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Controlling the plaque biofilm

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Aim: To examine the effect of supragingival plaque removal in conjunction with different periodontal therapies on subgingival plaque composition in different subject populations. **Method:** Four different studies are presented which examined the effect of repeated removal of supragingival plaque performed by professionals or by tooth brushing on subgingival plaque composition. The studies were performed in different populations including chronic periodontitis, periodontal maintenance and refractory subjects. For all studies, each subject was examined for clinical parameters at up to 168 sites and subgingival plaque samples were taken from the mesial aspect of each tooth and examined for their content of specific bacterial species using checkerboard DNA-DNA hybridisation techniques. **Results:** Repeated supragingival plaque removal used in conjunction with scaling and root planing only or combined with other periodontal therapies resulted in improvements in clinical parameters as well as significant decreases in the counts of subgingival species, including those associated with periodontal disease aetiology. Meticulous tooth brushing provided similar clinical and microbial improvements. **Conclusions:** Meticulous removal of supragingival plaque has beneficial effects on clinical parameters of periodontal disease and on the nature of the microbiota that colonises both above and below the gingival margin and appropriately has been a major focus in the prevention and control of dental diseases, particularly periodontal disease.

Key words: Periodontal diseases, supragingival plaque removal, supragingival plaque, subgingival plaque, periodontal therapy, periodontal pathogens, oral hygiene

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Periodontal diseases are infections that are caused by specific bacteria found in sub and also supragingival plaque[1]. Thus, the regular removal of the plaque biofilm above and below the gingival margin is essential and has been the principle cornerstone in the prevention and treatment of periodontal infections. Scaling and root planing (SRP) which involves the removal of plaque and hard deposits under the gum has been the major therapy employed in clinical practice to treat periodontal diseases. SRP has been shown to improve both clinical and microbiological parameters of periodontal disease[2-11] and is routinely employed during maintenance therapy to sustain improvements achieved by periodontal therapy.

Effect of supragingival plaque removal on subgingival microbial composition

The repeated removal of supragingival plaque has also been shown to affect the subgingival plaque biofilm as well as being a major aid in reducing clinical signs of inflammation. Smulow *et al.*[12] examined the effect of repeated supragingival plaque removal on specific cell or colony morphotypes in subgingival plaque. Fourteen subjects with chronic periodontitis and at least four sites with pocket depth ≥5mm were included in the study. The four sites were randomly assigned to receive a different therapy. The treatments included supra and subgingival plaque removal followed by 21 days of

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supragingival plaque removal; supra and subgingival plaque removal followed by no subsequent therapy; removal of supragingival deposits only followed by 21 days of supragingival plaque removal, or no therapy. Subgingival plaque samples were taken from the four sites in each subject and evaluated culturally for counts of *Bacteroides* sp., facultative and obligatory anaerobes and by darkfield microscopy for the presence of spirochetes. The results indicated that sites receiving the 21 days of professional supragingival plaque removal showed the greatest decrease in the test species. Clinical findings indicated that these sites also showed the greatest improvement in pocket depth reduction.

Hellstrom *et al.*[13] designed an experiment to determine if the change in the subgingival microbiota following careful supragingival debridement could be explained by a reduction in pocket depth. In their study, suprabony, infrabony and furcation sites in 12 subjects were examined clinically and samples of subgingival plaque were taken at baseline and at 30 weeks. Subjects received professional supragingival plaque removal 2 to 3 times per week for the 30 week period. The results indicated that reductions in total counts and

were observed at all site types including infrabony pockets where no pocket depth reduction occurred. Thus, the changes in the subgingival microbiota were due to persistent removal of the supragingival plaque rather than pocket depth reduction.

