Adsorption of Parotid Saliva Proteins and Adhesion of *Streptococcus Mutans* ATCC 21752 to Dental Fiber-Reinforced Composites

Johanna Tanner,[1] Anette Carlén,[2] Eva Söderling,[1] Pekka K. Vallittu[1]

[1] Institute of Dentistry and Biomaterials Research, University of Turku, Finland

[2] Department of Oral Microbiology, University of Göteborg, Sweden.

Received 7 May 2002; revised 11 October 2002; accepted 16 October 2002

Abstract: The use of fiber-reinforced composites (FRC) in dentistry has increased during recent years. In marginal areas of crowns and removable partial dentures the fibers may become exposed and come into contact with oral tissues, saliva, and microbes. To date, few articles have been published on oral microbial adhesion to FRCs. The aim of this study was to compare different FRCs, their components, and conventional restorative materials with respect to *S. mutans* ATCC 21752 adhesion and adsorption of specific *S. mutans* binding proteins. Surface roughness of the materials was also determined. Four different FRCs, a restorative composite, and a high-leucite ceramic material were studied. Polyethylene FRC was found to be significantly rougher than all other materials. Aramid FRC also showed higher surface roughness in comparison with all materials but polyethylene FRC. Without a saliva pellicle, adhesion of *S. mutans* coincided with surface roughness and polyethylene and aramid FRC promoted *S. mutans* adhesion better than the other smoother materials. In the presence of salivary pellicle, ceramic and polyethylene FRC bound more bacteria than the other materials studied. Higher quantities of *S. mutans* binding proteins in the pellicles may in part account for the higher *S. mutans* adhesion to saliva-coated ceramic and polyethylene FRC. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 66B: 391–398, 2003

Keywords: adherence; adsorption; salivary proteins; *Streptococcus mutans*; fiber-reinforced composite; restorative materials

INTRODUCTION

Fiber-reinforced composites (FRC) are metal-free materials and have good mechanical and cosmetic properties.[1,2] Their use in dentistry has increased during recent years in fixed partial dentures, periodontal splints, and as reinforcements of removable dentures. Different types of fibers, including glass,[3] UHMW polyethylene,[4] carbon/graphite,[5] and aramid fibers,[6] have been used to reinforce dental polymers. The reinforcing fibers are usually covered with the matrix polymer of the composite, particulate filler composite or denture base polymer. However, marginal areas of crowns and removable partial dentures and interdental spaces of periodontal splints often need adjustment after insertion into the mouth. This will result in exposure of the fibers, and they then come into contact with oral tissues, saliva, and microbes.

Development of dental caries requires adhesion and colonization of odontopathogens. The caries-associated *Streptococcus mutans* can colonize all solid surfaces in the mouth, tooth tissues, as well as restorative materials. Material surface physicochemical properties like surface free energy, hydrophobicity, and surface roughness have an influence on bacterial adhesion either directly or through adsorption of pellicle proteins. High-energy surfaces have been shown to collect more plaque than low-energy surfaces.[7,8] Several authors have found rough surfaces to promote bacterial adhesion.[9,10] The properties of reinforcing fibers may have an influence on bacterial adhesion.

Colonization of microbes is preceded by specific adsorption of salivary components and formation of an acquired pellicle. Pellicle components may act as receptors for oral microbes. High-molecular-weight glycoproteins, agglutinins, present in the pellicle are identified as the major receptor molecules for *S. mutans* adhesion,[11,12] Proline-rich proteins and secretory immunoglobulin A have also been found to mediate *S. mutans* binding.[11,13,14] Substratum surface properties have been found to influence the composition of the

Correspondence to: Johanna Tanner, Institute of Dentistry, University of Turku, 20520 Turku, Finland (e-mail: johanna.tanner@utu.fi)

TABLE I. The Composition and Manufacturers of Materials Used in this Study

Code	Material	Type of Fiber	Matrix Polymer	Commercial name	Manufacturer
Cer	High-leucite porcelain			Finesse All-Ceramic	Dentsply Ceramco Inc., Burlington, NJ
RC	Restorative composite		Bis-EMA[a]	Z250	3M dental, Minneapolis, MN
GF	Glass-fiber composite	Silanized E-glass[b]	Bis-GMA[c]/TEGDMA[d] (40/60 vol.%)	Experimental material	Fiber: Ahlström, Karhula, Finland
PE	Polyethylene-fiber composite	UHMW[e] polyethylene	Bis-GMA[c]/TEGDMA[d] (40–60 vol.%)	Experimental material	Fiber: DSH High Performance Fibers, Heerlen, The Netherlands
AR	Aramid-fiber composite	Aramid	Bis-GMA[c]/TEGDMA[d] (40/60 vol.%)	Experimental material	Fiber: DuPont, Engineering Fibers, Geneva, Switzerland
C/G	Carbon/graphite-fiber composite	Sizing-treated carbon/graphite	Bis-GMA[c]/TEGDMA[d] (40/60 vol.%)	Experimental material	Fiber: Zoltek Companies Inc., St. Louis, MO
E-glass	Bulk E-glass			Experimental material	Ahlström, Karhula, Finland
bPE	Bulk UHMW polyethylene			Experimental material	DSH High Performance Fibers, The Netherlands
Polymer	Bis-GMA/TEGDMA (40/60 vol.%)			Experimental material	Resins supplied by Sigma-Aldrich Co. Ltd., Gillingham, UK

