Effect of scaling and root planing on the composition of the human subgingival microbial flora

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The effect of a single session of scaling and root planing on the subgingival periodontal flora of 14 adult human subjects was investigated by darkfield microscopy. At baseline, one randomly selected periodontally diseased site in each subject was assessed for GI and PII scores, probing depth and the percentage distribution in subgingival debris of coccoid cells, spirochetes, motile cells and other microorganisms. Following a single full mouth scaling and root planing session, these criteria were reevaluated at other initially diseased sites, one per subject and time interval. The intervals tested were days 3, 7, 14, 21, 28, 35, 42, 49, 56, 70 and 90.

The PII and GI scores tended to decrease during the first 14 days, returning to baseline around days 21–28. After another transient decline around 35–42 days the values stabilized around baseline levels till the end of the experiment. Probing depth decreased below baseline during the first 7 days and with the exception of day 28, remained below baseline level till the end of the experiment. Coccoid cells increased from 25.1 % at baseline to 76.1 % on day 3. Return to the baseline level occurred by day 21. Spirochetes did not return to baseline until day 42. The percentage of motile cells decreased significantly from baseline on day 3 only (14.8 % to 3.8 %).

The results indicated that a single session of scaling and root planing is capable of disturbing the proportions of certain bacterial forms in the subgingival periodontal flora, and that it may require approximately 42 days for the proportions to return to baseline levels. Probing depth was significantly decreased by the debridement throughout most of the 90-day experimental period. The proportion of coccoid cells was negatively correlated with both GI and PII scores, while the percentage of spirochetes was positively correlated with GI and PII scores as well as probing depth measurements.

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Introduction

Recent cultural (Slots 1977a, b) and morphological studies (Listgarten 1976, Listgarten & Helldén 1978) have demonstrated that the periodontal microflora within healthy gingival sulci has a different composition than the flora within periodontal pockets. Proportional differences among certain bacterial groups can be readily detected by direct counting of cells in fresh preparations of sulcular or pocket scrapings under a darkfield microscope (Listgarten & Helldén 1978). This technique has been used to monitor the composition of the periodontal flora in periodontal pockets undergoing treatment (Listgarten, Lindhe & Helldén 1978). Thus it was possible to demonstrate that in comparison to untreated controls certain shifts in the microbial flora...
take place either following mechanical debridement or antibiotic therapy. Since microbial alterations induced by mechanical debridement could be detected 2 or more weeks after the last intervention, it seemed important to determine the effect in time of a single mechanical interference on the microbial composition of a periodontal pocket. Recently, Mousquès, Listgarten & Stoller (1980) examined the effect of taking a microbial sample from a periodontal pocket with a minimum amount of tissue disturbance. They concluded that sampling alone may contribute to detectable alterations, lasting 3–7 days, in the proportions of certain microbial groups, notably coccoid cells and spirochetes. However, superimposed behavioral changes such as improved oral hygiene may be partially responsible for longer lasting deviations from the baseline data.

In this investigation the effect of a single session of scaling and root planing on the periodontal flora and certain clinical parameters was examined in a patient population which had not been given any special instruction in oral hygiene. The main purpose of this study was to determine how long it takes for the microbial population to return to baseline following a single therapeutic intervention of this type.

**Method and Materials**

The population of patients who participated in a previous study (Mousquès, Listgarten & Stoller 1980) on the effect of bacterial sampling on the microbial population of untreated pockets was requested to volunteer for this study. Of the 18 patients who completed the first investigation, 17 accepted to enter the present study. Because of illness and absenteeism only 14 were able to complete the study. All 14 subjects, 2 males and 12 females ranging in age from 24 to 58, were in good general health, showed radiographic evidence of generalized bone loss and multiple pockets probeable to a depth of 4 mm or more. None of these subjects received any antibiotics in the 7½ months preceeding the study.

In the previous investigation, 6 tooth surfaces in each subject were examined at the beginning of the experiment and the Plaque Index, the Gingival Index and the probing depth at each site recorded. The pockets were sampled to determine the microbial composition of the pocket flora from fresh preparations of pocket scrapings examined by darkfield microscopy. Subsequently, each pocket was reexamined once at intervals of 3–42 days to determine the effect on the various parameters produced by the sampling procedure. Although a change from baseline was detectable in the bacterial proportions after the 3-day interval the values became more or less stabilized between days 3 and 42. The mean 42-day values for the subjects who volunteered for this study were selected as the new baseline.