Ximenez-Fyvie *et al.*[14] examined the effect of repeated supragingival plaque removal in 18 periodontal maintenance subjects. This study evaluated a wider range of 40 bacterial species and a larger number of plaque samples per subject using whole genomic DNA probes and the checkerboard DNA-DNA hybridisation tech-

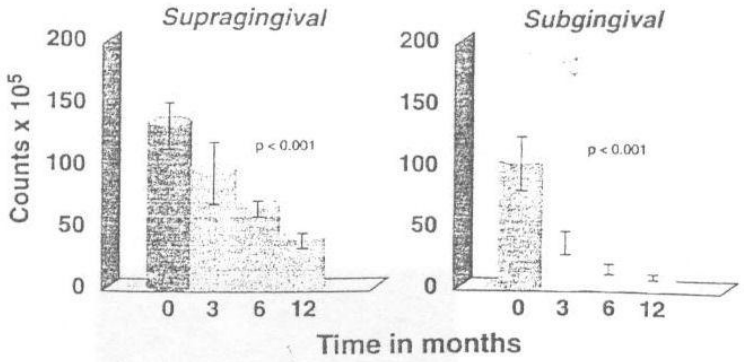

Figure 1. Mean total DNA probe counts (x10⁵, ±SEM) in supra and subgingival plaque samples taken at baseline, 3, 6 and 12 months in 18 periodontal maintenance subjects. Professional supragingival plaque control was performed between baseline and 3 months. Mean counts were computed for a subject for each visit and then values were averaged across the 18 subjects at each time point. The whiskers indicate the SEM. A total of 3,608 plaque samples were evaluated. Significance of differences over time was sought using the Quade test.

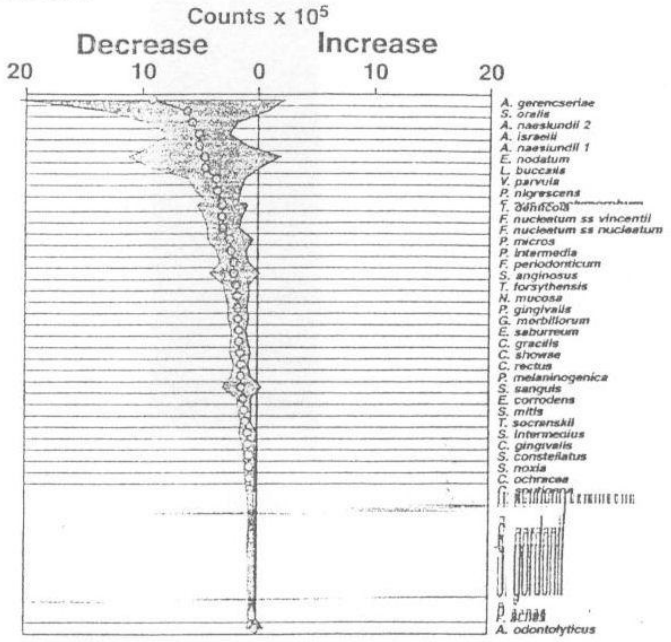

Figure 2. Mean change in counts (x10⁵) between baseline and 12 months. Mean counts of each species were computed within a subject at baseline and 12 months and the difference between the mean counts at the two visits computed. The circles represent the mean change for each species and the shaded area represents the 95% confidence intervals (CIs). Values to the left of the vertical '0' line represent a decrease in mean counts and 95% CIs that do not cross the '0' line indicate species where the decrease was significant. The species are ordered from greatest to least mean change.

and were seen at weekly intervals for a period of three months for professional removal of supragingival plaque using scalers and polishing instruments as well as flossing between the contact points. Clinical measurements of inflammation, pocket depth and attachment each tooth. Supra, and separately, subgingival plaque samples were taken from the mesial aspect of each tooth at baseline, 3 months (immediately following completion of professional supragingival plaque removal phase), 6 and 12 months. Total counts of both

Table 1 Mean (± SD) clinical parameters of the subjects in the four treatment groups at baseline and 3 months

