0.86

Table 5. Changes in clinical parameters from 3 to 6 months post-operative within groups

Recession width

There were no statistically significant differences in mean changes in RW (p>0.05) between the two groups at all time intervals. Only small clinical changes between the groups from baseline to 6 months (ES = 0.32) were observed.

Probing pocket depth

There were no statistically significant differences (p>0.05) in mean probing pocket depth changes between the groups throughout the study period.
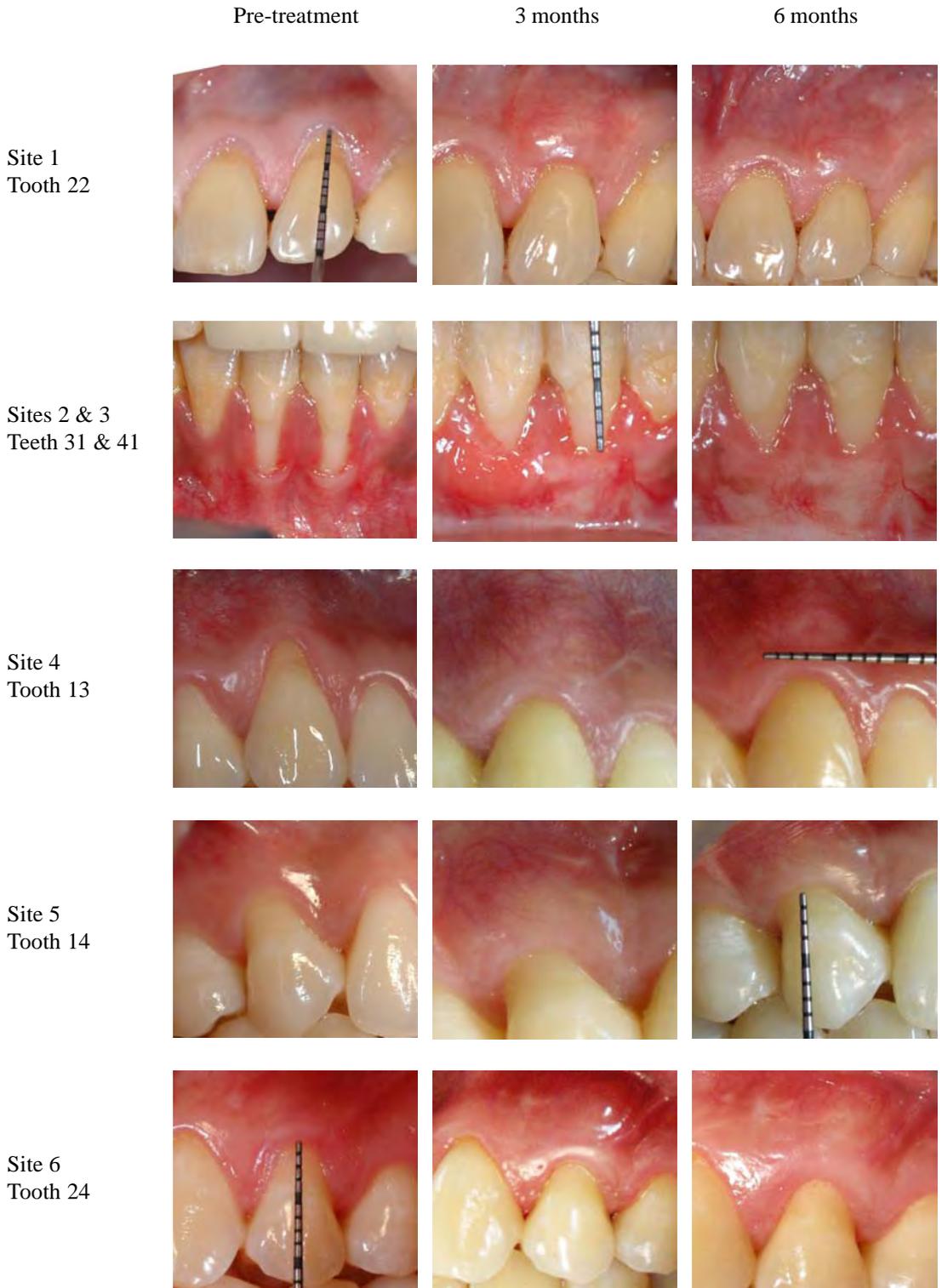
Clinical attachment gain

There were no significant differences in mean changes in clinical attachment gain between the ADM and sCTG groups throughout the study period. There were larger mean differences in clinical attachment gain between the two groups from baseline to 3 months (ES = 1.15) and to 6 months (ES = 1.39).

Keratinized gingiva

There were no statistically significant differences (p>0.05) in keratinized gingiva width increase between the two groups at all time intervals. Throughout the study period,

Clinical Parameter	Changes (mean±SD)		Difference in Changes (mean±SD)	
	BL-3mth	BL-6mth	BL-3mth	BL-6mth
Recession Height				
ADM	2.75±0.50	2.75±0.50	1.00±0.58	1.13±0.81
sCTG	1.75±0.65	1.63±1.03		
p value			0.049*	0.097
Effect Size			1.73	1.39
Recession Width				
ADM	1.00±1.41	1.00±1.41	0.00±1.15	0.50±1.58
sCTG	1.00±0.82	1.50±1.73		
p value			1	0.67
Effect Size			0	0.32
Probing Pocket Depth				
ADM	0.25±0.50	-	0.00±0.50	0.00±0.00
sCTG	0.25±0.50	-		
p value			1	-
Effect Size			0	-
Clinical Attachment Gain				
ADM	2.50±1.00	2.75±0.50	1.00±0.87	1.13±0.81
sCTG	1.50±0.71	1.63±1.03		
p value			0.154	0.097
Effect Size			1.15	1.39
Keratinized Gingiva				
ADM	3.00±1.41	3.00±1.41	1.00±1.83	1.25±1.67
sCTG	2.00±2.16	1.75±1.89		
p value			0.468	0.331
Effect Size			0.55	0.75
Plaque Score				
ADM	0.00±0.65	3.45±3.23	0.45±0.59	0.73±2.75
sCTG	0.00±0.65	2.73±2.17		
p value			0.32	0.722
Effect Size			0.77	0.26
Bleeding Score				
ADM	0.90±1.04	2.95±1.98	1.90±2.46	1.20±2.53
sCTG	2.80±3.33	4.15±2.99		
p value			0.317	0.528
Effect Size			0.77	0.47

Table 6. Comparison of means in clinical parameters at baseline and mean changes at 3 to 6 months post-operatively between test and control groups

	Pre-treatment	3 months	6 months
Site 1 Tooth 22			
Sites 2 & 3 Teeth 31 & 41			
Site 4 Tooth 13			
Site 5 Tooth 14			
Site 6 Tooth 24			

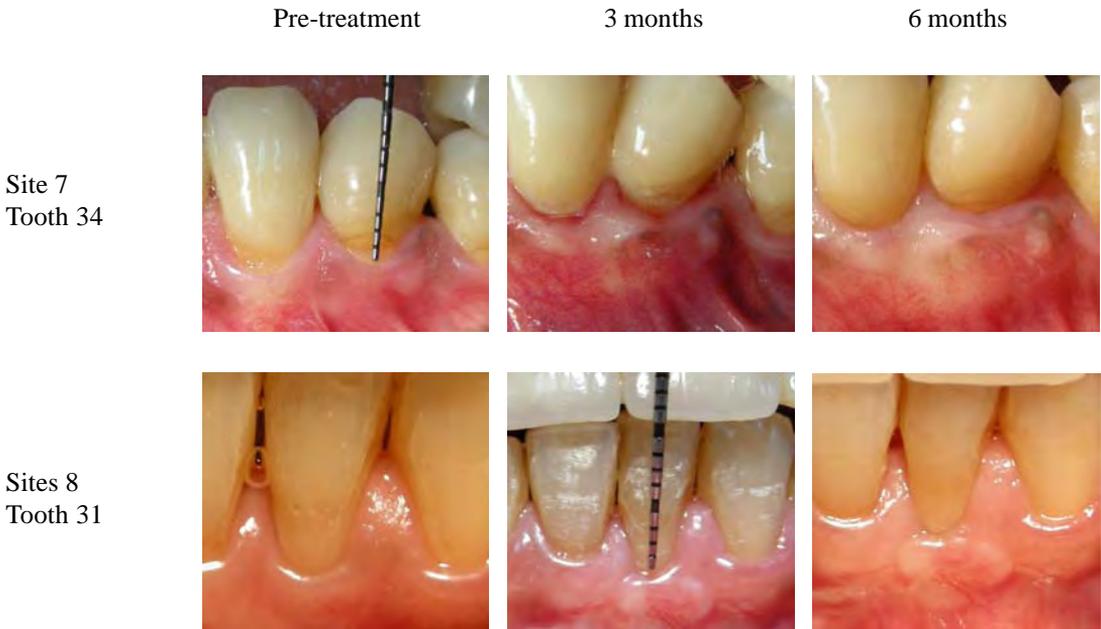|	Pre-treatment	3 months	6 months

Figure 16. Baseline and post-surgical clinical photographs of eight test and control sites

there were moderate clinical differences in mean changes (ES = 0.55-0.75).

Full mouth plaque score and bleeding scores

There were no significant differences in mean percentage changes for full mouth plaque score and bleeding scores between the groups throughout the study period

Discussion

Study design and sample selection

Due to the strictly defined criteria for sample selection, difficulty was experienced in obtaining an adequate sample size within the time frame of one and a half years. The main obstacle encountered was locating patients who were willing to treat recessions surgically. Patients rarely presented with bilateral recession defects that would meet the

inclusion criteria, thus a split mouth design could not be adhered to and a parallel design study was resorted to. Some potential patients opted out because of personal reasons, an inability to commit time for the postoperative review appointments and a lack of awareness of surgical solutions. It is often difficult to anticipate the success rate of the surgical approach since root coverage depends on several factors such as type and location of the defect and the technique used. Clinical measurements were done using a UNC15 periodontal probe because it has more distinct graduated and zone marking and standardized diameter.

Surgical procedures

The sCTG combined with coronally repositioned flap techniques were chosen as the treatment of choice as they have yielded a higher percentage of root coverage compared with other surgical techniques (Wennström

and Zucchelli 1996, Oates *et al* 2003). The use of coronally repositioned flaps was first reported by Bernimoulin *et al* (1975) and the requirements for success include shallow probing pocket depths on proximal surfaces, reduction of any root prominence within the plane of adjacent alveolar bone, adequate release of the flap to prevent retraction during healing and normal interproximal bone height. The flap was positioned coronally in an attempt to cover the graft so it could benefit from a double blood supply.

Langer and Langer (1985) reported an increase of 2 to 6 mm of root coverage over four years. Wennström and Zucchelli (1996) compared a coronally advanced flap (CAF) to a combination of CAF and CTG procedure in which Miller's I and II defects were treated. They found the success rate for the combination group to be higher (98.9%), as compared to CAF (97.0%). Placement of sCTG under a coronally repositioned flap allows the connective tissue of the palatal masticatory mucosa to differentiate the epithelial cells of the thin covering flap into keratinizing cells (Karring *et al* 1971).

The acellular dermal matrix (ADM), an alternative, was oriented so that the connective tissue side was placed against the denuded root surface, with the basement membrane facing up, as recommended by the manufacturer. Henderson *et al* (2001) conducted a study to determine which side of the ADM graft should be close to the root surfaces and found that there was no difference between the two surfaces of the soft tissue graft with a mean root coverage of 93%.

Statistical analysis and result

In this study, there was no difference in clinical parameters at baseline; showing that no bias was introduced into the study with respect to the initial defects in each groups. Due to a very small sample size, calculation of the effect size was done along with investigation of statistical significance. Unlike significance testing, effect size measures the magnitude of a treatment effect irrespective of sample size. It was reported to assess the strength of the relationship of the treatment rendered and to determine whether a statistically significant difference is a difference of practical concern. Zakzanis (2001) concluded that it is crucial to appreciate that 'non-significance' finding is not the same as 'no effect', as it is quite possible for a meaningful effect to be present although the statistical test lacks sufficient power to detect it at the desired significance level due to a small sample size or imprecise research design.

Post-operative healing and complications

All the patients tolerated the surgical procedures well, reported minimal discomfort and all patients in sCTG group did not experience painful palatal wound following the surgery. There was no major complications encountered during the surgery. However, a hematoma developed on site 8 following the surgery and site 7 was accidently hit by the patient's child a week and a half after surgery. All results were considered to be clinically and esthetically acceptable to the patients and the clinician (Figure 16). It was observed that areas treated with sCTG tended to be more bulky than those treated with the ADM. In all cases, including sites where root coverage was not completely obtained, patients were satisfied and claimed that hypersensitivity experienced in the area was significantly reduced. It was noted for all cases with the exception of the patient with site 5, patients were willing to undergo the procedure again if it became necessary to treat another area in the mouth.

Root coverage and reduction in recession height

In this study, mean root coverage was higher in ADM group (71.75%) at 6 months post-operatively compared to sCTG group (54.25%). However the difference between groups was not statistically significant. Thus it implies that both surgical procedures are useful in clinical practice and almost equally effective in its objective. In addition, these rates of mean root coverage may largely be influenced by the small sample size.

It was noted that a hematoma formed in one of the sCTG patients (site 8) which could have compromised the healing, thus affecting the outcome. Since the gingival tissue in the area was thin, the capillary beds could have been traumatized during the surgery when blood was extravasated into the perivascular connective tissue. This resulted in poor circulation at the site which allowed pooling of the blood, prostaglandin release, and a more profound inflammatory reaction. The presence of a hematoma (thick clot) would prevent the direct apposition of the flap to the bone and denuded root surfaces. This could probably have resulted in a lack of early wound strength recovery that could have been achieved by epithelium of a new dentogingival junction (Wirthlin *et al* 1980).

