CHAPTER 9

In vitro adherence of oral streptococci to zirconia core and veneering glass-ceramics.

Keywords: bacterial adhesion, zirconia, ceramics, streptococcus
9.1 Abstract

Objective. Plaque formation on dental ceramics may cause gingival inflammation and secondary caries. This in vitro study compared the susceptibility of various dental ceramics to adhere oral streptococci and verified the influence of substratum surface roughness and hydrophobicity.

Materials and Methods. Three zirconia ceramic materials and their veneering glass-ceramics were investigated. Fifteen test specimens were prepared, polished, and surface roughness and surface hydrophobicity were determined. Samples were incubated with suspensions of Streptococcus gordonii, S. mutans, S. oralis or S. sanguinis, respectively, in a thermo-shaking device for 2 h at 37°C. Adherent bacteria were quantified using a fluorescence dye for viable cell quantification (Alamar Blue/Resazurin). Statistical analysis: Mann-Whitney-U test (α=0.05).

Results. Median surface roughness ranged between 0.08 μm and 0.42 μm, median water contact angles between 63.9° and 91.8°. Low relative fluorescence intensities indicating low adhesion of streptococci were found for all ceramics compared to a glass reference. Few significant differences were determined either between the different zirconia ceramics or between the glass-ceramics. Only individual differences were found between the glass-ceramics and zirconia.

Conclusion. Rather similar adhesion of streptococci to the zirconia and glass-ceramics was found, which suggests that no restrictions in the clinical performance of exposed zirconia ceramic restorations concerning plaque formation are to be expected.
9.2 Introduction

Ceramic materials are extensively used in current dentistry for restoring lost tooth substance. Today, the scope of this material class ranges from restoration of single crowns to widespread bridge- and implant-restorations. Special ceramics either made of lithium disilicate, aluminium oxide or zirconia oxide have been developed for meeting high fracture resistance requirements and are commonly referred to core ceramics. Zirconia ceramic frameworks are fabricated using CAD/CAM techniques, and their strength allows indications reaching to the application in posterior fixed partial dentures. These zirconia ceramics differ in terms of doping and grain size as well the milling process, where soft pre-sintered or hard finally sintered materials are manufactured. The opaque oxide-ceramic frameworks are veneered for aesthetical and protective purposes using conventional glass ceramics. However, settings are thinkable where core ceramics are exposed to the oral environment. For example, in cases of insufficient space requirements dental technicians may renounce veneering (anterior resin bonded bridges, gingival areas of FPDs, implants, abutments). In addition, after polishing marginal areas of dental ceramic restorations, core layers may be exposed. That exposure, particularly, may take place in areas near the preparation limit where dental restorations need to be thin and gracile. Framework ceramics may rarely be exposed because of veneering chipping, too; however, these surface defects are usually small and may allow for leaving the restoration in situ. Exposed core ceramic areas may promote enhanced deterioration [1, 2] and provide an interface between the ceramic framework and the oral environment.

It is desirable that all dental restorative materials feature low susceptibility to adhere oral micro-organisms since plaque formation on dental restorations may lead to secondary caries and periodontal inflammation [3]. The adsorption of saliva constituents to tooth and restorative surface is considered as the first step in oral biofilm formation that is followed by the adhesion of facultative anaerobic pioneer bacteria [4, 5] such as Streptococcus gordonii, Streptococcus oralis and Streptococcus sanguinis [6]. Streptococcus mutans has been discovered in early plaque, too, and has furthermore been found to be one of the major causative agents for dental caries [7].

Compared with other dental materials, such as composites or methacrylate systems, numerous in vitro and in vivo studies found low adhesion of oral bacteria to ceramic surfaces [8, 9], but to date there is little information in the literature dealing with potential differences in bacterial adhesion to different types of ceramics and, in particular, high-strength oxide ceramics. Along with conventional material properties, bacterial colonization may be regarded as a further factor determining the clinical performance of dental materials. This research intended to evaluate some surface properties such as roughness and wetability of dental ceramics and to
rank the adhesion of early colonizing streptococci to these substrata. High-strength zirconia core materials were compared to their corresponding veneering ceramics.