[a]2,2-bis[(4-methacrylyloxy)phenyl]-propane.
[b]Electrical glass, composition (%): SiO_2 (55); CaO (22); Al_2O_3 (15); B_2O_3 (6).
[c]2,2-bis(4-[2-hydroxy-3-methacrylyloxypropoxy)phenyl]-propane.
[d]Triethyleneglycol dimethacrylate.
[e]Ultra-high molecular weight.

acquired pellicle as well.[15,16] In previous studies it was found that the salivary pellicle formed on glass fibers promoted *S. mutans* ATCC 21752 adhesion in comparison with the pellicle formed on the surrounding polymer matrix.[17]

To date, few articles have been published on oral microbial adhesion to FRCs and no studies comparing different FRCs with respect to bacterial adhesion can be found. The aim of this study was to compare different FRCs, their components, and conventional restorative materials with respect to *S. mutans* ATCC 21752 adhesion and adsorption of specific *S. mutans* binding proteins.

MATERIALS AND METHODS

Materials

Four different fiber-reinforced composites, a restorative particulate filler composite (Z-250; 3M dental products, St. Paul, MN), and a high-leucite ceramic material used for ceramic fillings and crowns (Finesse All-Ceramic; Dentsply, York, PA), were studied. Pellicle protein adsorption was studied, in addition to the aforementioned materials, with bulk E-glass, bulk polyethylene, and polymer of bis-GMA/TEGDMA. This was done to better understand the influence of individual materials of the composites on selective protein binding and pellicle mediated adhesion. Glass and polyethylene were selected for the closer investigation because of the wide variety of clinical applications using these materials as reinforcements.

The materials used in this study are described in Table I. The fibers used were E-glass fibers, UHMW-polyethylene fibers, aramid fibers, and carbon/graphite fibers. The polymer matrix of FRCs was made of monomer resin (2.2-bis(4-(2-hydroxy-3-methacrylyloxypropoxy)phenyl)-propane (Bis-GMA) (40 vol.%) and triethyleneglycol dimethacrylate (TEGDMA) (60 vol.%) with bentzoylperoxide (BPO) (1.5 wt.%) as the polymerization initiator. Fibers were wetted overnight in the resin mixture and manually laminated in a silicone mold, forming a bar of the size 50 × 4 × 5 mm. The bars were polymerized at 80 °C for 1 h. The bars were cut perpendicular to the long axis of the fibers, resulting in specimens of the size 2 × 4 × 5 mm. Specimens with the same size were also fabricated from restorative composite and high-leucite ceramic. A silicone mold was filled with restorative composite, which was light polymerized by first using a hand piece for 40 s. After removal from the mold, the composite specimens were further polymerized in a light-curing oven for 15 min (LicuLite; Dentsply, York, PA). Ceramic specimens were

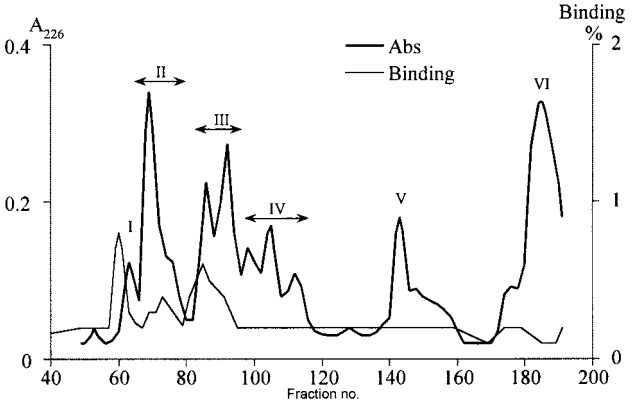


Figure 1. Binding of *S. mutans* ATCC 21752 to pellicles formed from factions of parotid saliva obtained as previously reported.[20] The fractions contain agglutinin (I), proline-rich glycoproteins (II), acidic (III), and basic (IV) proline-rich proteins, statherin (V), and amylase (VI).

TABLE II. Surface Roughness (R_a) of the Studied Materials. Mean ± SD of Triplicate Measurements

Materials	R_a (μm)
Cer	0.05 ± 0.01
RC	0.05 ± 0.01
GF	0.07 ± 0.02
PE	0.51 ± 0.02[a]
AR	0.18 ± 0.04[b]
CG	0.05 ± 0.01

[a]Significantly rougher than all other materials ($p < 0.001$).
[b]Significantly rougher than all other materials but PE ($p < 0.001$).

heat pressed according to the manufacturer's instructions. Eight specimens with equal surface area were made of each material were made. All surfaces of the specimens were wet ground and polished with the use of silicon carbide papers (grit 800 and 2400) (Struers RotoPol). After polishing the specimens were ultrasonically cleaned in distilled water for 10 min followed by washing with ethanol for 5 min. All specimens were stored in distilled water at room temperature for 24 h before testing.

