

The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota

Laurie Ann Ximénez-Fyvie,
Anne D. Haffajee, Sovanda Som,
Maureen Thompson, Gay Torresyap
and Sigmund S. Socransky

Department of Periodontology, The Forsyth
Institute, Boston, MA, USA

Ximénez-Fyvie LA, Haffajee AD, Som S, Thompson M, Torresyap G, Socransky SS. The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota. J Clin Periodontol 2000; 27: 637–647. © Munksgaard, 2000.

Abstract

Background, aims: The purpose of the present investigation was to determine the effect of weekly professionally administered supragingival plaque removal on the composition of the supra and subgingival microbiota.

Methods: 18 adult subjects with periodontitis who had been treated and were in a maintenance phase of therapy were clinically and microbiologically monitored at baseline, 3, 6 and 12 months. After the baseline visit, the subjects received scaling and root planing followed by professional supragingival plaque removal every week for 3 months. Clinical measures of plaque accumulation, bleeding on probing (BOP), gingival redness, suppuration, pocket depth and attachment level were made at 6 sites per tooth at each visit. Separate supra ($N=1804$) and subgingival ($N=1804$) plaque samples were taken from the mesial aspect of all teeth excluding third molars in each subject at each time point and evaluated for their content of 40 bacterial taxa using checkerboard DNA-DNA hybridization. Significance of changes in mean counts, prevalence and proportions of bacterial species over time in both supra and subgingival samples were determined using the Quade test and adjusted for multiple comparisons.

Results: Mean % of sites exhibiting plaque, gingival redness and BOP were significantly reduced during the course of the study. Significant decreases in mean counts were observed in both supra and subgingival samples. Mean total DNA probe counts ($\times 10^5$, \pm SEM) at baseline, 3, 6 and 12 months were: 133 ± 19 , 95 ± 25 , 66 ± 6 , 41 ± 6 ($p < 0.001$) for supragingival samples and 105 ± 22 , 40 ± 10 , 19 ± 4 , 13 ± 3 ($p < 0.001$) for subgingival samples. Mean counts of 22 of 40 and 34 of 40 species tested were significantly reduced in the supra and subgingival samples respectively over the monitoring period. For example, mean counts of *Porphyromonas gingivalis* $\times 10^5$ at baseline, 3, 6 and 12 months in the subgingival plaque samples were 2.0 ± 0.4 , 0.5 ± 0.2 , 0.6 ± 0.3 , 0.3 ± 0.1 ($p < 0.001$); *Bacteroides forsythus* 2.0 ± 0.6 , 0.4 ± 0.1 , 0.4 ± 0.2 , 0.1 ± 0.2 ($p < 0.001$); *Treponema denticola* 3.4 ± 1.1 , 0.8 ± 0.3 , 0.4 ± 0.2 , 0.3 ± 0.3 ($p < 0.01$). Similar reductions were seen in supragingival plaque samples. While counts were markedly reduced by professional plaque removal, the proportion and prevalence of the 40 test species were marginally affected.

Conclusions: Weekly professional supragingival plaque removal profoundly diminished counts of both supra- and subgingival species creating a microbial profile comparable to that observed in periodontal health. This profile was maintained at the final monitoring visit, 9 months after completion of therapy.

Key words: microbiology; periodontal health; periodontal disease; supragingival plaque; subgingival plaque; periodontal pathogens; bacteria; DNA probes; treatment

Accepted for publication 15 October 1999

The regular, meticulous removal of supragingival plaque has been and continues to be the cornerstone in the maintenance of a healthy periodontium. Indeed, it has been estimated that over 9 billion dollars are spent annually worldwide on products to remove supragingival plaque. Further, large sums of money are spent to develop new methods to decrease dental plaque including the development of better toothbrushes and interdental cleansing devices as well as more effective toothpastes and mouthrinses.

This financial investment in methods to reduce dental plaque is based on the recognition that professional treatment and well performed home care procedures are of clinical benefit to all, particularly the periodontal patient. Many studies have demonstrated the clinical importance of supragingival plaque removal. The classic studies of Løe et al. (1965) demonstrated that if plaque was allowed to accumulate undisturbed by oral hygiene procedures for a period of 21 days in periodontally healthy subjects, gingival inflammation increased. Further, removal of the accumulated plaque and a return to regular home care procedures reversed these effects. Other studies have shown the importance of meticulous plaque control after periodontal therapy. For example, Nyman et al. (1975) evaluated the effects of periodontal surgery in subjects in whom supragingival plaque control was scrupulously maintained by professional cleaning compared with subjects whose self-performed plaque control was less than optimal. The professionally maintained subjects exhibited mean attachment level gains of 0.3 mm, while the subjects with poor plaque control exhibited mean losses of >1 mm at 6–24 months post-therapy. Similar relationships between supragingival plaque control and therapeutic success were noted in studies of bone regeneration in infrabony pockets (Rosling et al. 1976a), the use of systemically administered antibiotics (Kornman et al. 1994), the use of barrier material in periodontal regeneration (De Sanctis et al. 1996a, b) and long-term maintenance after surgical procedures (Rosling et al. 1976b, Axelsson & Lindhe 1981, Lindhe et al. 1982, 1984, Westfelt et al. 1985). Lindhe & Nyman (1975) demonstrated that the periodontium of patients with advanced periodontitis could be maintained for 5 years if the patients exhibited meticu-

lous self-performed plaque control. Thus, in general, effective supragingival plaque control leads to an improved clinical status for the periodontal patient.

The effect of supragingival plaque control on the microbial composition of subgingival plaque has been examined. For the most part, these studies evaluated relatively small numbers of sites in small numbers of subjects. Treatment protocols varied, with a number of studies utilizing SRP prior to professional cleaning (Tabita et al. 1981, Smulow et al. 1983, Magnusson et al. 1984, Muller et al. 1986, Lavanchy et al. 1987, McNabb et al. 1992), while others evaluated the effect of repeated professional cleaning (Tabita et al. 1981, Smulow et al. 1983, Kho et al. 1985, Beltrami et al. 1987, Dahlen et al. 1992, Al-Yahfoufi et al. 1995, Hellstrom et al. 1996) or self-performed plaque control (Tabita et al. 1981). While some studies found no microbiological effect (Kho et al. 1985, Beltrami et al. 1987, Lavanchy et al. 1987), others suggested that careful supragingival plaque control decreased the amount of subgingival plaque and/or the levels of specific subgingival species or morphotypes. For example, studies utilizing darkfield microscopy, indicated that professional plaque removal at multiple visits reduced the proportions of motile organisms and spirochetes in subgingival plaque samples (Smulow et al. 1983, Magnusson et al. 1984, Muller et al. 1986, Katsanoulas et al. 1992, McNabb et al. 1992). Further, studies employing cultural techniques indicated that professional supragingival plaque removal decreased total counts and/or levels of specific species or bacterial groups including the “black-pigmented *Bacteroides*”, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (Dahlen et al. 1992, McNabb et al. 1992, Smulow et al. 1983, Hellstrom et al. 1996). In addition, the microbial changes were accompanied by an improvement in clinical parameters of disease and in some cases long-term stability.

In all of the studies outlined above, the microbiological assessment was limited in terms of one or all of the following: the number of sites evaluated, the number of species evaluated and the nature of the microbiological technique(s) employed. It is clear that supragingival plaque control by whatever means is important in the control

of periodontal disease progression. However, in order to understand the biological basis of this therapy, a more comprehensive evaluation of its effect on the subgingival microbiota is warranted. Thus, the purpose of the present investigation was to evaluate the effect of weekly professional supragingival plaque removal for a period of 3 months on the composition of the supra and subgingival microbiota assessed at each tooth in 18 subjects using 40 DNA probes and checkerboard DNA-DNA hybridization.

