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Adhesion and Early Colonization of S. *Mutans* on Lithium Disilicate Reinforced Glass-Ceramics, Monolithic Zirconia and Dual Cure Resin Cement

Keywords

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ABSTRACT

Objective: Monolithic zirconia and glass ceramics are increasingly used in implant crowns. Limited data is available on bacterial adhesion and early biofilm formation on these materials. Methods: Four different materials were investigated: (1) Lithium disilicate glass-ceramics (LDS), (2) Fully stabilized zirconia (FSZ), (3) Partially stabilized zirconia (PSZ), and (4) Dual curing cement (DCC). The materials' surfaces were characterized with spinning disc confocal microscopy and by water contact angle and surface free energy (SFE) measurements. For the adhesion tests the materials were rolled in suspensions of Streptococcus mutans. Early biofilm formation was studied on the materials and allowing the biofilms to form for 24 h. S. mutans cell counts were determined by plate culturing. ANOVA and post-hoc Tukey's tests ($p<0.05$) were used for statistical evaluation. Results: The LDS surfaces were clearly hydrophilic with the highest SFE value ($p<0.001$). For S. mutans adhesion, the ranking of the materials from lowest to highest was: LDS = FSZ < DCC < PSZ ($p<0.05$). No significant differences among the materials were noticed in biofilm formation. Conclusions: LDS has lower S.mutans adhesion than other materials examined in this study, but the difference was not reflected in early biofilm formation.

INTRODUCTION

Lithium disilicate reinforced glass-ceramic and zirconia are currently the most commonly used indirect restorative materials. The advantages of these materials comprise their esthetic and mechanical properties, which resemble natural tooth structures. Moreover, both materials are biocompatible in the oral environment.[1,2] Fractures of zirconia substructures are uncommon,[3] although some studies show that cracking and minor loss of material can occur in the structural ceramic layers of zirconia-based restorations.[4] Recently, monolithic zirconia crowns and fixed dental prostheses have gained attention because of their good fracture strength, low wear of the enamel antagonist and pleasant color.[5,6] Lithium disilicate glass-ceramics and monolithic zirconia are also increasingly used in implant dentistry. Both materials can be used in implant crowns and zirconia is also frequently used as an abutment material in the esthetic zone.[7,8]

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Screw retained monolithic zirconia crowns can be fixed directly on implants or by cementing them on titanium bases and abutments. The brittleness of glass ceramic materials, however, does not allow for direct fixation on implants without cementation on a titanium or zirconia abutment.[9] Zirconia structures in implant applications are mostly made with partially stabilized zirconia, where yttria content varies between 4 and 6%. Fully stabilized zirconia was recently introduced in which yttria content is clearly higher (<12%) containing more cubic form zirconia and hence a better translucency bringing its esthetical properties closer to class ceramic materials.

Peri-implantitis and implant mucositis are increasing concerns in implant dentistry.[10] Esthetic demands have increased the use of monolithic zirconia and lithium disilicate reinforced glass ceramics also in subgingival parts of implant restorations. Although both materials are biocompatible per se, their fixation on titanium bases or abutments with resin-based cements may hold a risk of microbial accumulation on the crown margins.[11]

Biofilms formed on tooth surfaces have been described as dental plaque. However, the term plaque has been expanded to encompass biofilms on all oral surfaces. Biofilm in the oral cavity consists of complex microbial communities embedded in a matrix of polymers, primarily of bacterial and salivary origin. Bacteria from dental plaque are the major etiologic factors in caries, gingivitis, periodontitis, peri-implantitis and stomatitis. Well-developed biofilms on dental implant surfaces and prosthetic restorations become the main source of microbes causing peri-implantitis, a condition that has been described as one of the main causes of dental implant failure.[12,13] There is no biofilm without an extracellular polysaccharide matrix.[14] The virulence of *S. mutans* resides in its superior ability to produce an insoluble polymer matrix with a high affinity for solid surfaces.[15] Many other microbes in dental plaque biofilms are potent acid producers and acid-tolerant, but *S. mutans* has in addition the ability to assemble an insoluble polysaccharide matrix.[16] In addition to supragingival plaque, *S. mutans* can also be found in subgingival and submucosal plaque.[17-19] Therefore, *S. mutans* biofilm on the surface of implant components is an important pre-requisite for the attachment of other micro-organisms (e.g. *Aggregatibacter Actinomycetemcomitance*) that are better known as causative factors of peri-implantitis.