Surface Roughness

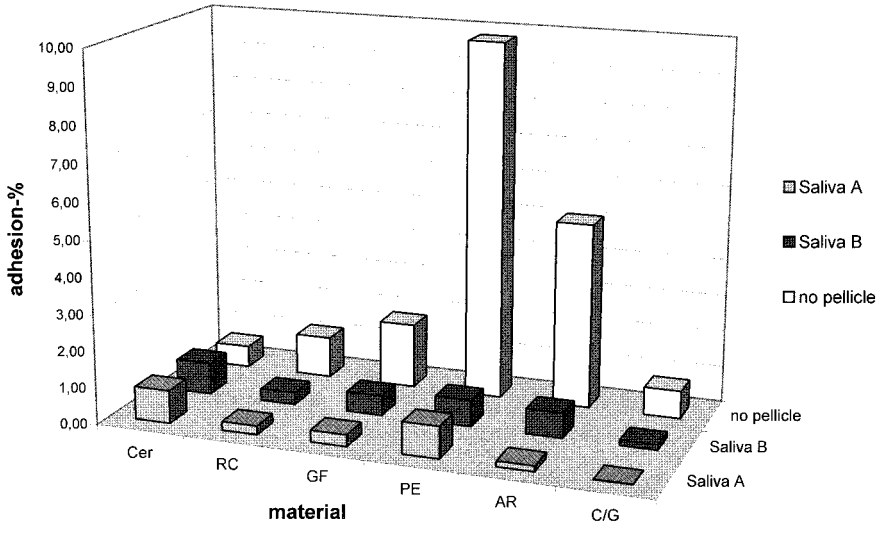
Surface roughness was determined on polished test specimens with the use of a two- dimensional height parameter R_a (Mitutoyo surftest 301, Mitutoyo Corporation, Kanagawa, Japan). The average of measurements obtained from triplicate specimens was calculated.

Collection and Pretreatment of Parotid Saliva

Citric acid stimulated parotid saliva was collected into an ice-chilled tube from two healthy adult donors (A and B) with the use of a Lashley cup. Filtered saliva (Millex HA 0.45 μm) was diluted (1:1) with phosphate-buffered saline (PBS) and used immediately for pellicle formation. Saliva from one individual at a time was used in the experiments.

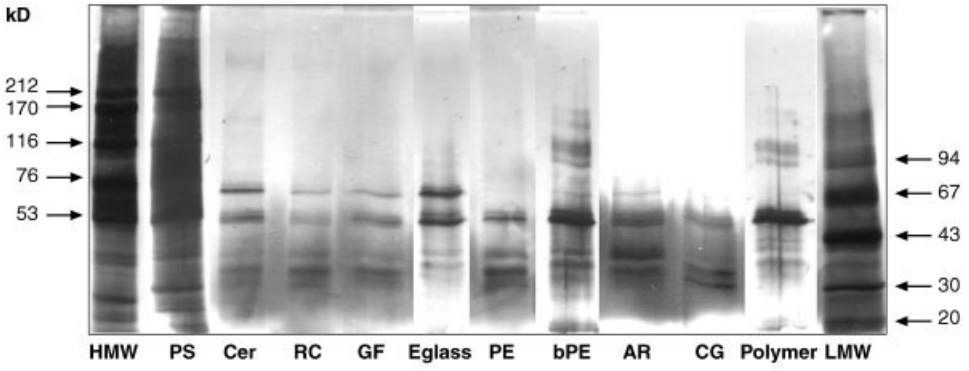
Pellicle Formation and Analysis

Saliva pellicles were formed by incubating the test specimens in diluted parotid saliva under continuous rolling for 30 min at room temperature. Thereafter the specimens were washed twice with PBS for 2 min. The protein collection was made according to Carlén et al.,[15] with some modifications. Proteins bound to the specimens were desorbed by rubbing the top and bottom surfaces of each specimen with three applicator sticks (Quick-Stick, Dentonova AB, Huddinge, Sweden) wetted with 4 μl of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) buffer (-1-mM Na-phosphate buffer, 2% SDS, 0.003% bromophenolblue) and finally with one dry applicator stick. The tips of the sticks were collected in an Eppendorf-tube to which 20 μl of buffer was added. The tube was heated in boiling water for 7 min. After the tube was perforated with a needle, the sample solution was recovered in a second outer tube by centrifugation for 2 min with a tabletop microcentrifuge (Sigma 201 M, Sigma, Germany). Samples of duplicate specimens were collected in the same tube. The protein solutions were analyzed