Material and Methods

Subject population

The subjects who participated in this study were a subset of the subjects described previously (Ximenez-Fyvie et al. 2000a). 21 adult periodontitis subjects who had received periodontal therapy and were in a maintenance program were selected for study. Data for 21 subjects were available to 6 months and for 18 subjects to one year. For simplicity, data will be presented for the 18 subjects who completed the study. However, analysis of data for either the 18 or 21 subjects at 6 months provided essentially the same results. The subjects had ≥ 20 teeth and at least 4 sites with pocket depth >4 mm and/or 4 sites with attachment loss >4 mm. Exclusion criteria included pregnancy, lactation, active periodontal or antibiotic therapy in the previous 3 months, any systemic condition which could influence the course of periodontal disease or which would require pre-medication for monitoring procedures. No subjects with localized juvenile periodontitis, rapidly progressive periodontitis or acute necrotizing ulcerative gingivitis were included in the study.

Clinical monitoring

Subjects were screened for suitability and, if accepted, signed informed consent. Clinical measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars (a maximum of 168 sites per subject) as previously described (Haffajee et al. 1983) at baseline, 3, 6 and 12 months. Clinical assessment included plaque accumulation (0/1), overt gingivitis (0/1), bleeding on probing (0/1), suppuration (0/1), probing pocket depth and probing attachment level.

Table 1. Baseline clinical characteristics of the subject group (N=18)

	Mean (\pm SD)	Range
age (years)	52 \pm 12	32–74
no. missing teeth	2.6 \pm 2.3	0–7
% males	61	
mean pocket depth (mm)	2.8 \pm 0.4	2.2–3.6
mean attachment level (mm)	2.7 \pm 0.8	1.5–4.3
% sites with:		
plaque	68 \pm 26	5–100
gingival redness	59 \pm 33	0–100
bleeding on probing	30 \pm 16	4–52
suppuration	0.7 \pm 1.3	0–4.6

Pocket depth and attachment level measurements were taken twice by the same examiner at each visit and the average of the pair of measurements was used for analysis. Such measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL). All measurements for a given subject were performed by the same examiner at each visit. The examiners performing the measurements did not treat the subjects. The baseline clinical features of the 18 subjects are presented in Table 1.

Microbial assessment of plaque samples

Samples of supra and separately subgingival plaque were collected from up to 28 supra and 28 subgingival sites in each subject at baseline, 3, 6 and 12 months. A total of 1804 supra and 1804 subgingival samples were taken for the 4 visits and individually analyzed for their content of 40 species using checkerboard DNA-DNA hybridization (Socransky et al. 1994). After drying and isolation with cotton rolls, supragingival plaque was sampled from the mesio-buccal aspect of each tooth excluding third molars, using sterile Gracey curettes. Each plaque sample was placed in individual tubes containing 150 μ l of TE buffer (pH 7.6). After removal of the supragingival sample and any remaining supragingival plaque, subgingival plaque samples were taken from the same sites (i.e., the mesio-buccal aspect of each tooth) using sterile Gracey curettes and placed in similar individual tubes. 100 μ l of 0.5 M NaOH were added to each tube and the samples were dispersed in a vortex mixer. Samples were boiled for 10 min and neutralized using 800 μ l of 5 M ammonium acetate. The released DNA was then placed into the extended slots

of a Minislot-30 apparatus (Immunetics, Cambridge MA), concentrated onto a 15 \times 15 cm positively-charged nylon membrane (Boehringer Mannheim, Indianapolis, IN) and fixed to the membrane by cross-linking under ultraviolet light followed by baking at 120°C for 20 min. Two lanes on each membrane had standards that consisted of a mixture at 10⁵ and 10⁶ cells of each bacterial species tested. The preparation of the plaque samples and evaluation of the plaque samples using checkerboard DNA-DNA hybridization were described in detail previously (Ximenez-Fyvie et al. 2000a).

Treatment

After the initial monitoring visit, subjects received scaling and root planing

(SRP) performed under local anaesthesia. Quadrants were treated at approximately weekly intervals, so that treatment was completed in one month. In addition, subjects were instructed in oral hygiene procedures. After the SRP phase, the subjects were seen weekly for professional supragingival plaque removal for 3 months. Supragingival plaque was revealed using disclosing solution. Visible supragingival plaque was removed using scalers on all accessible surfaces followed by polishing of the teeth using a rubber cup and dentifrice. Finally, all interproximal surfaces were cleaned using dental floss. When performing any of these procedures, care was taken to limit plaque removal to the supragingival area only. All subjects received subgingival maintenance scaling at 3, 6, 9 and 12 months after the monitoring and sample taking visit.

Data analysis

Clinical data available for each subject were mean full mouth pocket depth and attachment level measurements, the % of sites with gingival redness, bleeding on probing, supragingival plaque accumulation and suppuration at baseline, 3, 6 and 12 months. Values for each parameter were averaged across subjects for each time point and significance of differences over time were

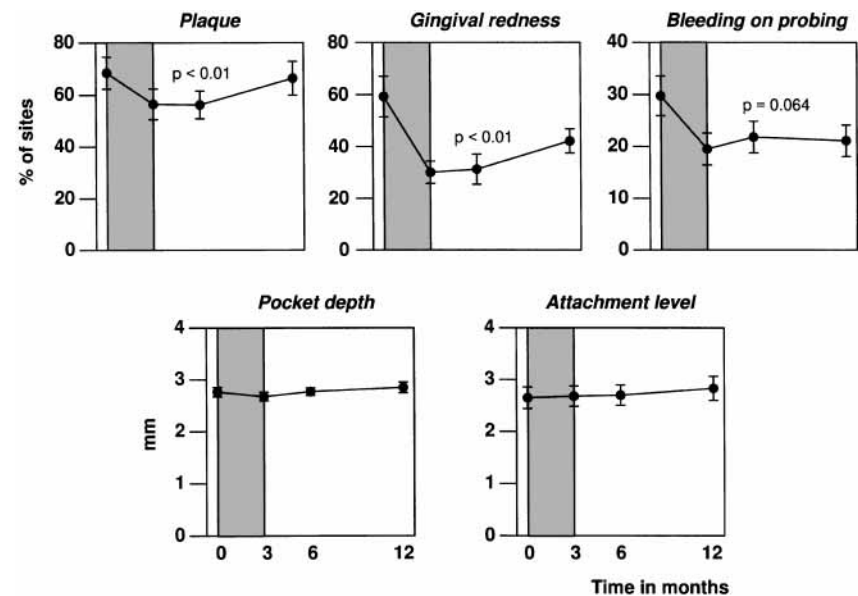


Fig. 1. Mean clinical characteristics (\pm SEM) of the subject group at baseline, 3, 6 and 12 months. Professional supragingival plaque control was performed between baseline and 3 months (shaded area). The % of sites or the mean values were computed for a subject for each visit and then values were averaged across the 18 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Quade test.

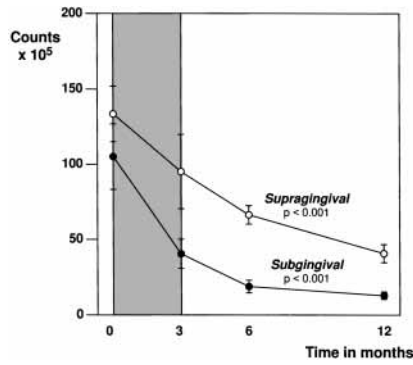


Fig. 2. Mean total DNA probe counts ($\times 10^5$, \pm SEM) in supra and subgingival plaque samples taken at baseline, 3, 6 and 12 months. Professional supra-lingival plaque control was performed between baseline and 3 months (shaded area). Mean counts were computed for a subject for each visit and then values were averaged across the 18 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Quade test.

sought using the Quade test (Conover 1980).

Microbiological data available for each subject were the counts of each of the 40 test species from up to 28 supra-lingival and, separately, up to 28 sub-lingival plaque samples per subject at baseline, 3, 6 and 12 months. The analyses compared microbial data expressed as counts $\times 10^5$ (levels), % DNA probe count (proportion) and % sites colonized (prevalence). In order to compare the counts of each of the bacterial species, the data were expressed as counts $\times 10^5$ at each site, averaged within a subject and then averaged across subjects at each time point for supra and subgingival counts separately. In a similar fashion, the % DNA probe count and prevalence of each species were computed at each site, averaged across sites within each subject and then across subjects for each time point. Changes in supra-lingival and subgingival plaque composition over time were evaluated using the Quade test. Significance of differences between pairs of time points for each species were sought using the Wilcoxon signed ranks test. For all microbial analyses, adjustments were made for multiple comparisons as described by Socransky et al. (1991).