9.3 Material and Methods

Sample preparation

Three oxide ceramics and their corresponding veneering glass-ceramics were used in this research (Table 9.1). Fifteen rectangular specimens (5x10 mm) of each product were prepared according to the manufacturers’ instructions and polished using silicone carbide grinding paper (grain 1,000 and 4,000, successively; Buehler GmbH, Düsseldorf, G) and a rotating grinding disc apparatus (Motopol 8, Buehler Ltd., Coventry, UK). Peak-to-valley surface roughness (Ra) was determined at three spots for each sample using a profilometric contact surface measurement device (Perthometer S6P, Feinprüf-Perthen, Göttingen, G). For evaluating surface wetability, water contact angles were measured using an automated contact angle measurement device equipped with a video camera and an image analyzer (OCA 15 plus, Dataphysics Instruments GmbH, Filderstadt, G). For each substratum, three drops of deionised water (500 µL) were analyzed on five randomly selected specimens (fifteen measurements in total per each product), and the left and the right contact angle of each drop were averaged.

Table 9.1: Material, manufacturer, application and type of ceramic.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Application</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercon Base</td>
<td>DeguDent, Hanau, G</td>
<td>Core</td>
<td>Zirconia</td>
</tr>
<tr>
<td>Digizon</td>
<td>AmannGirrbach, Pforzheim, G</td>
<td>Core</td>
<td>Zirconia</td>
</tr>
<tr>
<td>Inceram Y-TZP</td>
<td>Vita Zahnfabrik, Bad Säckingen, G</td>
<td>Core</td>
<td>Zirconia</td>
</tr>
<tr>
<td>Cercon Ceram S</td>
<td>DeguDent, Hanau, G</td>
<td>Veneering</td>
<td>Glass-ceramic silicate based</td>
</tr>
<tr>
<td>Omega 900</td>
<td>Vita Zahnfabrik, Bad Säckingen, G</td>
<td>Veneering</td>
<td>Glass-ceramic leucite based</td>
</tr>
<tr>
<td>GC Zirconia</td>
<td>GC, Alsip, IL, USA</td>
<td>Veneering</td>
<td>Glass-ceramic</td>
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</tbody>
</table>
Bacterial adhesion
The strains *S. gordonii* (DSMZ 6777), *S. mutans* (DSMZ 20523), *S. oralis* (DSMZ 20627) and *S. sanguinis* (DSMZ 20068) (all by DSMZ Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ, Braunschweig, Germany) were grown the day before the experiment in sterile DSMZ-medium (#92, Trypticase Soy Yeast Extract Medium, containing 30 g tryptic soy broth (Becton Dickinson Microbiology Systems, Sparks, USA) and 3 g yeast extract (Sigma-Aldrich, St. Louis, USA)). For inoculation of bacteria cultures a pre-culture was used, which had been freshly inoculated in medium 92 every week. 100 μL of this pre-culture were mixed with 25 mL of medium 92 and incubated for 12 h at a temperature of 37°C. Subsequently, the various cells were harvested by centrifugation (2200 rpm, 19°C, 5 min; Hettich Rotixa P, Tuttlingen, G), washed twice with Phosphate Buffered Saline (PBS, Sigma-Aldrich, USA) and re-suspended in the same buffer. Cell suspensions were subjected to low intensity ultrasonic energy to disperse streptococcal chains, and the optical density of the suspensions was adjusted to 0.3 at 550 nm with a spectrophotometer (Genesys 10S; Thermo Spectronic, Rochester, NY, USA), which corresponds to a microbial concentration of 3.65x10^8 microorganisms/mL.

For quantification of adherent bacteria Resazurin reduction (Alamar Blue) was used as described earlier; measurements were carried out using a fluorometric measurement device (Fluostar Optima, BMG Labtech, Offenburg, G). In brief, Resazurin reduction is based on the reduction of the blue, non-fluorescent redox indicator Resazurin (maximum absorbance at 605 nm) into the violet, fluorescent pigment Resorufin (maximum absorbance at 573 nm) by metabolically active, viable cells.

Specimens were equilibrated with ethanol for removing traces of lipids and proteins from the substratum surfaces and auto-fluorescence was measured. The specimens were incubated with streptococcal suspension (1 mL) and Resazurin (15 μL; Resazurin, Sigma-Aldrich, USA) for 2.5 h and, subsequently, carefully rinsed twice with PBS for removing non-adhering bacteria. Adherence of streptococci was quantified by measuring the dimensionless fluorescent signal. Fluorescence intensity linearly correlates to the number of adhered bacteria.

Medians and 5%/95% were calculated and displayed using SPSS 13.0 for Windows (SPSS Inc., USA). Statistical analysis was performed using the Mann-Whitney-U test (α<0.05).

