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Plaque development in relation to the periodontal condition and bacterial load of the saliva

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Abstract

Aim: To investigate the influence of the oral bacterial load on plaque development in various groups of periodontitis patients and in healthy subjects.

Material and Methods: This study included subjects with a healthy periodontium, a healthy reduced periodontium after treatment, an inflamed reduced periodontium after treatment and untreated periodontitis. At the start of the study, subjects were instructed to rinse with 10 ml reduced transport fluid (RTF) for 10 s in order to evaluate the oral bacterial load. The microbiological evaluation included anaerobe culture and phase-contrast microscopy. Next, the amount of plaque and the clinical condition were evaluated. Thereafter, all supragingival plaque was removed and patients were instructed to refrain from all oral hygiene procedures for 19 h. Subsequently, the rinsing procedure and the evaluation of the amount of plaque were repeated.

Results: The amount of plaque that developed in 19 h was significantly higher in the untreated periodontitis group as compared with the two healthy groups. In case of an inflamed reduced periodontium, sites with deep pockets developed more plaque in 19 h than sites with shallow pockets. The number of bacteria present in the rinsing samples of the two inflamed groups was considerably higher than of the two periodontally healthy groups. A significant correlation was found between the bleeding index at intake and the plaque index at 19 h. No correlations were found between gingival recession and the bacterial counts at intake, and the plaque index at 19 h.

Conclusion: The present findings support the concept that the periodontal condition is the dominating factor in relation to the rate of plaque formation. The number of bacteria present in the oral cavity as ascertained by means of a rinsing sample does not seem to play a role.

Key words: bacteria; periodontitis; plaque development; saliva

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The role of dental plaque in the initiation and development of inflammation of the periodontal tissues has been well documented (Löe et al. 1965, Theilade et al. 1966, Lindhe et al. 1973). Since the tooth has a solid non-shedding surface, colonisation of a tooth surface by bacteria will lead to a rapid development of plaque. For example, Alexander et al. (1969) showed that after thorough cleaning of a tooth surface, plaque accumulation reached its original level within 24 h. Several studies have shown that the development of plaque may be dependent on a number of factors including diet (Rateitschak-Pluss & Guggenheim 1982), surface free energy (Quirynen et al. 1989, 1990) and surface roughness (Quirynen et al. 1990, Siegrist 1991).

In the early 1970s, Saxton et al. (1973) showed that the start of the initial colonisation of bacteria on a previously cleaned tooth surface varied from 3 h adjacent to healthy gingivae, to 5 min where the gingival tissues were inflamed. This initial observation has been confirmed by several other studies. For example, Hillam & Hull (1977) showed in an experimental gingivitis study that the amount of plaque that developed in 24 h in gingival health at baseline was considerably less as compared with the amount of plaque that developed in 24 h at the end of the experimental gingivitis period. More extensive studies performed by Brecx et al. (1980), Goh et al. (1986), Quirynen et al. (1991), Ramberg et al. (1994, 1995) and Daly et al. (1996) all confirmed the phenomenon that dental plaque develops more rapidly at inflamed sites.

Hillam & Hull (1977) suggested that not only the inflammatory state of the gingiva but also the total number of oral bacteria may account for a more rapid plaque growth. This hypothesis could not be confirmed by Ramberg et al. (1994). They studied plaque development and numbers of microorganisms in the saliva in a group of 10 young adults in an experimental gingivitis model. Four days of plaque development was studied at baseline when the subjects had healthy gingivae and relatively few salivary bacteria, as well as at the end of the trial when that subjects had inflamed gingivae and an increased number of microorganisms in the saliva. The results of that study showed that plaque development was more influenced by the degree of gingivitis than by the number of bacteria in the saliva.

Up to now, little is known about plaque development in periodontitis patients. Goh et al. (1986) studied the influence of inflammation and pocketing on plaque development during non-surgical periodontal treatment. On surfaces associated with relatively healthy periodontal conditions and shallow pockets they found that the amount of plaque that developed in 24 h was less as compared with surfaces associated with inflamed tissues and deeper pockets. Unfortunately, counts of the number of salivary bacteria were not part of their study protocol. The purpose of the present study was to investigate the influence of the bacterial load in the saliva on plaque development in various groups of periodontitis patients and in periodontally healthy subjects.

