The role of surface free energy in the early in vivo formation of dental plaque on human enamel and polymeric substrata

Weerkamp, A. H.; Quirynen, M.; Marechal, M.; Van Der Mei, H. C.; Steenberghe, D. Van; Busscher, H. J.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

[Link to publication in University of Groningen/UMCG research database](#)
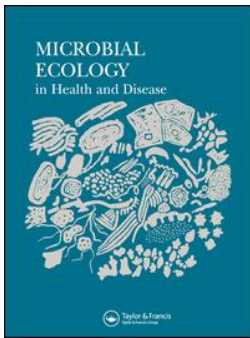
The Role of Surface Free Energy in the Early *In vivo* Formation of Dental Plaque on Human Enamel and Polymeric Substrata

A. H. Weerkamp, M. Quirynen, M. Marechal, H. C. Van Der Mei, D. Van Steenberghe & H. J. Busscher

The Role of Surface Free Energy in the Early *In vivo* Formation of Dental Plaque on Human Enamel and Polymeric Substrata

A. H. WEERKAMP*†, M. QUIRYNEN‡, M. MARECHAL‡, H. C. VAN DER MEI†, D. VAN STEENBERGHE‡ and H. J. BUSSCHER§

†*Dental School, Departments of Oral Biology and §Materia Technica, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands. ‡School of Dentistry, Oral Pathology and Maxillofacial Surgery, Department of Periodontology, Catholic University of Leuven, Leuven, Belgium.*

Strips of teflon and cellulose acetate were glued to the upper lateral incisors of human volunteers in a split mouth, double blind study on the influence of the substratum surface free energy (s.f.e.) on supragingival dental plaque accumulation during a three day period of no oral hygiene. Plaque accumulation, microbial composition of the plaque and s.f.e. of the microorganisms were determined and compared to plaque developed on natural enamel surfaces. Significantly less microorganisms colonised the polymer surfaces ($p < 0.002$). *Streptococcus sanguis* I was the predominant microorganism found in enamel samples, comprising about one-third of the total microflora, whereas it was recovered infrequently and in lower numbers from the polymeric surfaces, which predominantly contained *Streptococcus sanguis* II. Only on cellulose acetate sometimes high numbers of *Streptococcus mitis* and *Streptococcus morbillorum* were detected. The mean s.f.e. of the total plaque flora was lowest on teflon (84.5 mJ m^{-2}) followed by cellulose acetate (86.0 mJ m^{-2}), whereas enamel harboured a microflora with a significantly higher mean s.f.e. (93.0 mJ m^{-2}; $p < 0.05$). Also within the same bacterial species lower s.f.e. strains were isolated from the polymer surfaces compared to enamel. The results conform to a previously postulated model in which the interfacial free energy is the driving force for adhesion of microorganisms to solid surfaces.

KEY WORDS—Surface free energy; Bioadhesion; Oral microflora; Bacteria; Foreign Bodies.

INTRODUCTION

The formation of dental plaque is the primary step in the development of caries and periodontal disease. Plaque formation on smooth surfaces, like most natural surface-associated microbial communities, follows the sequence of acquisition of an organic surface film, followed by selective adhesion and proliferation of the microorganisms.[1,7,19,20] Numerous investigations have therefore been devoted to studying the adhesion of the early plaque-forming microorganisms. Generally such studies have focused on either specific molecular interactions between bacteria and host components[7] or on non-specific physicochemical aspects.[8] Other models have been developed with the aim to include both approaches.[2,6]

In order to adhere firmly to exposed flat surfaces in the oral cavity a microorganism has to approach the surface close enough to form chemical bonds, either through parts of the cell or by excreted polymeric material. Although plaque often grows from small irregularities in the enamel surface[14] offering increased resistance to shear forces operating in the oral cavity, physico-chemical parameters such as the surface free energy, surface charge and hydrophobicity are expected to play an important role.[2] According to a previously postulated model,[2] low surface free energy bacteria would be expected to adhere preferentially to low surface free energy solid substrata, in contrast to high surface free energy bacteria. This would offer a possibility to manipulate the formation of dental plaque and other microbial deposits on natural and foreign surfaces.

It was the aim of this study to explore whether a relation exists between the rate of plaque formation, microbial composition of the plaque and surface

free energy of the bacteria in the plaque, and the surface free energy of solid substrata exposed to the human oral environment.