Beginning at 4 days following completion of the first study, each subject received a thorough full-mouth scaling and root planing administered in a single session, each session averaging approximately 3 hours. No attempt was made to alter the existing oral hygiene habits of the subjects, in order to avoid affecting the outcome of the single session of mechanical debridement.

Following the session of mechanical debridement, each subject was reexamined at the following intervals: days 3, 7, 14, 21, 28, 35, 42, 49, 56, 70 and 90.

At each of the above time intervals one pocket in each patient was sampled according to a schedule based on a random assignment of the available experimental sites to the various experimental intervals. Because it was not possible to find 11 suitable pockets in all patients, the sites sampled at the 3-day interval were resampled to obtain the 90-day data in all subjects.
At each examination, the data collected at each site included a Plaque Index (PII) score (Silness & Loe 1964) and Gingival Index (GI) score (Loe & Silness 1963) for the individual experimental site, a probing depth measurement recorded to the nearest millimeter with a standard periodontal probe, and an estimate of the proportions of various microbial forms at the experimental site as described previously (Mousquès, Listgarten & Stoller 1980). For the purposes of this study, the microbial flora was subdivided into 4 groups, notably coccoid cells, motile cells, spirochetes and others. This list is a condensed version of the microbial categories described in a previous report by Listgarten & Helldén (1978).

The experimental design provided for sets of 14 measurements, one per subject, for each criterion, and at each time interval. Because of illness only 13 subjects were examined at the 49 and 70 day intervals. The data were analyzed by the Method of Fitting Constants (Yates 1934, Heiberger & Laster 1978) for unbalanced data (Type II sums of squares from the general linear model program in the SAS76 package (Barr et al. 1976). Comparisons between various paired time intervals were considered significant if the difference between the means was greater than \( t_{\nu/2k} \sqrt{\frac{MSE}{1/n_1 + 1/n_2}} \), where \( t_{\nu/2k} \) represents the value of the Bonferroni \( t \) statistic for a joint \( \alpha = 0.05 \), MSE is the mean square error term from the analysis of variance, and \( n_1 \) and \( n_2 \) are the number of subjects at each time interval.

The percentages were not transformed prior to statistical analysis, since none of the percentages were extremely high or extremely low. It should also be noted that prior experience with similar data failed to show any appreciable difference in the significance of the results when the data were analyzed either in raw form or following angular transformation (Listgarten & Helldén 1978, Listgarten, Lindhe & Helldén 1978, Mousquès, Listgarten & Stoller 1980). Correlation coefficients were calculated from the mean values per subject for the various criteria being correlated (i.e. from the values listed in Tables 1 and 2).

### Results

The clinical data collected during the experimental period are presented in Fig. 1. The PII and GI scores tended to decrease rapidly in the first week following debridement, thereafter gradually returning to baseline levels around day 28. A secondary decrease appeared to occur after day 28 with a return to baseline level around days 49 to 56.

The analysis of the PII scores indicated no significant change from baseline at any of the time intervals following day 28. However, the mean PII score at day 70 was significantly elevated over the scores at days 3, 7, 14 and 42 days. The mean score on day 28 was also significantly higher than the scores on days 3 and 7 (Fig. 1). No significant variations were noted among subjects in the overall mean plaque scores (Table 1).
Table 1
Mean values for various clinical parameters by subject for all time intervals

<table>
<thead>
<tr>
<th>Subject</th>
<th>N</th>
<th>PI</th>
<th>GI</th>
<th>PD (mm)</th>
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<tr>
<td>1</td>
<td>12</td>
<td>1.4</td>
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<td>2</td>
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N = Number of intervals at which each subject was examined.

With respect to the GI, the mean scores for day 3 to 35 included were significantly lower than the baseline values. The lowest GI score, which was recorded on day 7, was also significantly lower than the values recorded from the 49th to the 90th day included (Fig. 1). A significant variation was observed among the subjects in the study with respect to the overall GI scores (Table 1).