Material and Methods

For the present study, four groups of 10 subjects each were selected on the basis of their periodontal condition. This included a group with a healthy periodontium, i.e. no pockets $\geqslant 4$ mm and bleeding on probing $< 15\%$ (healthy (H)). A group with a healthy reduced periodontium after therapy, i.e. no pockets $\geqslant 4$ mm, bleeding on probing $< 15\%$ (healthy reduced (HR)). A group with an inflamed reduced periodontium after therapy, i.e. at least four pockets $\geqslant 4$ mm, bleeding on probing >15% (inflamed reduced (IR)) and a group with moderate to severe untreated periodontitis, i.e. at least four teeth with bone loss $\geqslant$ half of the root length as visible on full-mouth radiographs (untreated periodontitis (UP)). Exclusion criteria to enter the study were the use antibiotics during 6-month prior to the study, pregnancy and systemic diseases that could influence the condition of the periodontal tissues. The healthy subjects were recruited from members of the Department of Periodontology. Subjects with a healthy reduced periodontium or an inflamed reduced periodontium were selected from the pool of patients receiving periodontal maintenance care at the Department of Periodontology. The subjects with untreated periodontitis were newly referred patients. Informed consent was obtained after the background of the study and their involvement was explained.

Study design

At the start of the study (intake) the subjects were asked to rinse with 10 ml reduced transport fluid (RTF, Syed & Loesche 1972) for 10 s. Immediately after rinsing, 1 ml Fildes was added to the samples to preserve the motility of the motile microorganisms (Petit et al. 1991). In this way an initial rinsing sample (IRS) was obtained. Next, the following clinical parameters were assessed on all teeth: plaque index (Silness & Löe 1964), presence or absence of gingival recession, probing pocket depth (PPD) by means of a Florida probe set at a probing force of 20 g and bleeding on pocket probing (BOPP) recorded as present or absent. Third molars were excluded. After the completion of these measurements, a professional prophylaxis was provided to remove supragingival plaque and calculus. To study plaque development, the participants were asked to avoid any kind of self-performed oral hygiene measures for the next 19 h.

The rinsing samples were prepared for phase-contrast microscopic examination within 3 h after rinsing. For suspension of bacteria, samples were vortexed for 20 s, at the maximum setting and further dispersed by aspirating five times through a tuberculine syringe (1 ml Terumo syringe with a 0.45×12 mm neolus needle). One drop of the suspension was placed in a Thoma bright line counting chamber with squares covering 1/400 mm^2 and coverslipped. The samples were examined by means of a phase-contrast microscope at a magnification of $\times 1200$. Bacterial counting was performed in randomly selected squares until at least 100 bacteria were identified and classified into the four morphological categories (cocci, rods, motiles and spirochetes) as described by Listgarten & Helldén (1978). Furthermore, 0.1 ml of each sample was cultured under anaerobic conditions on blood agar plates for 1 week to assess the number of colony-forming units (CFU)/ml. At follow-up, after 19 h of plaque development, the plaque index was assessed and another rinsing sample was taken, designated the 19 h rinsing sample (19 h RS).

Statistical analysis

For each parameter, differences between groups were tested using a Kruskal–Wallis test, with post-testing corrected for multiple comparisons within each test. For each group, comparisons regarding microscopic and culture data between IRS and 19 h RS were performed by means of the Wilcoxon test. Spearman rank correlations were calculated to assess the relationship between the microbiological measurements and the plaque index for each group. To analyse the relation between plaque index, bleeding score and salivary bacterial counts, an analysis of variance (ANOVA) was used. Plaque index was entered as a dependent variable and bleeding scores and total bacterial counts both at intake and at 19 h as covariates. p-Values $\leqslant 0.05$ were accepted as statistically significant.

Results

The mean age, gender distribution and clinical parameters at intake for the four groups are presented in Table 1. Subjects of the H group were younger than that of the IR and UP groups. At intake, before supragingival cleaning, the plaque index ranged from 0.32 in the HR group to 0.69 in the UP group. Owing to the selection criteria, the two healthy groups (H and HR) showed low bleeding scores and no pockets $\geqslant 4$ mm, whereas the diseased groups (IR and UP) showed high bleeding scores and the presence of deep pockets. Also, the UP group had significantly more deep pockets than the IR group. Gingival recession was hardly present in the H group, whereas in the other groups, at least 20% of the surfaces displayed recession.