MATERIALS AND METHODS

Sixteen healthy students of the Dental School at the University of Leuven (11 males, 5 females, age 21 to 23 y) participated in a split mouth, double blind study of plaque formation on three different materials. The participants were selected for having clinically very smooth enamel surfaces,[14] no carious lesions, restorations, erosions or other enamel defects on the selected teeth, no crowding of the teeth and a normal implantation in the dental arch. None of the participants used mouth rinses or followed any antibiotic therapy in the year preceding the study or wore any orthodontic or prosthetic device during the experimental period. There were no signs of periodontitis nor mouth breathing.

In a period of fourteen days preceding the experiment the participants received a professional tooth cleaning and oral hygiene instruction until a healthy periodontium was obtained. Just before the start of the experiment all teeth were perfectly cleaned and the surfaces of the upper front teeth were treated for 30 sec with pumice slurry to remove pellicle and upper enamel layer.[5] The resulting tooth surfaces had an average stylus surface roughness of approximately 0·15 μm and a surface free energy (s.f.e.) of $88 \pm 9 \, \text{mJ m}^{-2}$.[28]

Strips of fluorethylenepropylene-Teflon (Fluorplast, Raamsdonkerveer, The Netherlands; s.f.e. $20 \pm 1 \, \text{mJm}^{-2}$, stylus surface roughness 0·10 μm) or cellulose acetate (Hawe Neos Dental, Switzerland; s.f.e. $58 \pm 3 \, \text{mJm}^{-2}$, stylus surface roughness 0·13 μm) with a width of 2 to 3 mm and a length of 4 to 6 mm, were attached in a random cross-over distribution to the buccal surface of the left and right upper lateral incisors using a cyanoacrylate glue (Histoacryl®). Measurements of s.f.e. and surface roughness of the polymers were made in accordance with Busscher *et al.*[3] The strips were centred and placed 0·5 mm subgingivally. To enhance the subgingival installation, the gingival sulci were slightly widened by means of dental floss. The control sides received the same manipulation. As a control side the upper canines were used. To avoid contamination the investigators wore gloves during all clinical procedures.

Plaque sample processing

Plaque samples were taken 72 h after final cleaning of the teeth and positioning of the test substrata in the oral cavity. Immediately before taking the samples the participants rinsed the oral cavity with approximately 20 ml sterile distilled water to remove debris and excess saliva. Small sterile surgical blades were used to collect all plaque from the dentogingival margin to the incisal edge of the test pieces and from a similar surface area of the control tooth surface. Care was taken to achieve as high as possible reproducibility. The tip of the blades containing the plaque sample was broken off and deposited in a screw-capped plastic vial containing 1 ml reduced transport fluid, RTF[16] and immediately processed for enumeration of the microflora. To this end the samples were dispersed by carefully moving them four times up and down a 27 gauge hypodermic needle, followed by vortexing for 15 sec. Serial 10-fold dilutions were subsequently made in RTF and 0·1 ml amounts of appropriate dilutions were spread on non-selective and selective agar media. All fluids and plates had been preincubated in an anaerobic atmosphere for 24 h.

Total anaerobic counts were made on ETSA-agar[22] incubated anaerobically in an atmosphere of 85 per cent N_2, 10 per cent H_2, and 5 per cent CO_2 for 5 to 7 d, in plastic jars (BBL GasPak) filled by repeated evacuation and gassing cycles and containing cold palladium catalyst. From plates containing 30 to 200 colonies the up to six predominant colony types, representing at least 90 per cent of the total microflora, were isolated and restreaked on ETSA plates to check for purity and for identification and surface free energy determination, and the proportion of the total count was recorded. If available, additional colonies of the same morphological types were isolated and stored to serve as back-up and to enable comparison with the further processed isolates.

The original ETSA plates were also used to screen colony types not included in the set of predominant microorganisms by Gram-stained smears and colony characteristics, thus supplying approximately 1 per cent detection level values for *Actinomyces odontolyticus*, *Propionibacterium acnes*, Gram-negative rods (including *Capnocytophaga* species, black-pigmented *Bacteroides*, fusobacteria) and *Streptococcus salivarius*.

Selective plates were used for low-dilution detection of *S. mutans* (TYS20B[17]) and lactobacilli (Rogosa SL-agar; Difco Laboratories).