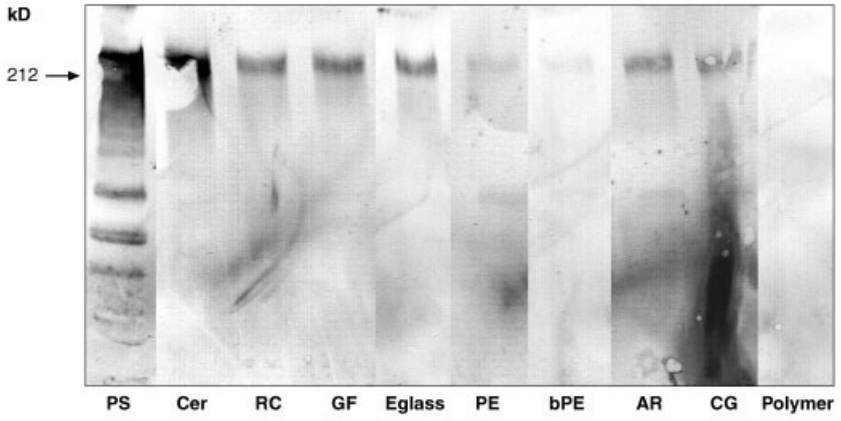


adhesion-% ± SD	treatment	N	Cer	RC	GF	PE	AR	CG
	no pellicle	4	0.6 ± 0.70	1.1± 0.52	1.8 ± 0.77	9.8 ± 2.58	5.0 ± 0.9	0.7± 0.54
	saliva B	3	0.9 ± 0.14	0.3 ± 0.01	0.5 ± 0.18	0.7 ± 0.18	0.6 ± 0.16	0.1 ± 0.12
	saliva A	4	0.9 ± 0.22	0.2 ± 0.13	0.3 ± 0.18	0.9 ± 0.19	0.2 ± 0.17	0.01 ± 0.01

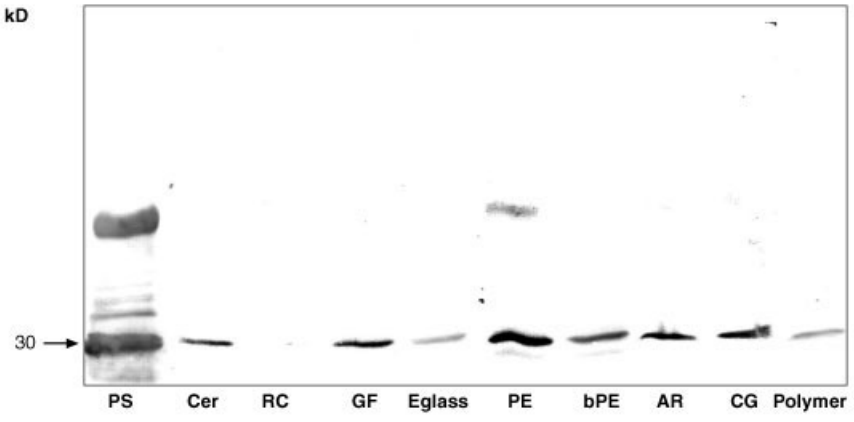
Figure 2. Adherence of *S. mutans* to uncoated materials and materials coated with parotid saliva pellicles of two different saliva donors (A and B). Bars represent mean values. Mean values ± standard deviations are indicated in the table.


kD
212
170
116
76
53
94
67
43
30
20
HMW
PS
Cer
RC
GF
Eglass
PE
bPE
AR
CG
Polymer
LMW

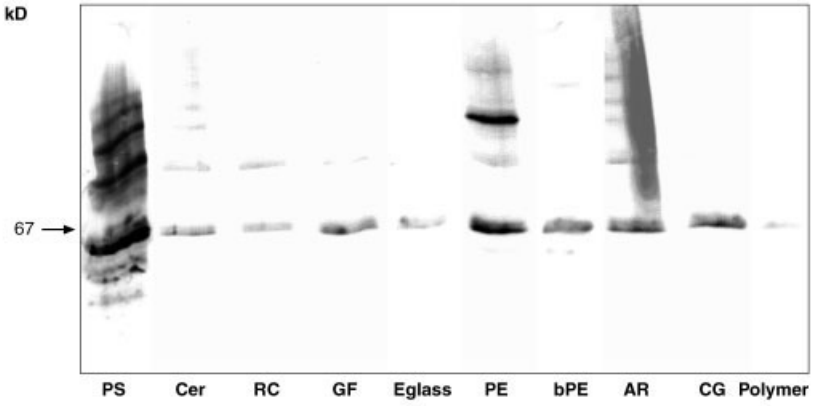
(a)


kD
212
PS
Cer
RC
GF
Eglass
PE
bPE
AR
CG
Polymer

(b)


kD
30
PS
Cer
RC
GF
Eglass
PE
bPE
AR
CG
Polymer

(c)


kD
67
PS
Cer
RC
GF
Eglass
PE
bPE
AR
CG
Polymer

(d)

by SDS-PAGE and silver staining with the use of the Phast-System™ (Pharmacia LKB Biotechnology AB, Bromma, Sweden) and by immunoblotting with specific antibodies against agglutinin, proline-rich proteins, and amylase, as described earlier.[15]

Microorganism

Streptococcus mutans ATCC 21752 was used for the adherence tests. In previous studies (unreported data) it was found that this strain adhered to parotid saliva fractions containing agglutinin, and glycosylated and acidic proline-rich proteins (Figure 1). Bacterial cells were labeled by overnight culture in Brain Heart Infusion broth (BHI, Unipath LTD, England) supplemented with (^{35}S)-methionine (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, UK). Five milliliters of the precultured organism was inoculated into 45 ml of the same broth, also supplemented with methionine, and cultured at 37 °C. Log phase cells were diluted in PBS to an optical density of $A_{660} \simeq 0.35$, corresponding to $\approx 1 \times 10^8$ colony-forming units. The suspension was gently sonicated to disrupt long streptococcal chains.

