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The effect of periodontal treatment on the salivary bacterial load and early plaque formation

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Abstract

Aim: The purpose of the present study was to investigate in periodontitis patients the relationship between the number of bacteria in the saliva and the amount of de novo plaque formation before and after treatment.

Methods: At baseline, before any treatment was provided, patients rinsed with 10 ml sterile saline. After professional tooth cleaning the patients were instructed to abolish all tooth cleaning procedures for the subsequent 24 h. After this period, the rinsing procedure was repeated and the amount of de novo plaque was assessed. Three months after the initial periodontal therapy was completed the experiment was repeated. The microbiological evaluation of the rinsing samples was carried out by means of phase contrast microscopy and anaerobic culturing.

Results: After treatment the amount of de novo plaque was less compared to before treatment, 0.40 and 0.65 respectively. Both before and after treatment more de novo plaque was present at sites with inflammation than at healthy sites. In order to evaluate the contribution of the numbers of salivary bacteria to the amount of de novo plaque formation an analysis was carried out for healthy sites. This analysis included only healthy sites as determined before treatment and the same sites after treatment. The results showed a significant reduction in the de novo plaque formation after treatment (0.49 before and 0.22 after treatment). Phase contrast microscopic evaluation showed that the number of bacteria in the rinsing samples after treatment was less than before treatment. After treatment also a reduction was found in the prevalence of *Prevotella intermedia*, *Tannerella forsythensis* and *Peptostreptococcus micros* in the rinsing samples.

Conclusion: The present study confirms the observation in the literature that the periodontal condition is of major importance in the rate of de novo plaque formation. In addition, the results suggest that the number of bacteria in the saliva may play a role.

Key words: bacteria; periodontal treatment; periodontitis; plaque development; saliva

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Bacterial plaque has been well established as the primary etiologic cause of gingival inflammation (Löe et al. 1965). It has been shown that the quantity of plaque, which forms after a tooth surface has been properly cleaned, is influenced by a number of factors including diet (Sheinin & Mäkinen 1971, Rateitschak-Plüss & Guggenheim 1982, Rykke & Sonju 1991), salivary factors (de Jong et al. 1986, Simonsson et al. 1987, Jalil et al. 1992) and surface characteristics (Quirynen et al. 1989, 1990, Siegrist et al. 1991). In addition, several studies have reported on increased plaque accumulation in the presence of gingival inflammation (Lang et al. 1973, Goh et al. 1986, Ramberg et al. 1994, 1995, Rowshani et al. 2004). Also in experimental gingivitis studies it has been shown that subjects develop plaque more rapidly in the presence of gingivitis (Quirynen et al. 1991, Ramberg et al. 1994, Daly & Highfield 1996).

Since a period of no oral hygiene leads to increased numbers of salivary bacteria (Rönström et al. 1979, Ramberg et al. 1994) it has been suggested that phenomenon may also account for the increased plaque formation (Brecx et al. 1980, Quirynen et al. 1991, Daly & Highfield 1996). However, in an

experimental gingivitis study by Ramberg et al. (1994), this could not be established. In this study it was shown that ''the early development of marginal plaque is more influenced by the local environment in the dento-gingival region than by the number of microorganisms in saliva''. The question remained whether this result applies to all periodontal conditions. The above-mentioned observations on de novo plaque formation in experimental gingivitis were made in individuals with longstanding healthy gingival tissues before the start of the experiment. Therefore these subjects may have had relatively low numbers of salivary bacteria compared to subjects with a longstanding history of periodontal inflammation. For example, Mantilla Gómez et al. (2001) found 9×10^8/ml bacteria in the saliva of periodontitis patients whereas Ramberg et al. (1994) reported 2×10^7/ml bacteria in the saliva at baseline of the experimental gingivitis study which increased to 7×10^7/ml bacteria after 3 weeks of no oral hygiene. In a recent study, we investigated the influence of the number of bacteria in saliva on plaque development in various groups of periodontitis patients and healthy controls (Rowshani et al. 2004). Patients included subjects with a healthy reduced periodontium after therapy, subjects with an inflamed reduced periodontium after therapy and an untreated periodontitis group. The results showed that the numbers of salivary bacteria present in the two inflamed groups was considerably higher than of the two periodontally healthy groups. However, although the two inflamed groups developed more plaque than the two healthy groups, no correlations were found between the numbers of bacteria in the saliva and the amount of de novo plaque formation. It was concluded that the periodontal condition is the dominant factor in relation to the rate of plaque formation. Nevertheless, it can be questioned whether the number of bacteria in the saliva does not play a role at all. For example, Goh et al. (1986) found in periodontitis patients that 24 h after tooth cleaning, 54% of the sites without inflammation showed the presence of plaque; whereas Ramberg et al. (1995), in gingivitis subjects, found that after 24 h 21% of the sites without inflammation had plaque. Unfortunately, in the study of Goh et al. (1986) counts of the number of salivary bacteria were not part of the study protocol. Consequently it would be interesting to study the number of bacteria in the saliva of periodontitis patients before and after treatment in relation to the rate of plaque accumulation at healthy sites before treatment and at the same sites after treatment. Therefore, the aim of the present study was to investigate the effect of non-surgical periodontal therapy on the bacterial load and the rate of plaque formation.