Identification

Following classification by colony morphology and Gram-stained smears the isolates were identified as follows: Streptococci were differentiated using the API-20STREP system and data base (API System, S.A., Montalieu-Vercieu, France), if appropriate supplemented with a test for dextran/levan production. Anaerobic rods and cocci were identified using the API-20A system, supplemented with tests for catalase, oxidase and nitrate-reductase.

Surface free energy determination

For determination of the s.f.e., pure cultures of the bacterial isolates were grown after the minimum number of transfers required to assure purity in Todd Hewitt broth (Oxoid Ltd, Basingstoke, England) supplemented with 0·2 per cent (w/v) glucose for 16 h at 37°C in anaerobic culture. A few isolates which failed to grow well in this medium were grown in Brain heart infusion broth (Oxoid) supplemented with 0·1 per cent (w/v) cysteine and 0·2 per cent (w/v) glucose, or in a Trypticase-soy broth-based medium supplemented with KNO_3, Na-formate, Na-succinate and Na-fumarate, all at 0·05 per cent (w/v) concentration. Veillonella parvula isolates were grown in Todd Hewitt broth containing 5 ml/l of a 60 per cent (w/v) Na-lactate solution. Control experiments demonstrated that the growth medium did not have a significant effect on the surface free energy.

Cells were harvested by centrifugation for 10 min at 7000 g, washed two times in phosphate-buffered saline, pH 7·0, and suspended in demineralised water. Contact angles were measured on lawns of the bacteria prepared on nitrocellulose membrane filters, as previously described[4,25] and on the polymeric surfaces. Water, water/n-propanol mixtures and α-bromonaphthalene were used as wetting liquids. Subsequently the s.f.e. and its polar and dispersion components were calculated from the contact angle data using the geometric mean equation.[3,4]

Handling of the data

In order to obtain a measure of the s.f.e. of a total plaque flora, composite s.f.e. were calculated as follows. The s.f.e. of each individual bacterial isolate in a plaque sample was multiplied by its proportion of the total plaque flora included in the s.f.e. determinations, yielding the proportional s.f.e. The composite s.f.e. of the plaque is the sum of the proportional

surface free energies. The microflora included in the calculations represented at least 70 per cent of the total flora at a given site (average over all samples $85\cdot5 \pm 9\cdot8$ per cent). Since in all types of surfaces tested the composite plaque s.f.e. were normally distributed, the Student's t-test for paired observations was applied to determine statistical differences between the test surfaces. The same test was also applied to compare the s.f.e. of bacterial species isolated from the various surfaces.

For comparison of the composition of the microflora, isolation frequencies as well as the mean percentages of the proportion of the total microflora and standard deviations were calculated. When a species was not encountered in a given sample, its detection limit was used in the statistical analysis. Differences between bacterial species on the various test surfaces were evaluated using Wilcoxon's matched signed rank test.

RESULTS

A total of 32 polymer strips was fixed to the dentition of the 16 subjects for the purpose of this study. Two participants lost both strips during the test period and were excluded from the study. Four participants lost either the teflon or the cellulose acetate strip. A total of 24 test strips (67 per cent) and 14 enamel surfaces were therefore evaluated, leading to the characterisation of 152 bacterial isolates. Due to loss of isolates in the course of the study another teflon and cellulose acetate surface had to be excluded, because of a too low proportion of characterised strains. In one subject the amount of plaque recovered from the polymer surfaces was too low to be included in the evaluation of the microflora.

Total bacterial counts on the test surfaces are presented in Table 1. On average, a 140 and 700-fold lower viable count was obtained on cellulose acetate and teflon, respectively, compared to enamel after three days of exposure to the oral environment. However, individual variation occurred, demonstrated by the finding that in one of the test subjects the difference was as extreme as 10^6 fold whereas in another subject similar high counts were made on all surfaces. A significantly lower viable count was also observed on teflon compared to cellulose acetate (Table 1).

The composition of the microflora on the various surfaces is presented in Table 2. Due to the low isolation frequencies of most species, statistical analysis could only be performed in a few cases.