The few existing clinical studies suggest that zirconia-based ceramics show low plaque accumulation, while lithium disilicate glass-ceramics showed higher plaque accumulation.[20,21] On the whole, however, little data is available on bacterial adhesion and biofilm formation on glass ceramic or zirconia implant crown materials. This lack of data also extends to the cements used to attach the crowns onto titanium bases. *S. mutans* has been found to be among the first colonizers on dental implant surfaces.[19] Thus, our aim was to compare adhesion and young biofilm formation of *S. mutans* on materials generally used in implant-crown restorations.

2. MATERIALS AND METHODS:

2.1. SPECIMEN PREPARATION

Four different groups of materials were investigated: (1) Lithium disilicate (LDS; IPS e.max CAD, Ivoclar Vivadent, Lichtenstein, control), (2) Fully stabilized zirconia (FSZ; Prettau Anterior, Zirkonzahn, Italy), (3) Partially stabilized zirconia (PSZ; Katana, Noritake, Japan) and (4) Dual curing resin cement (DCC; Multilink hybrid abutment cement, Ivoclar Vivadent, Lichtenstein). The specimens were square shaped (10×10×2 mm). Lithium disilicate, fully stabilized zirconia and partially stabilized zirconia specimens were prepared and sintered according to manufacturers' instruction. Dual curing resin cement samples were cast into molds (10×10×2 mm) and then photopolymerized. One surface of each specimen was ground sequentially using silicon carbide grinding paper. Subsequently, zirconia specimens were polished with Dialite by Brassler for zirconia polishers while lithium disilicate specimens were polished with Optrafine in an Ivoclar polishing system The specimens were cleaned ultrasonically in distilled water for 10 min before testing.

2.2. ROUGHNESS MEASUREMENTS

A spinning disk confocal microscope with a white light source (COM, µSurf explorer, NanoFocus, Oberhausen, Germany) was used to determine S_a (defined as the arithmetic mean of the absolute values of the surface departures above and below the mean plane within the sampling area and is measured in micrometers)[22] and S_q (defined as the root-mean-square value of surface asperity departures from the reference datum within the sampling area)[23] surface parameters. S_a/S_q values according to DIN EN ISO 4287 were measured at 100x magnification. In calculation of the S_a/S_q values, a Gaussian filter (ISO 11562) was used. At 100x magnification, the vertical resolution of the lens is 2 nm and the numerical aperture 0.8–0.95 for a measurement area of 160–158 µm. For the measurements done with the 100x lens, a cutoff wavelength of 80 µm was used. At least 5 readings per substrate were made at different randomly selected areas. The mean value was calculated and quantitatively expressed as a numerical value (in micrometers).

2.3. CONTACT ANGLE MEASUREMENTS

Equilibrium contact angles (θ_C) were measured using the sessile drop method (described in detail elsewhere[24]) with a contact angle meter (KSVCAM100 KSV, Instruments LTD, Finland). A drop was deposited on the surface and imaged for 20 seconds by collecting one image per 2 seconds. Determination of the contact angle was based on the Young-Laplace equation, yielding the contact angles on both sides of the droplet and their mean values. Three liquids were used as a probe for SFE calculations (*Table 1*). The result was the mean of at least 5 drops on each specimen (n=5).

Table 1. Test liquids and their surface tension components.