Results

Clinical findings

Weekly professional supra-lingival plaque removal for 3 months following

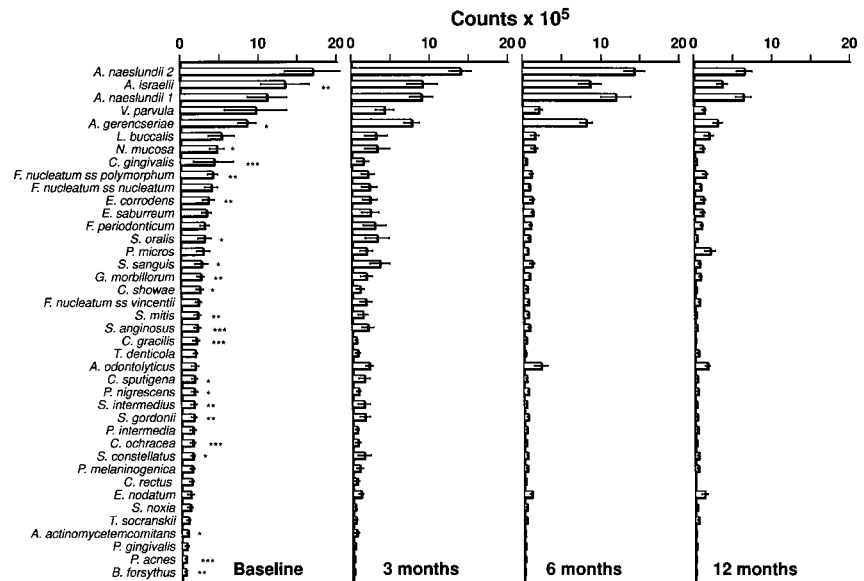


Fig. 3. Bar charts of the mean counts ($\times 10^5$, \pm SEM) of the 40 test species in supra-lingival plaque samples taken at baseline, 3, 6 and 12 months. Professional supra-lingival plaque control was performed between baseline and 3 months. Mean counts for each species were computed for a subject for each visit and then values were averaged across the 18 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Quade test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

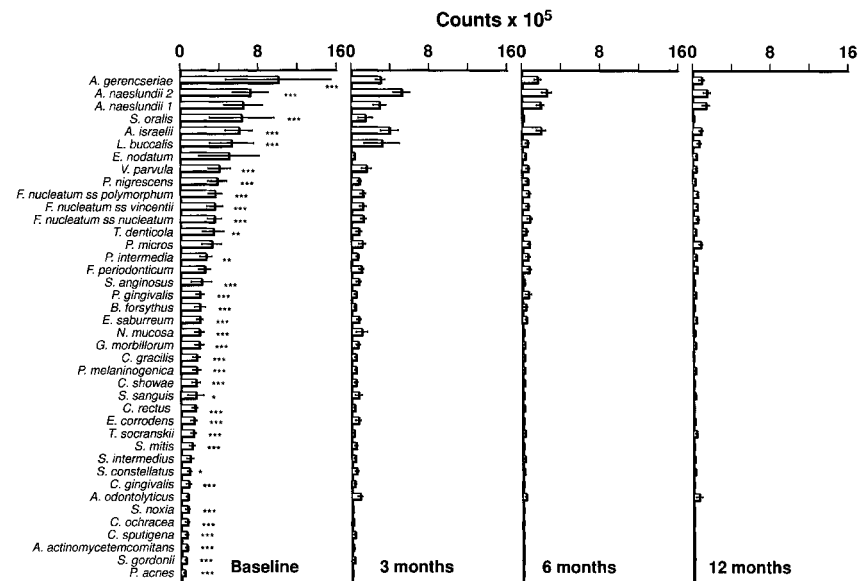


Fig. 4. Bar charts of the mean counts ($\times 10^5$, \pm SEM) of the 40 test species in sub-lingival plaque samples taken at baseline, 3, 6 and 12 months. Computation and analysis were performed as described in Fig. 3.

initial SRP significantly reduced the % of sites that exhibited plaque accumulation, gingival redness and bleeding on probing (Fig. 1). Mean pocket depth and mean attachment level measurements were not significantly changed from baseline to any time point.

Microbiological findings

Mean total DNA probe counts for both supra and subgingival plaque samples were significantly reduced after SRP followed by 3 months of professional cleaning (Fig. 2). The mean counts de-

creased after completion of the professional cleaning phase and continued to decrease for both the supra and subgingival samples even though the subjects returned to self-performed plaque control after the 3 month monitoring visit.

Mean counts of individual species were also reduced in both supra and subgingival plaque samples immediately after professional cleaning but also at the 6 and 12 month visits (Figs. 3, 4). 22 of 40 species were significantly reduced in the supragingival samples while 34 of 40 species were significantly reduced in the subgingival plaque samples. In the supragingival samples, less effect was observed for the *Actinomyces* species than for many of the gram-negative taxa particularly at 3 and 6 months. Fig. 5 presents the mean counts of 4 suspected periodontal pathogens for the supra and subgingival plaque samples at the 4 time points. At baseline, mean counts of *P. gingivalis*, *Treponema denticola* and *Bacteroides forsythus* were higher in subgingival plaque samples compared with supragingival samples, while the opposite was true for *A. actinomycetemcomitans*. All 4 species were significantly reduced over time in the subgingival plaque samples and counts of *B. forsythus* and *A. actinomycetemcomitans* in the supragingival plaque samples. The major effect was seen at 3 months, immediately after completion of the professional cleaning phase, for *P. gingivalis*, *T. denticola* and *B. forsythus*. Mean counts of *A. actinomycetemcomitans* were decreased at 3 months but continued to decline between 3 and 6 months. The overall decrease in mean counts of these 4 species after professional cleaning was also observed when sites were subset into those with initial pocket depths ≤ 4 mm and >4 mm (Fig. 6).

The reduction in mean counts observed in Figs. 3, 4 was due in part to a decrease in prevalence (% of sites colonized), but more to a reduction in the % of sites exhibiting high counts of the test species (Figs. 7, 8). For example, both the prevalence and the proportion of sites exhibiting high counts of *Streptococcus anginosus*, *Veillonella parvula* and *Prevotella nigrescens* decreased significantly in subgingival plaque (Fig. 8). For other species, such as *Actinomyces israelii*, *Fusobacterium nucleatum* ss *polymorphum* and *Streptococcus intermedius*, the % of sites colonized did not

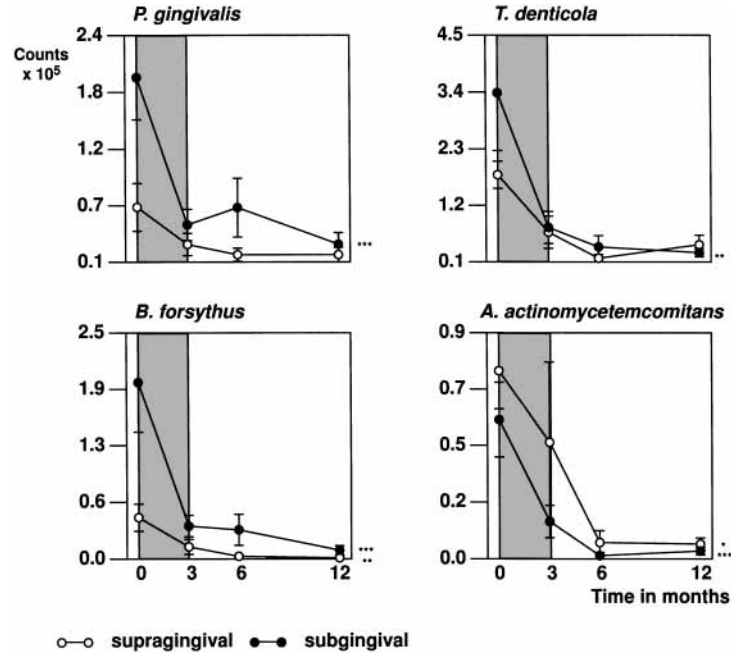


Fig. 5. Mean counts ($\times 10^5$, \pm SEM) of *P. gingivalis*, *T. denticola*, *B. forsythus* and *A. actinomycetemcomitans* in supra- and subgingival plaque samples taken at baseline, 3, 6 and 12 months. Professional supragingival plaque control was performed between baseline and 3 months (shaded area). Mean counts for each species 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. Significance of differences over time was sought using the Quade test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