Following mechanical debridement, mean probing depth recordings reached a significantly lower value on the 7th day. With the exception of the return toward baseline levels on day 28, probing depth measurements remained significantly lower than the mean baseline value through the remaining experimental period (Fig. 1). From a mean value at baseline of 6.4 mm the probing depth measurements fell to below 5.0 mm on day 7 and stayed at 5.00 mm or less through days 35 to 90. The overall probing depth measurements varied significantly among subjects (Table 1).

The variations in the proportions of the various bacterial categories are illustrated in Fig. 2. Following debridement, the proportion of coccoid cells immediately rose from a baseline mean of 25.1% to an experimental high of 76.1% on day 3. Coccoid cells remained significantly elevated through day 14, thereafter dropping to levels not significantly different from baseline (Fig. 2).

The proportion of spirochetes decreased significantly from the mean baseline value...
of 33.6% to a low of less than 2% on day 7. The percentage values remained significantly lower than baseline from day 3 to 35 included. The proportion of spirochetes on day 42, was no longer significantly different from baseline (21.4%). Thereafter the proportion of spirochetes became stabilized for the remainder of the experiment at mean levels of 25.5 to 33.3% through days 49 to 90, (Fig. 2). From the analysis of variance it was evident that the overall proportions of spirochetes among subjects varied significantly, with some subjects showing consistently higher or lower values than others (Table 2).

The proportion of motile cells dipped temporarily on day 3 from a baseline mean of 14.8% to 3.8%. This 3-day value was also significantly lower than the values on days 21, 28 and the final value on day 90 (Fig. 2). Only one subject had a significantly lower proportion of motile cells in his microbial flora, in comparison to the other subjects (Table 2).

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The other microorganisms which at baseline accounted for 26.5% of the flora also showed a small, transient proportional decrease on day 3 to 18.1%. From day 3 the percentage value gradually rose to peak at 46.1% on day 70. The 3-day value was significantly lower than all but the 0-, 3- and 7-day values, while the 70-day figure was significantly higher than the 0-, 3- and 7-day values (Fig. 2). The overall mean values for the clinical and microbiological criteria for each subject during the entire experimental period are shown in Table 1 and 2. A comparison of the data in these tables revealed several significant correlations (Table 3). Moderately strong positive correlations were noted between GI and PII scores, and between percentages of spirochetes on the one hand and probing depths, GI scores and PII scores on the other. A moderately strong inverse correlation was noted between the percentages of coccoid cells and both the GI and PII scores. As might be expected a strong inverse correlation could also be demonstrated between the percentages of spirochetes and coccoid cells.

Discussion

It is not the usual practice during initial therapy to carry out the scaling and root planing procedures in a single 3-hour session; nor is it usual to do a scaling and root planing procedure without attempting to improve the quality of the patient's oral hygiene habits. However, in order to test the effect of scaling on various clinical and microbiological parameters, altering the oral hygiene of the subjects or carrying out the scaling in several sessions may have made it more difficult to interpret the observations. It is likely that the effect of a single session of mechanical debridement on the clinical parameters measured is not as good as a series of repeated interventions. The reduction in the degree of gingival inflammation resulting from the initial session facilitates the complete removal of residual calculus and further resolution of the gingival inflammation during subsequent visits.

Imperfect as it may have been, the sing-
le session of full-mouth mechanical debridement probably removed the bulk of the calculus and the microbial flora associated with the sites being investigated. This is in contrast with the careful sampling of individual sites which was carried out in the study by Mousquès, Listgarten & Stoller (1980), where no calculus was removed and a considerable amount of microbial debris was left behind. In that study, the effect of the sampling procedure per se was also affected by suspected changes in the oral hygiene habits of the subjects. Altered oral hygiene was most likely responsible for stabilizing the clinical and microbiological criteria at the levels reached 3 days after the sampling procedure. While these levels differed significantly from baseline, the proportions of the various bacterial types at all time intervals (0-42 days) remained well within the range for periodontally diseased samples reported by Listgarten & Helldén (1978). Presumably, the remaining bacterial flora quickly gave rise to a similar flora within a few days of the sampling procedure.