Adherence Test

Bacterial adherence to uncoated and to pellicle-coated materials was studied. Following a 30-min preincubation in PBS, half of the specimens were incubated in diluted parotid saliva for 30 min. After being washed with PBS, the saliva-treated specimens were incubated in a suspension of human serum albumin (0.5% HSA) for 15 min to block uncoated surface areas. After washing again with PBS, the saliva-coated as well as the noncoated specimens were incubated for 60 min with (^{35}S)-methionine labeled bacteria. All incubations were done under continuous rolling at room temperature. To remove unbound bacteria the specimens were washed twice with PBS. The test specimens and the bacterial suspension used were subjected to liquid scintillation (MicroBeta Trilux, Wallac Oy, Turku, Finland). Adhesion was expressed as the number of adhered cells as a percentage of the number of cells added in the assay.

Scanning-Electron Microscopy

Scanning-electron micrographs were taken from the surfaces of specimens after incubation in the bacterial suspension with the use of a scanning electron microscope (JSM 5500, JEOL Ltd., Tokyo, Japan). The specimens for SEM were fixed with 0.25% glutaraldehyde for 5 min, dried with an ascending series of ethanol, and covered with a layer of gold. The detector was held in a 90° angle to the surface. Pictures of polyethylene FRC were taken also with a 45° detector angle to better illustrate the surface topography.

Statistical Analyses

Statistical analyses were performed with SPSS for Windows (Rel. 10.0.5, 1999, SPSS Inc., Chicago). First the data were subjected to a two-way ANOVA. Subsequent pairwise comparisons were made with the use of Tukey's post hoc analysis. The level of statistical significance was considered to be 0.05.

RESULTS

Surface Roughness

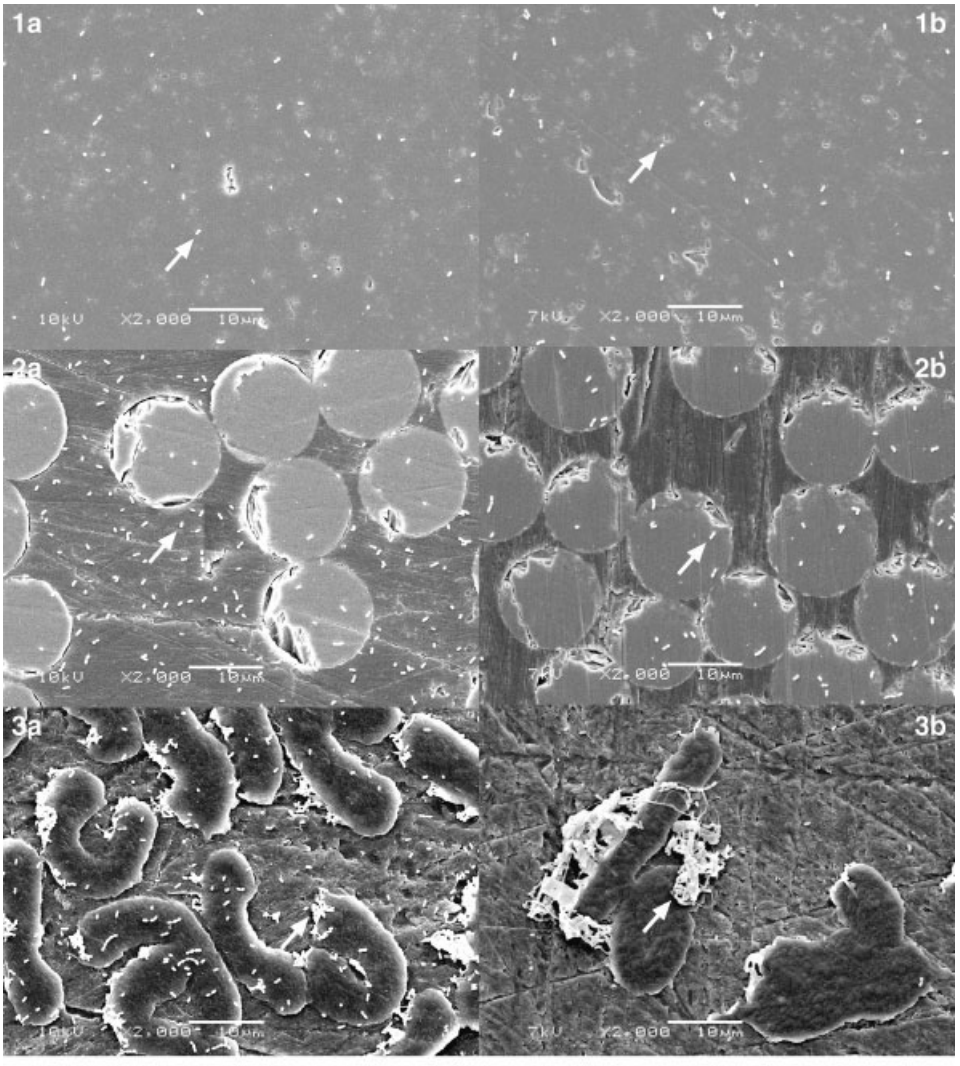
Significant differences were detected in the surface roughness of studied materials (Table II). The surface of polyethylene FRC was significantly rougher than all other materials, having an average R_a value of 0.5 μm ($p < 0.001$). Aramid FRC showed higher surface roughness (R_a of 0.18 μm) in comparison with all materials ($p < 0.001$) but polyethylene FRC. The other materials studied had an average roughness < 0.1 μm.

