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Adhesion of oral streptococci to all-ceramics dental restorative materials in vitro

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Abstract In recent years, patients have benefited from the development of better and more esthetic materials, including all-ceramics dental restorative materials. Dental plaque formation on teeth and restorative materials plays an important role in the pathogenesis of oral diseases. This study investigates initial adhesion of stationary phase streptococcal species to different all-ceramics dental restorative materials. The saliva-coated materials were incubated with the bacteria for 1 h in an in vitro flow chamber which mimics environmental conditions in the oral cavity. Number and vitality of adhering bacteria were determined microscopically after staining. Surface roughness and the composition of the materials had no distinctive influence on bacterial adhesion. However, *S. mutans* and *S. sobrinus* adhered about tenfold less numerous to all materials than the other streptococcal species. Further, there was a correlation between bacterial vitality and materials' glass content. The results showed that early plaque formation was influenced predominantly by the presence of the salivary pellicle rather than by material dependent parameters whereas the composition of the all-ceramics appeared to have influenced the percentage of viable cells during the adhesion process. This presented in vitro technique may provide a useful model to study the influence of different parameters on adherence of oral streptococcal species.

1 Introduction

In the oral cavity all exposed surfaces are rapidly coated with a salivary pellicle to which early colonizers, mostly oral streptococci, adhere [1]. These are the first steps in the formation of the oral biofilm, called dental plaque, the cause of caries and periodontal diseases [2].

Dental plaque is present on human tooth tissues as well as on restorative materials [3]. Accumulation of bacteria on marginal areas of enamel and restorative material may lead to bacterial plaque formation and secondary caries [4]. Since caries formation around existing restorations represents a primary reason for replacement there are efforts to minimize or prevent plaque formation on restorative materials [5]. Several in vitro and in vivo models exist to investigate adhesion of various oral microorganisms to dental restorations and the mechanisms involved [6–8].

The applications of all-ceramic restorations for medical and dental purposes have become very favoured owing to their high strength, biocompatability and excellent esthetic properties [9]. They are a metal-free alternative to the widely used metal–ceramic structures [10]. In vitro investigations on the mechanical properties as well as clinical studies have been published [9, 11, 12]. However, information on bacterial adherence to these materials is scarce.

The aim of this study was to investigate bacterial adhesion and vitality of two early colonizing (*S. sanguinis*,

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S. oralis) and two caries-associated (*S. mutans* and *S. sobrinus*) species of streptococci to four different all-ceramic dental materials after salivary coating in an experimental model which mimics environmental conditions in the oral cavity [13]. Particularly the effect of surface roughness, hydrophobicity, and glass content of the materials were examined. A glass surface served as the control.

2 Materials and methods

2.1 Materials

Table 1 lists the dental ceramics tested with the corresponding glass content. Rectangular test specimens ($14.4 \times 14.4 \times 0.2$ mm^3) were used as obtained from the manufacturer (Vita Zahnfabrik, Bad Säckingen, Germany). The surface roughness was measured by a Hommel tester (T 1000, Hommelwerke GmbH, VS-Schwenningen, Germany). Glass (borosilicate glass, ultrapure, Labor Vetter, Ammerbuch, Germany) was chosen as the reference because it behaves similarly to enamel with regard to microbial adhesion in vitro [14] (and our own data). Before the adhesion experiments the slides were decontaminated with ethanol and exposed to the sterile human saliva at room temperature for 15 min. Contact angles as an index of hydrophobicity were measured using a Processor Tensiometer K100 (Krüss GmbH, Hamburg, Germany).

2.2 Bacterial adherence

The bacteria used for this study were: *Streptococcus sanguinis* DSM 20068 (German collection of microorganisms and tissue culture cells, Braunschweig, Germany), *Streptococcus oralis* ATCC 35037 (American Type Culture Collection), *Streptococcus mutans* DSM 20523, and *Streptococcus sobrinus* OMZ 176 (Oral Microbiology, Zürich, Switzerland). All species were grown aerobically at 37°C overnight until stationary phase in Schaedler broth (BBL^{TM} Becton Dickinson, Basel, Switzerland), sonicated for 1 min then harvested by centrifugation, washed with physiological saline and suspended in human saliva to a final colony forming unit (CFU) of 10^8–10^9 ml^{-1}. Whole saliva was pooled from two healthy volunteers and processed as described previously [13].