Liquid	Source	Surface tension (mN/m)*			
		γ^{TOT}	γ^{D}	γ^{+}	γ^{-}
Distilled water, ultrapure water Milli-Q	Produced in-house	72.8	21.8	25.5	25.5
Diiodomethane > 99% purity	Sigma–Aldrich, St. Louis, USA	50.8	50.8	0	0
Formamide, pro analysis	Merck, Darmstadt, Germany	58	39	2.28	39.6

2.4. SURFACE FREE ENERGY CALCULATIONS

The SFE was calculated using the Owens-Wendt (OW) approach. The OW model approach gives the long-range dispersion (Lifshitz-van der Waals) (γ^D) and the short-range polar (hydrogen bonding) (γ^P) components of SFE.[25] The spreading pressure was not taken into account as it contributes to SFE and has to be considered if the SFE is higher than 60 mJ/m^2.[26] In the present work, SFE values were lower than this limit and thus the spreading pressure can be disregarded.

2.5. ADHESION TESTS

For the adhesion tests the reference strain *S. mutans* Ingbritt was grown overnight in Brain Heart Infusion medium (BHI; Becton,Dickinson and Company, Sparks, MD, USA), washed once (5000 x*g*, 10 min) in saline, and suspended in saline, $A_{550} = 0.35$. The materials were used in the experiments without presoaking or precoating. Precoating of the specimens with saliva would interfere with the properties of the materials contributing to the adhesion of *S. mutans*, thus no precoating with saliva was used. The procedure has been described in detail in a previous publication.[27] In short, the discs were rolled in the cell suspensions for 30 min, washed three times in saline, after which all attached cells from one side of the specimen were scraped to 0.5 ml of transport medium (Tryptic Soy Broth, Becton, Dickinson and Compan). Three applicators (Quick-Stick, Dentsolv AB, Saltsjö-Boo, Sweden) dipped into fresh transport medium before the procedure were used to collect the cells from the surfaces. The brush ends of the applicators were cut into the transport media. This cell collection method has been tested earlier and it is highly reproducible.[27,28] The experiments were performed with five replicates and repeated at least once. The adhesion test was done in room temperature.

For enumeration of the cells on the specimen surfaces as colony forming units (CFU) the vials with the microbe samples were thoroughly Vortex-treated and after serial dilutions the samples were grown anaerobically for two days at 37˚C on Mitis Salivarius agars (Becton, Dickinson and Company).

2.6. BIOFILM EXPERIMENTS

For the biofilm experiments *S. mutans* Ingbritt was grown in BHI as described above. After washing, the cells were suspended in BHI containing 1% sucrose ($A_{550} = 0.05$). This suspension was further diluted 1:50 and 2 ml was pipetted onto the experimental discs placed in 24-well culture plates. The plates were incubated anaerobically for 24 h at 37˚C. The collection of the biofilm and culturing of cells was performed as described above. The method has been described in detail earlier.[29] The experiments were performed with four replicates and repeated at least once.

2.7. SCANNING ELECTRON MICROSCOPY

The materials with the 24-hour biofilm were rinsed in saline, then fixed for 5 min (1.425 ml formaldehyde and 2 ml glutaraldehyde in 25 ml of phosphate-buffered saline), rinsed once in distilled water and then successively dehydrated at increasing alcohol concentrations. Subsequently, the materials were sputtered by 20 nm of carbon using a sputter coater (Bal-Tec SCD 050, Balzers, Liechtenstein) and the surfaces were analyzed through images taken using a scanning electron microscope (SEM) (Model JSM 5500, Jeol Ltd, Tokyo, Japan).

2.8. STATISTICAL ANALYSIS

Results are presented as means ± standard deviations. Statistical analyses were performed using IBM SPSS (version 23.0; SPSS. Inc, Chicago, IL, USA). Data was analyzed with one-way ANOVA followed by the Tukey's post-hoc test. Differences were considered significant at a 95% confidence level. Correlation coefficients were calculated to evaluate associations between contact angles and the rate of bacterial adhesion.

3. RESULTS

Polished PSZ surfaces showed lower S_a and S_q values than FSZ or LDS, although the differences were not statistically significant. The S_a and S_q values for PSZ were 0.009 (±0.001) and 0.014 (±0.002); for FSZ 0.013 (±0.002) and 0.021 (±0.004); and for LDS 0.011(±0.002) and 0.017 (±0.003).