Material and Methods

For the present study 23 patients were selected who had been referred to the periodontal department of ACTA (Academic Centre for Dentistry Amsterdam) for treatment of advanced periodontal disease. Exclusion criteria to enter the study were pregnancy, systemic diseases such as diabetes, HIV and other viral, fungal or bacterial infections, and the use of antibiotics during the past 6 months prior to the study and recent tooth extractions. Inclusion criteria were presence of deep pockets, generalised bleeding on probing, presence of >20 teeth and >35 years of age. Smoking was not an exclusion criterium, resulting in a sample of 10 smokers and 13 non-smokers.

Study design

At baseline, before any treatment was provided, patients rinsed with 10 ml of sterile saline (0.85% NaCl) for 30 s after which they expectorated the rinse into a vial. Next, the following clinical parameters were assessed at six sites per tooth on a full mouth level: (1) probing pocket depth (PPD), (2) bleeding on probing (BOP) both by means of the florida probe (Florida Probe®, Gainesville, FL, USA) at a probing force of 20 g, (3) plaque index (PlI, Silness & Loë 1964) and (4) gingival recession. All teeth except third molars were included in the examination.

After the completion of clinical measurements, the supragingival calculus and plaque were removed by means of scaling and polishing. Next the participants were instructed to abolish all tooth-cleaning procedures for the subsequent 24 h. After this period the rinsing procedure was repeated. Directly after rinsing the amount of plaque was evaluated. Thereafter the patients received initial periodontal therapy consisting of meticulous oral hygiene instructions and supra- and subgingival debridement. Three months after the completion of the therapy, the 24 h plaque accumulation experiment was repeated. Again, an initial rinsing sample was obtained and the above-mentioned clinical parameters were assessed. The patients received professional supragingival tooth cleaning after which they were instructed to abolish all tooth-cleaning procedures for the next 24 h. After this period, the rinsing procedure was repeated. Thereafter the amount of plaque was evaluated.

Microbiological evaluation

Immediately after rinsing, the saline sample was supplemented with 1 ml Fildes extract in order to preserve the bacterial motility (Petit et al. 1991). The rinsing samples were evaluated by phase-contrast microscopic examination within 3 h after rinsing. In order to minimise clumping of bacteria, each sample was vortexed for 20 s. to create a homogenous distribution of bacteria in the sample. A disposable tuberculin syringe (1 ml Terumo syringe with a 0.45×12 mm neolus needle) was used to apply one drop of the suspension on a Thoma counting chamber (TCC) and cover-slipped. Each TCC contains squares with a surface of 1/400 mm^2 and a depth of 0.02 mm. The sample was examined by a phase-contrast microscope at a magnification of $\times 1200$. At each assessment a minimum of 100 bacteria was counted in random squares. A differentiation was made into four morphological categories cocci, rods, motile microorganisms and spirochetes as described by Listgarten & Helldén (1978).