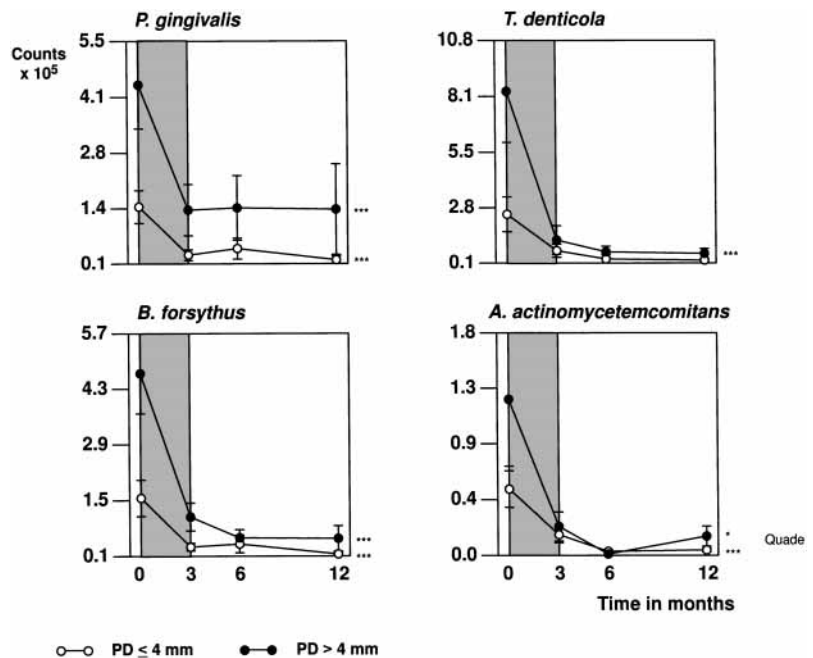


Fig. 6. Mean counts ($\times 10^5$, \pm SEM) of *P. gingivalis*, *T. denticola*, *B. forsythus* and *A. actinomycetemcomitans* in subgingival plaque samples taken at baseline, 3, 6 and 12 months from sites with initial pocket depths of ≤ 4 mm and >4 mm. Professional supragingival plaque control was performed between baseline and 3 months (shaded area). Mean counts for each species were computed for a subject for each visit in the 2 baseline pocket depth categories and then values were averaged across the 18 subjects in the 2 groups at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Quade test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

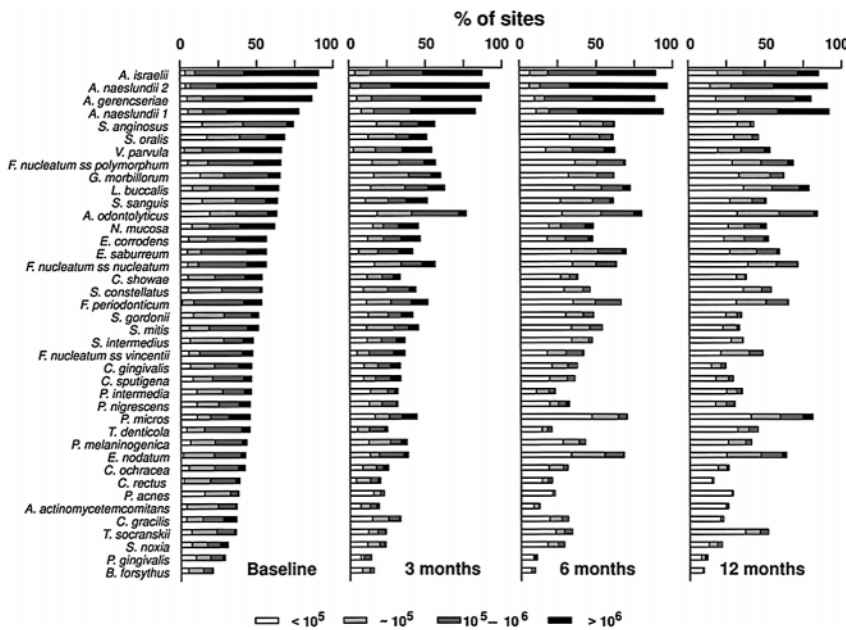


Fig. 7. Stacked bar charts of the mean prevalence and levels of the 40 subgingival species evaluated in *supragingival plaque* samples at baseline, 3, 6 and 12 months. The % of sites colonized by each of the 40 species examined was computed for each subject and then averaged across subjects at each time point. There were no significant difference in prevalence (% of sites colonized) over time using the Quade test after adjusting for multiple comparisons. Significance of differences in mean levels was presented in Fig. 3.

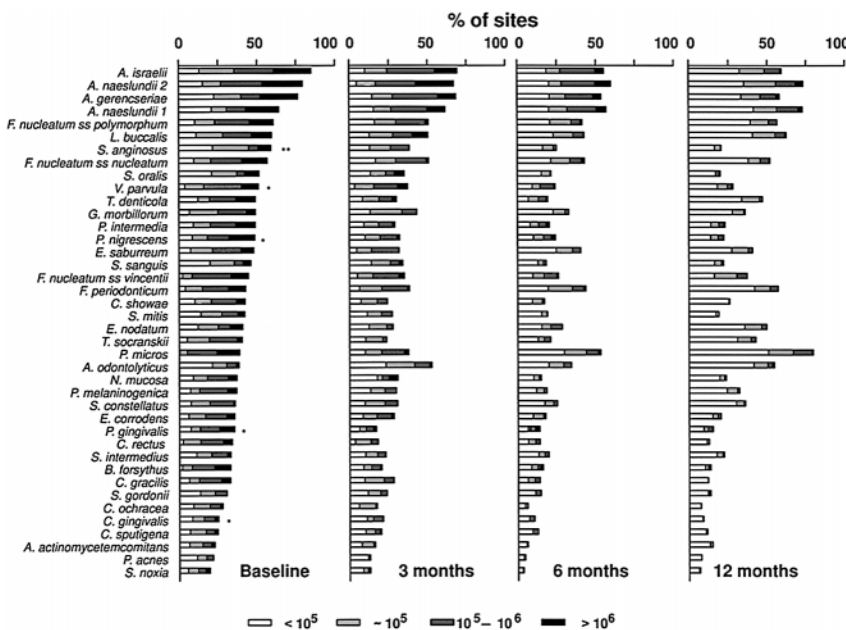


Fig. 8. Stacked bar charts of the mean prevalence and levels of the 40 subgingival species evaluated in *subgingival plaque* samples at baseline, 3, 6 and 12 months. The % of sites colonized by each of the 40 species examined was computed for each subject and then averaged across subjects at each time point. Significance of differences over time was sought using the Quade test. * $p < 0.05$, ** $p < 0.01$ after adjusting for multiple comparisons. Significance of differences in mean levels was presented in Fig. 4.

change significantly over time, but the proportion of sites exhibiting high counts decreased markedly (Fig. 7). No

species was significantly reduced in prevalence (% of sites colonized) in the supragingival plaque samples (Fig. 7),

although 5 species were significantly decreased in subgingival plaque (Fig. 8). These species, *S. anginosus*, *V. parvula*, *P. nigrescens*, *P. gingivalis* and *Capnocytophaga gingivalis* were found not only at significantly fewer sites but in significantly lower numbers (Fig. 4).