After 19 h of plaque development, the mean values of the plaque index varied

Table 1. Mean values (standard deviation) of age, gender, plaque index, percentages of sites with BOPP and recession, number of sites with PPD ⩾ 4 mm at intake

	H	HR	IR	UP	*p*-Value*
age	35.5 (8.2)	43.3 (7.6)	50.3 (9.0)[3]	48.1 (9.4)[1]	0.0066
gender	7♀, 3♂	7♀, 3♂	3♀, 6♂	5♀, 5♂	
plaque index – intake	0.50 (0.15)	0.32 (0.16)	0.45 (0.17)	0.69 (0.19)[2]	0.0021
BOPP %	5.0 (3.6)	6.7 (4.3)	28.9 (10)[3,4]	43 (14.9)[1,2]	<0.0005
recession %	0.8 (1.6)	21.7 (16.60)	24.8 (27.9)[3]	32.6 (24.0)[1]	0.0053
PPD ⩾ 4 mm	0 (0)	0 (0)	8.9 (8.4)	32.9 (21.4)	0.0019

BOPP: bleeding on pocket probing, PPD: probing pocket depth, H: healthy group, HR: healthy reduced group, IR: inflamed reduced group, UP: untreated periodontitis group.
*Kruskal–Wallis test.
The numbers 1, 2, 3, 4 indicate where significant differences exist between the various groups: [1]UP > H; [2]UP > HR; [3]IR > H; [4]IR > HR.

Table 2. Mean values (standard deviation) of the plaque index after 19 h of plaque accumulation for all, shallow and deep sites

	H	HR	IR	UP	*p*-Value*
all sites	0.58 (0.15)	0.55 (0.23)	0.77 (0.22)	0.89 (0.11)[1,2]	0.0014
PPD ⩽ 3 mm	0.58 (0.15)	0.55 (0.23)	0.75 (0.22)	0.88 (0.12)[1,2]	0.0017
PPD ⩾ 4 mm	–	–	0.90 (0.27)[†]	0.90 (0.11)	–

For abbreviations see Table 1.
*Kruskal–Wallis test.
[†]Plaque index at sites with PPD ⩾ 4 mm higher than at sites PPD ⩽ 3 mm (Wilcoxon-test $p = 0.008$).
The numbers 1, 2 indicate where significant differences exist between the various groups: [1]UP > H; [2]UP > HR.

Table 3. Mean values ($\times 10^6$/ml) and (standard deviation) of the number of colony-forming units (CFU) as assessed by anaerobe culturing and the number of bacteria as assessed by means of phase-contrast microscopy (total microscopic count) for the initial rinsing samples (IRS) at intake and the19 h rinsing samples (19 h RS)

	H	HR	IR	UP	*p*-Value*
IRS (CFU)	112.2 (35.0)	159.3 (113.1)	235.8 (164.1)	427.7[1,2] (183.5)	0.0003
IRS (total count)	342.5 (34.7)	432.3 (78.3)	625.9[3,4] (115.2)	710.6[1,2] (83.0)	<0.00005
19 h RS (CFU)	111.5 (47.7)	178.2 (171.4)	302.0** (152.8)	451.2[1,2] (212.4)	0.0005
19 h RS (total microscopic count)	426.0** (91.5)	418.3 (85.0)	714.8[3] (152.8)	773.5[1,2,**] (87.4)	<0.00005

For abbreviations see Table 1.
*Kruskal–Wallis test.
**Significant differences between IRS and 19 h RS within a group for CFUs and total microscopic counts.
The numbers 1, 2, 3, 4 indicate where significant differences exist between the various groups: [1]UP > H; [2]UP > HR; [3]IR > H; [4]IR > HR.

between 0.55 and 0.89 (Table 2). Analysis showed that the UP group had higher plaque scores than the two healthy groups. In order to study the relationship between plaque development and pocket depth, the material was analysed for shallow (PPD ⩽ 3 mm) and deep sites (PPD ⩾ 4 mm) separately. Shallow sites were present in all groups, whereas deep sites were only present in the IR and UP groups. At shallow sites, the plaque scores after 19 h of plaque development ranged from 0.55 in the HR group to 0.88 in the UP group. Analysis showed that the UP group developed more plaque than the H and HR group. At deep sites, the IR and UP group developed comparable amounts of plaque. Only in the IR group it was found that the deep sites developed more plaque than the shallow sites.