Figure 1 illustrates the study design. The flow rate of the suspension was 0.8 ml min^{-1}, which corresponds roughly to physiological oral conditions of low shear [15]. The system was placed on a shaker adjusted at 260 impulses min^{-1} to maintain the homogeneity of the bacterial suspension. The bacteria were allowed to adhere to the surfaces during 1 h at room temperature. The test specimens were removed, washed, stained by applying a dual fluorescent staining (Live/Dead BacLight Bacterial Viability Kit; MoBiTec, Luzern, Switzerland) and analyzed microscopically (Provis AX70, Olympus AG, Volketswil, Switzerland). The two fluorescent dyes allowed differentiation between vital (green) and dead (red) microorganisms [16]. Each material was tested with each streptococcus species in at least five independent experiments. In addition the optical density, CFU, and the pH of the bacteria–saliva mixture at the beginning and the end of the experiment were determined.

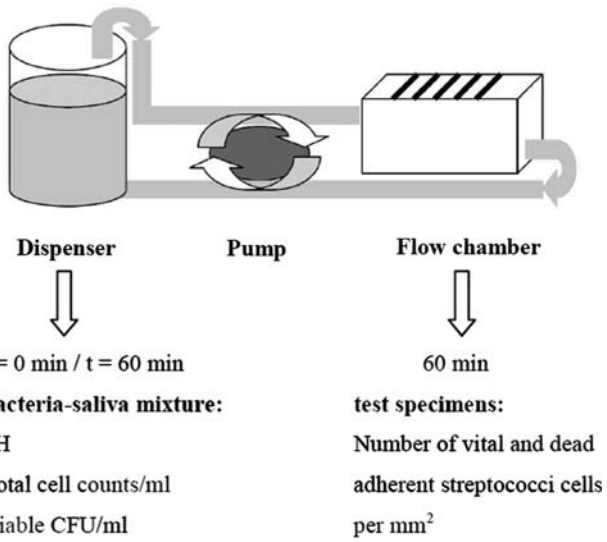

Fig. 1 Study design: starting from the dispenser, the bacteria–saliva suspension circulated via a peristaltic pump to the flow chamber containing the test specimens mounted in parallel. The different dental ceramic and glass surfaces were analyzed after 60 min (see text for details)

Table 1 List of glass and dental ceramics used

Type of material	Code	Chemical composition	Glass content (vol%)	Manufacturer
Glass		Borosilicate, ultrapure	100	Labor Vetter, Ammerbuch/D
Vita Mark II	MK	Feldspathic ceramics	96	Vita Zahnfabrik, Bad Säckingen/D
In-ceram aluminia	ICA	Glass-infiltrated aluminia	25	Vita Zahnfabrik, Bad Säckingen/D
In-ceram zirconia	ICZ	Zirconia-reinforced Glass-infiltrated aluminia	19	Vita Zahnfabrik, Bad Säckingen/D
In-ceram YZ	YZ	Tetragonal stabilized zirconia	0	Vita Zahnfabrik, Bad Säckingen/D

For measurements of hydrophobicity the streptococci were grown in Schaedler broth, washed and resuspended in PSB or human saliva. The measurements were done as described by Grivet et al. [17] using partitioning into hexadecane (Sigma-Aldrich GmbH, Buchs, Switzerland).

2.3 Data analysis and statistics

A total of 12 digital images (ColorView, Olympus AG, Volketswil, Switzerland) using two filters [blue excitation at 450–490 nm (FITC) and green excitation at 546 nm (rhodamine)] were obtained for each sample and the adherent bacteria enumerated from 12 fields of view (each of 0.0239 mm^2).

