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Adhesion of streptococci to saliva-coated and uncoated composite-based resins

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This study was designed to evaluate the bacterial adhesion to five types of experimental composite-based resins and a commercial composite resin used as a control. Physicochemical surface characteristics of composite resins with and without an artificial saliva coating were measured. The relationship between the numbers of adhering cells (*Streptococcus sanguis, S. mutans* and *S. sobrinus*) and surface characteristics was analysed. The values of contact angles and the number of adhering cells were small with saliva coating. *S. sanguis* ATCC 10557 showed a positive correlation ($r=0.835$, $p<0.05$) with the contact angles of uncoated resins, whereas no relationship was observed for saliva-coated resins. With *S. mutans* Ingbritt the cell numbers adhering to resins correlated strongly ($p<0.01$) with the values of zeta potential of resins for either saliva coated or uncoated. Electrical repulsion forces had a strong contribution to adherence of cells such as *S. mutans* and *S. sobrinus* which show a high absolute zeta potential.

1. Introduction

Dental composites have been widely used as aesthetic materials because of good mechanical properties [1] and adhesiveness [2]. It is, however, important to develop anti-plaque effects in restorative composite materials if they are to be used in clinical studies [3]. Although such anti-plaque effects were found for dental amalgam and cement [4, 5], no such properties were found for dental composite resins. Adherence of oral bacteria to restorative surfaces or pellicles was considered to initiate the formation of dental plaque which is implicated in the aetiology of dental caries and peridontal disease, because bacterial accumulation was related to the plaque-retaining capacity of the dental material's surface.

Bacterial adhesion to solid surfaces was prolonged with such physico-chemical interactions as van der Waals forces [2], electrostatic forces and polar interactions [6], hydrophobic forces [7] and hydrodynamics forces [2], and with specific interactions by lectin-like substances in the adhesion process to saliva-coated surfaces [8].

Bacterial adhesion to dental materials has been attributed to electrostatic forces [6, 9, 10], and also to hydrophobic interactions [1, 5, 11, 12].

On the other hand, commercial composite resins contain widely divergent materials in different proportions. No studies have been undertaken on the influence of these materials on the adhesion of bacteria. The aim of the present study is thus to examine the effect of composition of resins with and without a saliva coating on the process of adhesion, and to compare the results with those obtained using a commercial dental composite resin.

2. Materials and methods

Five visible-light-cured experimental resins without fillers, and a commercial composite resin were used in this study (Table I). All experimental resins contain 0.5% dl-camphoroquinone (CQ) as a photosensitizer and 1.95% N,N-dimethylaminoethylmethacrylate (DMAEMA) as a tertiary amine for polymerization promoter, and 0.05% butylated hydroxy toluene (BHT) as a polymerization prohibitor. They were placed on a stainless steel mould with an internal thickness of 1.00 ± 0.05 mm and a diameter of 20.0 ± 1.0 mm, and were left to cure by visible light for 30 min against acid-washed glass slides.

Unstimulated whole saliva was collected into chilled tubes and centrifuged for 20 min at 4 °C and 20 000 g to remove debris. The clear whole saliva was then frozen at −30 °C for 1 month. Then, the saliva was kept fresh without bacterial accumulation. Six samples of each resin were immersed in whole saliva for 1 h at 37 °C and washed with distilled water and used immediately for measuring surface characterization and for adhesion tests.

Streptococcus sanguis ATCC 10556, *S. sanguis* ATCC 10557, *S. mutans* Ingbritt and *S. sobrinus* OMZ 176 were used in this study. All strains were grown in trypticase soy broth (BBL; Microbiology Systems, Cockeysville, MD, USA) supplemented with 0.5% yeast extract in an aerobic atmosphere. The cells were harvested during the exponential growth phase by

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TABLE I Materials used and monomer composition

Code	Composite-based resins Main monomer (wt %)		Diluent (wt %)	
A	Bis-GMA	63 4	TEGDMA	34.1
B	Bis-GMA	68.3	GDMA	29 2
C	Bis-GMA	67.2	NPGDMA	30.3
D	Bis-MPEPP	65.7	NPGDMA	31 8
E	IUPAC	97.5	—	—

Code	Composite resin Material	Manufacture	Lot. No.
F	Silux plus	3M	1EB2

centrifugation, washed twice with 0.05 M Tris-HCl buffered saline (pH 7.2) and resuspended in Tris-HCl buffer (pH 7.2). The cell suspensions were subjected to low-intensity ultrasonic treatment in tubes held on crushed ice, to disperse bacterial chains and aggregates [13]: 40 s of minimum-intensity ultrasound was sufficient to produce predominantly single cells and pairs. Cell suspensions were used immediately to avoid reaggregation. The optical density of the suspensions was adjusted to give an OD_{550} of 0.3 (3.65×10^8cells/ml) using a spectrophotometer (Model 100–50; Hitachi Co., Tokyo, Japan).