In addition to the phase-contrast evaluation, the samples were cultured for further microbiological analysis. The samples were processed in the laboratory within 6 h in order to avoid a drop in viable counts. Tenfold serial dilutions were prepared and aliquots of 0.1 ml were plated onto 5% horse-blood agar plates (oxoid no. 2, Oxoid Ltd, Basingstoke, UK) supplemented with haemin (5 mg/l) and menadione (1 mg/l) for isolation and growth of obligate anaerobic bacteria and on TSBV for selective isolation and growth of *Actinobacillus actinomyctemcomitans* (Slots 1982). Blood agar plates were incubated anaerobically in 80% N_2, 10% H_2 and 10% CO_2 for up to 14 days and tryptic soy-serum-bacitracin-vancomycin

(TSBV) plates were incubated in air +5% CO_2 for 5 days (Van Steenbergen et al. 1986).

Data analysis

A Wicoxon test was used to test for differences before and after treatment for both clinical and microbiological data. The McNemar test was used to test the effect of treatment on the prevalence of specific bacterial species before and after treatment. Values of $p < 0.05$ were accepted as statistically significant.

Results

The study population consisted of 15 males and 8 females with a mean age of 45.2 years (range 35–64). Table 1 presents the clinical data before and after treatment. Due to treatment a significant reduction for all clinical parameters was found. This reduction was for plaque 1.08, BOP (bleeding on probing) 0.88, PPD (probing pocket depth) 1.08 mm and for the percentage of sites $\geqslant$5 mm 17.54%.

The mean values of the plaque index after 24 h of de novo plaque formation before and after treatment can be seen in Table 2. The analysis showed that the amount of de novo plaque on a full mouth level was reduced after treatment. Both at sites with and without gingival recession, treatment resulted in a reduced amount of plaque formation. Before and after treatment, sites with recession harboured more plaque than sites without recession but this difference was only statistical significant after treatment. At tooth surfaces adjacent to sites with gingivitis more plaque was formed than at healthy sites. This was found both before and after treatment. A further evaluation was performed by analysing separately the amount of plaque that developed at healthy sites with shallow pockets (PD (pocket depth) $\leqslant$ 4 mm and absence of BOP). This analysis included only sites which were diagnosed as healthy and shallow before treatment and the same sites after treatment. The results showed that at these sites the amount of plaque formed after treatment was less compared to before treatment, i.e. 0.22 and 0.49, respectively. No differences between smokers and non-smokers were found with regard to treatment response as well as de novo plaque formation.

Table 1. Mean values (standard deviation) of the clinical parameters before and after treatment

	Before treatment	After treatment
PI	1.53 (0.43)	0.45 (0.25)*
BOP	1.25 (0.32)	0.37 (0.22)*
PPD (mm)	3.46 (1.10)	2.38 (0.55)*
% of sites PPD $\geqslant$5 mm	23.55 (16.1)	6.01 (6.08)*

*$p \leqslant 0.00005$.
PI, plaque index; BOP, bleeding on probing; PPD, probing pocket depth.

Microbiological parameters

The results of the phase-contrast microscopic evaluation of the rinsing samples are presented in Table 3. Before treatment all 23 patients were positive for cocci and rods whereas motile microorganisms and spirochetes were found in 13 and 10 subjects, respectively. After treatment all patients remained positive for cocci and rods. The number of patients positive for motile microorganisms and spirochetes decreased; however, this reduction failed to reach the level of significance. On the basis of the initial rinsing samples the analysis showed that periodontal treatment had resulted in a significant reduction of the total numbers of bacteria in the rinsing samples as well as of the various morphotypes. The same phenomenon was found with regard to the 24 h rinsing samples. Twenty-four hours of no oral hygiene resulted both before and after treatment in an increase of the number of bacteria in the 24 h rinsing sample compared to the initial rinsing sample. This increase was mainly due to an increase of the number of cocci before treatment and cocci and rods after treatment. No differences were found between smoking and non-smoking subjects.

The culture results revealed that all periodontal pathogens were present in relatively low proportions (Table 4). The patients showed the lowest prevalence for *A. actinomycetemcomitans* and *Porphyromonas gingivalis* and the highest prevalence for *Fusobacterium nucleatum* and *Peptostreptococcus micros.* On the basis of the initial rinsing samples it was found that treatment had resulted in a reduction of the prevalence of *Prevotella intermedia, Tannerella forsythensis* and *P. micros*. In general, the 24 h rinsing samples showed lower prevalence's of periodontal pathogens than the initial rinsing samples, however this was only in a limited number of cases statistical significant. Again no differences were found between smokers and non-smokers.