The statistical analysis was performed using the open source programming language R version 1.6.1. The Wilcoxon-test was used to compare data of each material and the corresponding bacteria with those for glass. The level of significance was set at $\alpha \leq 0.05$. Regression analysis was used to correlate percentages of vital adherent cells and materials' glass contents of the all-ceramic materials.

3 Results

During the experimental period of 1 h, bacterial density and vitality in the bacteria–saliva suspension of the flow chamber system remained nearly constant. Although the pH slightly increased at the end of the test period, the bacteria–saliva suspension can be considered as a resting cell suspension [13].

3.1 Properties of the surface substrata

The different R_a values for surface roughness are presented in Table 2. Values for glass, MK, and YZ were similar. The all-ceramic ICA and ICZ yielded a fivefold higher value.

Substratum surface hydrophobicities were evaluated by measuring water contact angles. Glass and the all-ceramic restorative materials showed a hydrophobic surface (Table 2). Coating with human saliva drastically reduced hydrophobicity of all test specimens.

3.2 Properties of cell surfaces

Bacterial surface hydrophobicities were evaluated by quantifying partitioning to hexadecane (Table 3). All four streptococci cultured in Schaedler broth and resuspended in PBS were highly hydrophobic. However, suspension of the streptococcal cells in human saliva resulted in <1% partitioning to hexadecane, meaning that these bacterial suspensions behaved hydrophilic.

3.3 Streptococcal adherence to substrata surfaces

The results of the adhesion experiments are summarized in Fig. 2 and Table 4. *Streptococcus sanguinis* and *S. oralis* were not significantly different and revealed the greatest adherence whereas *S. mutans* and *S. sobrinus* showed significantly lower adherence (Fig. 2a) to all the materials investigated.

Any given streptococcal species adhered to the different materials in similar numbers (Fig. 2a; Table 4), although the surface roughness R_a of ICA and ICZ was fivefold higher than that of MK, YZ, and glass. This indicates little material-related or R_a-related differences in adherence.

3.4 Vitality of adhered bacteria in relation to glass content

The percentages of vital adherent cells are presented in Fig. 2b. Overall they were significantly lower on ICA, ICZ, and YZ with *S. sanguinis* and *S. oralis*. *Streptococcus mutans* and *S. sobrinus* showed no significant differences in the percentage of vital adherent cells compared to the glass surface except for YZ with *S. mutans*. The linear regressions between the percentages of vital adherent cells

Table 3 Bacterial partitioning to hexadecane

Solution	*S. sanguinis*	*S. oralis*	*S. mutans*	*S. sobrinus*
PBS	90.6% ± 3.3	90.3% ± 4.3	85.1% ± 4.0	85.6% ± 5.3
Human saliva	<1%	<1%	<1%	<1%

Means and standard deviations of bacteria suspended in PBS or saliva partitioning into the hexadecane phase ($n = 10$)

Table 2 Surface roughness R_a (μm) and contact angles CA (°) of the dental ceramics and glass used

	MK	ICA	ICZ	YZ	Glass
R_a	0.26 ± 0.01	1.33 ± 0.08	1.34 ± 0.13	0.26 ± 0.02	0.24 ± 0.05
CA					
Uncoated	82.9 ± 2.9	86.6 ± 2.4	83.5 ± 4.3	81.4 ± 4.2	81.5 ± 1.3
Saliva-coated	44.3 ± 3.9	44.1 ± 3.3	46.0 ± 4.2	44.8 ± 1.7	43.8 ± 1.8

Shown are means and standard deviations for R_a ($n = 4$ for each material) and for CA ($n = 3$ for each material with and without saliva-coating)

and materials' glass contents of the all-ceramic materials are given in Fig. 3. Positive correlations were obtained for *S. sanguinis* ($r = 0.63$), *S. oralis* ($r = 0.86$), *S. mutans* ($r = 0.79$), and *S. sobrinus* ($r = 0.19$).

