Adhesion of Streptococcus mutans to Zirconia, Titanium Alloy and some other Restorative Materials: "An in-vitro Study"

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Abstract

Introduction: Bacterial adhesion on restorative materials may lead to gingival inflammation and secondary caries.

Objectives: The aim of this in vitro study was to evaluate the adhesion of streptococcus mutans to zirconia, Feldespatic porcelain, titanium alloy and Indirect composite resin In-vitro. The effect of surface roughness on bacterial adhesion was also studied.

Materials and Methods: 10 specimens (5mm diameter, 1mm thickness) of each material, Zirconia, Indirect composite resin, Titanium alloy and Feldespatic porcelain were fabricated. Enamel was used as reference. Specimens were covered with artificial saliva and bacterial suspension (10^9 CFU/mL). Bacterial adhesion was determined using scanning electron microscope and culturing the specimens in blood agar. Data were analyzed with One way ANOVA followed by Tukey post hoc test for roughness and Kruskal-wallis test for adhesion values.

Results: The highest bacterial adhesion was recorded for composite specimens and the lowest was seen in Ziconia group ($p<0.5$). The mean value of adhesion for zirconia, feldespatic peocelain, Titanium alloy and indirect composite were 28±6.32, 40.80±8.40, 75±4.47 and 386±13.75, respectively. The differences between zirconia and titanium alloy and also zirconia and indirect composite and porcelain and indirect composite were statistically significant ($p<0.5$).

Conclusion: Zirconia showed the lowest bacterial adhesion in comparison to other tested materials and Enamel. The difference between zirconia and titanium alloy and also zirconia and indirect composite was statistically significant ($p<0.5$). No correlation was found between surface roughness and bacterial adhesion.

Keywords: Bacterial adhesions; Streptococcus mutans; Dental caries

Introduction

Biofilm formation on tooth surfaces is an important factor in the development of caries and periodontal diseases. Biofolm accumulation occurs on all surfaces in the mouth including natural, artificial or dental materials (1). *Streptococcus mutans* (*S. mutans*)

is isolated in plaque samples from intraoral carious surfaces (2). Surface roughness and surface free energy are among the factors that have been identified to influence oral biofilm formation (3). Some *In vitro* studies have shown increased degree of bacterial adhesion on surfaces with roughness greater than 0.2 µm in fixed restorations (4). Today many different restorative materials are available in dentistry (5). Nowadays, patients benefit from tooth-colored materials such as all-ceramic materials. All-ceramic restorations are preferred because of their high strength and esthetic properties and biocompatibility. However, there is little information on bacterial adherence to these materials (6).

Titanium (Ti) and titanium alloys have been used for prosthetic superstructures. Adequate mechanical properties, corrosion resistance, biocompatibility, high strength-to-weight ratio, high ductility, and low density in comparison to other alloys are among the characteristics which make this alloy a proper choice for dental means; however, biofilm formation on their surfaces is a common clinical problem with these materials (7,8).

Today, composite resins are widely used in restorative dentistry because of the improvement of bonding and polymerization systems, and mechanical and physical properties of the resins. It has been shown that some composite resins stimulate bacterial adherence. Polymerized composite resins release residual monomers which may increase the adhesion and growth of microorganisms including S. mutans. However, there is a lack of enough scientific information about the adhesion of cariogenic bacteria on the surface of indirect composite resins (9).

The present study evaluated the surface characteristics and also the effect of surface roughness of 4 restorative materials (Zirconia, Titanium alloy, Feldespatic porcelain and indirect composite) on bacterial (S. mutans) adhesion using scanning microscope and culturing.

Material and Methods

Four commonly used dental materials were selected. Enamel was used as reference (Table 1). The enamel samples were prepared from the recently extracted third molars.