However, the complete debridement carried out in this investigation altered the composition of the flora dramatically, so that after 3 days the bacterial proportions within the experimental pockets were more typical of those reported by Listgarten & Helldén (1978) for periodontally healthy sites, with coccoid cells predominating while the proportions of spirochetes and motile cells were markedly reduced. The time required for the various bacterial types to return to their baseline levels differed among the various groups, with spirochetes taking as long as 42 days, coccoid cells 21 days, and motile cells only one week. We know from previous studies of the development of the periodontal flora (Theilade et al. 1966, Listgarten, Mayo & Tremblay 1975) that in the first week the microbial flora is dominated by a coccoid flora which, beginning in the second week, is gradually displaced by filaments, fusiforms and motile forms. Spirochetes appear in significant numbers during the third week and form an important component of the subgingival flora from thereon. This pattern of microbial successions seemed to play a role in determining the manner in which the lesions became repopulated with bacteria, following the session of mechanical debridement. However, while motile cells only required approximately 1 week to return to baseline levels, spirochetes required approximately 6 weeks.

If one considers the general trend in the return of the microbial proportions toward baseline levels illustrated in Fig. 2, it would seem that most values tend to approach baseline levels by day 28. However, beyond day 28 a slight deviation seems to occur from the 28-day values and the more or less stabilized values which appear between days 49 to 90. Thus, a slight increase in the proportion of coccoid cells around days 35-42 seems to interrupt the normal rate of return of the proportions of coccoid cells from day 28 to the stabilized values seen in the latter part of the experiment. Similarly, the rate of return of the proportions of spirochetes seems to decrease somewhat between days 28 and 56. It is interesting to note that these deviations in the direction of values seen at periodontally healthy sites are accompanied by concomitant decreases in the GI PII scores during the same time intervals. Probing depth measurements also show a slight decrease on day 35 to the lowest mean values observed for any of the experimental time intervals. While statistically these deviations are not all significant, there is an apparent trend for microbiological and clinical criteria to shift back toward healthy values in the interval between days 28 to 49.

The reasons for the deviations recorded around the 35th day are not clear. One
possible explanation is based on the known existence of humoral as well as cell-mediated immunity against a number of microbial species in the periodontal flora of human subjects (see review by Nisen- gard 1977). The mechanical debridement by its very nature may introduce microorganisms into the tissues and produce an anamnestic immune response during the immediate post-operative period. Korn & Schaffer (1962) reported that following root planing the incidence of bacteremia may reach over 80%. Conner et al. (1967) demonstrated a bacteremia in over half of a group of adult patients with periodontitis following scaling. Similar results were also reported by DeLeo et al. (1974) in children. Even toothbrushing can induce bacteremias, particularly in patients with periodontitis (Sconyers, Crawford & Moriarty 1973, Silver, Martin & McBride 1977). It is conceivable, therefore, that the repopulation of the scaled sites by the microflora is modulated to some extent by the immune response of the host to these organisms. Since little is known about the effect of scaling or other periodontal therapeutic procedures on the immune response of human subjects to periodontal microorganisms, such a hypothesis will require further testing.

It is of interest to note that the fluctuations in the proportion of spirochetes closely approximated those reported for the GI and PII scores. On the other hand probing depth measurements decreased from baseline and remained at lower values throughout the experimental period. This may be due initially to a reduction in the degree of gingival inflammation and consequent tissue shrinkage. As shown by Listgarten, Lindhe & Helldén (1978) mechanical debridement may also be accompanied by a diminution in the size of the inflammatory infiltrate in the gingival tissue and a laying down of new collagen. These histological alterations may be responsible for the reduced probing depth measurements, because they contribute to making the tissues less penetrable by the probe.

If a single episode of scaling and root planing were capable of inducing an immune response which could subsequently affect the composition of the periodontal flora, it might be interesting to speculate on the effect of exposing the patient to a series of scaling and/or root planing sessions as is usually done in practice. It is conceivable that such appointments strategically spaced could act not only in disturbing the periodontal flora directly, but also as boosters of the immune status of the host to its periodontal flora. Oral hygiene practices may have a similar influence by contributing both to the mechanical removal of bacterial deposits as well as the periodic resensitization of the host to some of the inhabitants of the periodontal pocket.

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