Table 1. Log bacterial counts on the test surfaces after three days

Test surface	Number of surfaces tested	Mean Log_{10} count	Standard deviation	Range
Teflon	12	4·20*	1·56	1·70–6·86
Cellulose acetate	12	4·93**	1·71	<1·0 –7·50
Enamel	14	6·97	0·97	4·15–7·90

*Significantly lower than enamel ($p < 0·0001$) and cellulose acetate ($p < 0·05$)
**Significantly lower than enamel ($p < 0·002$)

Table 2. Bacterial composition of three day old plaque as mean of total flora (per cent) and isolation frequency (per cent)

Bacteria	Teflon (n = 10) IF*	M**	Cellulose acetate (n = 10) IF	M	Enamel (n = 14) IF	M
S. sanguis I	50	11·6 (14·6)	20	4·0 (8·3)	93	33·5 (20·0)
S. sanguis II	90	37·5 (23·4)	80	27·8 (27·1)	50	18·9 (23·4)
S. mitis	30	4·4 (9·8)	40	24·0 (38·3)	8	0·9 (3·0)
S. morbillorum	10	1·1 (3·5)	60	12·7 (14·9)	25	3·2 (5·8)
S. mutans	0	=†	0	=	0	=
S. salivarius	0	—††	0	—	0	—
A. viscosus	0	—	0	—	15	2·8 (9·6)
A. odontolyticus	30	3·6 (7·8)	40	1·2 (2·5)	71	3·4 (3·9)
P. acnes	10	4·2 (12·5)	10	7·8 (22·1)	0	=
Lactobacillus sp.	0	=	0	=	8	2·7 (9·9)
V. parvula	70	11·1 (11·1)	80	11·6 (11·3)	85	14·8 (10·0)
Gram-neg. rods	10	3·3 (10·5)	20	0·7 (1·5)	29	3·6 (6·7)

*IF = isolation frequency in percentage of samples
**M = mean percentage of total flora with standard deviation
† = not detected at 0·01 per cent level
†† = not detected at 1 per cent level

However, some clear differences can be seen. *S. sanguis* was isolated from 92 per cent of the surfaces and was the proportionally predominant microorganism, followed by *V. parvula*. Statistically significant differences were however observed between the two biotypes of *S. sanguis*, which are readily distinguished in the API-20Strep system. Type I predominated on enamel, but was present in much lower numbers on the polymeric substrata ($p < 0·01$), whereas type II was less frequently isolated from enamel than from the plastic surfaces.

S. mitis and *S. morbillorum* were only sporadically found on teflon or enamel, but comprised 36 per cent of the microflora on cellulose acetate. The absence of *S. salivarius* in the samples indicates that the plaques were not 'contaminated' with saliva, even when only very little plaque was present. *S. mutans* was also not found, notwithstanding the use of a selective medium, presumably because of the relatively low incidence of this organism in the test subjects and because it is a weak primary coloniser of smooth surfaces. Similarly, lactobacilli were not

Table 3. Surface free energy (γ_b) bacterial species isolated from plaques formed on enamel and plastic surfaces

| Bacterial species | Surface free energy (mJ m^{-2}) | | | | | |
| | Teflon + cellulose acetate | | | Enamel | | |
	Mean	Median	Range	Mean	Median	Range
S. sanguis I	85·0* (9·0)†	80·7	73–97	94·2 (7·0)	94·1	82–107
S. sanguis II	77·2 (23·9)	79·1	38–110	85·4 (25·0)	88·7	41–110
V. parvula	93·8 (21·0)	101·2	55–115	104·9 (8·6)	106·0	94–114

*Significantly different from enamel ($p < 0.02$)
†Standard deviation of the mean

Table 4. Composite surface free energy (γ_b) and its polar (γ_b^p) and dispersion component (γ_b^d) of the microflora isolated from three day old plaque developed on different surfaces

| Test surface | Mean surface free energy (mJ m^{-2}) | | | Number of surfaces tested | Percentage of total microflora included |
	γ_b	γ_b^p	γ_b^d		
Teflon	84·5* (11·5)†	47·9 (11·1)	36·7 (0·9)	10	83·2
Cellulose acetate	86·0* (11·2)	49·4 (11·8)	36·6 (1·5)	10	81·9
Enamel	93·0 (8·9)	56·5 (9·3)	36·6 (1·0)	14	82·9

*Significantly different from enamel, $p < 0.05$; t-test for paired observations
†Standard deviation of the mean

detected except for one sample that contained a high proportion of *L. fermentum*. *A. odontolyticus* was the predominant pleomorphic Gram-positive rod and was more frequently isolated from enamel than from the plastic surfaces, although no significant difference was found in the proportions of this species. *A. viscosus* was only encountered in two of the enamel plaque samples. Gram-negative anaerobic rods were infrequently isolated, consistent with the early stage of plaque development,[19,24] although in two samples *F. nucleatum* comprised 15 to 20 per cent of the plaque flora.

