The purpose of this study was to investigate the effects of various titanium and zirconia polishing protocols on the colonization of oral bacteria. Titanium and zirconia discs were divided into five groups: unpolished (control, UNP) and polished with Brownie only (BRO), Brownie plus Greenie (BPG), Brownie plus Greenie plus Supergreenie (BGS), and CeraMaster Coarse plus CeraMaster polishing tips (CER). The samples were sterilized and immersed in unstimulated saliva, then incubated in a liquid suspension of Streptococcus gordonii (S gordonii). The number of attached bacteria were counted 48 hours after the diluted suspensions were inoculated. Data were analyzed with ANOVA and Tukey test (P < .05). For titanium discs, the average number of bacteria from each group (CFU/mm²) was 1.51 x 10³ for UNP; 3.71 x 10³ for BRO; 5.65 x 10³ for BPG; 8.99 x 10² for BGS; and 8.49 x 10² for CER. For zirconia, the averages were 2.87 x 10² for UNP; 3.16 x 10² for BRO; 3.50 x 10² for BPG; 1.83 x 10² for BGS; and 8.73 x 10¹ for CER. Inadequate polishing roughens surfaces and promotes microbial adhesion to titanium and zirconia. Sequential polishing to the finest-finish polishing tips minimizes bacterial adherence to abutment surfaces. Zirconia exhibited less bacterial adhesion than titanium.

References:
1. Department of Preventive and Restorative Sciences, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania, USA.
2. Private Practice, Philadelphia, Pennsylvania, USA.
3. Department of Prosthetic Dentistry, Faculty of Dentistry, Istanbul University, Istanbul, Turkey.
4. Professor Emeritus, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania, USA.

Correspondence to: Dr Eva Anadioti, WELNOX Studio, 1512 Sansom st, suite 200, Philadelphia, PA 19102, USA. Email: eva@welnoxstudio.com

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has high strength, compression resistance, and corrosion resistance. Implant-abutment surfaces are prone to biofilm formation due to their supra- and submucosal locations. Therefore, a surface configuration that limits bacterial adhesion and reduces plaque accumulation is highly desirable. Metal alloys typically accumulate thick biofilms that have low viability, while ceramic materials form thin biofilms that have high viability.

The surface roughness of abutment materials has a major impact on the retention of oral microorganisms. Several studies have shown that an increase in surface roughness facilitates enhanced bacterial adhesion and biofilm formation by providing sheltered locations within surface irregularities and increasing the surface area for better adhesion. Conversely, smoother surfaces generally display reduced bacterial adhesion. It is important to reduce the number of initial adhering bacteria to prevent late colonization by pathogens that may ultimately cause implant-associated complications such as peri-implantitis, a major cause of implant failure. Surface modification by polishing is a simple and economic way to reduce biofilm formation on implant abutment materials.

The literature is still not clear on the possible effects of different abutment materials and connections on biofilm formation and maturation. Laboratory and clinical studies have shown conflicting results, mainly due to different experimental models and assessment methods. Previous studies regarding biofilm formation on abutment materials predominantly addressed the impact of titanium surface roughness on biofilm formation rather than the impact of the abutment material itself. Studies on biofilm formation on zirconia as an implant abutment material are scarce. Also, the effect of polishing the abutment material on biofilm formation was not thoroughly investigated. Therefore, the aim of this study was to investigate the effects of various polishing protocols on biofilm formation by the oral bacterium S. gordonii on titanium and zirconia. The null hypothesis was that polished abutment surfaces have decreased bacterial adhesion compared to that of unpolished surfaces.

Materials and Methods

Sample Preparation

For preparation of the titanium samples, a standardized disc with a 6-mm diameter and 1-mm thickness was machine-cut. This was duplicated with polyvinyl siloxane putty (Aquasil Ultra, Dentsply Sirona) to make an index. Standardized disc wax patterns were made by pouring inlay wax into the putty index. A total of 30 specimens were cast in a semi-automatic titanium-casting machine (Titec F210M, Orotig) according to the manufacturer’s instructions. For preparation of zirconia samples, 30 discs were fabricated from non–hot isostatic procedure Cercion blocks (DeguDent) and sintered to full density in a furnace according to manufacturer’s instructions.

Sample Groups and Treatment

The titanium and zirconia sample surfaces were finished with a handpiece (MASTERmatic LUX M25, Kavo) and a brown stone point (Dentaurum) at a speed ranging from 15,000 to 20,000 rpm to obtain a standardized surface.