3.1. CONTACT ANGLE

Table 2 shows the equilibrium contact angle values obtained by the sessile drop method on different surfaces after the polishing. LDS exposed the lowest contact angles for all test liquids ($p<0.05$), whereas polished zirconia had the highest contact angles with no differences between PSZ and FSZ surfaces.

Table 2. Mean values and standard deviations (SD) of contact angle measurements.

Groups	Water	Formamide	Diiodomethane
Dual cure resin cement	65.7 (6.0)^{a}	43.4 (3.9)^{a}	33.0 (3.7)^{a}
Lithium disilicate	30.3 (4.3)^{b}	27.9 (14.3)^{b}	41.4 (6.2)^{b}
Fully stabilized zirconia	104.1 (1.2)^{c}	87.2 (3.8)^{c}	73.9 (2.8)^{c}
Partially stabilized zirconia	97.2 (2.8)^{c}	85.5 (1.9)^{c}	72.4 (4.1)^{c}

Different superscript letters indicate statistically significant differences in contact angle measurements within the same liquid (p<0.05).

3.2. SURFACE FREE ENERGY

The surface free energies were calculated using the Owens-Wendt method. Polished LDS showed highest total SFE followed by DCC while both PSZ and FSZ expressed lowest total SFE values (p<0.001). The polar component of SFE followed the same trend, but dispersive SFE was the highest on DCC surfaces (p<0.001) *(Table 3)*.

Table 3. Mean values (mJ/m^{2}) of surface free energy (SFE) components calculated using the Owens-Wendt approach.

Groups	Polar SFE (γ^{P})	Dispersive SFE (γ^{D})	Total SFE (γ^{TOT})
Dual cure resin cement	8.07 (5.78)*	38.78 (6.45)*	46.85 (4.66)*
Lithium disilicate	30.00 (2.80)*	32.12 (3.00)*	62.13 (4.02)*
Fully stabilized zirconia	1.28 (1.10)	19.38 (2.54)	20.67 (1.99)
Partially stabilized zirconia	2.22 (1.05)	19.57 (2.78)	21.75 (2.20)

Compared with other groups * p<0.001.

3.3. *S. MUTANS* ADHESION

Some differences were found in *S. mutans* adhesion among the materials. LDS showed lower *S. mutans* adhesion as compared to DCC (p<0.05) and PSZ (p<0.005), with FSZ being lower than PSZ (p<0.05)*(Figure 1)*.

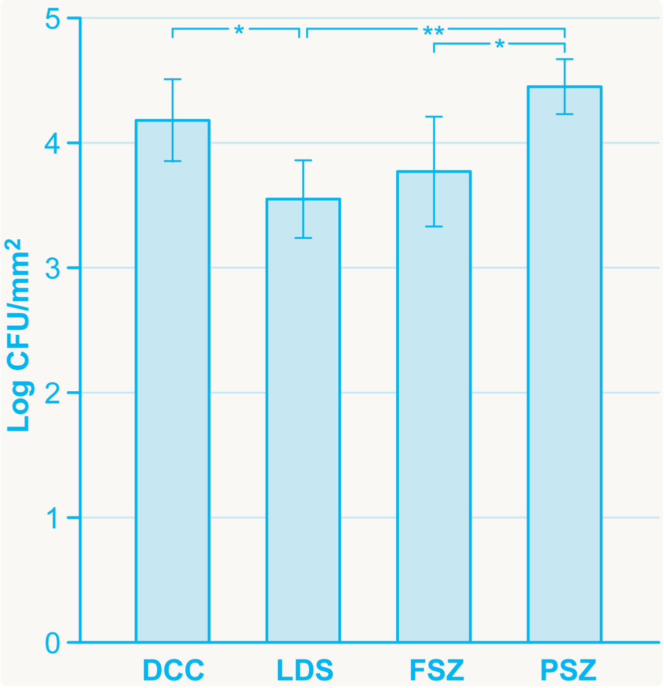

Figure 1: Adhesion of *S. mutans* to dual cure resin cement, lithium disilicate reinforced glass ceramics and fully or partially stabilized zirconia. Statistical significance, ** <0.01, * <0.05.