Adhesion of *S. Mutans*

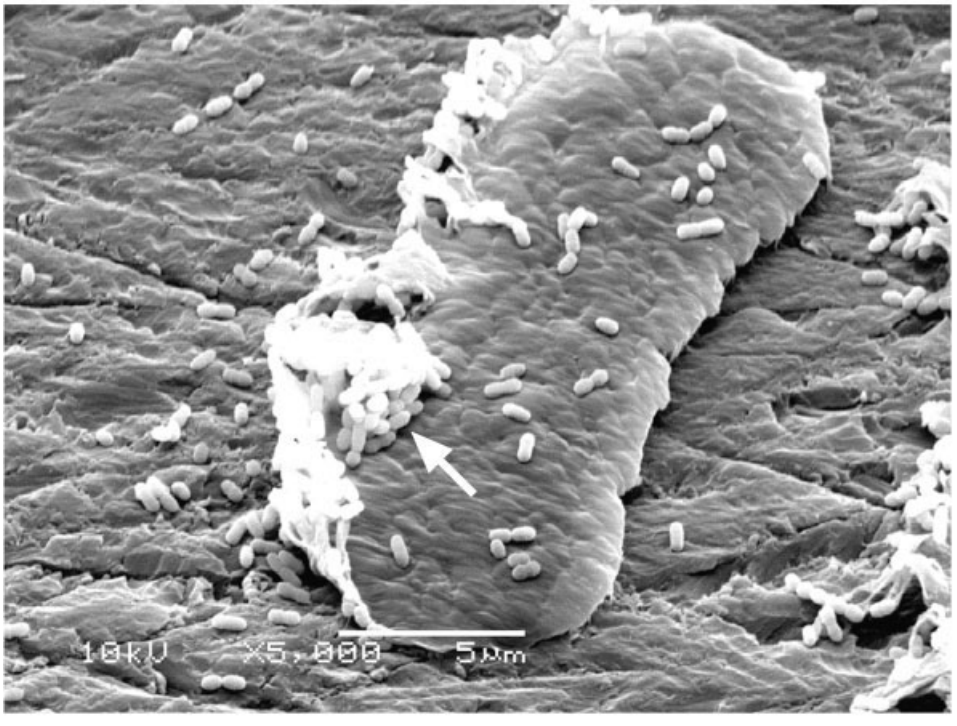
The results of the adhesion experiments are shown in Figure 2. Without pellicle, the adhesion of *S. mutans* ATCC 21752 coincided with the surface roughness of the adherent materials. The rougher polyethylene FRC ($p < 0.001$) and aramid FRC ($p \leq 0.01$) bound significantly more bacteria than the other materials. Binding to polyethylene FRC (9.8%) was significantly higher than to aramid FRC (5%) ($p < 0.001$). The adhesion to the other materials in no case exceeded 2%.

For both salivas used, pellicle coating resulted in strongly decreased adhesion on all materials but the ceramic. Pellicle-coated ceramic bound somewhat more bacteria than uncoated ceramic. With Saliva A, ceramic and polyethylene FRC showed adhesion percentages of approximately 1%. They

Figure 3. (a) Silver-stained proteins of samples recovered after parotid saliva incubation of studied materials. The borderlines in the gel show staining of high- (HMW) and low- (LMW) molecular mass (kD) proteins and proteins stained from parotid saliva (PS). The molecular mass proteins myosin (212), b-galactosidase (116), fosforylase B (94), bovine serum albumin (67), glutamic dehydrogenase (53), ovalbumin (43), and carbonic anhydrase (30) are indicated. (b) High-molecular-weight glycoprotein, agglutinin (> 212 kDa), in immunoblot of parotid saliva (PS) and in the parotid saliva pellicles recovered from the studied materials. The 212-kDa molecular mass protein Myosin is indicated. (c) Bands of acidic proline-rich proteins (≃ 30 kDa) in the immunoblot of parotid saliva (PS) and in the parotid saliva pellicles recovered from the studied materials. The 30-kDa molecular mass protein Carbonic anhydrase is indicated. (d) Bands of amylase (50–60 kDa) in immunoblot of parotid saliva (PS) and in the parotid saliva pellicles recovered from the studied materials. The 67-kDa molecular mass protein bovine serum albumin is indicated.


1a
1b
10kV X2,000 10µm
7kV X2,000 10µm
2a
2b
10kV X2,000 10µm
7kV X2,000 10µm
3a
3b
10kV X2,000 10µm
7kV X2,000 10µm

(A)


10kV X5,000 5µm

(B)

bound significantly more bacteria than restorative composite, glass FRC, and aramid FRC, which bound 0.15–0.3 % of the bacteria added. Hardly any bacteria were bound to pellicle-coated carbon/graphite FRC. With Saliva B the differences between materials were smaller but the overall adherence pattern was similar.