	SRP (N=14)		Metronidazole (N=14)		Professional cleaning (N=13)		Both (N=13)	
	Baseline	3 months	Baseline	3 months	Baseline	3 months	Baseline	3 months
Plaque Index	1.24 ± 0.81	0.93 ± 0.56*	1.27 ± 0.90	0.76 ± 0.32*	0.99 ± 0.46	0.57 ± 0.38**	0.92 ± 0.39	0.66 ± 0.58*
Gingival Index	0.66 ± 0.35	0.56 ± 0.29	0.90 ± 0.52	0.51 ± 0.26**	0.60 ± 0.23	0.45 ± 0.25	0.77 ± 0.26	0.42 ± 0.23**
% sites with BOP	26 ± 16	20 ± 14*	33 ± 22	20 ± 11**	25 ± 16	14 ± 8**	36 ± 18	18 ± 11**
% sites with suppuration	0.97 ± 2.03	0.89 ± 2.38	1.65 ± 3.12	0.04 ± 0.16*	0.18 ± 0.36	0.0 ± 0.0**	0.56 ± 1.69	0.26 ± 0.72
Mean PD (mm)	2.70 ± 0.34	2.55 ± 0.28**	2.91 ± 0.57	2.64 ± 0.34*	2.93 ± 0.30	2.69 ± 0.41*	2.93 ± 0.52	2.61 ± 0.40**
Mean AL (mm)	2.99 ± 0.74	2.97 ± 0.73	3.07 ± 0.90	2.94 ± 0.76	3.29 ± 0.79	3.17 ± 0.88	2.96 ± 0.69	2.83 ± 0.75*

* $p < 0.05$, ** $p < 0.01$; Significant difference from baseline to 3 months using the Wilcoxon signed ranks test.

decreased significantly from baseline to 3 months and continued to decrease at 6 and 12 months (*Figure 1*). Further, counts of 34 of the 40 test species in subgingival plaque samples were decreased significantly at 3 months and continued at the reduced counts to 12 months (*Figure 2*). Indeed, at 12 months the microbial profile of the subjects in the study was similar to that seen in periodontal health[14].

Supragingival plaque removal as an adjunct to other periodontal therapies

The study of Ximenez-Fyvie *et al.*[14] was performed in chronic periodontitis subjects who were in the maintenance phase of therapy and who had few pockets >5mm. Westfelt *et al.*[16] in a study of subjects with moderate to severe periodontal disease, indicated that professional supragingival plaque control alone was insufficient to prevent disease progression in subjects with advanced destructive periodontitis. However, it was conceivable that professional repeated supragingival plaque removal may be useful as an adjunct to SRP in the treatment of moderate to advanced periodontitis if combined with other periodontal therapies. In a study carried out at The Forsyth Institute, 54 subjects with moderate periodontitis were recruited in order to examine the adjunctive effect of systemically administered metronidazole, repeated professional supragingival plaque removal, neither or both. Subjects were measured clinically at six sites per tooth at up to 28 teeth at baseline and at 3 months. In addition, samples of subgingival plaque were taken from the mesial aspect of each tooth and evaluated for the levels of 40 bacterial species using checkerboard DNA-DNA hybridisation. After baseline monitoring, subjects were randomly assigned to one of four treatment groups. All subjects received full mouth SRP together with one of: systemically administrated metronidazole at the dosage of 250mg four times a day for 14 days (N = 14), repeated professional supragingival plaque removal weekly for 3 months (N = 13), both therapies (N = 13) or neither therapy (N = 14). At 3 months, subjects in all treatment groups showed improvement in clinical parameters (*Table 1*), but the greatest reduction in mean pocket depth was observed in the group receiving the combined therapy of metronidazole and professional cleaning (*Figure 3*).

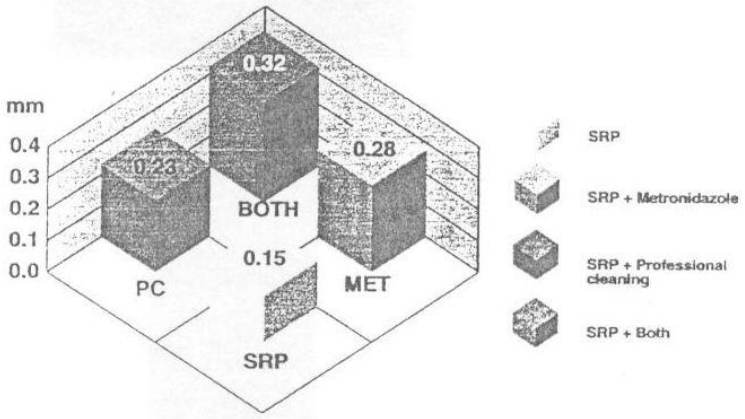

Figure 3. Mean full mouth pocket depth reduction from baseline to 3 months in subjects in the four treatment groups. The changes in pocket depth were averaged at six sites per tooth for all teeth excluding third molars in each subject and then averaged across the subjects in the four treatment groups separately. The values at the top of each bar indicate the mean reductions in mm. The treatment groups have been colour coded: yellow = SRP only; turquoise = SRP + repeated professional supragingival plaque removal; purple = SRP + systemically administered metronidazole; red = SRP + both repeated professional supragingival plaque removal and systemically administered metronidazole.