3.4. BIOFILM FORMATION

S. mutans biofilm was formed equally on all materials as judged by the viable cell numbers of the 24-hour biofilms *(Figure 2)*. The SEM evaluation revealed uniform bacterial biofilm on all material surfaces *(Figure 3)*.

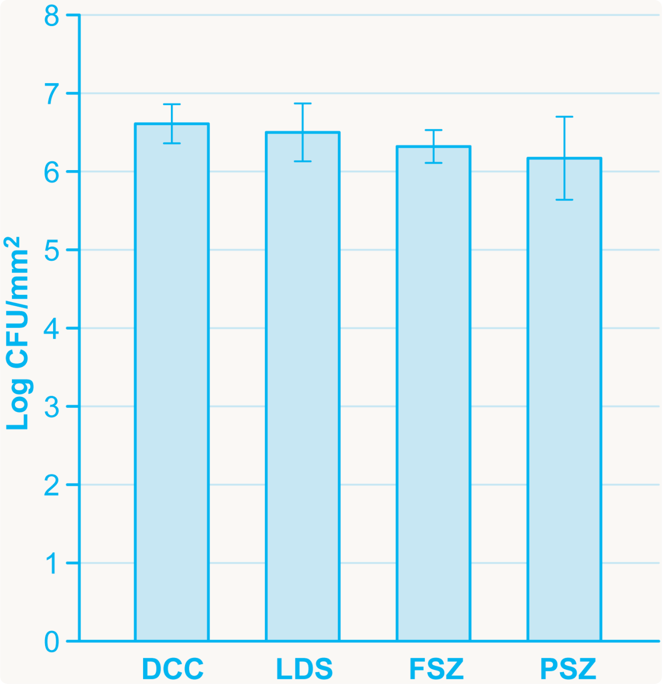

Figure 2: Young *S. mutans* biofilm formation on dual cure resin cement, lithium disilicate reinforced glass ceramics and fully or partially stabilized zirconia. No statistically significant differences were detected.

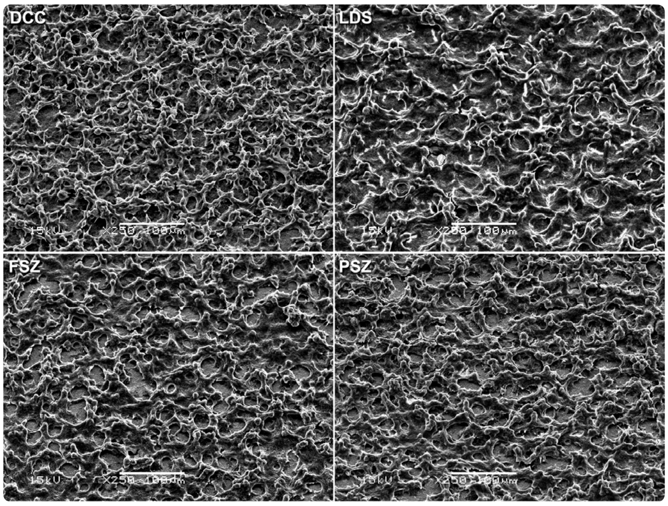

Figure 3: SEM micrograph of the young *S. mutans* biofilms on dual cure resin cement, lithium disilicate reinforced glass ceramics and fully or partially stabilized zirconia.

4. DISCUSSION

Inert biocompatible materials that are used in oral implant structures present hospitable environments for oral pathogens. Highly polished surfaces facilitate effective biofilm removal from prosthetic structures, but all subgingival surfaces and structural areas, which are difficult to clean are potential bacterial colonization sites. This study was set out to evaluate bacterial adhesion and biofilm formation on ceramic materials and dual cure resin cement, which are frequently used in implant structures including their subgingival components. According to this study lithium disilicate glass ceramic possesses lower bacterial adhesion than either fully or partially stabilized zirconia materials. Subgingival cement margins of implant restorations have been indicated as potential bacterial colonization sites and sources for the development of periimplantitis.[11] In our study polished dual cure resin cement per se did not promote bacterial adhesion or biofilm formation when compared with lithium disilicate or monolithic zirconia materials.