In site 7, even though the site suffered trauma, the root coverage obtained was considerably acceptable (67%). The tissue was traumatized at week 1.5, thus primary wound strength could have been achieved at the site, perhaps partially if not completely.

Overall, root coverage obtained in this study ranged from 17-100% in eight sites, with mean coverage ranging from 54.2% for sCTG to 71.75 for ADM. If site 8 were to be excluded, higher root coverage was obtained ranging from 50% to 100% for both ADM and sCTG approaches. Hence, the results of the present study compare relatively well with other studies using connective tissue graft combined with pedicle graft, in which mean root coverage of 69.2% to 98.9% have been reported by various authors (Raetzke 1985, Nelson 1987, Harris 1992, Allen 1994, Wennström and Zucchelli 1996, Harris 1997, Harris 1998).

Despite incomplete root coverage as seen in most sites in the present study, an increase in gingival height, independent of the number of millimetres, is considered to be successful outcome of gingival augmentation procedures (Wennström and Zucchelli 1996) as reflected by the effect size values obtained in this study.

Further reduction in apico-coronal dimensions in soft tissue graft cases may be obtained in later phase of healing and is referred to 'creeping attachment' which was described as 'postoperative migration of the gingival margin in a coronal direction over a previously denuded root (Goldman *et al* 1964). This migration is often seen to continue for long periods postoperatively until a constant marginal level is reached (Goldman *et al* 1964). In the present study, optimal creeping attachment may not have occurred as the final data collection was at the 6 month interval where this phenomenon may not have been achieved as most authors have reported this 'creep' at approximately 1 year postoperatively (Matter 1980, Borghetti and Gardella 1990).

Site 1 (treated with sCTG) showed a gingival increase over the recession of 0.5 mm from 3 months to 6 months post-operatively thereby resulting in 100% coverage at 6 months. This may be explained by the creeping attachment that could have occurred at a later phase of healing. Borghetti and Gardella (1990) stated that any root coverage that occurs 1 month postoperatively should be considered as the result of creeping attachment. Various amounts of creeping attachment have been documented for soft tissue autografts: 0.43 mm, 0.85 mm and 0.89

mm (Piniprato *et al* 2000, Borghetti and Gardella 1990, Harris 1997, Matter 1980).

Both ADM and sCTG groups showed significant reduction in recession height throughout the study. At sites treated with ADM grafts, mean reduction in recession height at 3 and 6 months were higher (2.75 mm) as compared to sCTG (1.75 mm at 3 months, 1.63 mm at 6 months). These reductions in recession height within each group were statistically significant. In addition, the effect size values reflected that these were large clinical changes that occurred within the groups. However, there were no changes that took place in recession height after 3 months interval in sites treated with ADM whereas in the sCTG group, the amount of reduction in recession height decreased. This was probably due to similar reasons described earlier for root coverage (i.e. hematoma formation), since root coverage was reported based on recession height level.

There were statistically significant differences in mean recession height reduction from baseline to 3 months ($p = 0.049$) between the groups. However, the mean reduction in recession height was not significant at 6 months postoperatively from baseline between the groups. This revealed that the ADM and sCTG procedures gave slight difference in a short term period of 3 months but over 6 months, they provided similar outcomes. The clinical significance of these findings needs to be considered. The differences in recession height reduction of approximately 1.00 mm at 3 and 6 months between the two techniques are probably not large enough to be detected or considered important in most clinical situations.

The changes in mean recession reduction from 3 to 6 months in both groups did not show any significant difference. Furthermore, the effect size value indicated that considerably small changes occurred clinically within and between the groups from 3 to 6 months. This suggests that the new gingival levels were relatively stable by 3 months in both groups.

Increase in keratinized gingiva width and clinical attachment gain

The gain in the width of keratinized gingiva and clinical attachment level is of clinical importance. There was increase in width of keratinized gingiva and large clinical improvements were observed within both ADM and CTG groups. The sites treated with ADM showed greater statistically significant increases in keratinized gingiva. However, no significant difference in the increase in keratinized gingiva width was seen postoperatively when the two approaches were compared.

It is commonly believed that a connective tissue graft will contribute to keratinization of the overlying epithelium. However, in this study, an increase in the amount of keratinized gingiva also occurred in the defects treated with ADM, also observed by Harris (2000). This may possibly be due to the fact that the skin from which the ADM is derived is naturally a keratinized tissue and hence may possess the inherent property to induce keratinisation.

Although the gingival thickness was not determined in the present study, several investigators have highlighted this issue. Woodyard *et al* (2004) found a coronally positioned flap over ADM produced significantly greater mean coverage and gingival thickness then a coronally positioned flap alone. Côrtes Ade *et al* (2004) indicated that acceptable root coverage can be achieved in Miller's Class I recession with or without ADM, however a greater thickness of keratinized tissue can be achieved with ADM. In the present study, it was observed that the quantity and quality of keratinized gingiva in all cases were adequate, although with sCTG

the gingiva appeared to be more bulky than those treated with ADM.

There were significant clinical attachment gains within both groups at 3 and 6 months post-operatively. In sites treated with ADM grafts, mean clinical attachment gain at 3 and 6 months were higher (2.50 mm and 2.75 mm, respectively) as compared to sCTG (1.50 mm and 1.63 mm, respectively). In addition, the effect size values reflected that these were large clinical gain within the groups, with greater changes seen in the ADM study group. In the sCTG group, the amount of attachment gain was less. This was probably due to similar reasons described earlier for root coverage and recession height since clinical attachment levels are related to those two measurements. However, there was no significant difference in clinical attachment gain between the two groups throughout the study.

Changes in recession width, probing depth, full mouth plaque and bleeding scores

In this study, there were no significant changes observed in recession width (RW) throughout the study within and between the ADM and sCTG groups. Hence, soft tissue grafting does not seem to significantly improve RW (Rahmani and Lades 2006). Both ADM and sCTG groups did not differ for probing pocket depths measurements, full mouth plaque score and bleeding scores at baseline and throughout the study indicating satisfactory gingival health conditions.

The scores for both full mouth plaque and bleeding scores in both groups were low throughout the study reflecting that the oral hygiene and gingival health of the patients were maintained and improved throughout the study period in both groups. Constant motivation and reinforcement of the oral hygiene during the postoperative recalls could be contributory.

Clinical implications

The most obvious advantage of the acellular dermal matrix is probably the ability to treat multiple defects at the same time, without regard for the size and thickness of the donor area making it possible to treat as many defects as desired. The important advantage of ADM is the exclusion of a second surgical site to acquire the donor tissue for root coverage. This option may be more acceptable to patients and use of allografts will also contribute to a significant reduction in patient morbidity. Irrespective of the financial cost that may be involved, ability to successfully and predictably cover gingival recessions with an allograft rather than an autograft may well serve medically compromised patients and patients with anatomical limitations.

Conclusion

Both ADM and sCTG techniques were effective in the treatment of gingival recessions from the findings of the present study. The amount of root coverage was higher with ADM graft as compared to that obtained with sCTG but the difference was not statistically significant.

Both the ADM and sCTG groups exhibited significant reductions in RH throughout the study. Between the groups, there was a significant difference in RH reduction during the 3 month interval but over 6 months, similar outcomes were obtained. There were small insignificant changes that occurred in mean RH reduction from 3 to 6 months within and between both groups. There was an increase in the width of keratinized gingiva and clinical attachment gain within both groups, with a greater increase in the ADM grafts throughout the study. However, the amount of keratinized gingiva and clinical attachment level did not differ significantly between the groups. There were no significant changes seen in recession

width, probing pocket depth, full mouth plaque scores and bleeding scores throughout the study.

The surgical procedure involving sCTG or ADM in combination with a coronally repositioned flap is considered periodontal plastic surgery, which is technique sensitive and requires clinical skill and experience. As these were performed by a young graduate student, whose experience and skill may not have been equal that of experienced periodontists who have reported their findings, hence the outcomes may be reduced in comparison. The present study was a randomized clinical pilot trial, with a small sample size. A larger sample size will represent better outcomes. The outcomes for root coverage should not only consider the quantitative results (mm and percentage), but to also include patient-oriented outcomes such as aesthetics, satisfaction, root sensitivity, and postoperative morbidities. Histological evaluation may also be considered in future studies.

References

AAP Glossary of Periodontal Terms. (2001) 4th edn. Chicago: American Academy of Periodontology. In: *Concise Encyclopedia of Periodontology*. Vandersall DC, ed. Blackwell Munksgaard; 2007: pp 59-60.

Addy M, Absi EG, Adams D. Dentine hypersensitivity. The effects in vitro of acids and dietary substances on root planed and burred dentine. *J Clin Periodontol* 1987b;14:274-279.

Addy M, Griffiths G, Dummer P, *et al*. The distribution of plaque and gingivitis and the influence of toothbrushing hand in a group of South Wales 11-12 year old children. *J Clin Periodontol* 1987a;14:564-572.

Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. II. Clinical results. *Int J Periodontics Restorative Dent* 1994;14:302-315.

Beck JD, Koch GG. Characteristics of older adults experiencing periodontal attachment loss as gingival recession or probing depth. *J Periodontal Res* 1994;29:290-298.

Bernimoulin JP, Lüscher B, Mühlemann HR. Coronally repositioned periodontal flap. Clinical evaluation after one year. *J Clin Periodontol* 1975;2:1-13.

Borghetti A, Gardella J. Thick gingival autograft for the coverage of gingival recessions: A clinical evaluation. *Int J Periodontics Restorative Dent* 1990;10:216-229.

Callan DP, Silverstein LH. Use of acellular dermal matrix for increasing keratinized tissue around teeth and implants. *Practical Periodontics and Aesthetic Dentistry* 1998;10:731-734.

Cecchi L, Daprile G, Gatto MRA, Pelliccioni GA. Gingival recession and tooth-brushing in an Italian School of Dentistry: a pilot study. *J Clin Periodontol* 1999;26:276-280.

Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd edn. Lawrence Erlbaurn Associates Inc, Hillsdale, NJ. 1988.

Côrtes Ade Q, Martins AG, Nociti FH Jr, *et al*. Coronally positioned flap with or without acellular dermal matrix graft in the treatment of Class I gingival recessions: a randomized controlled clinical study. *J Periodontol* 2004;75:1137-1144.

Davis WB, Winter PJ. The effect of abrasion on enamel and dentine after exposure to dietary acid. *Br Dent J* 1980;148:253-256.

Goldman H, Schluger S, Fox L, Cohen DW. in: *Periodontal. Therapy, 3rd ed*. CV Mosby Co, St Louis. Harris RJ (ed) 1997, pp 560.

Gorman WJ. Prevalence and etiology of gingival recession. *J Periodontol* 1967;38:316-322.

Greene PR. The flexible gingival mask: an aesthetic solution in periodontal practice. *Br Dent J* 1998;184:536-540.

Harris RJ. A comparative study of root coverage obtained with an acellular dermal matrix versus a connective tissue graft: result of 107 recession defects in 50 consecutively treated patients. *Int J Periodontics Restorative Dent* 2000;20:51-59.

Harris RJ. A short term and long term comparison of root coverage with an acellular dermal matrix and subepithelial graft. *J Periodontol* 2004;75:734-743.

Harris RJ. Creeping attachment associated with the connective tissue with partial-thickness double pedicle graft. *J Periodontol* 1997;68:890-899.

Harris RJ. Root coverage with a connective tissue with partial thickness double pedicle graft and an acellular dermal matrix graft: a clinical and histological evaluation of a case report. *J Periodontol* 1998;69:1305-11.

Harris RJ. The connective tissue and partial thickness double pedicle graft: a predictable method of obtaining root coverage. *J Periodontol* 1992;63:477-486.

Henderson RD, Greenwell H, Drisko C, *et al*. Predictable multiple site root coverage using an acellular dermal matrix allograft. *J Periodontol* 2001;72:571-582.

Hirsch A, Goldstein M, Goultschin J, Boyan BD, Schwartz Z. A 2-year follow up of root coverage using subpedicle acellular dermal matrix allograft and subepithelial connective tissue autografts. *J Periodontol* 2005;76:1323-1328.

Holbrook T, Ochsenbein C. Complete coverage of the denuded root surface with a one-stage gingival graft. *Int J Periodontics Restorative Dent* 1983;3:9-27.

Joshipura KJ, Kent RL, DePaola F. Gingival recession: Intraoral Distribution and Associated Factors. *J Periodontol* 1994;65:864-871.

Källestål C, Uhlin S. Buccal attachment loss in Swedish adolescents. *J Clin Periodontol* 1992;19:485-491.

Karring T, Ostergard E, Löe H. Conservation of tissue specificity after heterotopic transplantation of gingiva and alveolar mucosa. *J Periodont Res* 1971;6:282-293.

Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontal* 1985;56:715-720.

Lindhe J, Nyman S. Alterations of the position of the marginal soft tissue following periodontal surgery. *J Clin Periodontol* 1980;7:525-530.

Lindhe J, Socransky SS, Nyman S, Westfelt E. Dimensional alteration of the periodontal tissues following therapy. *Int J Periodontics Restorative Dent* 1987;7:9-21.

Löe H, Åneud A, Boysen H. The natural history of periodontal disease in man: prevalence, severity, extent of gingival recession. *J Periodontol* 1992;63:489-495.

Löst C. Depth of alveolar bone dehiscence in relation to gingival recession. *J Clin Periodontol* 1984;11:583-589.

Matter J. Creeping attachment of free gingival grafts: A five year follow-up study. *J Periodontol* 1980;51:681-685.

Maynard JG. Coronal positioning of a previous placed autogenous gingival graft. *J Periodontol* 1977;48:151-155.

Miller NA. Sutureless gingival grafting. A simplified procedure. *J Clin Periodontol* 1982;9:171-177.