Large differences were observed in the surface free energy of the individual bacterial isolates, ranging from 37·8 to 122·6 mJ m^{-2}, which is similar to the range of s.f.e. observed previously using oral streptococci.[30] Comparisons between the s.f.e. of strains belonging to the same species isolated from various surfaces could only be made in three cases, due to low isolation frequencies. Table 3 shows that the s.f.e. of *S. sanguis* I, *S. sanguis* II and *V. parvula* strains recovered from the polymeric substrata was considerably lower than those from enamel. However, only in the case of *S. sanguis* I was this difference statistically significant. To obtain a measure of the s.f.e. of an entire plaque population, composite s.f.e. were calculated. Table 4 presents the average composite s.f.e. calculated for the various surfaces tested. The results show that plaques formed on teflon and cellulose acetate strips contain a bacterial

population which has a slightly, but statistically significant lower s.f.e. than on natural enamel. The difference was completely due to the polar component of the s.f.e., denoting that predominantly the short range interactions between bacterial and substratum surface are affected.[2] Since Gram-negative bacteria generally show a slightly lower dispersion component of the s.f.e. than Gram-positives (unpublished observation) the finding is in line with the similar ratios of Gram-positive over Gram-negative bacteria found on the various surfaces (Table 2). No difference in composite s.f.e. was observed between both artificial surfaces.

DISCUSSION

Three important differences in the plaque formation on artificial polymeric substrata and native enamel after 72 h were noted in this study: firstly, plaque accumulates to much lower numbers on the artificial substrata, with teflon being lower than cellulose acetate, secondly, the bacterial composition of the plaques differs significantly, and thirdly, the s.f.e. of bacteria in plaques formed on the polymer surfaces is lower than those in enamel-associated plaque, both as a total and within individual species. Early plaque formation is thought to be influenced by the selective acquisition of bacteria and by their subsequent proliferation,[1,7,19] which in itself is a function of a variety of host-bacterial and interbacterial relationships. It should be noted therefore that the greater accumulation of plaque on the enamel surface may have created an environment more suitable for e.g. *S. sanguis* biotype I. Although this possibility cannot be totally excluded, our data did not show a relationship between the total amount of plaque accumulated on enamel and the prevalence of *S. sanguis* I (data not shown), and conversely between the low plaque accumulations and the presence of the other streptococci. It has also been shown that *S. sanguis* I strains adhere better to saliva-coated spheroidal hydroxyapatite than type II strains.[31] This suggests that the differences in incidence of the bacteria are primarily a function of differences in surface properties of the test substrata and the available bacteria in the oral cavity.

The significantly lower bacterial counts made on the polymeric surfaces are consistent with the previous findings made by measuring the planimetric plaque index.[15] Several possibilities may explain the observed effect. Surface roughness has been recognised as an important parameter in the retention of plaque on flat surfaces,[14,21] but is not likely to be

the main factor here, since only clinically extremely smooth tooth surfaces were selected,[14] which show a stylus surface roughness very similar to the polymer strips. Moreover, the observed lower plaque accumulation on teflon compared to cellulose acetate and the differences in the composition and properties of the microflora cannot be explained in this way.

The results in our study are very much in agreement with expectations based on a thermodynamic model of bacterial adhesion incorporating the s.f.e. of bacteria, solid surface and suspension liquid.[2,30] According to this model, low s.f.e. bacteria are expected to adhere preferentially to low s.f.e. surfaces, and high s.f.e. bacteria to high s.f.e. substrata. *In vitro* measurements done with oral bacteria[13,29] and yeasts[11] as well as preliminary *in vivo* experiments carried out in beagle dogs,[27] have presented evidence for the validity of the model. Because of the relatively high s.f.e. of most common oral bacteria it has previously been suggested[30] that the s.f.e. of a solid present in the oral cavity should be lower than 60 mJ m^{-2} to effectively reduce their adhesion and consequently plaque formation. It is remarkable in this respect that distinct effects were seen in our study notwithstanding the fact that in the presence of saliva surfaces rapidly acquire a proteinaceous pellicle which increases the s.f.e. of low energy substrata and bacteria[26,30] and precedes bacterial attachment.[9,20] Thus it appears that properties of the underlying substratum are transferred to the adsorbed pellicle, a phenomenon which has been noted previously *in vitro* with respect to streptococcal adhesion[13] and spreading of tissue cells,[18] either by affecting the amount and conformation of protein adsorbed or by qualitative differences in pellicle composition.[10,20] Similarly, the reflection of the substratum surface properties in the surface properties of the overall plaque microflora could result from a 'transfer' of properties to each subsequent layer of microorganisms, but more likely results from proliferation of the primary adhering microflora.[1]