The small but significant differences in the adhesion of *S. mutans* to the ceramic materials and the dual cure cement were not reflected in the early biofilm formation. This is not a surprise since in the biofilm model we used only materials with a rather strong antimicrobial activity can disturb the biofilm formation.[28,29] In vivo, the materials we studied are exposed to blood or saliva creating a protein pellicle. Serum and saliva coating significantly decreases the adhesion of *S. mutans* to dental materials and adhesins specific to pellicle proteins mediate the binding of *S. mutans*.[27,30] However, *S. mutans* can also bind to dental materials without the involvement of a pellicle. In our study all materials were polished in order to minimize the influence of surface roughness on bacterial adhesion. It has been shown that 0.2 µm is the R_a value below which surface roughness has no further impact on bacterial colonization.[31] In our study all materials had lower R_a values. R^a is an arithmetic mean of 2D deviations from the surface plane and has been used as an indicator of surface topography, while the 3D area roughness parameters (S_a and S_q) give more meaningful values. In our study no differences were found in S_a or S_q or S_a/S_q values either, which indicate that all materials were highly polished. With highly polished materials properties like hydrophobicity and surface free energy influence adhesion of *S. mutans*. The lithium disilicate glass ceramic with the low hydrophobicity and high surface free energy showed the lowest adhesion of *S. mutans* which is in accordance with earlier studies.[32,33] As for hydrophobicity the results speak both for and against its importance for adhesion of *S. mutans* to material surfaces.[30,33,34] With surfaces which are not exceptionally smooth, surface roughness is a confounding factor, which may explain the contradictory results in the literature.[33]

In vitro studies like the present one, are strongly influenced by the experimental conditions, for example the presence of shear, and the particular *S. mutans* strain used.[35] In vivo, the conditions supragingivally and subgingivally differ considerably especially with regard to shear. Supragingivally hydrophobic surfaces attract less plaque than hydrophilic ones, while subgingivally there is little difference in the accumulation.[34] In vivo, ceramic materials appear to show low plaque accumulation.[20,21,36] Our study did not find any difference in the young biofilm formation between the highly polished ceramic materials and the dual cure resin cement, which indicates that there would be no difference in the in vivo plaque accumulation either.

Biofilms are often studied using incubation times that last for several days. This may be purposeful when studying multispecies biofilms or the structure of the biofilm, but has little clinical relevance. We have shown earlier that a young biofilm of *S. mutans* is sensitive to antimicrobial and other plaque formation influencing agents.[29,37] The materials used in this study had no antimicrobial properties, however, the young biofilm should better reflect differences seen in the bacterial adhesion to materials as compared to several-days-old biofilms.

In this study we found differences in the adhesion of *S. mutans* to the ceramic materials and the dual cure resin cement, however, this was not reflected in the formation of young *S. mutans* biofilm. Thus, if the materials are properly polished, *S. mutans* should form biofilm similarly on these materials also in vivo.

All material surfaces were exceptionally smooth and no differences were observed in surface texture after the polishing procedure. Therefore, the differences in hydrophilicity and SFA values among the materials are possibly related with different chemical compositions of the polished surfaces. No differences were noticed in this regard between structurally similar zirconia materials, which turned out to be hydrophopic with the lowest SFE. Lithium dilsilicate glass ceramic, on the other hand, was clearly hydrophilic and had the highest surface free energy of the examined material surfaces. Although the water contact angle and SFE could not explain differences in *S. mutans* adhesion, these surface properties can play a significant role in bacterial adhesion strength. It has been indicated that *S. mutans* adhesion can be stronger on hydrophilic surfaces while the attached bacteria are easier to remove from hydrophopic surfaces.[38]

The findings of this study indicate that although the super smooth material surfaces show differences in SFE and hydrophilicity they are not directly linked with *S. mutans* adhesion properties.

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