Miller PD. A classification of marginal tissue recession. *Int J Periodontics Restorative Dent* 1985a;5:9-13.

Miller PD. Root coverage using a free soft tissue autograft following citric acid application. III . A successful and predictable procedure in areas of deep-wide recession. *Int J Periodontics Restorative Dent* 1985b;4:14.

Modéer T, Odenrick L. Post-treatment periodontal status of labially erupted maxillary canines. *Acta Odontol Scand* 1980;38:253-256.

Nelson S. The subepithelial connective tissue graft, a bilaminar reconstructive procedure for root coverage of denuded root surfaces. *J Periodontol* 1987;58:94-102.

Novaes AB, Grisi DC, Molina GO, *et al*. Comparative 6 months clinical study of a subepithelial connective tissue graft and acellular dermal matrix graft for the treatment of gingival recession. *J Periodontol* 2001;72:1477-1488.

Oates TW, Robinson M, Gunsolley JC. Surgical therapies for the treatment of gingival recession. A systemic review. *Ann Periodontol* 2003;8:303-320.

Paolantonio M, Dolci M, Esposito P, *et al*. Subepithelial acellular dermal matrix graft and autogenous connective tissue graft in treatment of gingival recessions: a comparative 1-year clinical study. *J Periodontol* 2002;73:1299-1307.

Pini Prato G, Pagliaro U, Baldi C, *et al*. Coronally advanced flap procedure for root coverage. Flap in tension versus flap without tension: a randomized controlled clinical study. *J Periodontol* 2000;71:188-201.

Pini Prato GP, Tinti C, Vincenzi GP, *et al*. Guided tissue regeneration versus mucogingival surgery in the treatment of human buccal gingival recession. *JPeriodontol* 1992;63:919-928.

Raetzke PB. Covering localized areas of root exposure employing the "envelope" technique. *J Periodontol* 1985;56:397-402.

Rahmani ME, Lades MA. Comparative clinical evaluation of acellular dermal matrix allograft and connective tissue graft for treatment of gingival recession. *J Contemp Dent Pract* 2006;7:63-70.

Renton-Harper P, Midda M. NdYAG laser treatment of dentinal hypersensitivity. *Br Dent J* 1992;172:13-16.

Sangnes G, Gjermo P. Prevalence of oral soft and hard tissue lesions related to mechanical tooth cleansing procedures. *Community Dent Oral Epidemiol* 1976;4:77-83.

Serino G, Wennstrõm J, Lindhe J, Eneroth L. The prevalence and distribution of gingival recession in subjects with a high standard of oral hygiene. *J Clin Periodontol* 1994;21:57-63.

Steiner GG, Pearson JK, Ainamo J. Changes of the marginal periodontium as a result of labial tooth movement in monkeys. *J Periodontol* 1981;52:314-20.

Sullivan HC, Atkins JH. Free autogenous gingival grafts I. Principles of successful grafting. *Periodontics* 1968a;6:121-129.

Sullivan HC, Atkins JH. Free autogenous gingival grafts III. Utilization of grafts in the treatment of gingival recession. *Periodontics* 1968b;6:152.

Tal H, Moses O, Zohar R, *et al*. Root coverage of advanced gingival recession: A comparative study between acellular dermal matrix allograft and subepithelial connective tissue graft. *J Periodontol* 2002;73:1405-1411.

Tinti C, Vincenzi GP, Cortellini P. Guided tissue regeneration in the treatment of human recession. A 12-case report. *J Periodontol* 1992;63:554-560.

Trott JR, Love B. An analysis of localized recession in 766 Winnipeg high school students. *Dent Pract* 1966;16:209-213.

Tugnait A, Clerehugh V. Gingival recession – its significance and management. *J Dent*

2001;29:381-394.

Wennström JL, Zucchelli G, Pini Prato GP. Mucogingival therapy – periodontal plastic surgery. In: *Clinical periodontology and implant dentistry 5ᵗʰ edn*. Lang NP, Lindhe J, eds. Blackwell Munksgaard 2008; p 955-964

Wennström JL, Zucchelli G. Increased gingival dimensions. A significant factor for successful outcome of root coverage procedures? A 2-year prospective clinical study. *J Clin Periodontol* 1996;23:770-777.

Wennström JL. Lack of association between width of attached gingiva and development of soft tissue recession. A 5-year longitudinal study. *J Clin Periodontol*. 1987;14:181-184.

Wirthlin MR, Yeager JE, Hancock EB, Gaugler RW. The healing of gingival wounds in miniature swine. *J Periodontol* 1980;51:318-327.

Woodyard JG, Greenwell H, Hill M, *et al*. The clinical effect of acellular dermal matrix on gingival thickness and root coverage compared to coronally positioned flap alone. *J Periodontol* 2004;75:44-56.

Yoneyama T, Okamoto H, Lindhe J, *et al*. Probing depth, attachment loss and gingival recession. *J Clin Periodontol* 1988;15:581-591.

Zachrisson BU. Tooth movements in the periodontally compromised patient. In: *Clinical periodontology and implant dentistry 5ᵗʰ edn*. Lang NP, Lindhe J, eds. Blackwell Munksgaard 2008; p 1241-1277.

Zaher CA, Hachem J, Puhan MA, Mombelli A. Interest in periodontology and preferences for the treatment of localized gingival recessions: A survey among Swiss Dentists. *J Clin Periodontol* 2005;32: 375-382.

Zakzanis KK. Statistics to tell the truth, the whole truth, and nothing but the truth: formulae, illustrative numerical examples, and heuristic interpretation of effect size analyses for neuropsychological researchers. *Arch Clin Neuropsychol* 2001;16:653-667.

Zalkind M, Hochman N. Alternative methods of conservative esthetic treatment for gingival recession. *Journal Prosthet Dent* 1997;77:561-563.

Chapter 14

Application of BMP and GDF-5 for periodontal regeneration: Experimental histometric observation

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Introduction

Four essential components are known to be required for successful tissue engineering; cells, growth factors, a delivery system (carrier or scaffold), and blood supply (Lynch *et al* 2008). The cell types involved in periodontal regeneration include mesenchymal cells such as fibroblasts, which are involved in regeneration of soft connective tissue, cementoblasts involved in cementogenesis, osteoblasts involved in bone regeneration, endothelial cells involved in angiogenesis, and epithelial cells involved in epithelial regeneration. Several growth factors are known to coordinate the regeneration process as follows:

- transforming growth factor-α (TGF-α) including bone morphogenetic proteins (BMP) and growth and differentiation factor-5 (GDF-5)
- epidermal growth factors
- fibroblast growth factors
- Insulin like growth factor-1 (IGF-1)
- Platelet-derived growth factor (PDGF).

Recombinant human BMP (rhBMP) is currently seen as one of the most promising growth factors and its efficiency of periodontal regeneration has been evaluated by many researchers. The primary action of BMPs are to differentiate mesenchymal stem cells into cartilage and bone forming cells. Two study models have been utilized to evaluate the effect of the growth factors. A rat calvarial defect model (Ahn *et al* 2003, Hyun *et al* 2005, Jung *et al* 2006, Pang *et al* 2004, Schmitz and Hollinger 1986) is being used for the evaluation of new bone formation (Figure 1) and a canine intrabony defect model (Dines *et al* 2007, Herberg *et al* 2008, Kim *et al* 2004, Kim *et al* 2009) has been used to evaluate the periodontal regeneration.

Rat calvarial defect models have been used mainly for evaluating the effect of bone formation. A cranial incision is made and an 8 mm diameter defect is surgically created with a trephine bur on the rat calvarium (Figure 2). If this process is not performed with caution severe brain damage can follow surgery. After conventional microscopic examination, a computerized analysis system is used for histometric analysis. Three parameters; augmented area, newly formed bone area and bone density, are measured for evaluation. Augmented area (mm^2) can be measured as all tissues within the boundaries of newly formed bone. New bone area can be measured as newly formed mineralized bone, excluding marrow and fibrovascular tissue. Bone density percentage was determined by the percentage of new bone area in the augmented area.
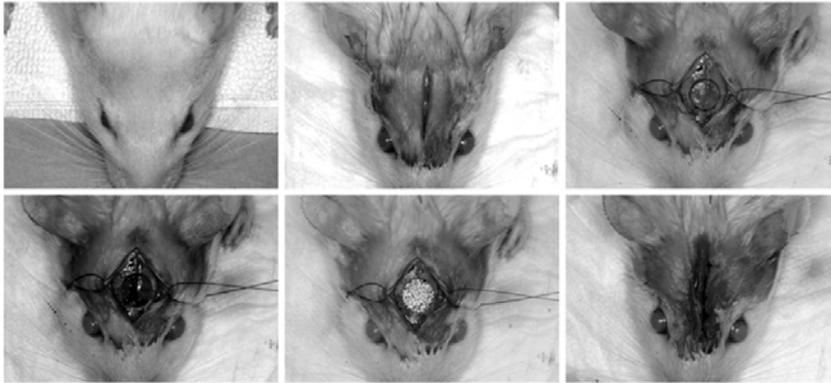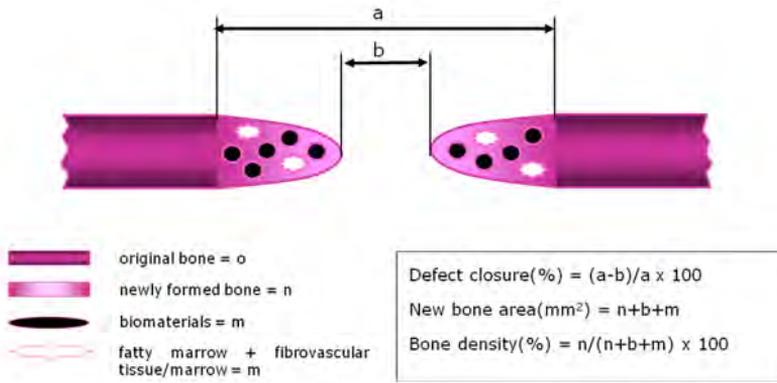
Figure 1. Preclinical rat calvarial defect mode

original bone = o
newly formed bone = n
biomaterials = m
fatty marrow + fibrovascular tissue/marrow = m

Defect closure(%) = (a-b)/a x 100
New bone area(mm²) = n+b+m
Bone density(%) = n/(n+b+m) x 100

Figure 2. Schematic drawing of osteotomy calvarial defect showing histometric analysis

Evaluation of BMP

Hyun *et al* (2005) evaluated and compared the osteogenic potentials of rhBMP-2, 4 and 7 with ACS (absorbable collagen sponge) at 2 week and 8 week wound healing phases in a critical sized rat calvarial defect model. It was concluded that within the selected rhBMP types used, concentration and observation interval, there appears similar bone regenerative potential. Hence, BMP-2, 4 and 7 with ACS used in this study can be considered to be effective factors for inducing bone formation.

Ahn *et al* (2003) evaluated the effect of recombinant human bone morphogenetic protein-4 with carriers in rat calvarial defects. The purpose of this study was to evaluate the

bone regenerative effect of rhBMP-4 delivered with ACS or β-TCP in a rat 8 mm calvarial defect model and the potential of β-TCP compared to ACS as carrier systems for rhBMP-4 was compared. The conclusion was that the bone regenerative effect of rhBMP-4/ACS was greater than that of rhBMP-4/β-TCP, and surgical implantation of rhBMP-4/ACS may be used to support bone regeneration in the rat calvarial critical sized defect without complication. In addition, rhBMP-4/β-TCP may have the potential to regenerate bone in rat calvarial critical sized defects without side effects. Therefore, both ACS and β-TCP may be considered as effective carriers for rhBMP-4.

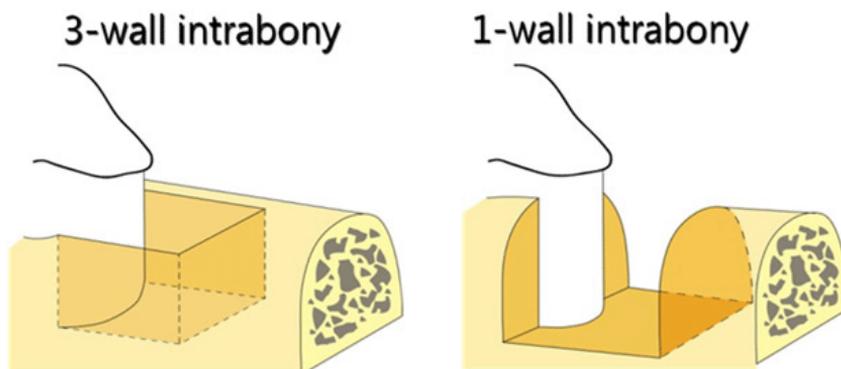Pang *et al* (2004) performed an experiment on the effect of rhBMP-4 concentration on

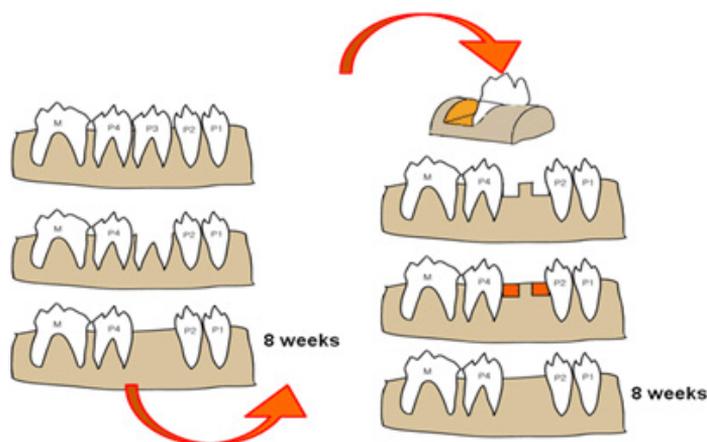**Figure 3.** Canine intrabony defect

Figure 4. Schematic drawings of the procedures of the 1 wall intrabony defect surgery

bone regeneration in rat calvarial defects. The purpose of this study was to evaluate the effect of rhBMP-4 dose within ACS (2.5 µg and 5 µg) or β-TCP (2.5 µg and 5 µg) on bone formation in the rat calvarial critical size defect model. The result showed that rhBMP-4 using ACS or β-TCP carrier has significant potential to induce bone formation in rat calvarial critical size defects. Within the selected rhBMP-4 dose and observation interval, no meaningful differences in *de novo* bone formation were observed. Therefore it can be concluded that both ACS and β-TCP may be considered effective carriers for rhBMP-4.