The overall composition of the microflora was essentially similar to the predominantly coccal early plaque flora found supragingivally on natural teeth,[19] dentures,[23] dental implants[12] and other smooth artificial surfaces.[24] In specific aspects our results may diverge from those of Theilade *et al.*[24] who did not observe differences in the composition of plaque formed during 8 h on enamel and Mylar® plastic film, respectively. However, the proportional differences in plaque flora observed in our study

demonstrates the importance of the surface proper-ties of the solid material, since this could facilitate the establishment of a potential pathogenic plaque microflora.[12] It seems also important to distinguish between the two biotypes of *S. sanguis* in adhesion and plaque studies, since a significant difference in s.f.e. was observed (type I $90 \cdot 6 \pm 8 \cdot 7$ mJ m^{-2}, type II $79 \cdot 9 \pm 22 \cdot 2$ mJ m^{-2}; $p < 0 \cdot 02$), connected with a different colonisation behaviour.

In conclusion, the results of our present work demonstrate that plaque accumulation on smooth surfaces may be strongly retarded by lowering the s.f.e. of the attachment substratum. In addition, this affects the qualitative microbial composition of the plaque. Preventive measures may thus be aimed at lowering the s.f.e. of the tooth surface or of materials used as restoratives or dental implants. Analogously, adhesion to implants in other parts of the body may be reduced by using low s.f.e. materials.

REFERENCES

1. Brecx M, Theilade J, Attström R. (1983). An ultra-structural quantitative study of the significance of microbial multiplication during early dental plaque growth. *Journal of Periodontal Research* **18**, 177–186.

2. Busscher HJ, Weerkamp AH. (1987). Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiology Reviews* **46**, 165–173.

3. Busscher HJ, van Pelt AWJ, de Boer P, de Jong HP, Arends J. (1983). Effect of spreading pressure on surface free energy determinations by means of contact angle measurements. *Journal of Colloid and Interface Science* **95**, 23–28.

4. Busscher HJ, Weerkamp AH, van der Mei HC, van Pelt AWJ, de Jong HP, Arends J. (1984). Measure-ment of the surface free energy of bacterial cell sur-faces and its relevance for adhesion. *Applied and Environmental Microbiology* **48**, 980–983.

5. Davidson CL. (1980). De conditionering van de tandarts voor gecond008itioneerd glazuur. *Nederlands Tijdschrift voor de Tandheelkunde* **87**, 2–4.

6. Doyle RJ, Nesbitt WE, Taylor KG. (1982). On the mechanism of adherence of *Streptococcus sanguis* to hydroxylapatite. *FEMS Microbiology Letters* **15**, 1–5.

7. Gibbons RJ. (1984). Adherent interactions which may affect microbial ecology in the mouth. *Journal of Dental Research* **63**, 378–385.

8. Glantz P-O, Baier RE. (1986). Recent studies on nonspecific aspects of intraoral adhesion. *Journal of Adhesion* **20**, 227–244.

9. Leach SA, Saxton CA. (1966). An electron micro-scopic study of the acquired pellicle and plaque formed on the enamel of human incisors. *Archives of Oral Biology* **11**, 1081–1094.

10. Mandel ID. (1974). Relation of saliva and plaque to dental caries. *Journal of Dental Research* **53**, 246–266.

11. Minagi S, Miyake Y, Inagaki K, Tsuru H, Suginaka H. (1985). Hydrophobic interaction in *Candida albi-cans* and *Candida tropicalis* adherence to various denture base resin materials. *Infection and Immunity* **47**, 11–14.