The species were grouped in the complexes described by Socransky et al. (1998). Fig. 9 presents the proportion that each complex comprised of the total DNA probe count in both supragingival and subgingival plaque samples at each time point. The areas of the pies have been adjusted to reflect the mean total DNA probe count at each visit. The major change in proportions of microorganisms occurred in supragingival plaque samples where the “red” ($p < 0.001$), “orange” ($p < 0.001$) and “green” complexes ($p < 0.01$) and *Actinomyces* species ($p < 0.05$) were significantly changed over time. In the subgingival samples only the proportions of the *Actinomyces* species ($p < 0.05$) changed significantly over time. Typically, the “red” and “green” complex species decreased in proportion at 3 and 6 months and increased at 12 months. In contrast, *Actinomyces* species increased in proportion at 3 and 6 months and decreased at 12 months. It is of interest to note that the mean total DNA probe counts decreased more over time for the subgingival plaque samples.

Fig. 10 presents a comparison between mean counts in the subgingival plaque samples for the 40 test species at baseline and 12 months for the 18 periodontitis subjects and 22 periodontally healthy individuals. The data from the healthy subjects were obtained from Ximenez-Fyvie et al. (2000b) and were derived from periodontally healthy subjects who had not been treated. While the majority of species were significantly reduced between baseline and 12 months, no statistically significant differences in mean counts were seen between the 12 month data of the periodontitis subjects and data for the periodontally healthy individuals.

Discussion

The purpose of the present investigation was to evaluate the effect of professionally administered supragingival plaque removal on clinical parameters of periodontal disease as well as on the composition of the supra and subgingival plaque. The aim of this study was

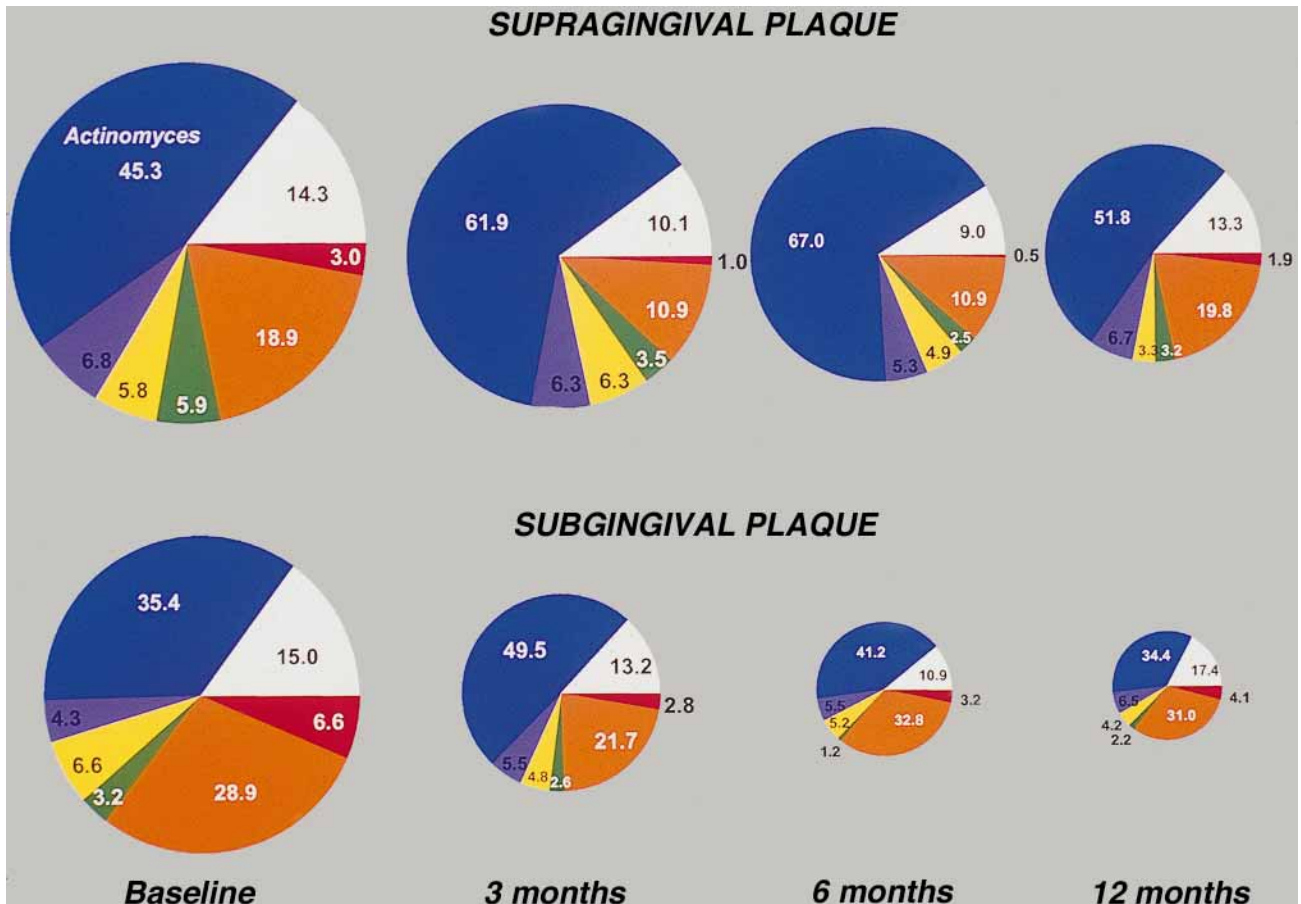


Fig. 9. Pie charts of the mean % DNA probe count of microbial groups in supra and subgingival plaque samples at baseline, 3, 6 and 12 months. The species were grouped into 7 microbial groups based on the description of Socransky et al. (1998). The areas of the pies were adjusted to reflect the mean total counts at each of the time points relative to the mean count at baseline for the supragingival plaque samples. Significance of differences over time was sought using the Quade test for the supra and subgingival samples separately. For the supragingival samples, the "red" and "orange" complex species were significantly different at $p < 0.001$, the "green" complex species differed at $p < 0.01$ and *Actinomyces* species at $p < 0.05$ after adjusting for 7 comparisons. Only the *Actinomyces* species differed significantly ($p < 0.05$) in the subgingival plaque samples.

not novel in that it has been recognized for decades that supragingival plaque removal is important in maintaining a healthy periodontium in periodontally healthy subjects and probably more so in subjects who have periodontal disease. Further, it has been shown in a number of studies that regular professional removal of supragingival plaque does have an effect on the subgingival microbiota. What the current investigation hoped to add to the wealth of data in this area was a somewhat more comprehensive microbiological evaluation which consisted of the examination of supra and subgingival plaque samples from every tooth in each of 18 subjects for their content of 40 bacterial species at four time points. Further, the microbiological data were presented in 3 different ways; as counts,

percent of sites colonized and proportions.

While a number of earlier investigations indicated that repeated professional removal of supragingival plaque had an effect on specific bacterial species and bacterial groups ("black-pigmented *Bacteroides*"), or morphotypes (e.g. spirochetes), the results of the present investigation were somewhat surprising. The previous data indicated that repeated removal of supragingival plaque lowered levels of certain organisms; however, the extent of this effect and its duration in the current investigation were unexpected. The major effect of supragingival plaque removal was to reduce the total number of organisms in both supragingival and, more dramatically, in subgingival plaque. This reduction appeared to be

for the majority of species evaluated. In planning this experiment, it was expected that counts of supragingival plaque would be lowered at the end of the professional cleaning phase for obvious reasons; the subjects had just completed 3 months of weekly plaque removal. It was thought that this procedure would lower counts of species in subgingival samples particularly certain periodontal pathogens like *P. gingivalis*, other members of the "red" complex and *A. actinomycetemcomitans*, since these effects had been demonstrated in studies performed by other investigators. It was also expected that this effect would diminish over time and that samples taken 3 and 9 months after the completion of professional cleaning (the 6 and 12 month data points) would show a return towards baseline levels.