The sites were subset into initial pocket depth categories of <4mm or ≥4mm (*Table 2*). Sites with initial pocket depth ≥4mm showed a significant decrease in both pocket depth and attachment level in all treatment groups at 3 months. In contrast, little improvement was seen in mean attachment level at sites with initial pocket depth <4mm. However, a significant decrease in mean pocket depth was seen for sites in the <4mm category in subjects receiving SRP and professional cleaning in the presence or absence of systemically administered metronidazole.

Inspection of the data in *Table 2*

Table 2 Mean (± SD) pocket depth and attachment level at baseline and 3 months at sites with baseline pocket depths <4mm and ³4mm

	SRP		Metronidazole		Professional cleaning		Both	
	Baseline	3 months	Baseline	3 months	Baseline	3 months	Baseline	3 months
Baseline Pocket Depth <4mm								
Mean PD (mm)	2.27 ± 0.21	2.23 ± 0.19	2.32 ± 0.19	2.21 ± 0.18	2.48 ± 0.17	2.35 ± 0.25*	2.34 ± 0.21	2.21 ± 0.22*
Mean AL (mm)	2.65 ± 0.70	2.71 ± 0.71	2.51 ± 0.65	2.48 ± 0.61	2.89 ± 0.72	2.86 ± 0.80	2.45 ± 0.48	2.42 ± 0.53
Baseline Pocket Depth ³4mm								
Mean PD (mm)	4.59 ± 0.33	3.93 ± 0.52**	4.77 ± 0.50	3.95 ± 0.50**	4.78 ± 0.49	4.10 ± 0.59**	4.71 ± 0.32	3.83 ± 0.56**
Mean AL (mm)	4.57 ± 0.83	4.15 ± 0.83**	4.80 ± 0.96	4.24 ± 0.96**	4.97 ± 1.15	4.48 ± 1.24**	4.52 ± 0.71	3.98 ± 0.93**

* $p < 0.05$, ** $p < 0.01$; Significant difference from baseline to 3 months using the Wilcoxon signed ranks test.

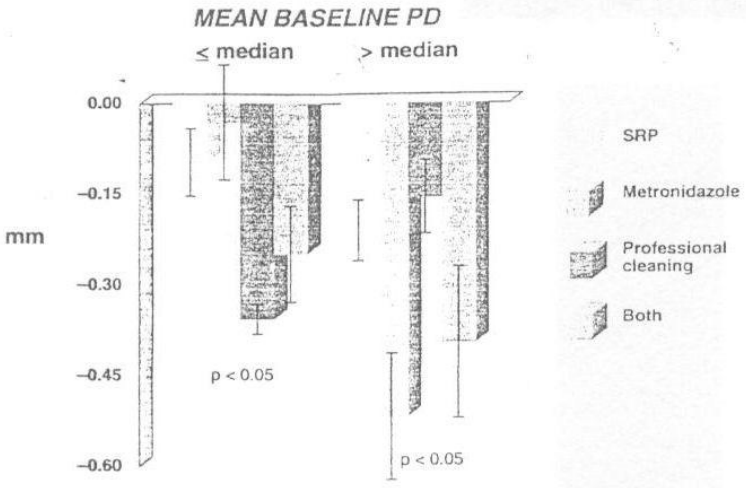

Figure 4. Mean full mouth pocket depth reduction (±SEM) from baseline to 3 months in subjects in the four treatment groups subset according to their mean full mouth baseline pocket depth. The four bars to the left represent the subjects whose mean baseline pocket depth was less than or equal to the median of 2.82mm for the entire group of 54 subjects. The four bars to the right represent the subjects whose baseline mean pocket depth was greater than the median. For each category of subjects, the changes in pocket depth were averaged at six sites per tooth for all teeth excluding third molars in a given subject and then averaged across subjects in the four treatment groups. Significance of differences in mean pocket depth reduction among treatment groups in the two baseline pocket depth categories was tested using the Kruskal-Wallis test. The treatment groups are colour coded as described in *Figure 3*.