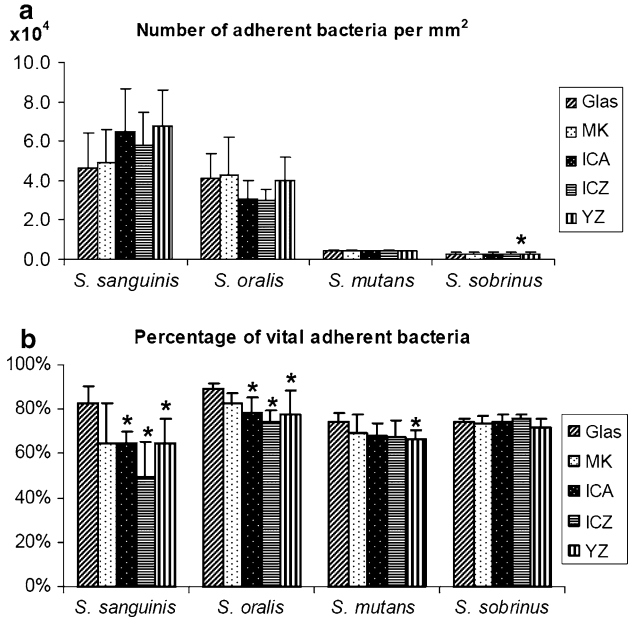

Fig. 2 Streptococci adhered to different dental ceramics and glass. Shown are means and standard deviations ($n = 5$). Values significantly different from the respective value for glass are marked with an asterisk. (**a**) Total number of cells per mm^2. (**b**) Percentage of vital adherent streptoccoci

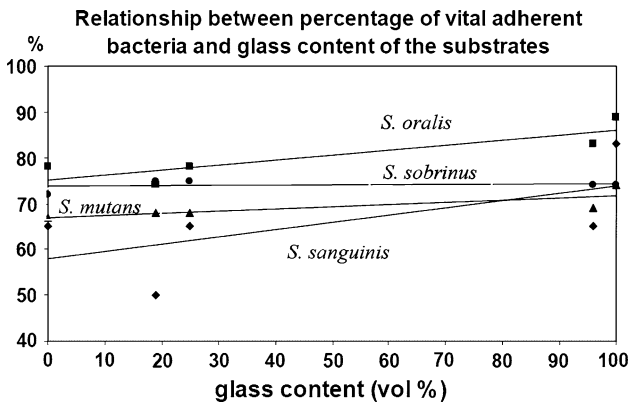

Fig. 3 Relationship between materials' glass content and percentage of vital streptococci. ◆, *S. sanguinis* ($r = 0.63$); ■, *S. oralis* ($r = 0.86$); ▲, *S. mutans* ($r = 0.79$); ●, *S. sobrinus* ($r = 0.19$)

4 Discussion

The in vitro model mimics environmental conditions in the oral cavity such as human saliva, the selected bacteria and some shear forces in the circulating medium. Although in the oral cavity an average temperature of 34–36°C prevails, the experiments were conducted at room temperature for practical reasons. Several authors have pointed out, that adhesion or co-adhesion kinetics was similar at 22–35°C [6]. The all-ceramic dental materials used differ in their mechanical properties like strength, reliability, and the fracture mechanism due to their glass content [10]. The purpose of this investigation was to investigate adhesion of four streptococcal species to these different materials with regard to number and vitality. Factors like type of the culture medium, culture conditions, and growth phase of the bacteria may influence in vitro the early bacterial adhesion [18]. To minimize the effects of different growth conditions all strains were prepared identically so that differences in bacterial adhesion would result from the salivary pellicle, or material properties like hydrophobicity, roughness, or glass content.

4.1 Evaluation of bacterial adhesion among the streptococcal species

The composition of the materials and their physico-chemical properties like hydrophobicity are known to modulate initial bacterial adhesion [3]. This initial unspecific adhesion was facilitated if bacteria and surfaces involved had similar hydrophobic properties [3, 17]. The initial layer deposited on the dental all-ceramic specimens and glass was human saliva. This coating reduced the contact angles measured and made all surfaces more hydrophilic which is in accordance to the findings of Quirynen and Bollen [19] who concluded that coating has a drastic effect on hydrophobicity of the substratum. Since the four streptococci species suspended in saliva showed similar hydrophilic nature similar adhesion profiles to the pellicle-coated all-ceramic slides were expected. However, the results revealed about tenfold differences in cell adhesion. Therefore, hydrophobic interactions are not the only mechanism involved in the adherence of these streptococci to the surfaces.