Jung *et al* (2006) evaluated the effects of rhBMP-4 and different particle sizes of β-TCP (Cerasorb®) on bone regeneration in rat calvarial defects. It was concluded that rhBMP-4 combined with either small (50-150 µm), or large (150-500 µm) β-TCP had a significant effect in inducing bone regeneration compared to β-TCP controls, or a sham-surgery control in a rat calvarial defect model. It was concluded that the particle size of β-TCP, large or small, both induced similar bone regeneration.

The experiments above which dealt with rhBMPs mainly produced by the Chinese Hamster Ovary cell expression system. Recently, an *E. coli* expression system for BMP-2 production was developed. Since both rhBMPs have similar bone regeneration
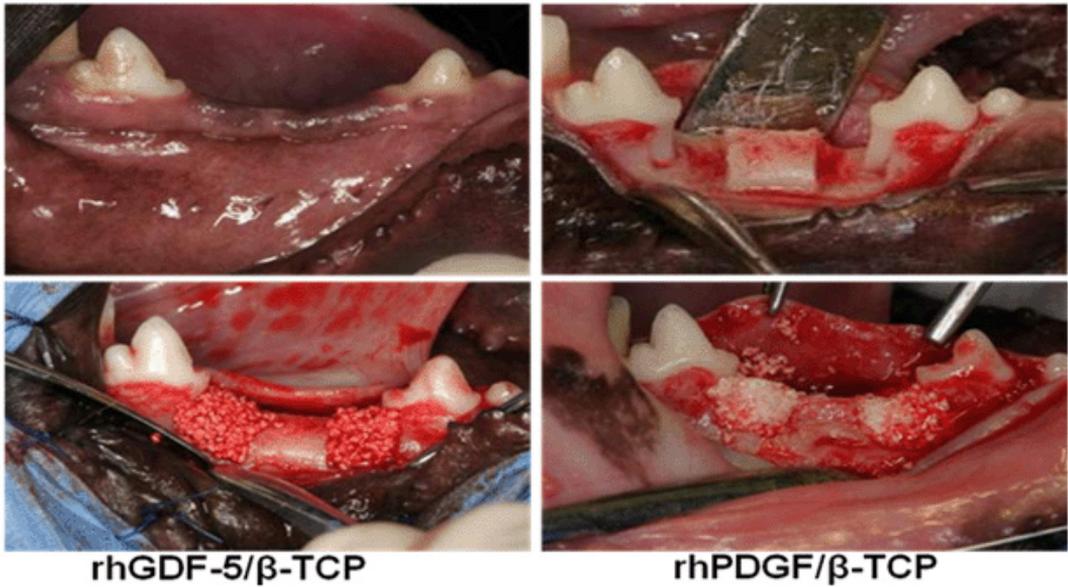
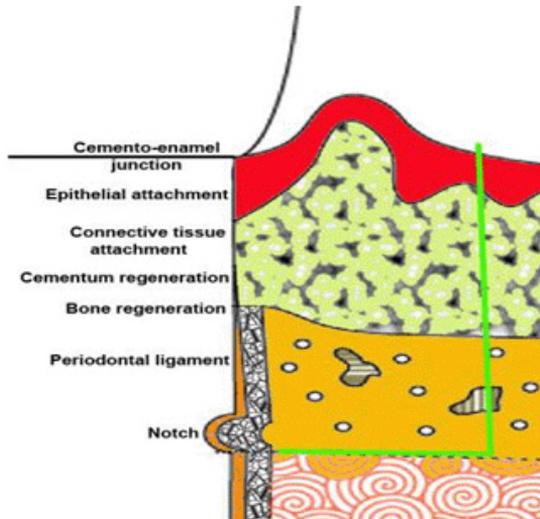**Figure 5.** Surgical protocol for 1 wall intrabony defect model

Figure 6. Histometric analysis

potential, it would be wise to use the *E. coli* expression BMP because of its lower cost**.**

BMP for periodontal regeneration

The procedures for treating 1 wall intrabony defects in a periodontal defect model are as follows (Figures 3 and 4).

After extraction of the third premolar carefully a healing period of 8 weeks is needed. A 4 mm x 5 mm one wall intrabony defect can be surgically created in the mesial aspect of the fourth premolar, and distal aspect in second premolar (Figure 5).

The test materials can be inserted into the defects and left for another 8 weeks before,

the histologic process is performed.

The following parameters for histometric analysis are used (Figure 6):

- epithelial attachment
- connective tissue attachment
- cementum regeneration
- bone regeneration (height)
- bone regeneration (area).

PC-based image analysis (Image-Pro Plus, Media Cybernetics, Silver Spring, MD,USA) was used.

Wikesjo *et al* (1999) reported that ankylosis was observed in supra-alveolar periodontal defects in dogs receiving rhBMP-2/ with ACS. Sigurdsson *et al* (1995), King *et al* (1997), Kinoshita *et al* (1997) and King *et al* (1998) also reported ankylosis in animal models receiving BMP-2, rhBMP-7. Therefore, BMP-2 and periodontal regeneration including cementum and PDL needs to be further investigated with regards to the incidence and extent of ankylosis.

Choi *et al* (2002) performed an experiment on the effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing of 3 wall intrabony defects in dogs. The objective of this study was to evaluate the regeneration of alveolar bone and cementum and the incidence and extent of ankylosis in 3 wall intrabony periodontal defect model in the dog following surgical implantation of rhBMP-2/ACS 8 and 24 weeks with histometric observation (Figures 7 and 8). It was found that surgical implantation of rhBMP-2/ACS may be used safely to support regeneration of alveolar bone in intrabony periodontal defects in dogs without aberrant events such as root resorption or ankylosis complicating the regenerative procedure. However, rhBMP-2/ACS does not appear to have a significant effect on cementum regeneration and formation of a functional periodontal ligament in this model.

Evaluation of GDF-5

GDF-5 is another kind of growth factor which can be used for periodontal regeneration. GDF-5, also called cartilage derived morphogenetic protein-1, is a member of the BMP superfamily, which is known as BMP-14. GDF-5, -6 and -7 are essential for normal formation/development of bone, joints, tendons and ligaments in the axial and appendicular skeleton (Erlacher *et al* 1998, Nakase *et al* 2002, Storm and Kingsley 1996). Cartilage formation appears to be controlled by GDF-5 (Francis-West *et al* 1999, Hatakeyama *et al* 2004). GDF gene expression in cells associated with PDL formation and in cells located along alveolar bone and cementum surfaces, and insertion sites of the PDL during root formation. Its expression is down-regulated upon completion of root formation (Sena *et al* 2003). GDF-5, -6 and -7 gene expression have all been shown to be present during tooth and periodontal tissue formation in bovine teeth (Morotome *et al* 1998). RhGDF-5 dose-dependently enhanced human PDL cell proliferation (Nakamura *et al* 2003) and GDF-5 induced neotendon/ligament formation and supports Achilles tendon repair (Dines *et al* 2007, Forslund *et al* 2003, Wolfman *et al* 1997). It also accelerates bone formation in maxillary sinus and alveolar augmentation procedures and it is being considered as a candidate therapy for periodontal indications (Gruber *et al* 2009, Weng *et al* 2009, Kim *et al* 2009).

Poehling *et al* (2006) has compared GDF-5 with conventional bone substitutes in the rat calvarial defect model in which GDF-/α-TCP, bovine bone mineral, bovine bone mineral plus collagen, bovine bone mineral plus synthetic peptide, β-TCP, sham control were tested. It was concluded that when assessed histomorphometrically new bone formation was about five times greater with GDF-5 than with the other bone substitutes tested. All
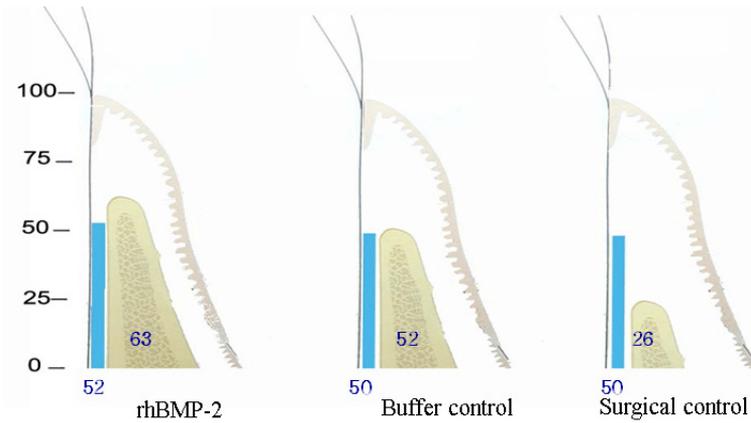
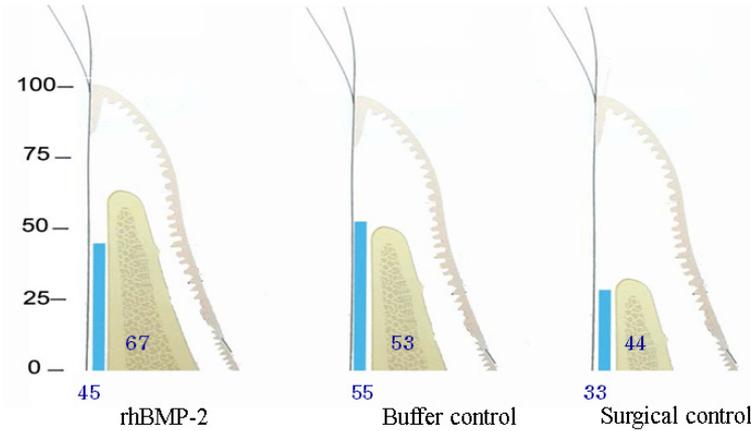**Figure 7.** Histometric observations at 8 weeks

Figure 8. Histometric observations at 24 weeks

implants showed good biocompatibility. GDF-5 in vitro and in the rat calvarial model showed acceleration of bone formation potency. Consequently periodontal regeneration in the intrabony defects was shown to have occurred.

rhGDF-5/ACS carrier significantly supports periodontal wound healing/regeneration

The rationale of this experiment was to investigate whether recombinant human growth/differentiation factor-5 (rhGDF-5) in ACS carrier may provide an environment conducive to regeneration of the structures encompassing the periodontal attachment.

15 young adult, male Beagle dogs were used. A bilateral, critical-size, 1 wall box-type defect of 4 mm x 5 mm was created. Five animals received rhGDF-5/ACS with 0 µg (buffer control) and 100 µg rhGDF-5 placed in contralateral defect sites. Five animals received 1 µg and five animals received 20 µg rhGDF-5 in unilateral defect sites. Contralateral sites received treatments reported elsewhere. A healing of 8 weeks occurred.

The objective of this study was to evaluate the effect of rhGDF-5/ACS (the selected dose interval: 0, 1, 20 and 100 µg) on periodontal wound healing/regeneration following implantation.
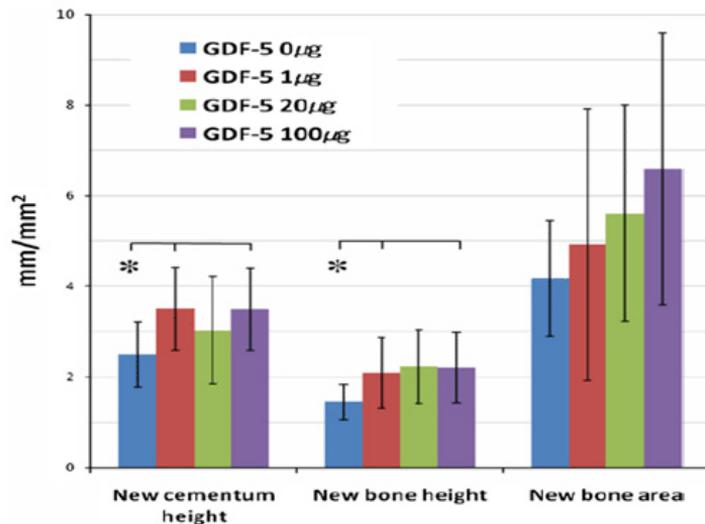
Figure 9. Histometric analysis of periodontal regeneration following implantation of rhGDF-5/ACS within the selected dose interval

The results showed that surgical implantation of rhGDF-5 stimulated significant periodontal regeneration and there were no significant or remarkable differences in bone and cementum formation within the selected dose interval (1, 20 and 100 mg rhGDF-5) (Figure 9). Therefore, it was concluded that surgical implantation of rhGDF-5/ACS may be used safely to support periodontal wound healing/regeneration in intrabony periodontal defects without complications.

Delivery systems (scaffolds)

To date various delivery systems for bioactive materials have been developed and clinically tested however more are still needed. Suggested delivery systems are as follows:
- Decalcified bone matrix (DBM)
- β-Tricalcium phosphate
- Hydroxyapatite
- bovine deorganised bone matrix (Bio-Oss)
- absorbable collagen sponge (ACS)
- Polylactic acid granule (PLA)
- PLA, polyglycolic acid (PLGA) copolymer

- fFbrin-fibronectin system
- gelatin
- composites of these materials.