suggested that shallow sites were most improved in subjects receiving adjunctive professional cleaning with or without adjunctive metronidazole, whereas the greatest improvement at the deeper sites occurred in subjects receiving adjunctive metronidazole with or without professional cleaning. This observation led to an analysis in which subjects were subset into those with less baseline periodontal disease or greater baseline periodontal disease based on the median pocket depth value (2.82mm) for all 54 subjects. *Figure 4* presents the mean change in pocket depth in subjects with mean full mouth baseline pocket depth ≤2.82mm or >2.82mm in the four treatment groups.

The data indicated that subjects receiving SRP and professional cleaning with or without metronidazole exhibited the greatest mean pocket depth reduction in subjects with less baseline periodontal disease. In subjects with more initial disease, the greatest improvement in mean pocket depth was seen in subjects receiving SRP plus metronidazole alone or in combination with professional cleaning. In both baseline disease level categories, the changes in pocket depth among treatment groups were statistically significant.

The clinical improvements observed in the different treatment groups were reflected by changes in counts of the species in subgingival plaque. The change in median counts ($\times 10^5$) of the 40 test species in the four treatment groups from baseline to 3 months post therapy is presented in *Figure 5*. The biggest decrease in mean counts occurred in subjects receiving both adjunctive professional cleaning and metronidazole. The most striking reductions were for members of the orange complex, including species thought to contribute to the initiation and progression of periodontal diseases such as members of the genera *Campylobacter*, *Eubacterium*, *Fusobacterium* and *Prevotella*. Importantly, members of the red complex, which includes two designated periodontal pathogens, *Bacteroides forsythus* and *P. gingivalis* were reduced in all treatment groups although the greatest reduction occurred in the combined adjunctive therapy group. Mean counts of the total red complex species were reduced in all treatment groups post therapy (*Figure 6*). However, the greatest reduction was seen in subjects receiving metronidazole, particularly those subjects receiving both the antibiotic and professional cleaning. When the sites were subset according to baseline pocket depth of <4mm or ≥4mm, greater reductions in mean counts were observed at sites with initially deep pockets in all treatment groups (*Table 3*). In