The microbiological examination of the rinsing samples included culture and phase-contrast microscopy. Table 3 shows the mean numbers of the CFU and total microscopic counts as obtained from the IRS and the 19 h RS. In the IRS, the number of CFU ranged from 112.2 $\times$ 10^6/ml in the H group to 427.7 $\times$ 10^6/ml in the UP group. Analysis showed that the UP group had a larger number of CFU than the two healthy groups. The total microscopic counts in the IRS ranged from 342.5 $\times$ 10^6/ml in the H group to 710.6 $\times$ 10^6/ml in the UP group. Statistical analysis showed that the total microscopic counts from the two diseased groups were higher than that from the H group. Furthermore, the total microscopic count from the UP group was also higher as compared with that from the HR group.

After 19 h of no oral hygiene, the numbers of microorganisms present in the rinsing samples tend to be higher. With regard to the CFUs, this was statistically significant for the IR group. For the total microscopic counts it was significant for the H and UP group. The numbers of CFU in the 19 h RS ranged from 111.5 $\times$ 10^6/ml in the H group to 451.2 $\times$ 10^6/ml in the UP group. It could be shown that the number of CFU from the UP group was higher as compared with that of the two healthy groups. The total microscopic counts in the 19 h RSs ranged from 418.3 $\times$ 10^6/ml in the HR group to 773.5 $\times$ 10^6/ml in the UP group. Statistical analysis showed that the total microscopic counts in the 19 h RS of the two diseased groups were higher as compared with that of the H

Table 4. Mean numbers ($\times 10^6$/ml) and (standard deviation) of cocci, rods, motils and spirochetes in the initial rinsing sampels (IRS) at intake and the19 h rinsing samples (19 h RS)

	H	HR	IR	UP	*p*-Value
Cocci					
IRS	220 (38)[2,3,a]	250 (77)[1]	367 (61)	347 (61)[b]	0.0001
19 h RS	279 (68)[3]	244 (46)[1,4]	398 (96)	456 (169)	0.0002
Rods					
IRS	122 (40)[2,3]	164 (61)[4]	246 (72)	331 (66)	<0.00005
19 h RS	146 (63)[2,3]	179 (51)[4]	303 (80)	338 (88)	0.0001
Motiles					
IRS	0.64 (1.4)[2,3]	4 (3)	7 (5)	9 (5)[c]	0.0006
19 h RS	0.9 (2)[3]	5 (3)	8 (8)	14 (6)	0.0002
Spirochetes					
IRS	0 (0)[3]	2 (3)	7 (18)	9 (5)	0.0333
19 h RS	0 (0)	2 (3)	5 (10)	3 (4)	0.1297

For abbreviations, see Table 1.

The numbers 1, 2, 3, 4 indicate where significant differences exist between the various groups: [1]IR > HR; [2]IR > H; [3]UP > H; [4]UP > HR.

The characters a, b, c indicate where significant differences exist between intake and 19 h: [a]$p = 0.0166$; [b]$p = 0.0125$; [c]$p = 0.0234$.

group. Furthermore, the total microscopic counts from the UP group were also higher than that from the HR group.

The results of the analysis of the prevalence of various microscopic morphotypes are presented in Table 4. The majority of microorganisms in all groups were cocci and rods and only spirochetes were not present in the rinsing samples of the H group. In general, no differences were found between the numbers and distribution of the various morphotypes in the IRS and the 19 h RS. Only in the H group and the UP group larger numbers of cocci could be assessed in the 19 h RS than in IRS. Furthermore, for the UP group the number of motile microorganisms was higher in the 19 h RS.

The results of the analysis of variance showed a strong correlation between plaque index at 19 h and total microscopic counts in the 19 h RS ($p = 0.0005$). Also, a correlation was found with the bleeding index at intake and the plaque index at 19 h ($p = 0.04$). No correlations were found between the plaque index at 19 h and the bacterial counts at intake, the presence of gingival recession or with the anaerobic CFU of the rinsing samples at intake and 19 h. The differences between groups were statistically explained by the two abovementioned significant correlations.