Six uncoated and saliva-coated resin samples were placed in a beaker, making a circle around the central area occupied by a magnetic stirring rod. Bacterial suspensions were poured into the beaker and stirred at a constant speed for specific times at 37 °C in an aerobic condition. Each sample was taken out, washed with 50 ml distilled water in a beaker, fixed with 2.5%(v/v) glutaraldehyde at 4 °C for 30 min, and stained with 1%(v/v) acridine orange. The number of adhered cells were obtained by counting bacteria directly under a fluorescence microscope (Model BHS; Olympus Co., Tokyo, Japan) according to the method of Ørstavik *et al.* [14]. The number of bacteria in each of 20 separate fields were counted for every sample and divided by the area of the field. From these values, the average number of cells per mm^2 of resin surface were calculated. All the numerical data obtained were analysed by Student's *t*-test. The contact angle was measured to determine the index of hydrophobicity of the uncoated resin samples described above. Streptococci were measured immediately within the dried streptococcal layer after 2 h [1] by the horizontal projection technique using a contact angle meter (Model CA-A; Kyowa Co., Tokyo, Japan) at 20 °C at three separate points on 10 samples. The contact angles of distilled water on each saliva-coated resin were measured by the suspending technique under wetting conditions at 18 °C. Ten samples of each resin were measured.

Zeta potentials of crushed resin samples and streptococci cells were studied with a particle micro-electrophoresis apparatus in Tris-HCl buffer (0.05 M/l, pH 7.2) at 25 °C using a face zeta potential meter (ZP-OM, Kyowa Kaimen Kagaku Co., Japan) using the Helmholts–Smoluchowsky formula [3]. The zeta potentials are effectively used as a measuring method to estimate an assay of bacterial accumulation [15].

3. Results

3.1. Surface characteristics

The results of contact angle and zeta potential measurements of the bacteria and resins are listed in Tables II and III, respectively. *S. sanguis* ATCC 10557 showed the highest contact angle value, while *S. mutans* Ingbritt showed the lowest ($p < 0.05$) . Zeta

TABLE II Contact angles and zeta potentials of streptococci

Streptococci	Contact angle* (degrees)	Zeta potential§ (mV)
S. sanguis ATCC 10556	$37.2 \pm 1.4_{a}$	$-27.6 \pm 3.5_{bc}$
S. sanguis ATCC 10557	$44.1 \pm 2.8_{a}$	$-23.5 \pm 1.7_{de}$
S. mutans Ingbritt	$15.8 \pm 2.2_{a}$	$-37.5 \pm 4.2_{bd}$
S. sobrinus OMZ 176	$32.0 \pm 0.8_{a}$	$-36.3 \pm 2.8_{ce}$

*Mean contact angle obtained from 10 determinations (at three separate points) ± standard deviation.
§The mean values and standard deviations of each strain were calculated from the mobilities of 10 cells in two directions measured on 10 occasions.
Differences between the values indicated by the same inferior letter are significant at the 5% level.

TABLE III Contact angles and zeta potentials of composite-based resins

Code	Contact angle* Saliva uncoated (degrees)	coated (degrees)	Zeta potential§ Saliva uncoated (mV)	coated (mV)
A	$60.2 \pm 1.4_{ab}$	$33.9 \pm 1.9_{de}$	$-27.3 \pm 1.7_{ij}$	-23.0 ± 3.2
B	$59.4 \pm 1.6_{c}$	$33.1 \pm 2.1_{fg}$	$-21.7 \pm 2.6_{ikl}$	$-19.1 \pm 1.5_{op}$
C	$66.6 \pm 1.9_{ac}$	$37.2 \pm 1.2_{df}$	$-20.1 \pm 1.7_{jmn}$	-19.8 ± 2.5
D	$69.6 \pm 2.0_{ac}$	$47.6 \pm 3.4_{egh}$	$-27.8 \pm 2.3_{km}$	$-22.3 \pm 1.8_{o}$
E	$65.7 \pm 4.2_{b}$	$34.3 \pm 2.6_{h}$	$-30.3 \pm 3.2_{ln}$	$-23.8 \pm 1.9_{p}$
F	62.8 ± 2.2	34.1 ± 2.7	-35.8 ± 2.2	-24.8 ± 1.8