12. Nakou M, Mikx FHM, Oosterwaal PJM, Kruijsen JCWM. (1987). Early microbial colonization of permucosal implants in edentulous patients. *Journal of Dental Research* **66**, 1654–1657.

13. Pratt-Terpstra IH, Weerkamp AH, Busscher HJ. (1987). Adhesion of oral streptococci from a flowing suspension to uncoated and albumin-coated sur-faces. *Journal of General Microbiology* **133**, 3199–3206.

14. Quirynen M, Thevissen E, van Steenberghe D. (1986). The role of gingivitis, tooth roughness and tooth type on plaque growth. *Journal of Dental Research* **65**, 549.

15. Quirynen M, Marechal M, Busscher HJ, Arends J, van Steenberghe D. (1988). The influence of the sur-face free energy on plaque growth in man. *Journal of Dental Research* **67**, 691.

16. Rundell BB, Thomson LA, Loesche WJ, Stiles HM. (1973). Evaluation of a new transport medium for the preservation of oral streptococci. *Archives of Oral Biology* **18**, 871–878.

17. Schaeken MJM, van der Hoeven JS, Francken HCM. (1986). Comparative recovery of *Strepto-coccus mutans* on five isolation media, including a new simple selective medium. *Journal of Dental Research* **65**, 906–908.

18. Schakenraad JM, Busscher HJ, Wildevuur CRH, Arends J. (1986). The influence of substratum sur-face free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. *Journal of Biomedical Materials Research* **20**, 773–784.

19. Socransky SS, Manganiello AD, Propas D, Oram V, van Houte J. (1977). Bacteriological studies of developing supragingival dental plaque. *Journal of Periodontal Research* **12**, 90–106.

20. Sönju T, Rölla G. (1972). Pellicle formation on mylar strips and other artificial surfaces. *Journal of Periodontal Research* Suppl. no. **10**, 20–21.

21. Swartz ML, Phillips RW. (1957). Comparison of bacterial accumulations on rough and smooth enamel surfaces. *Journal of Periodontology* **28**, 304–307.

22. Syed S, Svanberg M, Svanberg G. (1980). Predomi-nant cultivable dental plaque flora of beagle dogs with gingivitis. *Journal of Periodontal Research* **15**, 123–136.

23. Theilade E, Budtz-Jorgensen E, Theilade J. (1983). Predominant cultivable microflora of plaque on removable dentures in patients with healthy oral mucosa. *Archives of Oral Biology* **28,** 675–680.

24. Theilade E, Theilade J, Mikkelsen L. (1982). Microbiology of early dento-gingival plaque on teeth and mylar strips in man. *Journal of Periodontal Research* **17,** 12–25.

25. Van der Mei HC, Weerkamp AH, Busscher HJ. (1987). A comparison of various methods to determine hydrophobic properties of streptococcal cell surfaces. *Journal of Microbiological Methods* **6,** 277–287.

26. Van Dijk LJ, Goldsweer R, Busscher HJ. (1988). Interfacial free energy as a driving force for pellicle formation in the oral cavity: an *in vivo* study in beagle dogs. *Biofouling* **1,** 19–25.

27. Van Dijk J, Herkströter F, Busscher HJ, Weerkamp AH, Jansen H, Arends J. (1987). Surface free energy and bacterial adhesion; an *in vivo* study in beagle dogs. *Journal of Clinical Periodontology* **14,** 300–304.

28. Van Pelt AWJ, de Jong HP, Busscher HJ, Arends J. (1983). Dispersion and polar surface free energies of human enamel. *Journal of Biomedical Materials Research* **17,** 637–641.

29. Van Pelt AWJ, Weerkamp AH, Uyen MHWJC, Busscher HJ, de Jong HP, Arends J. (1985). Adhesion of *Streptococcus sanguis* CH3 to polymers with different surface free energies. *Applied and Environmental Microbiology* **49,** 1270–1275.

30. Weerkamp AH, van der Mei HC, Busscher HJ. (1985). The surface free energy of oral streptococci after being coated with saliva and its relation to adhesion in the mouth. *Journal of Dental Research* **64,** 1206–1210.

31. Wyatt JE, Hesketh LM, Handley PS. (1987). Lack of correlation between fibrils, hydrophobicity and adhesion for strains of *Streptococcus sanguis* biotypes I and II. *Microbios* **50,** 7–15.