Polishing was done with the handpiece (speed ranging from 2,000 to 5,000 rpm) for 60 seconds under water. The movement of the disc was unidirectional and held parallel to the specimen in the horizontal plane. Zirconia and titanium samples were divided into five groups for different polishing applications (n = 6 sample per material per group): unpolished (control); group UNP: only ground with 400-grit silicon carbide sandpaper), polished with Brownie (Shofu Dental; group BRO), Brownie + Greenie (Shofu Dental; group BPG), Brownie + Greenie + Supergreenie (Shofu Dental; group BGS), and CeraMaster Coarse + CeraMaster (Shofu Dental; group CER). Brownie is the most coarse finish, while Supergreenie and CeraMaster are the finest finish. All polishing procedures were carried out by a single investigator (Y.M.) with similar polishing pressure and the exact same duration, following a strict timeline and protocol.

Saliva Preparation

Unstimulated whole saliva was collected in a chilled tube immediately prior to the experiments by expectoration from a single healthy
25-year-old man who refrained from ingestion and oral hygiene for at least 2 hours prior. Saliva was sterilized via single-use filtration devices immediately before the experiments (Vacuflo, Whatman Schleicher & Schüll Microscience; 0.45-µm and 0.22-µm pore sizes, successively).

Biofilm Formation

Titanium and zirconia disc samples were placed in 24-well tissue culture plates. Plates were sterilized by ultraviolet irradiation for 1 hour prior to the addition of the saliva. Samples were immersed in filtered, unstimulated saliva to achieve pellicle formation on the surfaces for 1 hour at 37ºC.

*S. gordonii* ATCC 10558 was used in this study. Treated discs were removed from the saliva incubation and added to a culture of *S. gordonii* in Brain-Heart Infusion (BHI; BD) medium that had been adjusted to an optical density of approximately 4 × 10⁸ cells/mL by use of a spectrometer (Gene-sys 10UV UV-Vis, Thermo Fisher Scientific). The discs were then incubated for 1.5 hours at 37ºC in anaerobic conditions. The discs were then rinsed with distilled water for 3 seconds, then sonicated for 5 minutes in BHI broth to detach bacteria that adhered to the material surface.

The sonicates were serially diluted and spread on BHI agar to determine the number of colony-forming units (CFUs). The agar plates were incubated for 48 hours before counting CFUs. The average number of CFUs, adjusted for the surface area of the samples, was calculated.

Statistical Analysis

All statistical analyses were performed using standard software (SPSS, version 21.0 for Windows, IBM). The level of significance was set to .05. One-way analysis of variance was used to evaluate the polishing procedures and the average CFUs from the treated titanium and zirconia abutment materials. Tukey honest significant difference post hoc test was employed to highlight significant differences where appropriate.

Results

The bacterial adherence results for titanium and zirconia samples are shown in Tables 1 and 2. The average CFUs counted (calculated from six samples in each group) is listed for UNP, BRO, BPG, BGS, and CER groups. The graphs in Figs 1 and 2 depict the average CFU/mm² of each study group.

The number of bacteria that attached to material surfaces decreased significantly (*P* < .05) when surfaces were polished with the finest-grain polishing tips (Super-greenie and CeraMaster) compared to surfaces polished with coarser-grain silicon polishing tips (such as Brownie and Greenie).

When the materials for each polishing group were compared, zirconia had significantly lower (*P* < .05) bacterial adherence than titanium in each polishing group. The zirconia group with the lowest average (SD) CFU/sample counts of *S. gordonii* was the CER group, with a value of 7.04 x 10³ (4.50 x 10³). In comparison, the titanium group with the lowest average (SD) CFU/sample counts of *S. gordonii* was the CER group, with a value of 2.86 x 10⁵ (1.07 x 10⁶).

Discussion

The results of this study partially reject the hypothesis. Bacterial adherence to material surfaces polished to the finest finish, Super-greenie and CeraMaster, was decreased compared to unpolished surfaces. However, samples polished with a coarser finish (Brownie and Greenie) displayed higher biofilm accumulation than unpolished samples.

Oral biofilm formation is a highly complex process. Results in the laboratory may not be completely extrapolated to a clinical setting. To keep the experimental conditions reproducible, saliva from a single healthy donor was used rather than pooled saliva from a group of donors. The submersion time to saliva was 1 hour based on results from a previously published study that showed an increase in enamel pellicle thickness for ~1 hour, after which the rate of pellicle formation leveled off.²¹

Although previous studies evaluated bacterial adherence and biofilm accumulation on implant abutments,¹⁵,¹⁶ the effect and
Table 1  Titanium Discs: Average Number of Viable S. gordonii Recovered from Each Study Group