Discussion

This study investigated the relationship between plaque development, numbers of oral bacteria and the clinical condition of periodontal patients and periodontally healthy subjects. Overall, a relationship was found between the bleeding index at intake and the plaque index at 19 h. The analysis showed that the 19 h plaque index of the UP group was higher than that of the subjects with a healthy periodontal condition. This finding confirms the results of the study of Goh et al. (1986), which investigated the effect of periodontal treatment on plaque development in periodontitis patients. During treatment, the level of inflammation and the depths of the pockets decreased. At all time intervals, the rate of experimental plaque formation over a given 24 h period was less on surfaces associated with healthy gingivae and shallow pockets than on surfaces associated with diseased tissues and deeper pockets. In the present study, the relationship between plaque development and pocket depth could only be studied in the two diseased groups (IR and UP) since they showed the presence of pockets $\geqslant 4$ mm. At intake, both these groups had more plaque at deep sites ($\geqslant 4$ mm) than at shallow sites. With regard to the 19 h plaque index, no difference was found between sites with shallow or deep pockets in the UP group. However, in the IR group, sites with shallow pockets harboured less plaque than sites with deep pockets. It has been suggested that 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 (Rudiger et al. 2002). Furthermore, it has been shown that the quantity of pocket fluid increases with increasing pocket depth and gingival inflammation (Mann 1963). Therefore, it can be speculated that the shallow sites in the IR group produced less pocket fluid than the deep sites, resulting in less bacterial substrate and a slower rate of plaque formation. In case of the UP group, the data suggest that the shallow and deep sites produce a comparable amount of pocket fluid and have as a consequence the same plaque index after 19 h of plaque development.

In the present study, it was found that the untreated periodontitis patients had higher numbers of bacteria in the rinsing samples than the healthy subjects, 112×10^6 and 427×10^6, respectively, as assessed by means of anaerobic culturing and 342×10^6 and 710×10^6, respectively, as assessed by phase-contrast microscopy. This finding is in contrast to the results of a previous study at our department (Gomez et al. 2001). In that particular study, the values of the healthy/gingivitis group and untreated periodontitis patients as assessed by means of phase-contrast microscopy were 860×10^6 and 918×10^6, respectively, and no difference could be assessed. These higher values could be explained by: (1) a longer rinsing time i.e. 20 s in the study of Gomez et al. and 10 s in the present study, and (2) that the periodontal condition of the subjects in the healthy group of the present study was at a

higher level; considering that in the study of Gomez et al. subjects selected as healthy were subjects with periodontal pockets $\leqslant 5$ mm and consequently included subjects with various degrees of gingivitis.

Both the present study and the study of Gomez et al. (2001) do indicate much higher numbers of bacteria in the rinsing samples as compared with the study of Ramberg et al. (1994). In their experimental gingivitis study, the total viable count was 17.1×10^6 at the start of the experiment and increased to 77.3×10^6 at the end of the experiment. A basic difference between the present study and the study of Ramberg et al. (1994) is that in the latter the subjects were examined after 4 days of plaque development. It may be suggested that bacteria as observed in rinsing samples of the present study may be partly derived from bacteria of the plaque that are released into the sample by the action of rinsing. It has been shown that a spray of water removes the bulk of the loosely adherent bacteria during early plaque formation (Brecx et al. 1981). Established plaque as in the Ramberg et al. (1994) study may be of a more stable structure, since the adherence of the bacteria has become more consolidated, and therefore the release of bacteria while rinsing is smaller and more variable. So, in case of the 19 h old plaque, the action of rinsing may be more readily capable of releasing bacteria from the tooth surface into the sample than in case of the 4-day-old plaque. This is supported by the observed trend in the present study that the numbers of bacteria in the 19 h RS show higher counts than in the IRS. The ANOVA showed a strong correlation for the plaque index after 19 h and bacterial counts at 19 h. No significant correlation is found with the bacterial counts at intake. This corroborates to the suggestion that in case of 19 h plaque, the plaque bacteria are more easily released from the plaque by rinsing than from established plaque. The finding that no correlation was found between the numbers of bacteria in the IRS and the plaque index after 19 h of plaque development may indicate that the numbers of oral bacteria as ascertained by means of a rinsing sample do not play an important role in plaque development.

In conclusion, the present findings support the concept that the periodontal condition is the dominating factor in relation to plaque formation.

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