Type of material	Manufacturer	Composition provided by manufacturer
Ceramic	Ivoclar Vivadent AG, Schaan, liechtenstein	3Y-TZP (yttria stabilized tetragonal zirconia polycrystalline)
Feldespatic Porcelain	Noritake , Japan	Sio2,Al2O3,Na2O,K2O in glassy matrix
Titanium alloy(Tilite Omega Ceramic alloy)	Talladium, USA	Nickel (60-76%) Chromium (12-21%) Molybdenum (4-14%) Titanium (4-6%)

Indirect composite	GC America, Gradia	Monomer composition UDMA, Filler composition Sio2 (70%)

**Table 1:** Materials used in this study and their manufacturers

Ten samples of each material were prepared and polished according to the manufacturer's instructions in the form of disks (5*1mm).

3Y-TZP (Ivocular vivadent) samples were polished using 46μm (fine) and then 25μm (ultrafine) diamond rotary cutting instruments (DRCI) for 30 seconds, and Feldespatic porcelain (Noritake Japan) disks were polished by white stone and silicon carbide for 30 seconds and then glazed . Titanium disks (Tilite; Talladium Inc.) were polished using EVE (DIAPOL, diamond polisher, Ernst Vetter Gmbh Untere Felsentre.29D-75180 pforzheim Germany). Composite samples (GRADIA micro-hybrid ceramic) were lightcured for 3min with a light polymerizing unit (blue phase; Ivocular Vivadent AG, Schaan, Liechtenstein) from both sides. Wavelength range was 430-490 nm. The disks were then polished using the special CG GRADIA DIAPOLISHER for 60 seconds by single operator.

Roughness of each sample was measured using a surface profilometer (Mituytoyo surftest 301, Mitutoyo corporation, Kanagawa, Japan) with a standard cutoff of 0.8 mm, a transverse length of 0.8mm, and a stylus speed of 0.1 mm/s. Numerical average of three profilometer values was determined for each specimen (one in middle and two on sides). The bacteria used in this study were S. mutans NCTC.1683. The obtained bacteria from stock were incubated at 37˚C in a 10% CO_2 atmosphere for 24 hours. Then, the bacteria obtained from culture were used to make a bacterial suspension with concentration of 10^9 bacteria ml^{-1}. Samples were cleaned with ultrasonic device (Biosonic; Coltene/whaledent Inc, Ohio) for 15 minutes and then sterilized for 30 minutes using autoclave at 21˚C. Each group of materials was covered with a mixture of 0.5 cc sterile physiologic serum and 0.5 cc bacterial suspensions with concentration of 0.5 Mcfarland and 1cc sterile artificial saliva (Hypozalix; BIOCODEX Inc., France) for 10 minutes. Samples were then removed from the tubes and were rinsed with 1mL strill normal saline and the discs were placed on the plates containing solid blood agar culture media and incubated at 37˚C for 48 hours. In order to evaluate the samples under scanning electron microscope (FE SEM Hitachi) the disks were covered by 10 nm gold using DC sputtering (Technique USA). The specimens were also examined under scanning electron microscope by Three independent observers estimated the bacterial amount on the disk surfaces and for each material images under different magnifications in JPEG format were created (Figures 1-5).