### 9.4 Results

Surface Roughness
The investigated ceramics may be divided into three groups according to their different surface roughness (Fig. 9.1). Significantly highest surface roughness was found for *Vita Omega 900* (median 0.42 μm). Lower values could be determined for *YZ Cubes* (0.23 μm),
Cercon Ceram S (0.19 μm) and Cercon Base (0.19 μm). Significantly lowest surface roughness was detected for Digizon and GC Zirconia (both 0.08 μm).

**Fig. 9.1:** Surface roughness [μm]; (grey bars indicate core ceramics, hatched bar veneering ceramics) (median, 5%/95%).

Contact Angle Measurements
Significantly lowest contact angles were measured for Digizon (median 63.9°). Higher angles were determined for Cercon Base (71.1°) and GC Zirconia (73.7°). Intermediate contact angles were found for YZ-Cubes (89.1°), which were similar to Cercon Base (p=0.132), but significantly higher than for GC Zirconia (p=0.003). Significantly highest values were measured for Omega 900 (98.1°) and Cercon Ceram S (91.8°). Lowest contact angles were found for the glass control (61.4°) (**Fig. 9.2**).
Streptococcal adherence
A strong variation in the adherence of streptococci was found for the different ceramic materials. Furthermore, the adhesion was dependent on the streptococcal strain. For all strains evaluated, the glass control featured significantly higher values compared with all ceramic materials (Fig. 9.3).

Investigating *Streptococcus mutans* adhesion, the significantly lowest fluorescence intensity (indicating lowest adhesion) was determined for *GC Zirconia* (median 4288). Significantly higher values were measured for *Digizon* (6994), which were comparable to *Cercon Base* (9376; p=0.393) and *YZ Cubes* (10725; p=0.280), but significantly higher than values for *Omega 900* (18591; p=0.043). No significant differences were found between *Cercon Base*, *YZ Cubes* and *Omega 900*. *Cercon Ceram S* (18367) yielded significantly higher fluorescence than the other ceramics with the exception of *YZ Cubes* (p=0.075) and *Omega 900* (p=0.853). Lowest fluorescence intensities for *Streptococcus sanguinis* were found for *Omega 900* (2813), *GC Zirconia* (5565) and *Cercon Ceram S* (3666), which did not differ significantly from each other. Higher values were recorded for *Omega 900*. No significant differences were found between *YZ Cubes* (30988), *Digizon* (27571) and *Cercon Base* (14095). Relative fluorescence intensities measured for the core materials *YZ Cubes, Digizon* and *Cercon Base*
were significantly higher than values measured for the veneering ceramics Omega 900, Cercon Ceram S and GC Zirconia.

No significant differences in Streptococcus gordonii adhesion could be found between Cercon Base (1698) and Digizon (6875). Cercon Base revealed significantly lower values than YZ-Cubes (12011), Omega 900 (11051), Cercon Ceram S (8997) and GC Zirconia (14518). Similar values were found for Digizon compared with Omega 900 (p=0.089) and Cercon Ceram S (p=0.143), but the values were significantly lower than for YZ Cubes (p=0.029) and GC Zirconia (p=0.035). No significant differences in fluorescence intensities were determined between YZ-Cubes, Omega 900, Cercon Ceram S and GC Zirconia.

For Streptococcus oralis the lowest fluorescence intensities were measured for GC Zirconia (8258), Digizon (12175) and YZ-Cubes (12716). Significantly higher results were found for Cercon Base (22502), Omega 900 (12993) and Cercon Ceram S (10425). Similar values were determined for YZ Cubes, Omega 900 and Cercon Ceram S. Significantly higher values were found for Cercon Base than for Digizon (p=0.019), YZ-Cubes (p=0.015), Cercon Ceram S (p=0.002) or GC Zirconia (p=0.000).

The smoothed materials provided the lowest contact angles. No significant correlation could be determined between the contact angle or surface roughness and bacterial adhesion.

**Fig. 9.3:** Relative fluorescence intensity [ ] with different types of bacteria (median, 5%/95%).

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**Graph:**
- Bacterium types: Streptococcus mutans, Streptococcus sanguinis, Streptococcus gordonii, Streptococcus oralis
- Ceramics: Digizon, GC Zirconia, YZ-Cubes, Omega 900, Cercon Base, Cercon Ceram S, Glas
- Relative fluorescence intensity [ ]
- Contact angles
- Surface roughness
9.5 Discussion

Streptococcal adherence to dental restorative surfaces has often been addressed in dental materials science. In this study, the focus was set on the adhesion of representative oral streptococci to zirconia ceramics and their corresponding veneering ceramics with regard to substratum surface properties such as surface roughness and hydrophobicity.