Herberg *et al* (2008) developed Poly Lactic-Co-Glycolic Acid (PLGA) as an injectable composite carrier for growth factor-enhanced periodontal regeneration. They compared GDF-5 carriers, Polylactic-co-glycolic acid (PLGA), β-TCP, and absorbable collagen sponge (ACS).

They concluded that composite/GDF-5 will be conveniently injectable, biodegradable within 4 weeks, able to sustained release of GDF-5 over 4 weeks and have no appreciable negative effects on periodontal wound healing.

Various delivery systems can also be used in periodontal regeneration, however not all of the carriers are able to satisfy all of the conditions for periodontal regeneration. Each carrier has its own merits and flaws. More research is required to establish the most effective delivery device which meets all of the requirements.

Summary

The following conclusions can be made:
- BMP-2,4 and 7 have similar bone formation potential in the rat calvarial model
- BMP-2 has greater bone formation without ankylosis/root resorption, but limited cementum and PDL formation effect in intrabony defect model
- ACS and α-TCP have similar effects of bone formation on the rat calvarial model
- GDF-5 has bone, PDL and cementum formation potency in intrabony defect model.

References

Ahn SH, Kim CS, Suk HJ, *et al*. Effect of recombinant human bone morphogenetic protein-4 with carriers in rat calvarial defects. *J Periodontol* 2003;74:787-797.

Choi SH, Kim CK, Cho KS, *et al*. Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3 wall intrabony defects in dogs. *J Periodontol* 2002;73:63-72.

Dines JS, Weber L, Razzano P, *et al*. The effect of growth differentiation factor-5-coated sutures on tendon repair in a rat model. *J Shoulder Elbow Surg* 2007;16(5 Suppl):215-221.

Erlacher L, McCartney J, Piek E, *et al*. Cartilage-derived morphogenetic proteins and osteogenic protein-1 differentially regulate osteogenesis. *J Bone Miner Res* 1998;13:383-392.

Francis-West PH, Abdelfattah A, Chen P, *et al*. Mechanisms of GDF-5 action during skeletal development. *Development* 1999;126:1305-1315.

Gruber RM, Ludwig A, Merten HA, *et al*. Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a pilot study in the Goettingen miniature pig comparing autogenous bone and rhGDF-5. *Clin Oral Implants Res* 2009;20:175-182.

Hatakeyama Y, Tuan RS, Shum L. Distinct functions of BMP4 and GDF5 in the regulation of chondrogenesis. *J Cell Biochem* 2004;15;91:1204-1217.

Herberg S, Siedler M, Pippig S, *et al*. Development of an injectable composite as a carrier for growth factor-enhanced periodontal regeneration. *J Clin Periodontol* 2008;35:976-984.

Hyun SJ, Han DK, Choi SH, *et al*. Effect of recombinant human bone morphogenetic protein-2, -4, and -7 on bone formation in rat calvarial defects. *J Periodontol* 2005;76:1667-1674.

Jung UW, Choi SY, Pang EK, *et al*. The effect of varying the particle size of beta tricalcium phosphate carrier of recombinant human bone morphogenetic protein-4 on bone formation in rat calvarial defects. *J Periodontol* 2006;77:765-772.

Kim CS, Choi SH, Chai JK, *et al*. Periodontal repair in surgically created intrabony defects in dogs: influence of the number of bone walls on healing response. *J Periodontol* 2004;75:229-235.

Kim TG, Wikesjö UM, Cho KS, *et al*. Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge carrier into 1 wall intrabony defects in dogs: a dose-range study. *J Clin Periodontol* 2009;36:589-597.

King GN, King N, Cruchley AT, *et al*. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. *J Dent Res* 1997;76:1460-1470.

King GN, King N, Hughes FJ. Effect of two delivery systems for recombinant human bone morphogenetic protein-2 on periodontal regeneration in vivo. *J Periodontal Res* 1998;33:226-236.

Kinoshita A, Oda S, Takahashi K, *et al*. Periodontal regeneration by application of recombinant human bone morphogenetic protein-2 to horizontal circumferential defects created by experimental periodontitis in beagle dogs. *J Periodontol* 1997;68:103-109.

Lynch SE, Wisner-Lynch LA, Nevin M. Use of rhPDGF to improve bone and periodontal regeneration. *Tissue Engineering* 2008;6:87-102.

Morotome Y, Goseki-Sone M, Ishikawa I, Oida S. Gene expression of growth and differentiation

factors-5, -6, and -7 in developing bovine tooth at the root forming stage. *Biochem Biophys Res Commun* 1998;6:244:85-90.

Nakamura T, Yamamoto M, Tamura M, Izumi Y. Effects of growth/differentiation factor-5 on human periodontal ligament cells. *J Periodontal Res* 2003;38:597-605.

Nakase T, Sugamoto K, Miyamoto T, *et al*. Activation of cartilage-derived morphogenetic protein-1 in torn rotator cuff. *Cli Orthop Relat Res* 2002;399:140-145.

Pang EK, Im SU, Kim CS, *et al*. Effect of recombinant human bone morphogenetic protein-4 dose on bone formation in a rat calvarial defect model. *J Periodontol* 2004;75:1364-1370.

Poehling S, Pippig S, Hellerbrand K, *et al*. Superior effect of MDO5,beta- tricalcium phosphate coated with recombinant growth/differentiation factor-5, compared to conventional bone substitutes in the rat calvarial defect model. *J Periodontol* 2006;77:1582-1590.

Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Rel Res* 1986;205:299-308.

Sena K, Morotome Y, Baba O, *et al*. Gene expression of growth differentiation factors in the developing periodontium of rat molars. *J Dent Res* 2003;82:166-171.

Sigurdsson TJ, Tatakis DN, Lee MB, Wikesjö UM. Periodontal regenerative potential of space-providing expanded polytetrafluoroethylene membranes and recombinant human bone morphogenetic proteins. *J Periodontol* 1995;66:511-521.

Storm EE, Kingsley DM. Joint patterning defects caused by single and double mutations in members of the bone morphogenetic protein (BMP) family. *Development* 1996;122:3969-3979.

Weng D, Poehling S, Pippig S, *et al*. The effects of recombinant human growth/differentiation factor-5 (rhGDF-5) on bone regeneration around titanium dental implants in barrier membrane-protected defects: a pilot study in the mandible of beagle dogs. *Int J Oral Maxillofac Implants* 2009;24:31-37.

Wikesjö UM, Guglielmoni P, Promsudthi A, *et al*. Periodontal repair in dogs: effect of rhBMP-2 concentration on regeneration of alveolar bone and periodontal attachment. *J Clin Periodontol* 1999;26:392-400.

Wolfman NM, Hattersley G, Cox K, *et al*. Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6, and 7, members of the TGF-beta gene family. *J Clin Invest* 1997;15:321-330.

Chapter 15

Periodontal intervention in the enhancement of restorative results in implant dentistry

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Introduction

Much of the focus in implantology has been on the bone-to-titanium interface, as a successful osseointegrated implant requires direct bone contact to the implant surface. Emphasis, if any, on the soft tissue surrounding dental implants has been limited to the partially edentulous patient and more so, only when edentulousness involves the esthetic zone in the maxillary anterior dentition (Myshin and Wiens 2005). Few studies have evaluated soft tissue around dental implants in patients over time. However, the preservation and/or reconstruction of soft tissue around dental implants is more than an esthetic requirement, it is a functional biological necessity affecting longevity and treatment outcomes. Additionally, soft tissue around implants should be viewed from two perspectives; quantitative and qualitative, because it is equally important to generate the right type of tissue at the implant interface.

Natural teeth have a structural defense barrier around them that protects the periodontium. This zone, consisting of supracrestal attached epithelial and connective tissue, is referred to as the biologic width. When implants replace teeth, a new biologic width develops after connection of the implants with abutments. Overall blood supply to the newly forming gingival connective tissue is reduced due to the absence of a periodontal ligament which has important implications for the management of soft tissue at various stages during the implant restorative process. Reduction in blood supply first occurs after extraction and then follows implant placement, resulting in a possible predisposition for loss of soft tissue volume and increased risk of implant/abutment exposure (Small and Tarnow 2001). Therefore, the patient's preoperative tissue biotype and bone thickness are important diagnostic parameters that must be evaluated, keeping in mind expected treatment outcomes. The thicker the native hard and soft tissue, the more abundant the blood supply that can be expected after implant placement, with correspondingly heightened expectations for esthetic success (Makigusa 2009).

Several factors affect the final soft tissue result around dental implants (Table 1). This paper shall seek to highlight techniques that the clinician may consider to preserve or enhance the soft tissue result.

Soft tissue maintenance: Preserving soft tissue where it exists

Strategic implant placement that conserves

Pre existing biotype (Grunder *et al* 2005, Lee *et al* 2005)

Original bone levels (Tarnow *et al* 2000)

Design and diameters (Small *et al* 2000, Small *et al* 2001)

Cause and duration since tooth loss (Saadoun and Landsberg 1997)

Magnitude of bone resorption

Type of the intermediate prosthesis and duration

Number, Positioning and Angulations of the implants (Romeo *et al* 2008)

The number and type of surgeries carried out in that particular area

Location and type of the implant abutment interface (Rompen *et al* 2003)

Technique carried out for the uncovering of the implant head
during second stage (Bernhart *et al* 1998)

Soft tissue condition preceding final restorative impression (Small and Tarnow 2003)

Table 1. Factors influencing the soft tissue around dental implants

an existing soft tissue profile involves many elements. Satisfactory morphology of the papilla and of the gingival margin after final implant restoration depends ultimately on two factors: implant placement and implant restorative protocols (Esposito *et al* 1993, Grunder *et al* 2005).

Optimal surgical placement of the implant three dimensionally, especially in the esthetic zone, follows established guidelines. The tridimensional criteria for implant placement in the aesthetic zone are:

- mesio-distal: 1.5 - 2 mm between implant and adjacent tooth 3.5 - 4 mm between implant and adjacent implant
- bucco-lingual: 2.5 - 3 mm from the cervical height of contour of the adjacent teeth to the buccal surface of the implant platform
- corono-apical: 2.5 - 3 mm apical to the bucco gingival margin depending on the biotype.

Therefore, if immediately post extraction implant placement is indicated, the osteotomy

must be performed against the palatal wall to prevent any damage to the remaining (and usually thin) buccal cortical bone (Testori 2003).

From a restorative standpoint, optimal aesthetics will be promoted if the final abutment is installed at the time of implant placement and left in place undisturbed throughout the final restoration phase, avoiding disturbance of bone and soft tissue architecture (Rompen *et al* 2003). Disconnection and reconnection of the abutment disrupts the biologic zone, inducing the junctional epithelium to migrate apically beyond the implant abutment junction until it can adhere again. This often results in marginal bone loss, particularly in cases of thin gingival biotype. It is also important to minimize bacterial contamination in and around the implant-abutment junction.

Some advantages may exist for titanium or zirconium abutments, as hemidesmosome attachment has been demonstrated (Touati and

Guez 2002, Touati 2004).

In terms of restorative protocols that encourage an ideal soft tissue profile, it has been suggested that design of final crowns should comply with the following "norms" in order to optimize papillary form (Elian *et al* 2002):

- distance from interdental bony crest to contact point between natural crown and implant-borne crown: 4.5 mm
- distance from inter-implant bony crest to contact point between two implant-borne crowns: 3.4 mm
- distance between bony crest and connection point between an implant-borne crown and a pontic: 5.5 mm

If the use of a permanent abutment at surgery is contraindicated for any reason or if loading may not be possible, a custom gingival level healing abutment is easily fabricated with plastic temporary abutments. This kind of custom healing abutment serves to support papillary and marginal gingival tissue until a more definitive permanent or healing abutment can be placed (Pow *et al* 2004). If the custom healing abutment is made of composite resin, it is often possible to continually modify the profile to enhance 'guided' soft tissue healing.

Soft tissue control: Manipulating soft tissue for better results

The opportunity to manipulate existing soft tissue for better results presents itself at either the primary surgical stage or at the second stage surgery. A common example of this technique is the 'pouch' technique for soft tissue bulk enhancement. The technique involves the de-epithelisation of the soft tissue covering the implant platform with a coarse bur or a scalpel. The connective tissue is consequently exposed. Using a palatally biased paracrestal incision, this exposed connective tissue is elevated as part of a labial

flap. This can then be 'pouched' under the labial flap allowing two connective tissue surfaces to come in contact and create a doubled over bulk of tissue.

Transposition of keratinized tissue by paracrestal incisions in order to equally distribute keratinized tissue on either side of the implant platform is another common example of soft tissue control during second stage surgery.

Soft tissue grafting: Creating soft tissue where it does not exist

The employment of soft tissue grafting techniques that are commonly used in periodontics has seen much success in the past in implantology. Three techniques have become fairly commonplace in the management of soft tissue profiles around dental implants.

Connective tissue graft

Since introduction of the connective tissue graft for treatment of gingival recession, this technique has been employed in implantology as well (Langer and Langer 1985). It is commonly used for augmentation of tissue biotypes and general postoperative tissue thickness in implantology (El Askary 2002). Osseous and connective grafts can also be used to convert a thin gingival biotype into a thick gingiva, to enhance gingival marginal stability and simplify tissue management during the restorative treatment phase (Mathews 2000).

Free gingival graft

The free gingival graft, although limited in its application in periodontics today, is still a useful technique for the creation of keratinised tissue around dental implants in conjunction with an apically repositioned flap (Sevor 1992).

Acellular dermal graft

The use of autogenous gingival grafts has proved to be an effective and predictable way to increase the amount of keratinized gingiva. However, discomfort and pain at the graft donor site have been cited as issues leading to the alternative use of acellular dermal matrix allografts as donor tissue, eliminating the need for another surgical site and alleviating pain and trauma. The efficacy of these grafts in increasing the width of keratinized tissues however seems lower than autogenous gingival grafts (Yan *et al* 2006).