Figure 1: SEM view of enamel
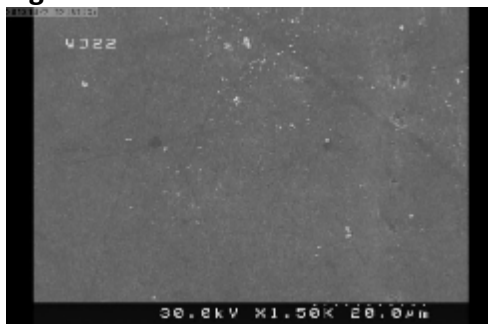
Figure 2: SEM view of Feldespatic porcelain
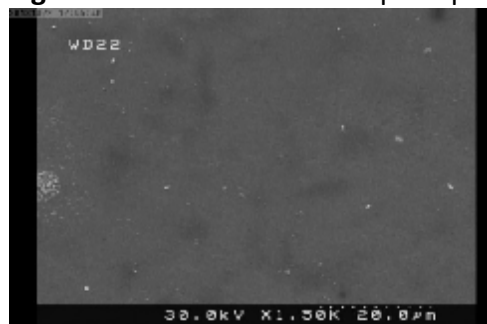
Figure 3: SEM view of zirconia
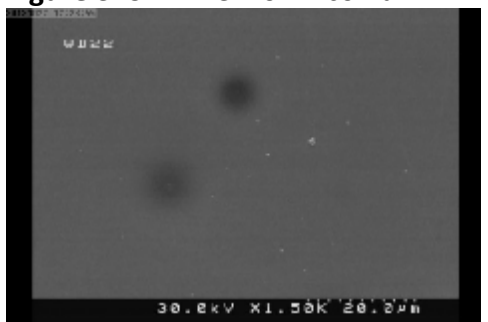
Figure 4: SEM view of Titanium
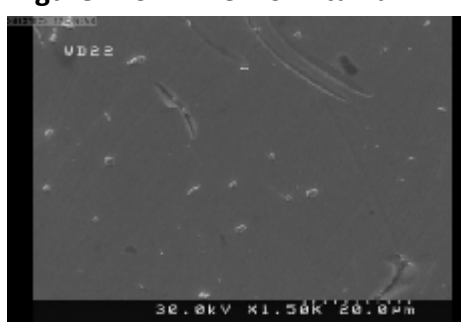
Figure 5: SEM view of Indirect composite
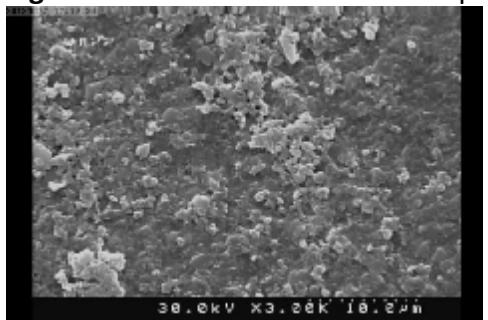
After 48 hours, bacterial colonies around each sample were counted by an experienced operator.

Statistical Evaluation

Statistical analysis was performed using analysis of variance (ANOVA) and post-hoc Tukey test for roughness values and Kruskal-Wallis test for adhesion values. Statistically significant differences were set at P<0.05.