Table 4 Means and standard deviations of adherent streptococci on dental ceramics and glass per mm^2 ($n = 5$)

	S. sanguinis	*S. oralis*	*S. mutans*	*S. sobrinus*
Glass	46,400 ± 18,300	41,100 ± 12,800	4,300 ± 700	2,500 ± 200
MK	48,600 ± 17,000	42,600 ± 18,700	4,100 ± 1,100	2,900 ± 700
ICA	65,400 ± 21,600	30,900 ± 8,600	3,800 ± 400	2,800 ± 300
ICZ	58,000 ± 16,600	30,100 ± 6,100	3,600 ± 1,000	2,700 ± 150
YZ	67,500 ± 18,300	39,900 ± 12,100	3,800 ± 300	3,100 ± 300*

* $P = 0.02$

A more firm adhesion can be established between a bacterium and a surface through specific interactions [8]. This is mediated by specific components on the surface of the adhering organism and receptor molecules of the pellicle on the substratum surface [20]. The observations suggest that there where fewer binding components for *S. mutans* and *S. sobrinus* than for *S. sanguinis* and *S. oralis*, even though saliva was prepared from two volunteers. Both, hydrophobic sites of the bacterial cells and sites complementary to saliva pellicle seemed contributing to bacterial adherence to the surfaces.

The specific adhesion process to the acquired pellicle is also mediated by extracellular polysaccharides [8]. In the presence of sucrose *S. mutans* and *S. sobrinus* synthesize extracellular glucans via glucosyltransferases [21]. These glucans promote adhesion of these two streptococcal species to the salivary pellicle and to other bacterial cells. The resting cells used in these experiments had been carefully washed to remove traces of the medium. After suspension in human saliva there was no or little sucrose available for synthesizing extracellular glucans de novo. This aspect could also be responsible for the low binding of the two species to the surfaces and emphasize the importance of glucans during the adhesion process of mutans streptococci.

4.2 Evaluation of bacterial adherence and vitality in relation to materials' properties

The effect of surface roughness on bacterial adherence is complex. It was found both in vivo and in vitro that bacteria accumulated to a greater degree on rough surfaces than on a highly polished surface [3]. According to Bollen et al. [22] $R_a \leq 0.2$ μm had a negligible impact on bacterial adhesion whereas higher values correlated with higher numbers of adhering cells. In the present study the significantly higher (fivefold) surface roughness of the dental ceramics ICA and ICZ did not result in a significantly higher number of adherent bacteria. Also no relationship was found between bacterial adherence and the glass content of the materials indicating that the composition of these materials exerted no influence on bacterial adhesion in saliva. It is conceivable that salivary proteins are adsorbed onto the surface of the materials in a similar adsorption pattern regardless of different surface roughness or glass content. The influence of the specific interactions with the bacterial surface was more important in this adhesion model than materials' properties.

We found a relationship between the percentage of vital adherent streptococci cells and the glass content of the dental ceramics. These results are in agreement with an earlier study [13] where a lower proportion of vital bacteria were adhering to dental restorative materials than to enamel. The question whether dead rather than vital *S. sanguinis* cells adhere preferentially to restorative materials has not been decided. The ceramic material used, Vita Omega 900, exhibited surface properties similar to MK in this study and showed similar percentages of vital adhered cells. Indeed, the vitality of adherent bacterial cells may be influenced by the composition of restorative materials as other in vitro and in vivo studies showed [23, 24].

5 Conclusions

The data reported in this study showed that specific interactions between streptococci cells and saliva-coated all-ceramic substrata predominate initial adhesion in this model. The materials' properties surface roughness and glass content had only a weak influence on adhesion. This in vitro technique may provide a useful model to study the influence of different parameters (materials, saliva component, interfering substances) on adherence of oral streptococcal species.

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