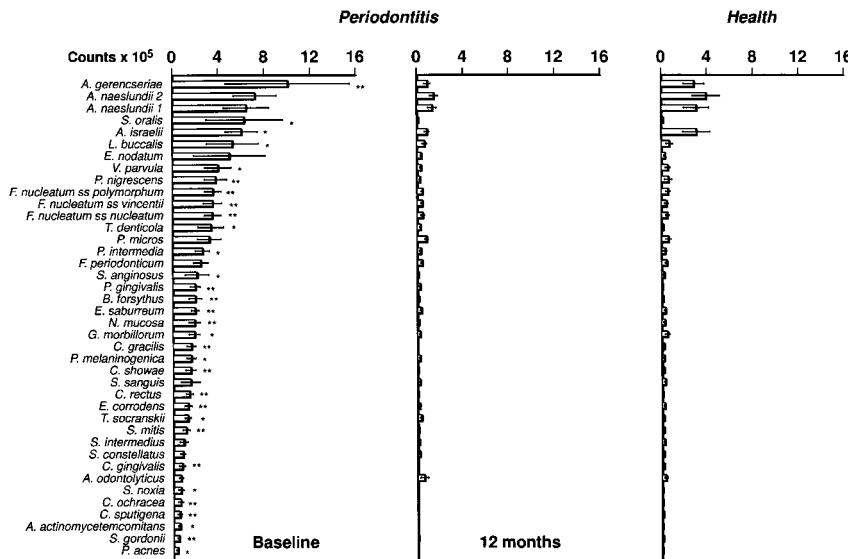


Fig. 10. Bar chart of mean counts ($\times 10^5$, \pm SEM) of individual species in subgingival plaque samples taken from the 18 periodontitis subjects at baseline and 12 months as well as from 22 periodontally healthy subjects at baseline (Ximénez-Fyvie et al. 2000b). The bars represent the mean counts and the whiskers indicate the standard error of the mean. Professional supragingival plaque control was performed between baseline and 3 months in the periodontitis group. Mean counts for each species were computed for a subject for each visit and then values averaged across the subjects at each time point. Significance of differences between baseline and 12 months were sought using the Wilcoxon Signed Ranks test. * $p < 0.05$, ** $p < 0.01$, after adjusting for multiple comparisons. Significance of differences between the healthy and 12-month periodontitis samples were sought using the Mann-Whitney test. No significant differences were observed after adjusting for multiple comparisons.

This did not occur. The reason for the prolonged efficacy of the professional cleaning is not entirely clear. At least 2 factors appear to have played a role. The first was a change in the oral hygiene habits of the subject group. The repeated visits for the removal of supragingival plaque and the weekly incidental reinforcement of the importance of this procedure may have consciously or subconsciously affected the subjects' attitude toward and performance of plaque control procedures. The second major factor that may have led to a prolonged reduction of bacterial species, particularly in subgingival plaque, may have been the disruption of the supragingival biofilm and a subsequent alteration of the periodontal ecosystem. The repeated removal of organisms in the supragingival biofilm may have directly affected bacterial cells in the adjacent subgingival biofilm. Furthermore, removal of supragingival plaque accompanied by a reduction in subgingival plaque may have altered the inflammatory state in the local gingival tissues. This reduction in inflammation would be accompanied by a decrease in gingival crevicular fluid flow and thus a decrease in plaque re-growth (Daly and

Highfield 1996, Ramberg et al. 1996). In accord with this notion, in the current investigation there was a significant, albeit modest, decrease in gingival redness over time and a decrease in bleeding on probing.

The major surprise in the results, as indicated above, was the long-term reduction of organisms in both the supra and subgingival plaque. The data presented in Fig. 10 indicate that the subgingival microbial profile of the maintenance subjects was, on average, converted to one similar to that found in periodontally healthy subjects who had received no periodontal therapy. Indeed, the mean counts of microorganisms in the test subjects were somewhat lower than those observed in the healthy subjects both in the subgingival plaque and in the supragingival plaque (data not shown). The examination of Figs. 3, 4, 7, 8 indicates that while mean counts of individual species had decreased, the % of sites colonized by the species was, for the most part, not substantially altered. The data in Figs. 7 and 8 reinforce that notion. A large % of sites had counts of individual species $\geq 10^5$ at baseline but the prevalence of such sites decreased markedly after

therapy. For example, *B. forsythus* was found at levels $\geq 10^5$, on average, at 31% of sampled sites at baseline, but at only 3% of sites at that level at 12 months (Fig. 8). Thus, counts decreased while prevalence was only slightly diminished for most species.

Proportions of species were also modestly affected (Fig. 9). In the supragingival plaque samples the proportion of the *Actinomyces* increased while "red" and "orange" complex species were decreased to 6 months. In the subgingival samples few changes in proportions were observed. However, Fig. 9 once more reinforces the decrease in the total numbers of bacteria at each time point in the 2 sample locations which is reflected in the decreasing areas of the pies.

It should be stressed that the subjects in this study were in a maintenance program. They had all received periodontal therapy, including SRP, surgery or antibiotics months to years prior to inclusion in the current investigation. However, each subject had at least 4 sites with pocket depth and/or attachment loss >4 mm to allow inclusion in the study. Although only 17% of sampled sites were >4 mm in pocket depth at baseline, these sites exhibited similar microbiological changes to sites of ≤ 4 mm at baseline. For example, mean counts of *P. gingivalis*, *B. forsythus* and *A. actinomycetemcomitans* were significantly reduced in pockets in both baseline pocket depth ranges, although the baseline counts were higher in the deeper periodontal sites. This is in agreement with a study by McNabb et al. (1992) in which they evaluated the effect of professional cleaning on the subgingival microbiota at moderate (4–5 mm) pockets. They found that a number of periodontal pathogens such as *P. gingivalis* and spirochetes were decreased in moderate depth pockets after professional cleaning. The effectiveness of supragingival plaque removal in reducing levels of bacterial species at deep periodontal pockets is questionable. Indeed, Westfelt et al. (1998) in a study of 12 subjects with advanced periodontitis showed that supragingival plaque control alone failed to prevent further attachment loss particularly at sites with pocket depth >6 mm at baseline.

The frequency and duration of professionally administered supragingival plaque control for optimum clinical and microbiological results is not clear. Axelsson & Lindhe (1974) first described

the use of professional supragingival plaque removal at 2 week intervals to control dental plaque and decrease the number of new carious lesions and gingivitis in school children. These studies led to the use of a similar protocol to control plaque in adult subjects after periodontal surgery. Three studies from the University of Gothenburg (Nyman et al. 1975, Rosling et al. 1976a, b) demonstrated that the professionally administered supragingival plaque control program was successful in controlling plaque, gingivitis, probing pocket depth and attachment level for the 2 years of its administration after different types of periodontal surgery. Studies from the same group demonstrated that supragingival plaque control every 2 weeks for a period of 6 months following surgery, followed by professional maintenance care at 3 month intervals was comparable in maintaining periodontal stability to the programs involving professional plaque control every 2 weeks for 2 years (Lindhe et al. 1982, Westfelt et al. 1983). The present investigation indicated that weekly supragingival plaque control for a period of 3 months was effective in maintaining a microbial profile similar to that observed in periodontally healthy subjects for periods of at least 1 year. This protocol may not be optimal or even necessary to achieve long lasting clinical and microbiological stability. However, it may be useful in its current form for the control of periodontal infections in a subset of periodontitis patients who are difficult to maintain by other means. Ideally, better methods of supragingival plaque removal performed by the patient can be designed to accomplish the same end without the necessity of repeated professional care. Studies in which high quality self-performed supragingival plaque control has been achieved have also led to long term periodontal stability (Lindhe & Nyman 1975).