Interdisciplinary techniques to enhance soft tissue profiles around dental implants

The use of a combination of periodontal, maxillofacial surgical and orthodontic techniques to enhance the postoperative soft tissue profile has led to the creation of a new clinical entity in implantology that is often referred to as 'site development'.

In addition to many soft and hard tissue grafting techniques, orthodontic treatment has been suggested as the best solution for patients who wish to limit the surgery required for the placement of implants to a single session, and to enhance the hard and soft tissue profile prior to extraction and implant placement (Salama *et al* 1993).

Conclusion

The understanding of importance of the soft tissue implant interface has gradually increased over the years. As osseointegration itself becomes more predictable and controllable, an understanding of the biology of the soft tissues and their wound healing response has helped the implant clinician provide better esthetics and function.

References

Bernhart T, Haas R, Mailath G, Watzek G. A minimally invasive second-stage procedure for single-tooth implants. *J Pros Dent* 1998;79:217-219.

El Askary AS. Use of connective tissue grafts to enhance the esthetic outcome of implant treatment: A clinical report of 2 patients. *J Pros Dent* 2002;87:129-132.

Elian N, Jalbout ZN, Cho SC, *et al*. Realities and limitations in the management of the interdental papilla between implants: Three case reports. *Pract Proced Aesthet Dent* 2003;15:737-744.

Esposito M, Ekestubbe A, Grondahl K. Radiological evaluation of marginal bone loss at tooth surfaces facing single Branemark implants. *Clin Oral Imp Res* 1993;4:151-157.

Grunder U, Gracis S, Capelli M. Implantation in the tridimensional space: aesthetic consequences. *Int J Perio Rest Dent* 2005;25:72-85

Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol* 1985;56:715-720.

Lee DW, Park KH, Moon IS. Dimension of keratinized mucosa and the interproximal papilla between adjacent implants; *J Perio* 2005;76:1856-1860.

Makigusa K. Histologic comparison of biologic width around teeth versus implants: The effect on bone preservation. *J Impl Recons Dent* 2009;1:20-24.

Mathews DP. Soft tissue management around implants in the esthetic zone. *Int J Periodont Rest Dent* 2000;20:141-149.

Myshin HL, Wiens JP. Factors affecting soft tissue around dental implants: A review of the literature. *J Pros Dent* 2005;94:440-444.

Pow EHN, McMillan SA. A modified implant healing abutment to optimize soft tissue contours: A case report. Impl Dent 2004;13:297-300.

Romeo E, Lops D, Rossi A, *et al*. Surgical and prosthetic management of interproximal region with single implant restorations: 1 year prospective study. *J Perio* 2008;79:1048-1055.

Rompen E, Touati B, Van Dooren E. Factors

influencing marginal tissue remodeling around implants. *Pract Proced Aesthet Dent* 2003;15:754-761.

Salama H, Salama MA. The role of orthodontic extrusive remodeling in the enhancement of soft and hard tissue profiles prior to implant placement. *Int J Perio Rest Dent* 1993:13:312-333.

Saadoun AP, Landsberg C. Classifications and timed sequence of different approaches to implant insertion after tooth extraction. *Pract Perio Aesth Dent* 1997;8:933-944.

Sevor JJ. The use of free gingival grafts to improve the implant soft tissue interface: Rationale and technique. *Pract Perio Aesthet Dent* 1992;4:59-64.

Small PN, Tarnow DP, Cho SC. Gingival recession around wide-diameter versus standard-diameter implants: A 3 to 5 year longitudinal prospective study. *Pratic Proced Aesthe Dent* 2001;13:143-146.

Small PN, Tarnow DP. Gingival recession around implants: A 1-year longitudinal prospective study. *Int J Maxillofacial Imp* 2000;15:527-532.

Tarnow DP, Cho SC, Wallace SS. The effect of inter-implant distance on the height of inter-implant bone crest. *J Periodontol* 2000;71:546-549.

Testori T. Ideal implant positioning in maxillary anterior extraction sockets. *Acad News* 2003;1-13.

Touati B. Biologically driven prosthetic options in implant dentistry. *Pract Proced Aesthet Dent* 2004;16:517-520.

Touati B, Guez G. Immediate implantation with provisionalization: From literature to clinical implications. *Pract Proced Aesthet Dent* 2002;14:699-707.

Yan JJ, Tsai AY, Wong MY, Hou LT. Comparison of acellular dermal graft and palatal autograft in the reconstruction of keratinized gingiva around dental implants: A case report. *Int J Periodontics Restorative Dent* 2006;26:287-92.

Poster Presentations

The following is a record of the posters presented at the
8th Meeting of the Asian Pacific Society of Periodontology

Periodontal regeneration by transplantation of human adipose tissue derived stem cells.

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Introduction: The complete reconstruction of periodontium destroyed by periodontal diseases is an ideal goal of periodontal therapy. Recent studies have reported the ubiquitous distribution of adult stem cells in various tissues and organs, including bone marrow, muscle, brain, skin, and adipose tissue. Because adipose tissue is abundant and easily accessible, adipose tissue-derived mesenchymal stem cells (ADSC) are anticipated to be beneficial in periodontal regenerative cell therapy. In this study, we examined the cellular characteristic of ADSC *in vitro*. In addition, using 2-wall intrabony defects model of beagle dogs, the regenerative efficacy of topical implantations of ADSC was analyzed.

Materials and Methods: Human ADSC were isolated from several donors at Osaka University Medical Hospital Translational Research Center. The phenotype of ADSC was determined by FACS analysis. The proliferative response was measured by population doublings. The chromosomal analysis was performed by G-Band and SKY methods. The alkaline phosphatase (ALPase) activities and the calcified nodule formation of ADSC cultured in mineralization-inducing medium were analyzed by p-nitrophenylphosphate as substrate, and alizarin red staining, respectively. Two-wall intrabony defects were surgically created on the mesial sides of bilateral mandibular fourth premolars in 5 beagle dogs. ADSC were isolated from subcutaneous adipose tissue of each beagle dog. The fibrin carrier alone or the carrier containing the ADSC was transplanted into the defects of each dog. Six weeks after the transplantation, the periodontal regeneration at each defect was analyzed radiographically and histologically.

Results: FACS analysis revealed that ADSC was CD34-, CD45-, CD73+, CD44+, CD105+. ADSC demonstrated a high proliferation rate until 8-passages and no abnormal chromosomes were shown until 14-passages at which point ADSC failed to proliferate. Cultured in mineralization-inducing medium for 28 days, the ADSC increased the ALPase activity and formed calcified nodules. Micro CT and histological analyses revealed greater periodontal regeneration at the ADSC-applied sites than in the carrier alone applied sites. A considerable amount of new bone growth, as well as new cementum on the instrumented root surface was observed at the ADSC-applied sites. Furthermore, new connective tissue fibers were vertically inserted into the newly formed bone and cementum.

Conclusion: In this study, we indicated that ADSC can be stably maintained in vitro and be induced to differentiate into osteoblast-like cells. Furthermore, a topical transplantation of ADSC can enhance considerable periodontal regeneration at the surgically created alveolar bone defects of beagle dogs. Thus, ADSC can be potentially used for cell therapy for periodontal regeneration.

*Recipient of Best Poster Presentation Award - First Place

Medium to long-term soft tissue health around osseointegrated titanium dental implants

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Introduction: To assess the soft tissue conditions around osseointegrated titanium dental implant after a minimum of five years in function.

Materials and methods: All patients in Prince Philip Dental Hospital, The University of Hong Kong who had received implants which had been in function for a minimum of five years were recruited. Those who had received resective or ablative therapy followed by major jaw reconstruction were excluded. All patients received clinical examination by two clinicians, who independently recorded their periodontal and prosthetic conditions, respectively.

Results: A total of 153 dental implants in 50 patients with a mean 8.7 ± 3.3 years of post-rehabilitation loading were investigated. All subjects were partially edentulous. The mean probing pocket depth (PPD) at remaining teeth was 2.21 ± 1.67 mm (range 0-9 mm) and the mean clinical attachment level was 2.82 ± 2.1 mm. On average, 35.2% of the sites in remaining teeth had plaque and 37% of sites exhibited bleeding on probing (BOP). The percentage of sites with PPD >5 mm was 1.4%. For soft tissue health around implants, the mean probing depth was 2.95 ± 1.59 mm (range 0-10 mm). On average 48% of the sites were covered with plaque and 67% of the sites exhibited bleeding on probing. For recession (measured from implant neck to mucosal margin), 10% of sites showed recession of 1 mm while 3% had recession of up to 2 mm. The percentage of sites around implants with suppuration was 0.5% and 0.3% of sites had rough surfaces exposed. The percentage of sites with probing >5 mm was 11.1%. At the patient level, there were significantly greater mean percentage of sites with plaque and BOP at implants than at teeth (43% versus 34%, $p < 0.05$; 62% versus 38%, $p < 0.01$). At the patient level, the mean probing pocket depth was also found to be significantly deeper at implants than at teeth (2.83 mm versus 2.28 mm, $p < 0.01$).

Conclusion: In this study, oral hygiene was found to be poorer and BOP more frequently observed around implants than around natural teeth. Patients appeared less able to control plaque around implants, and therefore clinicians should put more emphasis on oral hygiene education and in reinforcing patients' plaque control around dental implant reconstructions, which themselves should be amenable to being cleaned by patients.

Recipient of Poster Presentation Merit Award

The effects of full mouth SRP conjunction with Azithromycin for peri-implantitis

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Introduction: It has been reported that flora in peri-implant sulci was similar with flora in periodontal pocket, and that some bacteria spread in the same mouth, from natural teeth to implants. Recently, full mouth SRP (FM-SRP) using azithromycin (AZM) is performed in order to prevent propagation of the periodontal pathogen during treatment for serious periodontitis. There were some reports to show this procedure gave good improvement and stability for the treatments of serious periodontitis. The purpose of this study was to determine whether FM-SRP using AZM was also effective against peri-inplantitis.

Materials and methods: Seventeen adult subjects with severe chronic periodontitis and peri-implantitis were randomly divided into FM-SRP (test group, N=10) and seven received conventional SRP (control group, N=7). The subjects in the test group were given AZM 3 days before FM-SRP. After the tooth brushing instruction and supragingival scaling (base-line), clinical parameters such as periodontal probing depth, gingival index and bleeding on probing were examined all implants and natural teeth. Bacterial samples were taken from the implants and natural teeth, which had deepest PPD. Subsequently, measurement of clinical parameters were recorded and subgingival plaques were taken at 1, 4 and 12 weeks after FM-SRP. In order to perform bacterial examinations qualitatively and quantitatively, PCR and PCR-Invader methods were carried out to detect 5 periodontal pathogens (*Porphyromonas gingivalis, Treponema denticola, Tannerella forthysia, Prevotella intermedia* and *Aggregatibacter actionmycetemcomitans*).

Results: All clinical parameters of implants and natural teeth improved in the test group more than in the control and maintained an improved condition during the whole observation period. A lower detection rate and quantity of periodontal pathogens were found both implants and natural teeth in the test group at 12 weeks. The detection rate and number of *T. forthysia* and *A. actionmycetemcomitans* were decreased at one week after FM-SRP, however, those were increased in 12 weeks after FM-SRP. This tendency was remarkable in the implants compared with the natural teeth.

Conclusion: The results of this study suggested that full-mouth SRP using systemically administered AZM was both clinically and bacteriologically useful not only for periodontal treatment of severe periodontitis but also that of peri-implantitis. However, reinfection was observed in the implants at 12 weeks after FM-SRP, it was thought that the treatment for peri-implantitis required further consideration.

*Recipient of Poster Presentation Merit Award

Diode laser modulation of inflammation in subjects with chronic periodontitis

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Introduction: Dental lasers as an adjunct to non-surgical periodontal therapy may have a beneficial effect on the control of periodontal disease, due to anti-infective and anti-inflammatory properties. The present study was to evaluate the short-term effects of photodynamic therapy (PDT) with laser biostimulation as an adjunct in treatment of chronic periodontitis.

Materials and Methods: 20 non-smoking Chinese adults with untreated chronic periodontitis were selected and half-mouths were randomly assigned to receive scaling and root debridement with (test) or without (control) adjunctive PDT and biostimulation treatment by a diode laser (Ezilase™, BIOLASE Technology Inc), according to a split-mouth design. Outcome measures included clinical parameters of plaque, bleeding on probing (BOP), probing depth (PD) and gingival recession. The volume of gingival crevicular fluid (GCF) and the level of IL-1β in GCF (assessed by ELISA) were measured at baseline, one week and one month after treatment.

Results: At baseline, no significant differences were found in the mean/median clinical parameters or GCF data, between the test and control sites. Test sites receiving scaling and root debridement with PDT showed greater reduction in mean percentage of BOP ($p<0.05$) and mean PD ($p<0.05$) at one month as compared to the control sites. Both test and control sites showed a decrease in percentage of plaque and an increase in gingival recession, and no significant difference was found between them. A significant decrease in GCF volume from baseline to one week was observed in both test and control sites ($p<0.01$) with a further but statistically insignificant decrease from one week to one month. There was a significantly greater reduction of IL-1β levels at one week in the test sites when compared to the control sites ($p<0.01$).

Conclusion: Photodynamic therapy in combination with laser biostimulation appears to be beneficial in the short-term as an adjunct to scaling and root debridement in treatment of chronic periodontitis.

Chief complaint patterns of subjects attending periodontology clinic

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Introduction: Periodontal disease is generally 'asymptomatic' and hence may be overlooked by patients at different stages of the disease course. Therefore, patients may miss early opportunities for treatment, resulting in compromised therapeutic outcomes. This study investigated the patterns of patients' chief complaints related to periodontal disease.