Protein Adsorption

The protein profiles of the two salivas used were similar, with the exception that no adsorbed agglutinin could be detected from the pellicles formed from Saliva A. The antibody used recognizes a carbohydrate epitope on agglutinin. Individual variations in this structure may result in the antibody not recognizing the protein. The protein adsorption results presented below were obtained from experiments using Saliva B.

Both quantitative and qualitative differences in the protein-binding profiles of the studied materials were detected. According to repeated silver-stained gels aramid FRC seemed to bind quantitatively more proteins than the other materials. The protein profile of polyethylene FRC lacked a band around 67 kD, which was seen in the profiles of the other composite materials [Figure 3(a)]. This band was also missing in the profile of matrix polymer and was only weakly seen on bulk polyethylene.

The materials also seemed to adsorb specific adhesion-associated proteins differently [Figures 3(b)–3(d)]. The most extensively stained bands of agglutinin were seen in the immunoblot samples from the ceramic surfaces [Figure 3(b)]. Bulk E-glass, restorative composite, and glass-fiber composite showed a stronger band of agglutinin compared to aramid FRC and carbon/graphite FRC. No agglutinin was detected in samples from the polymer matrix, and only weakly stained bands were obtained from the polyethylene fiber composite and bulk polyethylene samples [Figure 3(b)]. Acidic proline-rich proteins and amylase were, however, detected in largest amounts on polyethylene fiber composite [Figure 3(c) and (d)]. Ceramic, glass, aramid, and carbon/graphite FRCs, as well as bulk polyethylene, showed moderately stained bands of these components. Weak bands were seen in the immunoblots of restorative composite, E-glass, and polymer.

Scanning Electron Micrographs

Scanning-electron microscopy of bacteria binding to non-coated and saliva-coated ceramic, glass, and polyethylene FRCs revealed less bacteria on the composites after saliva coating [Figure 4(A)]. The saliva-coated polymer matrix surfaces in particular bound less bacteria in comparison with the polymer matrix of noncoated specimens [Figure 4(A)]. However, in the case of glass FRC, more bacteria seemed to adhere to saliva-coated fibers than to noncoated fibers [Figure 4(A)]. During polishing, the edges of the polyethylene fibers appeared to melt and fray. This caused pronounced surface irregularities and distinct retention sites, which would promote bacterial adherence [Figure 4(B)].

DISCUSSION

In the present study adhesion of *S. mutans* ATCC 21752 to different FRCs, dental ceramics and restorative composites were studied in relation to surface roughness and adsorption of salivary proteins to the studied materials.

In the absence of a salivary pellicle, binding of *S. mutans* was found to correlate with surface roughness of the materials studied. Polyethylene FRC showed highest binding of *S. mutans*, and its surface was found to be much rougher than other materials studied. Further polishing of the polyethylene FRC (grit 4000) did not affect surface roughness (unreported data). Aramid FRC also had a comparably high surface roughness and bound more *S. mutans* than the other materials but less than polyethylene FRC. The smoother ceramic, restorative composite, glass, and carbon/graphite FRC surfaces promoted significantly less adhesion. These findings are in accordance with several previous reports stating that rough surfaces promote bacterial adhesion.[9,15] A rough surface provides bacteria with a large area available for adhesion, and retention sites where bacteria are protected from shear forces.

Physicochemical surface properties have also been found to influence bacterial adhesion. Materials with high surface free energy collect more plaque than materials with low-energy surfaces.[7,8] However, the influence of surface roughness has been said to overrule that of surface free energy.[9] This is supported by current observations, where rough low-energy polyethylene composite promoted adhesion better than smoother high-energy surfaces like ceramic- and glass-fiber composite.

A strong decrease in adhesion after pellicle coating was observed on all materials, except for the ceramic, where pellicle-mediated adhesion was of the same magnitude as without pellicle. Several authors, stating that pellicle coating generally results in reduced numbers of adhering bacteria, support this finding.[18,19] In the oral environment all materials are rapidly covered by an acquired pellicle.

In previous studies, *S. mutans* serotype c strains bound preferentially to high-molecular-weight agglutinin.[11,20] The *S. mutans* strain used in the present study binds, however, in substantial amounts also to proline-rich proteins (Figure 1). Agglutinin was found in largest amounts in pellicles recovered from inorganic ceramic and bulk E-glass surfaces, and

Figure 4. (A) SEM photomicrographs of (a) uncoated and (b) saliva-coated (1) ceramic-, (2) glass-, and (3) polyethylene-fiber composites with adhered *S. mutans* cells (arrows). Original magnification × 2000. (B) An SEM photomicrograph with a larger magnification (× 5000) of polyethylene-fiber composite surface with adhered *S. mutans* cells. Note the irregular fiber edges and large aggregates of bacteria (arrow).

from restorative composite and glass FRC surfaces containing inorganic particles. The present results support the findings of Carlén et al., who showed that polishing of restorative composite increased the exposure of inorganic filler particles, the adsorption of agglutinin, and *S. mutans* adhesion.[15] Thus the amount of agglutinin in the pellicle of ceramic could explain the higher adhesion of *S. mutans*. Bulk E-glass, one of the two components of glass FRC, also showed strong adsorption of agglutinin, whereas from the polymer surface, the other component of FRCs, no or comparably little bacteria binding proteins could be recovered. This may in part explain earlier findings of higher *S. mutans* adhesion to pellicle-coated glass fibers in comparison with the matrix polymer of the FRC.[17,21] Weak adsorption of bacteria binding proteins to polymer surfaces is also in line with the SEM observation that very little bacteria adhere to the polymer matrix of saliva-coated FRCs.