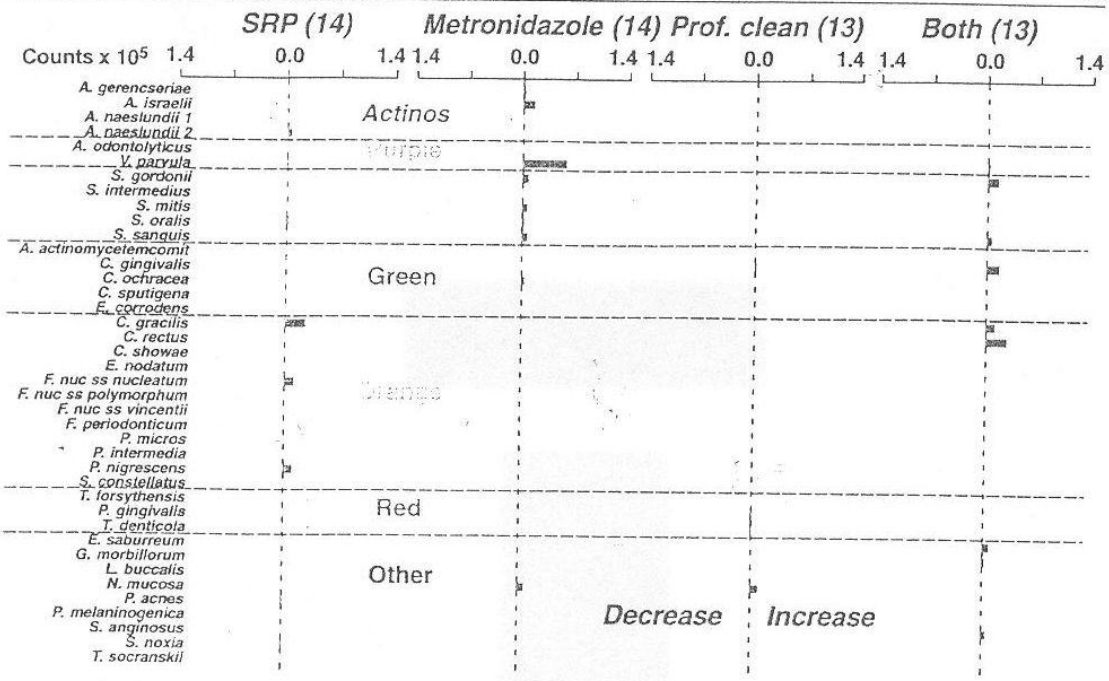

Figure 5. Median changes from baseline to 3 months in the counts (x10^5) of 40 subgingival species in subjects in the four treatment groups. The changes in counts of each species were averaged for all sampled sites in a subject and then the median values were determined for each species in each treatment group. The species are ordered according to the microbial complexes described by Socransky *et al.*[24]. The bars to the left in each panel represent species that decreased in counts, while the bars to the right represent species that increased in counts post-therapy. The treatment groups are colour coded as described in *Figure 3*.

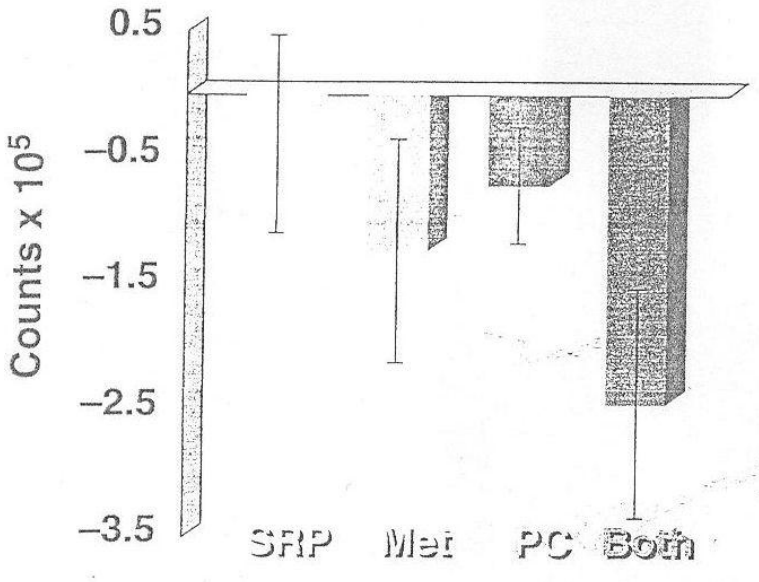

Figure 6. Mean changes from baseline to 3 months in the total counts (x 10^5, ±SEM) of the red complex species (*B. forsythus, P. gingivalis* and *T. denticola*) in subjects in the four treatment groups. The sum of the three species was computed at each site for each visit for all subjects. The change in total counts of red complex species were then averaged for all sites in a subject and then averaged across subjects in each treatment group. The treatment groups are colour coded as described in *Figure 3*.

both pocket depth categories, the greatest reduction in mean counts post therapy was seen in subjects receiving the combined therapies. Thus, the data from this investigation indicated that the clinical and microbial effects of SRP can be augmented by the systemic administration of metronidazole and by careful repeated supragingival plaque debridement.

Use of combined therapies in the treatment of 'refractory' periodontitis

The beneficial effect of repeated professional supragingival plaque removal may be seen in other studies. Studies by Colombo *et al.*[17] indicated that certain subjects responded well to periodontal therapies that included SRP, periodontal surgery and systemically administered antibiotics, while other subjects did not. The latter

Table 3 Change in mean counts (x10^5, ± SEM) of red complex species (*B. forsythus*, *P. gingivalis* and *T. denticola*) in samples from sites with different baseline pocket depths in the four treatment groups

	Baseline PD <4mm	Baseline PD >4mm
Scaling and root planing	−0.15 ± 0.72	−0.99 ± 1.57
SRP + Metronidazole	−0.52 ± 0.59	−1.43 ± 1.07
SRP + Professional cleaning	−0.54 ± 0.44	−1.53 ± 0.80
SRP + Both	−0.96 ± 0.57	−4.76 ± 1.15