Results

Results are summarized in charts 1 and 2. In this study, the highest bacterial adhesion was observed in indirect composite samples and the lowest in zirconia samples. According to pair analysis there was significant differences between zirconia and composite samples (p= 0.001), zirconia and titanium samples (p=0.014) and Feldespatic porcelain and composite samples (p=0.023). Among the restorative materials, Titanium revealed the highest surface roughness (0.468±0.03µm) and zirconia the lowest (0.292±0.04µm). All of the samples had surface roughness of lower than that of the enamel (0.646±0.05).
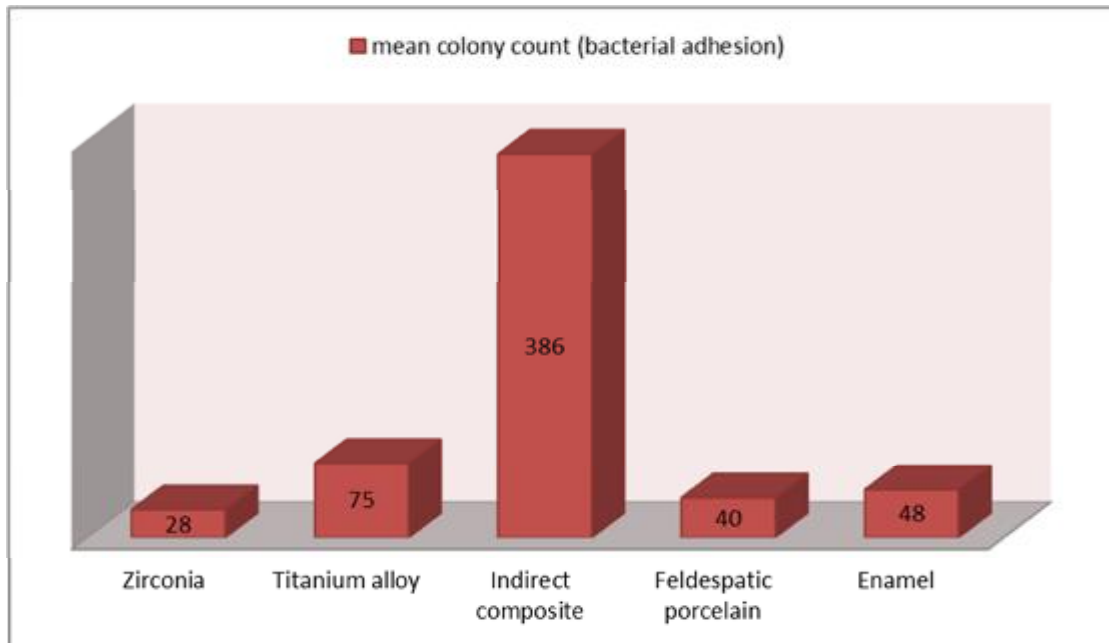
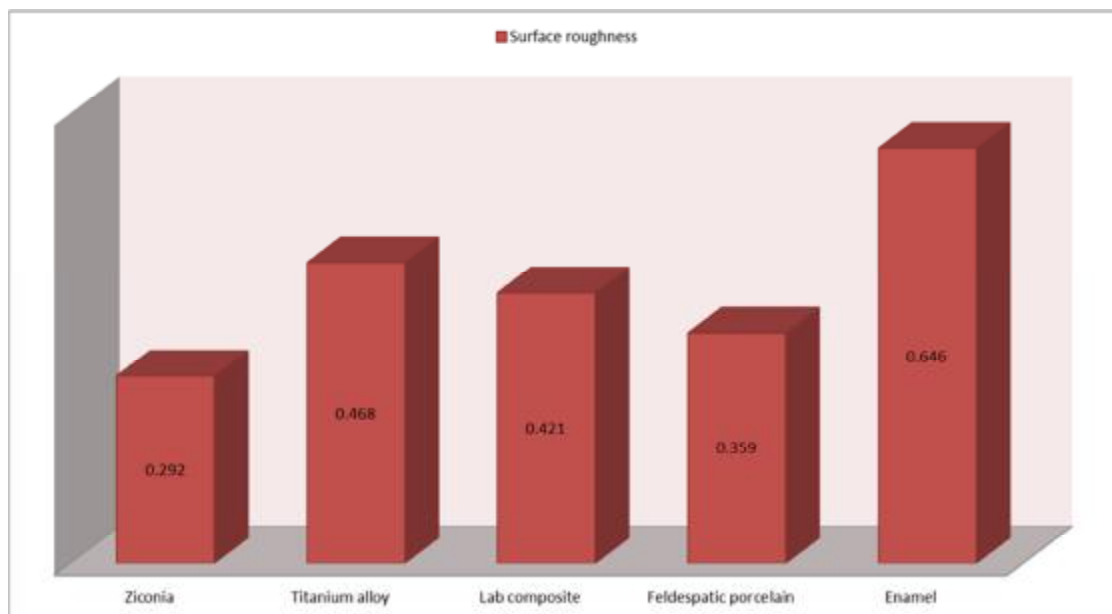**Figure 6:** Mean values of bacterial adhesion (colony counts)

Figure 7: Mean value of surface roughness (micrometer)

Discussion

Bacterial adhesion and biofilm formation on teeth and restorative materials plays an important role in pathogenesis of dental diseases. S. mutans is known as caries initiator and the pathogenesis of this bacteria is because of producing extracellular polysaccharides and lactic acids (10,11).