Materials and Methods: Dental records of patients treated in the Periodontology Clinic at the Prince Philip Dental Hospital, Hong Kong from 1981 to 2008 were randomly selected and screened for chief complaints reported at the first visit. Various complaints reported were classified according to their nature. The patterns of the chief complaints of different genders, age groups and reasons for attendance were recorded and analyzed using SPSS 16.0.

Results: 340 patient records of 190 females and 150 males, 177 referred patients and 163 walk-in patients, mean age 44.7±11.6 years were screened. The following complaints were identified, including gum problems (gum swelling, bleeding gum and gum discomfort), chewing problems (mobile teeth, chewing discomfort and food impaction), aesthetic problems (drifted teeth, spacing and gum recession), tooth sensitivity, halitosis, 'gum disease informed by other dentist', and others. Overall, mobile teeth was the most common chief complaint (42.1%), followed by gum swelling and/or gum discomfort (40.0%), bleeding gum (26.2%), chewing discomfort (21.8%) and tooth sensitivity (19.7%). On grouping, 55.3% of the complaints were gum related problems and 52.1% were related to chewing problems. Chewing problems and tooth sensitivity were more common in the walk-in group when compared to the referred group (65.0% versus 40.0% $p < 0.001$; 24.5% versus 18.0%, $p < 0.05$). No significant difference in complaint patterns was found between males and females, or among cases documented in the 1980s, 1990s and 2000s. More reports of 'gum disease informed by other dentist' were noted in subjects below 45 years of age than those above 45 (9.9% vs. 1.8%, $p < 0.01$).

Conclusion: Subjects attending the Periodontology Clinic at the Prince Philip Dental Hospital reported diverse problems related to periodontal disease. Gum or chewing problems were the most common chief complaints, especially in the walk-in patients. The present study shows that symptom-driven motives account for patients seeking dental care in this university dental hospital, and thereby further promotion of strategies of 'Prevention is better than treatment' and 'In-time for treatment' is suggested for control of periodontal disease in this community.

C-reactive protein measurement in aggressive periodontitis and chronic periodontitis

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Background: C-reactive protein (CRP) is an acute phase protein, synthesized in the liver and secreted in plasma. It could be used as a serologic marker on the systemic inflammation related to the risk of systemic diseases, including cardiovascular diseases. The increase of serum CRP level is related with periodontitis patients. The purpose of CRP measurement in this research is to examine the quality of host response to infection.

Objective: The purpose of this research is to examine serum CRP level in aggressive periodontitis and chronic periodontitis patients.

Materials and Methods: The study group comprised patients diagnosed with aggressive and chronic periodontitis who attended the Periodontal Clinic, Faculty of Dentistry Airlangga University. Samples of 3 ml of venous blood were taken from the inner elbow and mixed with EDTA as anticoagulant. The samples were then diluted with LCRZ reagent and Immunometric Assay was undertaken to measure the serum CRP level.

Result: The result of this research showed that CRP levels in aggressive periodontitis and chronic periodontitis patients were within a normal range (<1 mg/l). This condition showed that the quality of patient's immune response was still within the normal range.

Conclusion: The existence of optimal protein metabolism in a healthy body is able to support the regeneration and repair of the tissues; therefore, a good respond after periodontal treatment could be expected.

Cytotoxicity effect of crude toxin *Actinobacillus actinomycetemcomitans* Serotype B in gingival epithelium

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Introduction: :*Actinobacillus actinomycetemcomitans serotype b* has been associated with aggressive periodontitis, secreted protein toxin that inhibits the proliferation of wide variety of cell types. Eucariotic cells that are sensitive to the toxin are usually arrested at the G0/G1 or G2/M phase of the growth cycle through the action of a DNA-se I- like nuclease that causes double strand breaks in the host cell DNA.Gingival epithelial cell exquisitely sensitive to the toxin so that may lead to the epithel protective barrier disruption..

Aim: To examine the cytoxicity effect of serotype b *A. actinomycetemcomitans* toxin based on apoptosis mechanism which associated with DNA fragmentation, PARP-1, NFkB, Bax expression, and glutathione (GSH).

Materials and Methods: Thirty adult mice (Swiss Webster strain) were randomly divided into two groups (toxin and control). The mice were sacrified 24 hours after induced crude toxin *A. actinomycetemcomitans serotype b*, tissue samples of gingival epithelium were stained with tunnel assay and immunohistochemistry.

Result s: Treatment with these toxin induced apoptosis of gingival epithelium was associated with increase DNA fragmentation,PARP-1, NFkB, Bax expression and reduced gluthatione (GSH).

Conclusion: Toxin bacteria serotype b induces apoptosis in gingival epithelium.

Immediate implantation in maxillary and mandibular molars fresh sockets: Technique and preliminary results

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Introduction: The aim of this study was to evaluate the predictability of implantation at the time of maxillary and mandibular molar extraction.

Materials and Methods: Maxillary and mandibular molars were extracted with atraumatic technique (root separation and careful extraction with periotome) to preserve all remaining interradicular bone. 95 tapered and straight implants were then inserted in extraction sites. Interradicular bone was utilized to provide primary stability of implant. Regenerative therapy including placement of bone substitute and resorbable membrane was placed around all of the implants.

Results: All of the implants were stable 3 to 4 months post insertion and restored with fixed prostheses. A total of 95 implants have been in function up to 84 months (mean time of 60 months).

Conclusion: The combination of atraumatic extraction of hopeless molars, immediate implant placement and concomitant regenerative therapy is a predictable surgical procedure afforded implant stability for being restored with a single crown.

The expression of VEGF during wound healing of periodontal defects on diabetes rats

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Introduction: Diabetes mellitus attracts attention of risk factor of periodontal disease.The aim of this study was to investigate the expression of VEGF during early wound healing of periodontal defects on rats with type II diabetes mellitus.

Materials and Methods: Palatal dehiscence defects were surgically created on bilateral maxillary first molars in normal and type 2 diabetes model rats. The rats were put euthanasia at 3,5,7 days after surgical operation,and prefusion-fixed,followed by removal of the periodontium including maxillary first molar and preparation of paraffin sections. The paraffin sections were stained with hematoxilin-eosin(HE). VEGF were detected immunohistochemically.

Results: At 3 days and 5days,VEGF were localized in the vascular endothelium cells and around the vascular in all the sections. At 7 days, the expression of VEGF was stronger in the control group than the experimental group.

Conclusion: It is suggested that VEGF inhibit the normal vascularization during early wound healing of periodontal defects on rats with type II diabetes mellitus.

A cohort study for chronic disease: Periodontal status of elderly people in Korea

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Introduction: To assess the periodontal status of elderly people, Korea with regard to factors affecting relationship between chronic systemic disease and periodontal status.

Materials and Methods: For this study, 2044 out of 5823 elderly population (ages over 50) of Gyerim-dong and Sansu-dong, Dong-gu, Gwangju, Korea were questionnaired and clinically examined for their chronic diseases, systemic (blood pressure, physical measurement, body fat, bone density, echocardiography, EKG and blood test) and oral(salivary flow rate, OHI, probing depth, gingival recession, BOP, and root caries) health.

Results: Mean age of study population was 65.13¡¾7.89 years. Average salivary flow rate was 1.37¡¾0.69ml/min. There was no significant correlation with ages but male¡¯s were higher(p=0.0000). The rate of xerostomia was 5-8% and most frequent in 70s. Dentures were used by 8.7% of the people and most common in 70s. Eighty three subjects had complete dentures. One hundred forty nine (7.3%) had dental implants in their oral cavity. 12% were healthy or mild periodontitis, 57% had moderate, 27% had severe chronic periodontitis. Average number of remaining teeth was decreasing with ages. Average OHI was relatively low (male 24.56% and female 26.72%). BOP frequency was increasing with ages, highest in 80s. The rate of gingival recession over 3mm was highest in 60s. 15% of polpulation had root caries. In smokers, the proportion of severe chronic periodontitis was highest. In severe chronic periodontitis subjects, mean AST, ALT, GGT, HbA1C, Glucose, RBC, CHL level were significantly different to those of mild or moderate periodontitis (p<0.01).

Conclusion: Considering the results that 84% had moderate or severe chronic periodontitis and low OHI, almost all subjects need periodontal treatments, supportive periodontal therapy, and intensive oral hygiene education.

Antioxidant profile of whole saliva after scaling & root planing in periodontal disease

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Introduction: Generally reactive oxygen species (ROS) are caused by bacteria in tissue destruction and host-mediated pathway. Recently it has been discussed that inappropriate anti-oxidative defence accelerates periodontitis. The purpose of this study is to compare total antioxidant status (TAS) level and superoxide dismutase (SOD) activity in saliva of periodontally compromised patients before and after scaling and root planing and ultimately apply the results to diagnostic tool.

Materials and Methods: A test group included seven patients who were diagnosed with severe chronic periodontitis in the Dept. of Periodontology, Chonnam National University Hospital from May to October in 2008. In a control group, seven patients who did not have attachment loss and sites which showed 3 mm or more probing depth, and whose sulcus bleeding index was under 10% were chosen. At initial examination, each group was included after clinical and radiographic examination. After 1 week, each group was taken saliva sampling, clinical examination and scaling and root planing. From this baseline, a week, a month and 3 months later, saliva sampling and clinical examination were performed respectively. TAS level and SOD activity in each patient's saliva were measured by ELISA reader in 450nm with kits.

Results: The mean TAS levels of the test group were 341.7 ± 36.6 ìmol/l at baseline, 314.9 ± 50.2 ìmol/l at 1 week, 331.1 ± 46.0 ìmol/l at 1 month, 285.9 ± 53.2 ìmol/l at 3 months. Those of the control group were 282.7 ± 55.1 ìmol/l at baseline, 266.1 ± 59.3 ìmol/l at 1 week, 293.3 ± 54.2 ìmol/l at 1 month, 366.1 ± 17.6 ìmol/l at 3 months. At the baseline and 1 week, TAS level of the test was statistically higher than that of the control group($P<0.05$). There was no statistical difference for the times between the test group and the control group in TAS flow rate (TAS level/salivary flow rate). TAS level and flow rate in test group was decreased over time and those in control was increased overtime and 3 months after SRP those in both group were similar. The mean SOD activity of the test group was $36.2 \pm 15.9\%$ at baseline, $31.8 \pm 14.2\%$ at 1 week, $26.7 \pm 20.7\%$ at 1 month, $29.3 \pm 46.4\%$ at 3 months. The control group was $15.1 \pm 17.8\%$ at baseline, $26.6 \pm 21.0\%$ at 1 week, $15.3 \pm 19.1\%$ at 1 month, $55.3 \pm 4.2\%$ at 3 months. At baseline, SOD activity of the test group was statistically higher than that of the control group ($P<0.05$). However, SOD activity in the control group 3 months after SRP was higher than that of the test group.

Conclusion: Antioxidant level in saliva was higher in the patients who had severe chronic periodontitis than healthy or gingivitis control before SRP. The TAS flow rate in saliva of patients who had severe chronic periodontitis gradually decreased until 3 month after SRP. Meanwhile, antioxidant level in saliva of healthy control was gradually increased until 3 month after SRP. The salivary antioxidant level during experiment periods were showed by different profile in periodontitis and healthy or gingivitis group.

Pilot study on the prediction of periodontal disease from self reported periodontal health status

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Introduction: Self reported periodontal status can provide a diagnostic data to predict the actual clinical periodontal condition. If patients were able to recognise that they were affected by periodontal diseases this might give practical advantages in term of cost and time in estimating disease status. The aim of this study was to investigate the agreement between self reported periodontal status and periodontal clinical examinations in a selected adult population of healthy and periodontal patients.

Methods and Materials: A convenient sample of 101 patients (50 periodontally healthy and 51 patients with periodontitis) attending Universiti Kebangsaan Malaysia Dental Faculty were asked to complete a set of questionnaires concerning their perceived periodontal status and then underwent full periodontal pocket charting to determine actual periodontal status. Sensitivity and specificity values of particular responses to questions were calculated to determine the predictors to actual clinical status and values more than 0.8 would be reasonable predictors.

Results: Two items gave strong prediction of disease status which were "do you think you have gum disease" and "do you think that your teeth are loose or wobbly" with sum of sensitivity and specificity values were 15.2 and 14.8 respectively. Lower sensitivity values were found for items on knowledge of professional diagnosis of periodontal disease for example "has any dentist told that you have deep pockets" and "have you been told that you have gum disease" (0.28 and 0.50 respectively), suggesting patients with periodontal disease has not been informed by their dentists. Higher specificity values were found for most self reported questionnaires.

Conclusion: Self perceived statuses of "having gum disease" and "having mobile teeth" in this study population were successful in predicting disease presence. However, most patients with periodontal disease had not been informed of their disease status by their dentists. Better sampling methods with a different population base should be suggested for future studies.

Treatment of altered passive eruption: Periodontal plastic surgery of the dentogingival junction

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Excessive gingival display, frequently seen in adults and resulting in short clinical crowns, has been described in the literature by several authors as "altered passive eruption." It is defined as a dentogingival relationship wherein the gingival margin is positioned coronally on the anatomic crown and does not approximate the cementoenamel junction due to the disruption in the development and eruptive patterns of the dentogingival unit. This article describes how periodontal plastic surgery can remodel the attachment apparatus, reestablish the correct biologic width, eliminate the excessive show of gingiva, and expose the correct dimensions of teeth. Apically repositioned flaps with osseous recontouring can restore gingival health and the esthetic parameters of the smile line.