In addition to ceramic, pellicle-mediated adhesion of *S. mutans* ATCC 21752 was higher to polyethylene FRC than to the other materials studied. From this material only low amounts of *S. mutans* binding agglutinin, but the highest amounts of proline-rich proteins were recovered. These findings further emphasize the importance of pellicle receptors for bacterial adherence. However, SEM micrographs revealed distinct retention sites with adhering bacteria on the saliva-coated polyethylene FRC. The influence of higher surface roughness of the material most likely prevails in the case of pellicle-mediated adhesion as well.

To summarize, without a pellicle comparably rougher surfaces of polyethylene and aramic FRC promoted *S. mutans* adhesion better than other smoother materials studied. Comparably more *S. mutans* binding proteins in the pellicles may in part account for the higher *S. mutans* adhesion to saliva-coated ceramic and polyethylene FRC. Further studies on the materials are currently being performed in the oral cavity, where adhesion and colonization takes place under competition of different microbial species.

REFERENCES

1. Vallittu PK. Comparison of the *in vitro* fatigue resistance of an acrylic resin removable partial denture reinforced with continuous glass fibers or metal wires. J Prosthodont 1996;5:115–121.
2. Vallittu PK. A review of fiber-reinforced denture base resins. J Prosthodont 1996;5:270–276.
3. Vallittu P, Lassila V, Lappalainen R. Transverse strength and fatigue of denture acrylic-glass fiber composite. Dent Mater 1994;10:116–121.
4. Ladizesky N, Chow T. The effect of highly drawn polyethylene fibers on the mechanical properties of denture resins. Clin Mater 1990.
5. Ekstrand K, Ruyter I, Wellendorf H. Carbon/graphite fiber reinforced poly(methyl methacrylate): Properties under dry and wet conditions. J Biomed Mater Res 1987;21:1065–1080.
6. Mullarky R. Aramid fiber reinforcement of acrylic appliances. J Clin Orthodont 1985;19:655–658.
7. Van Dijk J, Herkströter F, Busscher H, Weerkamp A, Jansen H, Arends J. Surface-free energy and bacterial adhesion. An *in vivo* study in beagle dogs. J Clin Periodontol 1987;14:300–304.
8. Glantz PO. The adhesiveness of teeth. J Colloid Interface Sci 1971;37:281–290.
9. Quirynen M, Bollen CML. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. J Clin Periodontol 1995;22:1–14.
10. Nyvad B, Fejerskov O. Scanning electron microscopy of early microbial colonization of human enamel and root surfaces *in vivo*. Scand J Dent Res 1987;95:287–296.
11. Gibbons R, Hay D. Adsorbed salivary acidic proline-rich proteins contribute to the adhesion of *Streptococcus mutans* JBP to apatitic surfaces. J Dent Res 1989;68:1303–1307.
12. Carlén A, Olsson J. Monoclonal antibodies against a high-molecular-weight agglutinin block adherence to experimental pellicles on hydroxyapatite and aggregation of *Streptococcus mutans*. J Dent Res 1995;74:1040–1047.
13. Rundegren J, Arnold R. Differentiation and interaction of secretory immunoglobulin A and a calcium-dependent parotid agglutinin for several bacterial strains. Infect Immun 1987;55.
14. Oho T, Yu H, Yamashita Y, Koga T. Binding of salivary glycoprotein-secretory immunoglobulin A complex to the surface protein antigen of *Streptococcus mutans*. Infect Immun 1998;66:115–121.
15. Carlén A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. Biomaterials 2001;22:481–487.
16. Absolom D, Zingg W, Neumann A. Protein adsorption to polymer particles: Role of surface properties. J Biomed Mater Res 1987;21:161–171.
17. Tanner J, Vallittu PK, Söderling E. Adherence of *Streptococcus mutans* to an E-glass fiber-reinforced composite and conventional restorative materials used in prosthetic dentistry. J Biomed Mater Res 2000;49:250–256.
18. Pratt-Terpstra IH, Weerkamp A, Busscher H. The effects of pellicle formation on streptococcal adhesion to human enamel and artificial substrata with various surface free energies. J Dent Res 1989;68:463–467.
19. Weerkamp A, Uyen H, Busscher H. Effect of zeta potential and surface free-energy on bacterial adhesion to uncoated and saliva-coated human enamel and dentin. J Dent Res 1988;67:1483–1487.
20. Carlén A, Bratt P, Stenudd C, Olsson J, Strömberg N. Agglutinin and acidic proline-rich protein receptor patterns may modulate bacterial adherence and colonization on tooth surfaces. J Dent Res 1998;77:81–90.
21. Tanner J, Vallittu PK, Söderling E. Effect of water storage of E-glass fiber reinforced composite on adhesion of *Streptococcus mutans*. Biomaterials 2001;22:1613–1618.