The results of the current investigation suggest that the weekly professional removal of supragingival plaque for 3 months does have a major effect on the composition of the supra and subgingival plaque up to 9 months after completion of the therapy. Although counts for the majority of species were decreased, the species were still there but at levels that appeared to be tolerated by the host. It is not clear how long this situation will persist. Conceivably, the subjects were returned to a microbial state resembling health

that might persist for months to years. Indeed, long-term clinical studies where professional supragingival plaque control was employed every 2 weeks for periods of 6 months to 2 years demonstrated virtually no further attachment loss (Nyman et al. 1975, Rosling et al. 1976a, b, Lindhe et al. 1982, Westfelt et al. 1983). After professional cleaning has been withdrawn, it is conceivable that, in time pathogenic species may increase in numbers leading to disease progression and a new climax community. Nonetheless, the therapy employed in the current investigation in a group of maintenance subjects was able to establish a host compatible microbiota for a prolonged period of time. The regime was somewhat impractical, although no subject dropped out of the study during the professional cleaning phase. The data appear to provide the desired microbiological outcomes consistent with periodontal stability that might be achieved by more realistic methods.

Acknowledgments

This work was supported in part by research grants DE-12108, DE-10977 and DE-04881 from the National Institute of Dental and Craniofacial Research and by DGAPA, National Autonomous University of Mexico (UNAM).

Zusammenfassung

Die Wirkung einer wiederholten supragingivalen Plaqueentfernung auf die Zusammensetzung der supra- und subgingivalen Mikroflora
Der Zweck der vorliegenden Untersuchung war es, den Effekt einer wöchentlichen supragingivalen Plaqueentfernung auf die Zusammensetzung der supra- und subgingivalen Mikroflora zu bestimmen. 18 Erwachsene mit behandelter Parodontitis, die sich in der Erhaltungstherapie befanden, wurden klinisch und mikrobiologische zu Beginn der Studie sowie, 3, 6 und 12 Monate danach untersucht. Nach dem ersten Termin wurde bei den Teilnehmern Scaling und Wurzelglättung sowie jede Woche für einen Zeitraum von 3 Monaten eine professionelle supragingivale Plaqueentfernung durchgeführt. Zu jedem Termin wurde an 6 Stellen pro Zahn die Plaqueakkumulation, Sondierungsblutung (BOP), Gingivarötung, Pusentleerung, Taschentiefe und das Attachmentniveau bestimmt. Bei jedem Teilnehmer wurden zu jedem Zeitpunkt und von allen Mesialflächen aller Zähne, ohne 3. Molaren, separate supra- ($n=1804$) und subgingivale ($n=1804$) Plaqueproben entnommen. Sie wurden hinsichtlich des Vorkommens von 40 bakteriel-

len Taxa mittels Schachbrett-DNA-DNA-Hybridisierung untersucht. Mit dem Quade-Test, der für multiple Vergleiche adjustiert wurde ist die Signifikanz der zeitlichen Veränderungen der Mittelwerte der Anzahl, Prävalenz und Anteile der Bakterienspezies sowohl der supra- als auch der subgingivalen Proben bestimmt worden. Der durchschnittliche Prozentsatz von Stellen mit Plaque, Gingivarötung und Sondierungsblutung reduzierte sich im Laufe der Studie signifikant. Sowohl bei den supra- als auch der subgingivalen Proben wurde eine signifikante Reduktion der mittleren Bakterienanzahl beobachtet. Die mittlere Anzahl mit DNA-Sonden-test betrug ($\times 10^5$, \pm Standardabweichung) zu Beginn, nach 3, 6 und 12 Monaten: 133 ± 19 , 95 ± 25 , 66 ± 6 , 41 ± 6 ($p < 0.001$) für supragingivale Proben und 105 ± 22 , 40 ± 10 , 19 ± 4 , 13 ± 3 ($p < 0.001$) für subgingivale Proben. Die Anzahl von 22 der 40 bzw. 34 der 40 getesteten Spezies war in den supra- bzw. subgingivalen Proben während der Beobachtungsperiode signifikant reduziert. Zum Beispiel waren die durchschnittlichen Anzahlen für *Porphyromonas gingivalis* $\times 10^5$ zu Beginn, nach 3, 6 und 12 Monaten 2.0 ± 0.4 , 0.5 ± 0.2 , 0.6 ± 0.3 , 0.3 ± 0.1 ($p < 0.001$); für *Bacteroides forsythus* 2.0 ± 0.6 , 0.4 ± 0.1 , 0.4 ± 0.2 , 0.1 ± 0.2 ($p < 0.001$); für *Treponema denticola* 3.4 ± 1.1 , 0.8 ± 0.3 , 0.4 ± 0.2 , 0.3 ± 0.3 ($p < 0.01$). Ähnliche Reduktionen wurden bei den supragingivalen Plaqueproben beobachtet. Während die Anzahl der Keime durch die professionelle Zahnreinigung bemerkenswert reduziert wurde hatte sie auf die Proportion und Prävalenz der Keime nur einen marginalen Effekt. Eine wöchentliche professionelle supragingivale Plaqueentfernung führte zu einer bedeutenden Reduktion sowohl der supra- als auch der subgingivalen Spezies und schuf ein mikrobiologisches Profil, welches vergleichbar ist mit dem bei parodontaler Gesundheit. Dieses Profil wurde bis zum letzten Beobachtungstermin 9 Monate nach dem Ende der Therapie aufrechterhalten.

Résumé

Effet de l'enlèvement de la plaque dentaire sus-gingivale par des professionnels sur la composition de la flore sus- et sous-gingivale
Le but de cette étude a été de déterminer l'effet d'un nettoyage de la plaque sus-gingivale professionnel et hebdomadaire sur la composition de la flore tant sus- que sous-gingivale. 18 adultes avec parodontite qui avaient été traités et qui étaient dans la phase de maintenance ont été cliniquement et microbiologiquement suivis lors de la visite initiale et après 3, 6 et 12 mois. Après l'examen de départ, les sujets ont reçu un détartrage et un surfacage radiculaire suivi d'un prélèvement de plaque dentaire sous-gingivale tous les 3 mois. Les mesures cliniques de l'accumulation de la plaque dentaire, du saignement au sondage (BOP), de la rougeur gingivale, de la suppuration, de la profondeur de poche et du niveau d'attache ont été effectuées au niveau de 6 sites par dent lors de chaque visite. Des

échantillons de plaque dentaire séparés sus- ($n=1804$) et sous-gingivaux ($n=1804$) ont été prélevés de la partie mésiale de toutes les dents, excluant les dents de sagesse chez tous les sujets à chaque moment et évalués pour la présence de 40 types de bactéries en utilisant l'hybridation ADN/ADN en damier. La signification des variations dans les comptages moyens, la fréquence globale et les proportions d'espèces bactériennes dans le temps tant dans les échantillons sus- que sous-gingivaux ont été déterminées en utilisant le test Quade et ajustées pour les comparaisons multiple. La moyenne de % de sites avec de la plaque dentaire, de la rougeur gingivale et du BOP était significativement réduite durant le cours de cette étude. Des diminutions significatives dans les comptages moyens ont été observées dans les échantillons sus- et sous-gingivaux. Les moyennes totales des comptages par sonde ADN ($\times 10^5$, \pm EC) à l'examen initial et 3, 6 et 12 mois après étaient: 133 ± 19 , 95 ± 25 , 66 ± 6 , 41 ± 6 ($p < 0.001$) pour les échantillons sus-gingivaux et de 105 ± 22 , 40 ± 10 , 19 ± 4 , 13 ± 3 ($p < 0.001$) pour les sous-gingivaux. Les comptages moyens de 22 des 40 et de 34 des 40 espèces testées étaient significativement réduits dans les échantillons sus- et sous-gingivaux. Par exemple, les comptages moyens des *Porphyromonas gingivalis* $\times 10^5$ sous-gingivaux lors de l'examen initial et après 3, 6 et 12 mois étaient respectivement de 2.0 ± 0.4 , 0.5 ± 0.2 , 0.6 ± 0.3 , 0.3 ± 0.1 ($p < 0.001$); pour le *Bacteroides forsythus* de 2.0 ± 0.6 , 0.4 ± 0.1 , 0.4 ± 0.2 , 0.1 ± 0.2 ($p < 0.001$); pour le *Treponema denticola* 3.4 ± 1.1 , 0.8 ± 0.3 , 0.4 ± 0.2 , 0.3 ± 0.3 ($p < 0.01$). Des réductions semblables ont été aperçues dans les échantillons de plaque sus-gingivale. Tandis que ces comptages étaient fortement réduits par le nettoyage dentaire professionnel, la proportion et la fréquence globale des 40 espèces testées n'étaient que très peu touchées. L'enlèvement de la plaque dentaire sus-gingivale de manière professionnelle et hebdomadaire diminue sévèrement les comptages des espèces tant sus- que sous-gingivales créant un profil microbien comparable à celui observé en présence de parodontite sain. Ce profil était maintenu lors de la visite finale 9 mois après la fin du traitement.