Retrospective analysis of tooth loss in chronic periodontitis patients on maintenance

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Introduction: It has been well-established that supportive periodontal therapy (SPT) is important in the maintenance of oral health. There has been increasing emphasis on the use of "true" endpoints (e.g. tooth loss) rather than "surrogate" endpoints for the evaluation of effectiveness of periodontal therapy. There have been no studies done in an Asian population to date. It is our aim to investigate the prevalence of and reasons for tooth loss during active periodontal therapy (APT) and SPT in a group of patients treated for chronic periodontitis in an institutional practice (National Dental Centre Singapore, NDCS) who were followed up to 10 years after completion of APT.

Materials and Methods: Patients who were treated by periodontists in the Centre for chronic periodontitis between March 1997 and December 1998, inclusive, and who are still undergoing SPT in NDCS were identified from an electronic patient database. Their record folders were retrieved and reviewed by the principal investigator, who was not involved in the clinical treatment of these patients. Data was obtained from their clinical notes/charts/radiographs. The number of teeth present was determined at 3 time points: Initial examination, end of APT and the most recent SPT appointment visit. Total number of folders drawn was 478 and upon review, 273 patients met the inclusion criteria. Relevant information was collected and entered into an Excel spreadsheet. Descriptive statistics were done using SPSS.

Results: 273 patients (106 males, 167 females) were included in this study with mean age of 44.7 years (range 19-80). There were a total of 7086 teeth present at the start of treatment and 360 (5.1%) were extracted during APT. 218 teeth (60.6%) extracted during APT was due to periodontal reasons, having mean bone support of 27.2% (range 0-75%). The mean duration of SPT was 10.9 years (range 7.0-20.4). During SPT, 253 teeth (3.8%) were lost; out of which, 76 teeth (30%) were lost due to periodontal reasons. Other reasons for tooth loss during APT and SPT included non-functional teeth, prosthodontic reasons, non-restorable & tooth fracture. Multi-rooted teeth were extracted more frequently than single-rooted teeth during both APT (61% Vs 39%) and SPT (77.6% Vs 22.4%). Patients who complied with SPT lost 0.03 teeth/patient/year due to periodontitis.

Conclusion: Periodontitis-susceptible patients who were treated more than 7 years ago in NDCS and who complied with periodontal maintenance responded well to periodontal therapy, showing a low prevalence of tooth loss due to periodontitis.

A comparison of self reported periodontal disease in a hospital practice and a private practice setting

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Background: The purpose of this study was to compare self-reported periodontal disease in adults between a hospital and private practice setting, and to validate the use of self-reported items in the estimation of the prevalence of periodontal disease.

Materials & Method: 179 healthy subjects were recruited, with 116 from a hospital dental practice (HP) and 63 from a private dental practice (PP). Full mouth periodontal assessments were carried out and a self administered questionnaire was administered to find out subjects' perceptions and ratings of their oral health. Student's t test, ANOVA and Chi-square test were used to analyze the data. Logistic regression models tested demographic and questionnaire variable for significance with respect to severity of periodontitis and the subjects' self rating of their oral health condition.

Results: The PP subjects had significantly more severe periodontitis (82.5%) than the HP subjects (56.9%). 41.3% of the PP subjects rated themselves as having poor oral health as compared to 24.1% of the HP subjects. Amongst those that had "poor" self rating, the HP group had significantly higher mean percentage of plaque and bleeding (BOP), whereas the PP group had significantly higher mean percentages of BOP only. Self rating of poor oral health condition and some questionnaire variables were significantly predictive of the actual severity of periodontal disease.

Conclusion: The results suggest that self reporting could reliably predict subjects with more severe periodontitis. The combination of demographic, periodontal risk factors and self reported variables is the best method in this prediction.

Root surface characteristics after planing with diamond coated ultrasonic inserts

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Introduction: A variety of inserts are designed for ultrasonic devices, of which plain metal (PM) and diamond coated (DC) insert have been preferentially used in scaling and planing of the superficial root surfaces. This study was undertaken to compare the effectiveness of these two kinds of insert in calculus removal and the effect on periodontal ligament and cementum.

Materials and Methods: Six extracted periodontal involved teeth were used for calculus removal test. Sub-gingival calculus marking area were randomly debrided either by DC or PM insert until visually satisfactory clean. The residual calculus was determined by scanning electron microscope (SEM). Six non-periodontal teeth extracted for orthodontic purposes were used to evaluate the effect on periodontal ligament and cementum after planing with different number of strokes (10, 20 and 30 strokes). Soft tissue damage, surface roughness or scratches and the thickness of remained cementum were examined by SEM.

Results: For calculus removal effectiveness, the residual calculus per area in DC group was greater than PM group. The periodontal tissue damage and surface roughness or scratches were observed in DC group rather than in PM group. Finally, the thickness of remained cementum was slightly greater in PM group than in DC group. All effects were increasingly observed according to a higher number of planing strokes.

Conclusion: In ultrasonic root planing on extracted root surfaces, the results indicate that the effectiveness of diamond coated insert is slightly less than plain metal insert.

Screening of three Thai medicinal plants for potential antibacterial activity

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Introduction: It is proved that the destruction of the periodontium in periodontitis is associated with Gram-negative anaerobic bacterial endotoxins and the host immune responses. The eradication of the putative periodontopathic bacteria, which could be achieved by mechanical debridement and in some situations with an adjunct of chemicals (antibiotics/antiseptics), is obviously the promising method in treating this morbidity. Eventhough the use of chemicals have shown to be a good adjunct to mechanical debridement, the increased drug resistance activity of microorganisms has become a serious problem which seems to hinder the use of these. In order to find an alternatives, medicinal plants seems to be a potent one because of their widely acceptance to use in treating various diseases including infectious diseases for centuries, though little known about the correlation in scientific reports. According to scarcely known about their scientific activities, three Thai medicinal plants: *Murraya paniculata L.*, called in Thai "Kaew"; *Azadirachta indica var Siamensis*, Thai name "Sa-dao"; *Chromolaena odorata linn.*, called "Sarb-sua", were chosen to evaluate their antibacterial activities on the two common periodontopathic bacterial species; *Prevotella intermedia(Pi)* and *Aggregatibactor actinomycetemcomitans(Aa)*.

Materials and Methods: Leaves and branches of selected plants were cut and ground into powder then mercerated in organic solvents (n-hexane, ethyl acetate, chloroform, dichloromethane, methanol). The extracts were concentrated under reduced pressure, resulting in crude extract. Stock solutions were prepared using DMSO to dissolve crude extract to yield concentration of 100 mg/ml (w/v). An agar diffusion technique was used and all samples were tested in duplicate.

Results: Diameter of the inhibition zone (mm)(Average)

	M. paniculata (leaves)			*M. paniculata* (branches)			*Azadirachta indica* (leaves)			*Chromolaena odorata* (leaves)			CHX 2%
	H	E	M	H	E	M	H	C_1	M	H	C_2	M	
Pi	11.25	9.75	8.75	10.75	0	10	7	9.5	9	8.5	9	7.75	18.75
Aa	5	5.25	6.25	7	8	6	6.5	7.5	9	7	7.75	9	29.5

H = n-Hexane, E=Ethyl acetate, M=Methanol, C_1=Chloroform, C_2=Dichloromethane

Conclusion: Our study indicated that three Thai medicinal plants, Kaew, Sa-dao and Sarb-sua, demonstrated antimicrobial activities against the studied periodontopathic bacteria (*Pi* and *Aa*). The action in killing of microorganisms may minimized the development of bacterial resistance. The availability and low cost of these herbs should encourage further investigation leading to a better understanding of Thai traditional medicine for prevention and reducing the incidence of periodontal infections.

HMGB1 expression in human gingival and PDL fibroblast activated by *Porphyromonas gingivalis*

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Introduction: High mobility group box1 (HMGB1) protein is nuclear protein which is recognized as late inflammatory cytokine but plays important roles in many inflammatory diseases. This study aimed to investigate the expression of HMGB1 in human gingival and periodontal ligament (PDL) fibroblasts after activated by *Porphyromonas gingivalis.*

Materials and Methods: Both types of cell were cultured and activated by LPS or sonicated extract of *P. gingivalis*. 25 µg/ml and 50 µg/ml of LPS were used in the LPS treatment group and 0.25 µg/ml and 0.50 µg/ml of sonicated extract were used in the sonicated extract group. Sterile distilled water was used as a control. Duration of treatment was 24 hours for RNA extraction and 48 hours for cell lysate extraction. The expression of HMGB1 mRNA was determined by RT-PCR and HMGB1 protein in cell lysate was determined by Western blot.

Results: Both HMGB1 mRNA and protein expression were upregulated in human gingival fibroblast when activated by LPS of *Pg,* while only HMGB1 protein level was upregulated in PDL fibroblast. While limited change of HMGB1 mRNA and protein level in both cells were found when activated by sonicated extract of *P. gingivalis.*

Conclusion: The results showed that only the purified LPS of *P. gingivalis* can activate the expression of HMGB1 in gingival and PDL fibroblasts. HMGB1 may play important role in the pathogenesis of periodontal disease.

Expression of IL-1β, TNF-α and iNOS in diabetes induced rats with periodontitis

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Introduction: Diabetes mellitus is a chronic inflammatory disease that is closely related to periodontitis. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and inducible nitric oxide synthase (iNOS) have been reported to play a key role in pathogenesis of both diseases but the role of these cytokines in the severity of diabetes associated periodontitis remains unclear. Therefore, the influences of type 2 diabetes mellitus on the severity of periodontitis through the role of 3 inflammatory markers were investigated in this study.

Materials and Methods: Thirty male wistar rats were divided equally into 3 groups. Diabetes group (DM, n=10) were injected with nicotinamide (230 mg/kg, i.p.) and streptozotocin (65 mg/kg, i.v.) compared with periodontitis group (PD, n=10) in which 4-0 silk ligatures were placed around the cervical area of first upper molars and diabetes associated periodontitis group (DMPD, n=10). All of them were later sacrificed at 2, 4, 6 and 12 weeks and their maxilla including gingival tissues were evaluated histologically. Paraffin sections of gingival tissues were immunohistologically stained to investigate the expression of 3 inflammatory markers.

Results: The expression of all 3 inflammatory markers were increased with duration of time especially in PD and DMPD group, but there's no clearly difference in the expression between PD and DMPD groups. In DM group, the expressions of the inflammatory markers were decreased at 12 week.

Conclusion: The results suggested that TNF-α, IL-1β and iNOS may play an important role in local inflammatory response involving periodontal destruction in type 2 diabetes mellitus.

GCF osteoprotegerin level after ultrasonic debridement with essential oil solution

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Introduction: The piezoelectric ultrasonic root debridement in conjunction with antimicrobial agent as a coolant has become popular in recent years due to their bactericidal properties. The objective of this research was to assess the level of osteoprotegerin in gingival crevicular fluid (GCF) after using an essential oil solution as a coolant during piezoelectric ultrasonic root debridement in chronic periodontitis patients.

Materials and Methods: 120 periodontal pockets in 30 moderate to severe periodontitis patients were recruited. The samples were divided into 2 groups of 60 periodontal pockets each. Group 1 was debrided with piezoelectric ultrasonic scaler with an essential oil solution as a coolant and Group 2 debrided with piezoelectric ultrasonic scaler with distilled water as a coolant. Clinical changes were noted and GCF were collected at baseline, 6 weeks and 12 weeks after treatment. The level of osteoprotegerin was evaluated using osteoprotegerin instant ELISA kit.

Results: Pocket depths were significantly decreased and clinical attachment levels were significantly increased at 6 and 12 weeks ($p<0.05$) after treatment in both groups, but no significant difference was observed between groups. Osteoprotegerin concentrations were also significantly increased from 1.91 (pg/µl) at baseline to 3.29 (pg/µl) and 3.55 (pg/µl) after 6 and 12 weeks in the experimental test group ($p<0.05$). No significant difference was observed in control group**.**

Conclusion: The use of the essential oil solution as a coolant during piezoelectric ultrasonic root debridement is potentially an alternative strategy to improve periodontal health in periodontitis patients due to increased osteoprotegerin in GCF.

Cytodifferentiation activity of synthetic human enamel sheath protein peptides

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Introduction: Enamel sheath protein (ESP) is one of the porcine enamel matrix proteins involved in the construction of the enamel sheath during teeth development. 17-kDa ESP is one-step cleavage product processed by proteolysis from the N-terminal side of sheathlin (ameloblastin/amelin). ESP showed cementum regeneration activity in a buccal dehiscence model in dogs and promoted the cytodifferentiation of cultured human periodontal ligament (HPDL) cells. The aim of this study was to determine the enamel sheath protein peptide on the C-terminal side of the 17-kDa ESP, which possesses the cytodifferentiation activity for cultured HPDL cells.

Materials and Methods: Several peptides were determined based on the sequence of C-terminal side peptide of human and porcine ESPs. These peptides were synthesized by a commercial company. HPDL cells were obtained from extracted healthy premolars for orthodontic reasons. The cells were maintained in a-MEM containing 10% fetal bovine serum and 1% antibiotics (100U/m of Penicillin-G and 100mg/ml of Streptomycin sulfate) at 37°C in humidified 5% CO_2 atmosphere. Synthetic peptides corresponding to peptide increments along the 17-kDa human ESP C-terminal side sequence were tested the alkaline phosphatase (ALP) and mineralization activity of cultured HPDL cells.

Results: The synthetic peptides made from the 17-kDa human ESP increased ALPase activity and matrix mineralization activity in long-term culture of HPDL cells. The expression of osteocalcin and BSP was showed by RT-PCR compared with control. The peptide SDKPPKPELPGVDF showed the strongest cytodifferentiation activity among the synthetic peptides.

Conclusion: The results indicated that the SDKPPKPELPVDF, one of the peptides synthesized on the basis of the human enamel sheath protein sequence, accelerated the cytodifferentation activity of HPDL cells in the cell culture system. Since the cytodifferentitaon activity of HPDL cells may correlate with cementum regeneration promoting activity, these results may be clinically useful for periodontal ligament regeneration.