*Mean contact angle obtained from 10 determinations (at three separate points) ± standard deviation.
§The mean values and standard deviations of each resin or strain were calculated from the mobilities of 10 particles or cells in two directions measured on 10 occasions
Differences between the values indicated by the same inferior letter are significant at the 5% level.

potentials of bacteria were all negative. The absolute value was highest for *S. mutans* Ingbritt, while *S. sanguis* ATCC 10556 was the lowest ($p < 0.05$).

The values of contact angles on resins were reduced upon salivary coating. Bis-MPEPP/NPGDMA-based resin (code D) showed the highest value while Bis-GMA/GDMA-based resin (code B) showed the lowest. However, the difference between code B and the Bis-GMA/TEGDMA-based resin (code A) was not significant. The zeta potential of all resins were negative. While IUPAC (code E) showed the highest absolute value among the composite-based resins, the Bis-GMA based resins showed the lowest, and code D showed an intermediate value. A commercial composite resin (code F) showed a higher absolute value than the composite-based resins.

3.2. Streptococcal adherence to resin surfaces

The numbers of adherent bacteria differed from resin to resin. Fig. 1 shows the result obtained by incubating each strain on the six types of resin surfaces. Numbers of adherent bacteria varied according to the type and surface characteristics of the resins. All strains of bacteria adhered in the lowest numbers to the commercial composite resin (code F). In composite-based resins with *S. sanguis* no difference between adhesion of the two strains was observed except for the Bis-MPEPP/NPGDMA-based resin (code D) ($p < 0.05$). Both strains of *S. sanguis* adhered in highest numbers to code D and in lowest numbers to Bis-GMA/GDMA-based resin (code B) ($p < 0.05$). However, there were no significant differences between adhesion to code B and Bis-GMA/TEGDMA (code A), code D and IUPAC (code E). In contrast, both *S. mutans* and *S. sobrinus* strains showed the highest adherence capacity to Bis-GMA/NPGDMA (code C) and the lowest to code E, although there were no significant differences in adhesion of both strains to Bis-GMA based resins and between adhesion of *S. mutans* Ingbritt to code E and code D ($p < 0.05$).

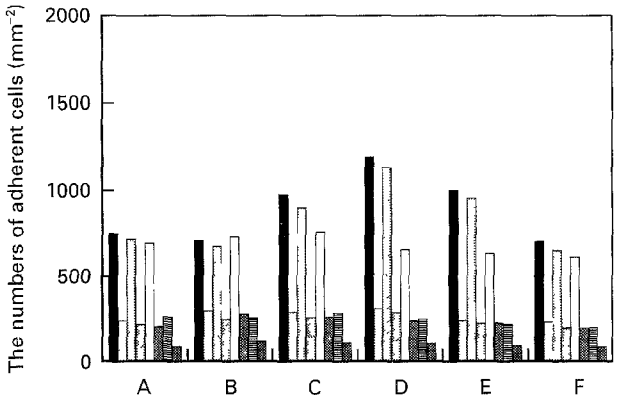

Figure 1 Adhesion of streptococcal cells to six types of composite-based resin surfaces (A–F) The values were obtained with six replicate samples, in which the number of bacteria adhered in 20 discretionary views was counted and the average number of bacteria adhered per mm^2 calculated ■ S. sanguis 10556, non-saliva, □ S. sanguis 10556, saliva; □ S sanguis 10557, non-saliva, □ S. sanguis 10557, saliva; □S. mutans Ingbritt, saliva. ▩ S. mutan Ingbritt, non-saliva, ▤ S. sobrenus OMZ 176, non-saliva; ▩ S. sobrinus OMZ 176, saliva.