References

- Al-Yahfoufi, Z., Mombelli, A., Wicki, A. & Lang, N. P. (1995) The effect of plaque control in subjects with shallow pockets and high prevalence of periodontal pathogens. *Journal of Clinical Periodontology* **22**, 78–84.
- Axelsson, P. & Lindhe, J. (1974) The effect of a preventive programme on dental plaque, gingivitis and caries in schoolchildren. Results after one and two years. *Journal of Clinical Periodontology* **1**, 126–138.
- Axelsson, P. & Lindhe, J. (1981) The significance of maintenance care in the treatment of periodontal disease. *Journal of Clinical Periodontology* **8**, 281–294.
- Beltrami, M., Bickel, M. & Baehni, P. C. (1987) The effect of supragingival plaque control on the composition of the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **14**, 161–164.
- Conover, W. J. (1980) Practical nonparametric statistics, 2nd edition. John Wiley & Sons, New York, pp 295–299.
- Dahlen, G., Lindhe, J., Sato, K., Hanamura, H. & Okamoto, H. (1992) The effect of supragingival plaque control on the subgingival microbiota in subjects with periodontal disease. *Journal of Clinical Periodontology* **19**, 802–809.
- Daly, C. G. & Highfield, J. E. (1996) Effect of localized experimental gingivitis on early supragingival plaque accumulation. *Journal of Clinical Periodontology* **23**, 160–164.
- De Sanctis, M., Zuchelli, G. & Clauser, C. (1996a) Bacterial colonization of barrier material and periodontal regeneration. *Journal of Clinical Periodontology* **23**, 1039–1046.
- De Sanctis, M., Zuchelli, G. & Clauser, C. (1996b) Bacterial colonization of bioabsorbable material and periodontal regeneration. *Journal of Periodontology* **67**, 1193–1200.
- Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1983) Comparison of different data analyses for detecting changes in attachment level. *Journal of Clinical Periodontology* **10**, 298–310.
- Hellstrom, M.-K., Ramberg, P., Krok, L. & Lindhe, J. (1996) The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **23**, 934–940.
- Katsanoulas, T., Renee, I. & Attstrom, R. (1992) The effect of supragingival plaque control on the composition of the subgingival flora in periodontal pockets. *Journal of Clinical Periodontology* **19**, 760–765.
- Kho, P., Smales, F. C. & Hardie, J. M. (1985) The effect of supragingival plaque control on the subgingival microflora. *Journal of Clinical Periodontology* **12**, 676–686.
- Kornman, K. S., Newman, M. G., Moore, D. J. & Singer, R. E. (1994) The influence of supragingival plaque control on clinical and microbial outcomes following the use of antibiotics in the treatment of periodontitis. *Journal of Periodontology* **65**, 848–854.
- Lavanchy, D. L., Bickel, M. & Baehni, P. C. (1987) The effect of plaque control after scaling and root planing on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **14**, 295–299.
- Lindhe, J. & Nyman, S. (1975) The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease. *Journal of Clinical Periodontology* **2**, 67–79.
- Lindhe, J., Westfelt, E., Nyman, S., Socransky, S. S. & Haffajee, A. D. (1984) Long-term effect of surgical/non surgical treatment of periodontal disease. *Journal of Clinical Periodontology* **11**, 448–458.
- Lindhe, J., Westfelt, E., Nyman, S., Socransky, S. S., Heijl, L. & Bratthall, G. (1982) Healing following surgical/non surgical treatment of periodontal disease. *Journal of Clinical Periodontology* **9**, 115–128.
- Löe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.
- Magnusson, I., Lindhe, J., Yoneyama, T. & Liljenberg, B. (1984) Recolonization of a subgingival microbiota following scaling in deep pockets. *Journal of Clinical Periodontology* **11**, 193–207.
- McNabb, H., Mombelli, A. & Lang, N. P. (1992) Supragingival cleaning 3 times a week. The microbiological effects in moderately deep pockets. *Journal of Clinical Periodontology* **19**, 348–356.
- Muller, H.-P., Hartmann, J. & Flores-de-Jacoby, L. (1986) Clinical alterations in relation to the morphological composition of the subgingival microflora following scaling and root planing. *Journal of Clinical Periodontology* **13**, 825–832.
- Nyman, S., Rosling, B. & Lindhe, J. (1975) Effect of professional tooth cleaning on healing after periodontal surgery. *Journal of Clinical Periodontology* **2**, 80–86.
- Ramberg, P., Furuichi, Y., Volpe, A. R., Gaffar, A. & Lindhe, J. (1996) The effects of antimicrobial mouthrinses on de novo plaque formation at sites with healthy and inflamed gingivae. *Journal of Clinical Periodontology* **23**, 7–11.
- Rosling, B., Nyman, S. & Lindhe, J. (1976a) The effect of systematic plaque control on bone regeneration in infrabony pockets. *Journal of Clinical Periodontology* **3**, 38–53.
- Rosling, B., Nyman, S., Lindhe, J. & Jern, B. (1976b) The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque-free dentitions. A 2-year clinical study. *Journal of Clinical Periodontology* **3**, 233–250.
- Smulow, J., Turesky, S. S. & Hill, R. G. (1983) The effect of supragingival plaque removal on anaerobic bacteria in deep periodontal pockets. *Journal of American Dental Association* **107**, 737–742.
- Socransky, S. S., Haffajee, A. D., Smith, C. & Dibart, S. (1991) Relation of counts of microbial species to clinical status at the sampled site. *Journal of Clinical Periodontology* **18**, 766–775.
- Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, Jr. R. L. (1998) Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology* **25**, 134–144.
- Socransky, S. S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) "Checkerboard" DNA-DNA hybridization. *Biotechniques* **17**, 788–792.

- Tabita, P. V., Bissada, N. F. & Mayberry, J. E. (1981) Effectiveness of supragingival plaque control on the development of subgingival plaque and gingival inflammation in patients with moderate pocket depth. *Journal of Periodontology* **52**, 88–93.
- Westfelt, E., Bragd, L., Socransky, S. S., Haffajee, A. D., Nyman, S. & Lindhe, J. (1985) Improved periodontal conditions following therapy. *Journal of Clinical Periodontology* **12**, 283–293.
- Westfelt, E., Nyman, S., Lindhe, J. & Socransky, S. (1983) Use of chlorhexidine as a plaque control measure following surgical treatment of periodontal disease. *Journal of Clinical Periodontology* **10**, 22–36.
- Westfelt, E., Rylander, H., Dahlen, G. & Lindhe, J. (1998) The effect of supragingival plaque control on the progression of advanced periodontal disease. *Journal of Clinical Periodontology* **25**, 2536–2541.
- Ximénez-Fyvie, L. A., Haffajee, A. D. & Socransky, S. S. (2000a) Microbial composition of supra and subgingival plaque in subjects with adult periodontitis. *Journal of Clinical Periodontology* **27**, in press.
- Ximénez-Fyvie, L. A., Haffajee, A. D. & Socransky, S. S. (2000b) Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *Journal of Clinical Periodontology* **27**, 648–657.

Address:

Anne D. Haffajee
 Department of Periodontology
 The Forsyth Institute
 140 The Fenway
 Boston, MA 02115
 USA