In this in vitro study, adhesion of streptococcus mutans was evaluated on zirconia, feldespatic porcelain, titanium alloy and indirect composite. Evaluated materials showed different surface roughness; however, all of them had roughness values lower than 1

micron which is considered clinically very smooth (12). No correlation was found between surface roughness and bacterial adhesion so other factor such as hydrophobicity or surface free energy of bacterium or oral surfaces, the ionic strength of the surrounding liquid medium and electrostatic interactions (7). Eick et al. also reported that there was no relationship between surface roughness and colony forming units of S. mutans in their in vitro study (5). Meier et al. showed that there is little Ra-related difference in bacterial adhesion between different ceramics (13). Lippo et al. also found no relation between surface roughness and S. mutans adhesion in their in vitro study (2).

Adhesion of S. mutans was significantly different between studied materials with Indirect composite showing the highest and the zirconia samples the lowest. However, bacterial adhesion on all samples was comparable to enamel (p>0.05). Byung et al. reported higher adhesion of S. mutans to composites compared with zirconia and titanium which is in agreement with present study (3). Rosentritt et al. evaluated the adhesion of S. mutans on different brands of composite resin, ceramics and some metal alloy and reported that high bacterial adhesion on some composite samples and the lowest adhesion was observed on metal alloys and ceramics showed intermediate values; these findings are in agreement with our study (14).

Antonio et al. conducted an in vitro study about the bacterial adhesion on zirconia and pure titanium, and concluded that the surface covered by bacteria was significantly higher in titanium samples which is similar to our results (15).

Byung et al. reported different results which may be because of different techniques used in two studies. In contrast to our study, they evaluated the bacterial adhesion on pure titanium but our samples were titanium alloy (3).

Rosentritt conducted an study about S. mutans adhesion on zirconia core and veneering ceramic and reported that there was only little difference between them which is in agreement with our results (16). Ssurface roughness and surface free energy are among the most important factors which may influence the bacterial adhesion (17-18). Some studies have demonstrated that the higher surface roughness values may enhance plaque formation (18). In this study, no correlation was found between surface roughness and bacterial adhesion in spite of different degrees of surface roughness as previously mentioned. The composites samples showed the highest adhesion whereas their roughness value was intermediate, and although the surface roughness of enamel was the highest; its adhesion values were intermediate. This is indicative of other important influencing factors on bacterial adhesion other than surface roughness.

Studies have shown that the surfaces with higher surface free energy enhance bacterial adhesion (18). Also, the bacteria with high surface energy will probably adhere more to surfaces with high surface energy (19). S. mutans shows less hydrophobicity compared to S. sanguis and S. oralis and adhere more to hydrophilic surfaces like composite resins (20). clinically, this increased bacterial adhesion on composite surfaces may lead to lower survival rate for composite restorations compared to ceramics or metal alloys (20-22).

In this study, enamel was used as reference which allows better comparison between materials. Very few studied have used enamel as reference (2,15).

According to this study, all materials showed comparable adhesion with enamel although ceramic samples showed less bacterial

adhesion and composites revealed higher adhesion; however, this difference was not statistically significant. This study suggests that all studied materials may be used in clinical situation when proper case selection is adopted and if the materials are highly polished. Nevertheless, because of lower bacterial adhesion, the use of dental ceramics including zirconia and feldespatic porcelain is a better choice in comparison to composite resins in patients with poor oral hygiene and who are susceptible to periodontal disease.

A limitation of this in-vitro study is evaluating one type of oral bacteria and using static technique. Therefore, further studies are needed to focus on other bacteria including P. gingivalis or S. sanguis and using dynamic techniques like flow chamber to simulate the oral environment.

Conclusion

Different restorative materials have different surface roughness and different bacterial adhesion. Zirconia has the lowest adhesion among the tested materials and composite the highest; yet no correlation was found between surface roughness and bacterial adhesion.

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