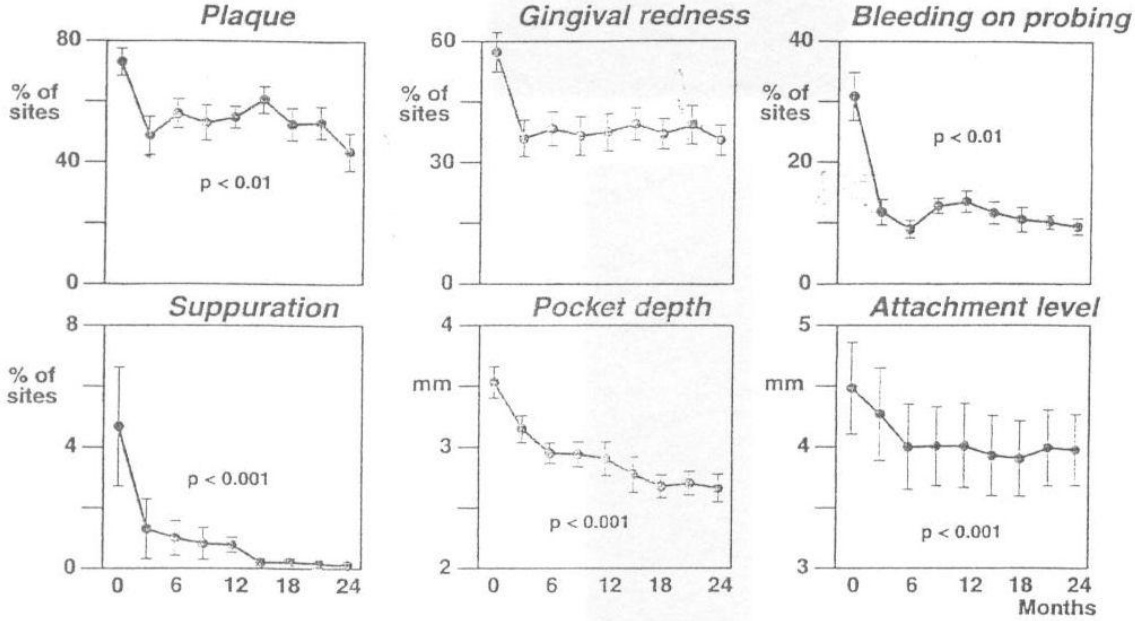

Figure 7. Mean clinical parameters (±SEM) at different time points in 10 refractory patients. Values were averaged within a subject for up to 168 sites (six sites/tooth for all teeth excluding third molars) and averaged across subjects for each time point. Significance of differences over time was sought using the Quade test.

group of subjects who were 'refractory' to a series of conventional periodontal therapies received maintenance therapy at the Forsyth Center but continued to show disease progression. The earlier studies indicated that conventional treatments diminished periodontal pathogen load comparably in successfully treated and 'refractory' subjects. Therefore, it was hypothesised that 'refractory' subjects may harbour more virulent pathogens, or be more susceptible to even low levels of these species. Thus, a study was undertaken to determine whether combined periodontal therapies, including repeated professional supragingival plaque removal, could further reduce periodontal pathogens leading to periodontal stability in refractory subjects. Ten subjects were identified as 'refractory' based on full mouth mean attachment loss and/or >3 sites with attachment loss ≥3mm within 1 year following SRP, periodontal surgery and systemic antibiotics. After baseline monitoring, the refractory subjects received SRP, locally delivered tetracycline at pockets ≥4mm, systemically administered amoxicillin (500mg, tid for 14 days) + metronidazole (250mg, tid for 14 days) and professional removal of supragingival plaque every week for 3 months. Subjects were monitored every 3 months post-therapy for 2 years.

The change in the mean levels of the clinical parameters are shown in *Figure 7*. All clinical parameters were significantly improved over time with the exception of gingival redness. Thus, the combined therapy was successful in controlling disease progression in 'refractory' periodontitis subjects for up to 2 years.

The microbial changes that occurred after this combined aggressive antimicrobial therapy in subjects identified as 'refractory' to conventional periodontal therapy were determined. Subgingival plaque samples were taken from the mesial aspect of each tooth and the levels of 40 subgingival taxa were determined using checkerboard DNA-DNA hybridisation. From baseline to 24 months,