Evaluating initial streptococcal adhesion to various ceramic surfaces, a representative selection of bacteria has been used. These have been either counted among early colonizing oral bacteria [6] or have been associated with the pathogenesis of caries [7]. The microbial suspension was adjusted to an optical density of 0.3, corresponding to $3.65 \times 10^8$ microorganisms/mL [11], which is in accordance with the total bacterial concentration per ml saliva [14, 15]. In order to achieve a better differentiation between the various ceramic substrata and in order to allow for a higher influence of individual surface properties on bacterial adhesion, it was decided not to coat the specimens with saliva, as the salivary pellicle is known to level substratum surface properties and reduce overall bacterial adhesion [16]. This approach is justified by a phenomenon called the “shine through effect”, which describes the transfer of original substratum properties through a levelling protein film on the substratum surfaces [17, 18].

Surprisingly, only a poor correlation between surface properties and bacterial adhesion could be determined. It has been reported that surface free energy, and in particular surface roughness, are regarded as the most decisive substratum properties influencing bacterial adhesion [19]. High values for substratum surface roughness have been associated with increased adhesion of oral bacteria [19]. Similar surface roughness was found for zirconia and glass-ceramic materials, which was in a range that can be achieved by polishing using ultra fine burs [20]. Surface roughness differed significantly among the various ceramics, but was not dependent on ceramic type. Although some ceramics exceeded the threshold value at 0.2 $\mu$m [21], our results provided no influence of substratum surface roughness on the bacterial adhesion. Bollen and co-workers found that a surface roughness lower than 0.2 $\mu$m does not influence adhesion of oral bacteria to solid surfaces [21]. It has been supposed that high substratum surface free energy, which corresponds to hydrophilic surface properties, is associated with increased plaque formation [22]. Although hydrophobicity varied significantly among the various ceramics, no correlation between substratum hydrophobicity and streptococcal adhesion could be determined.

Only small differences concerning streptococcal adhesion were found between the various materials. Identical \textit{Streptococcus mutans} adhesion to all zirconia ceramics was determined. For the other streptococci, few differences were found between the zirconia materials \textit{Digizon} and \textit{YZ Cubes}; only \textit{Cercon Base} showed higher adhesion of \textit{Streptococcus sanguinis} and
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Streptococcus gordonii but lower values for Streptococcus oralis. These differences may be attributed to minor differences in ceramic structure, which differ in grain size or yttria doping. In contrast to the industrially manufactured hot isostatic pressed (hip) Digizon, YZ Cubes and Cercon Base are fabricated in talcum-like “white” condition; however, stronger influences of different fabrication and quality of the zirconia ceramics on streptococcal adhesion could not be confirmed. If the materials are used directly after milling, variations in surface properties dependent on individual milling strategies may not be excluded. The few differences in streptococcal adhesion to the various ceramics that have been found in this study concur with other laboratory investigations using Streptococcus mutans, Streptococcus sanguinis, Actinomyces viscosus, Actinomyces naeslundii and Porphyromonas gingivalis, finding lower adhesion of bacteria to zirconia in comparison to titanium. In vivo studies found less accumulation of bacteria to zirconia than to titanium implant material, too [23].

It has been reported that glazed zirconia shows a tendency towards increased accumulation of bacteria than untreated zirconia [24]. These observations are merely in partial agreement with the results of this study, as adhesion of streptococci to the glass-ceramics was found to be similar to the zirconia ceramics. However, slight individual differences were found, observing similar results for Cercon Ceram S and Omega 900 but differences compared to GC Zirconia. These differences in streptococcal adhesion may be attributed to individual ceramic components, but there is no detailed information concerning ceramic composition available in the literature which helps for the interpretation of these results.

Within the limitations of this study, we found low bacterial adhesion on glass-ceramics as well as on zirconia ceramics. Although some differences in surface roughness and contact angle exist, the results indicate that there was no influence of these criteria on the bacterial adhesion. Generally, zirconia and glass-ceramics provided no strong differences in the bacterial adhesion. In the aspect of bacterial adhesion, there may be no limitations when zirconia ceramic is exposed in the oral cavity.
9.6 References