Table 2. Mean values (standard deviation) of the PI after 24 h of de novo plaque formation before and after treatment

Sites	Before treatment	After treatment	*p*-value
all	0.65 (0.33)	0.40 (0.16)	0.001
without recession	0.63 (0.32)	0.35 (0.18)	0.0005
with recession $\geqslant$1 mm	0.72 (0.49)	0.43 (0.21)†	0.003
without BOP	0.44 (0.37)	0.30 (0.14)	n.s.
with BOP	0.71 (0.33)*	0.64 (0.27)*	n.s.
PPD $\leqslant$4 mm; no BOP	0.49 (0.49)	0.22 (0.19)	0.002

*Significant difference between sites with and without BOP: $p < 0.0005$.
†After treatment significant difference between sites with and without recession: $p < 0.03$.
PI, plaque index; BOP, bleeding on probing; PPD, probing pocket depth; n.s., not significant.

Discussion

In this study the relationship was investigated between the number of salivary bacteria and the amount of de novo plaque that accumulated over a period of 24 h in periodontitis patients before and after treatment. The results showed that all clinical parameters improved significantly after initial periodontal therapy. The mean reduction in PPD was 1.08 mm and the % of sites $\geqslant$5 mm dropped to 6%; therefore, the treatment result can be considered adequate (Cobb 2002).

It has been shown that periodontal pathogens are present in saliva (Denepitiya & Kleinberg 1982, Asikainen et al. 1991, Danser et al. 1994). This phenomenon was confirmed in the present study. A recent study indicated that samples from saliva are a good approach to detect bacteria such as *P. gingivalis, P. intermedia and T. denticola* in the oral cavity (Umeda et al. 1998). Von Troil-Linden et al. (1995) showed that salivary levels of periodontal pathogens reflect the periodontal status of the patient, and that treatment of periodontitis generally eliminates or decreases the salivary levels of perio-

Table 3. Mean numbers of bacteria (10^7/ml) in the rinsing samples as assessed by means of phase- contrast microscopy and number of positive patients for the various morphotypes

	Before treatment		After treatment	
	initial rinsing sample numbers of bacteria (patients)	24 h rinsing sample numbers of bacteria (patients)	initial rinsing sample numbers of bacteria (patients)	24 h rinsing sample numbers of bacteria (patients)
total count	52.1 (23)	59.7† (23)	39.1* (23)	45.4†,‡ (23)
cocci	41.6 (23)	49.0† (23)	35.3* (23)	40.3†,‡ (23)
rods	8.4 (23)	9.5 (23)	3.6* (23)	4.9 (23)
motiles	1.0 (13)	0.7 (12)	0.3* (8)	0.4 (8)
spirochetes	1.6 (10)	1.0 (8)	0.1* (5)	0.3 (4)

*Number of bacteria in the initial rinsing sample after treatment lower than in the initial rinsing sample before treatment ($p<0.05$).
†Number of bacteria in the 24 h rinsing sample higher than in initial rinsing sample.
‡Number of bacteria in the 24 h rinsing sample after treatment lower than before treatment ($p<0.01$).

Table 4. Prevalence of periodontal pathogens and mean proportions for each species on the basis of positive subjects

	Before treatment		After treatment	
	initial rinsing sample	24 h rinsing sample	initial rinsing sample	24 h rinsing sample
A. actinomycetemcomitans	1 (0.10)	1 (0.06)	3 (0.22)	3 (0.11)
P. gingivalis	7 (5.13)	5 (1.52)	2 (1.10)	1 (0.3)
P. intermedia	11 (1.88)	11 (0.48)	4 (0.67)*	2 (0.15)
T. forsythensis	13 (0.61)	6 (0.78)†	6 (0.68)*	1 (0.2)
P. micros	18 (1.07)	11 (0.42)†	11 (0.64)*	10 (1.36)
F. nucleatum	23 (1.54)	23 (2.00)	23 (1.46)	21 (1.74)

*Number of positive subjects in the initial rinsing sample after treatment lower than in the initial rinsing sample before treatment ($p<0.05$).
†Number of positive subjects in the 24 h rinsing sample higher than in initial rinsing sample.

dontal pathogens. Except for *A. actinomycetemcomitans*, also in the present study a decrease in the prevalence of periodontal pathogens was found reflecting the improved periodontal condition.