TABLE IV Correlation coefficient between adherent cell numbers and contact angles or zeta potentials of composite-based resins

Adherent cell numbers	Contact angle of resins Saliva uncoated	coated	Zeta potential of resins Saliva uncoated	coated
S. sanguis ATCC 10556	0 790	0.420	0.001	0 044
S. sanguis ATCC 10557	0.835*	0.356	0 014	0 044
S. mutans Ingbritt	0.048	0.022	0.921**	0.945**
S sobrinus OMZ 176	0.000	0.001	0.860*	0.873*

*Significant: $p < 0.05$
**Significant: $p < 0.01$

3.3. The relationship between the surface characteristics of the resins and the number of adherent cells

Correlation coefficients between adherent cell numbers and contact angles and zeta potentials of composite-based resins are summarized in Table IV. A significant relationship was observed between the zeta potential of the resin samples and the adherent cell numbers of *S. mutans* Ingbritt with no saliva coating (correlation coefficient $r = 0.921$, $p < 0.01$) and with saliva coating ($r = 0.945$, $p < 0.01$), and also between zeta potential and *S. sobrinus* OMZ 176 with no saliva coating ($r = 0.860$, $p < 0.05$) and with saliva coating ($r = 0.873$, $p < 0.05$). However, no such relationship between the zeta potential of the resins and the number of adherent *S. sanguis* cells was observed. Similarly, a significant relationship was also evident between the contact angles of resins and the number of adherent *S. sanguis* ATCC 10557 cells with no saliva coating ($r = 0.835$, $p < 0.05$), whereas no relationship was observed for saliva-coated resins. In the case of *S. sanguis* ATCC 10556, no significant relationship was found.

4. Discussion

A method has been developed to assess the type of chemical bonds involved in bacterial adherence to solid surfaces, and this has shown that not only were hydrophobic interactions important, but electrostatic interactions also play an important role depending on the physico-chemical properties of the solid and the bacterial surfaces [15]. In this study, we prepared various types of composite-based resin surfaces having different characteristics. The two *S. sanguis* strains showed higher hydrophobicity than the *S. mutans* and *S. sobrinus* strains, which was consistent with data on contact angles. This was especially true for *S. sanguis* ATCC 10557 which had the highest contact angle. A strong correlation was found between the number of adhered *S. sanguis* ATCC 10557 and contact angles of

resins, indicating that hydrophobic interaction is vital for adhesion of the hydrophobic strain. The strains of *S. mutans* with higher negative charges than *S. sanguis* showed a close relationship between the zeta potential values and the number of adherent bacteria. This suggests that the adherence of these strains is influenced by electrostatic repulsive forces. These findings support the view that neither electrostatic forces nor hydrophobic interactions alone determine the adherence of streptococci to resin surfaces.

On the other hand, penta-hydroxymethylene groups may contribute less to bacterial adherence owing to the hydrogen bond [15]. Bis-GMA, which is widely used as the main monomer in composite-based resins, contains two hydroxyl groups in its chemical structure. Among the different diluent monomers, only GDMA contains a hydroxyl group. The combination of Bis-MPEPP (main monomer) and (NPGDMA) (diluent monomer) was more hydrophobic in nature than the combination of Bis-GMA and TEGDMA, which is more commonly used in commercial composite-based resin [16]. If hydrogen bonding contributes to the bacterial adherence, an increase in the number of adherent cells to resin surfaces having hydroxyl group might be expected. The high cell counts of *S. mutans* adhering to Bis-GMA suggest that hydrogen bonding contributes to the bacterial adherence to resins which have hydroxyl groups (Fig. 1). It is known that the phenomenon of adhesion is dependent on the electrostatic charges of these resins. The adherent cell count is inversely proportional to the negative charges of these resins. The higher the negative charges, the stronger the repulsive forces and hence the smaller the number of bacteria which adhere. A close relationship exists between zeta potential and the number of adherent high negative charged strains, *e.g. S. mutans.*

In Bis-GMA/GDMA-based resin, which has the most hydroxyl groups of all the resins examined, this result suggests that the lowest number of adherent cells found for all resins should be *S. sanguis.* It seems that the adhesion of hydrophobic strains to resin surfaces by hydrophobic interactions is stronger than that by hydrogen bonding. Thus, hydrogen bonding seems to contribute least to streptococcal adherence. The water molecule has a function as an acceptor or donor in the milieu of water. The hydroxyl group has a strong capacity to form hydrogen bonds if there are acceptors or donors present, and the strong interaction between hydroxyl groups and water molecules might prevent the adherence of bacterial cells.

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