Regarding the de novo plaque formation the results of the present study showed that (1) before therapy more plaque was formed than after therapy and (2) on tooth surfaces adjacent to sites with inflammation more plaque was formed than at healthy sites. These findings confirm the results of previous studies in periodontitis patients showing the same phenomenon (Goh et al. 1986, Rowshani et al. 2004). The main aim of the present study was to investigate whether or not the numbers of bacteria in the saliva play a role in de novo plaque formation. Previous studies concluded that although the number of bacteria in saliva is greater when the periodontium is inflamed this does not contribute to more de novo plaque formation. This was shown in an experimental gingivitis study by Ramberg et al. (1994) and in periodontitis patients by Rowshani et al. (2004). However, it remains difficult to explain why after 24 h of no oral hygiene in periodontitis patients 54% of the healthy sites showed presence of plaque (Goh et al. 1986) whereas this was 21% at healthy sites in subjects with gingivitis (Ramberg et al. 1995). Therefore in the present study the relationship was studied between number of bacteria in the saliva and the amount of de novo plaque formation before and after periodontal treatment at healthy sites. Moreover, for this specific aim an analysis was carried out which included only sites, which were healthy before treatment and the same sites after treatment. The results showed that at these sites the amount of plaque that was formed after treatment was less compared to before treatment, i.e. 0.22 and 0.49, respectively. Since the phase-contrast microscopic results showed a reduction of bacteria in the rinsing samples after treatment it seems likely that, to some extent, the numbers of bacteria in the saliva do play a role in the de novo plaque formation.

The bacteria as found in saliva are transients and derive from different sites within the mouth of which the dorsum of the tongue and the teeth are considered to be major contributors (Gibbons 1964). In particular, due to its papillary structure, the tongue dorsum provides a large surface area favouring accumulation of microorganisms. It has been shown that tongue brushing, which reduces the bacterial counts in the saliva, can reduce the rate of plaque formation (Gross et al. 1975). This finding supports the hypothesis that the number of bacteria in saliva plays a role in the rate of de novo plaque formation.

In the present study, it was found that before treatment the numbers of bacteria in the rinsing sample as assessed by phase-contrast microscopy amounted to 52.1×10^7. Previous studies at our department revealed somewhat higher values of 71.0×10^7 and 91.8×10^7 (Rowshani et al. 2004 and Mantilla Gómez et al. 2001 respectively). After treatment the numbers in the present study dropped to 39.1×10^7 which is in the range of the numbers as found in patients with a healthy reduced periodontium of the study of Rowshani et al. (2004), i.e. 43.2×10^7. Fewer bacteria in the saliva, as suggested before, could contribute to less de novo plaque formation in the following way. It has been shown that the concentration of bacteria has to exceed a certain level before colonisation of a tooth surface starts (Van Houte et al. 1970). For example, about 10^4 cells of *Streptococcus mutans* per ml saliva must be present before it becomes probable that one cell will attach to a previously cleaned tooth surface. It is conceivable that plaque formation starts with the attachment of individual bacteria on the tooth surface (Lie 1977, 1979, Nyvad & Fejerskov 1987). Since most bacteria require >4 h to multiply it is likely that the increasing numbers of bacteria

attached to the tooth surface in the early phase of colonisation are the result of the initial attachment of new bacteria rather than by multiplication of bacteria. Therefore the higher numbers of bacteria in the saliva as found before treatment in the present study, could have contributed to a higher initial colonisation rate.

Multiplication of the initially attached bacteria to the cleaned tooth surface is considered to account for the increase in the plaque mass over time (Socransky et al. 1977). In this respect, the available nutrients are highly important. Recently it has been shown that an increased gingival crevicular fluid flow due to gingival inflammation affects the pellicle formation and that the plasma proteins may modify bacterial attachment and early dental plaque composition (Rüdiger et al. 2002). Furthermore, the quantity of pocket fluid increases with increasing pocket depth and gingival inflammation (Mann 1963) both contributing to a more rapid de novo plaque formation.

In summary, the present study confirms the observation in the literature that the periodontal condition is of major importance in relation to the rate of de novo plaque formation. In addition, the results suggest that also the number of bacteria in the saliva may play a role.

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