<table>
<thead>
<tr>
<th>Sample group</th>
<th>Average (SD) CFU/sample</th>
<th>Average (SD) CFU/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNP</td>
<td>$5.08 \times 10^5$ (1.69 $\times 10^5$)α</td>
<td>$1.51 \times 10^4$ (5.02 $\times 10^3$)α</td>
</tr>
<tr>
<td>BRO</td>
<td>$1.25 \times 10^6$ (3.08 $\times 10^5$)β</td>
<td>$3.71 \times 10^3$ (9.13 $\times 10^2$)β</td>
</tr>
<tr>
<td>BPG</td>
<td>$1.90 \times 10^6$ (6.12 $\times 10^5$)β</td>
<td>$5.65 \times 10^3$ (1.81 $\times 10^3$)β</td>
</tr>
<tr>
<td>BGS</td>
<td>$3.02 \times 10^6$ (9.34 $\times 10^5$)β</td>
<td>$8.99 \times 10^3$ (2.76 $\times 10^3$)β</td>
</tr>
<tr>
<td>CER</td>
<td>$2.86 \times 10^5$ (1.07 $\times 10^5$)γ</td>
<td>$8.49 \times 10^2$ (3.14 $\times 10^2$)γ</td>
</tr>
</tbody>
</table>

CFU = colony-forming units; UNP = unpolished (control); BRO = Brownie polishing; BPG = Brownie + Greenie polishing; BGS = Brownie + Greenie + Supergreenie polishing; CER = CeraMaster Coarse + CeraMaster polishing.

Same letters indicate no significant differences between those groups (P > .05).

Table 2  Zirconia Discs: Average Number of Viable S. gordonii Recovered from Each Study Group

<table>
<thead>
<tr>
<th>Sample group</th>
<th>Average (SD) CFU/sample</th>
<th>Average (SD) CFU/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNP</td>
<td>$2.31 \times 10^4$ (1.50 $\times 10^4$)α</td>
<td>$2.87 \times 10^3$ (1.84 $\times 10^3$)α</td>
</tr>
<tr>
<td>BRO</td>
<td>$2.52 \times 10^4$ (1.63 $\times 10^4$)β</td>
<td>$3.16 \times 10^3$ (2.13 $\times 10^3$)β</td>
</tr>
<tr>
<td>BPG</td>
<td>$2.79 \times 10^4$ (1.46 $\times 10^4$)β</td>
<td>$3.50 \times 10^3$ (1.92 $\times 10^3$)β</td>
</tr>
<tr>
<td>BGS</td>
<td>$1.47 \times 10^4$ (6.12 $\times 10^3$)γ</td>
<td>$1.83 \times 10^3$ (7.28 $\times 10^2$)γ</td>
</tr>
<tr>
<td>CER</td>
<td>$7.04 \times 10^3$ (4.50 $\times 10^3$)δ</td>
<td>$8.73 \times 10^2$ (5.35 $\times 10^2$)δ</td>
</tr>
</tbody>
</table>

CFU = colony-forming units; UNP = unpolished (control); BRO = Brownie polishing; BPG = Brownie + Greenie polishing; BGS = Brownie + Greenie + Supergreenie polishing; CER = CeraMaster Coarse + CeraMaster polishing.

Fig 1  Average CFU/mm² per group for the titanium discs. Same letters indicate no significant differences between those groups (P > .05). UNP = unpolished (control); BRO = Brownie polishing; BPG = Brownie + Greenie polishing; BGS = Brownie + Greenie + Supergreenie polishing; CER = CeraMaster Coarse + CeraMaster polishing.

Fig 2  Average CFU/mm² per group for the zirconia discs. Same letters indicate no significant differences between those groups (P > .05). UNP = unpolished (control); BRO = Brownie polishing; BPG = Brownie + Greenie polishing; BGS = Brownie + Greenie + Supergreenie polishing; CER = CeraMaster Coarse + CeraMaster polishing.
comparison of different polishing procedures on abutment materials were limited.\textsuperscript{18} In the present study, polishing with only Brownie or Brownie + Greenie actually increased bacterial adhesion compared to the unpolished samples for both abutment materials. These results indicate that poor or insufficient polishing can have detrimental effects and unintentionally cause roughening, therefore promoting microbial adhesion. Polishing with the finest-grain polishing tips (Supergreenie or CeraMaster) showed the most dramatic decrease in bacterial adhesion, correlated with the smoothest surface finish. This finding is in accordance with a recent study that evaluated the effect of polishing protocols of zirconia on biofilm formation.\textsuperscript{20} In that study, the specimens were subdivided into four groups based on the surface treatment protocol used: coarse finishing alone, coarse finishing and medium polishing, coarse finishing and fine polishing, and coarse finishing with medium and fine polishing. The results showed that omission of a polishing step significantly influenced the surface roughness and recommended sequential application of polishing steps.

The present study confirms the findings of previous studies\textsuperscript{9,22} that demonstrated differences in biofilm formation on titanium and zirconia materials. Although others have reported no differences in biofilm formation between the material surfaces,\textsuperscript{23,24} the present study found that bacterial attachment to zirconia was significantly lower than titanium, regardless of the polishing procedure. This is in accordance with clinical and in vitro studies that reported less biofilm accumulation and less pronounced inflammation around zirconia abutments.\textsuperscript{6,25–27} Contrary to the present results, a recent clinical study found that the zirconia abutment with surface roughness ($Ra = 0.74 \mu m$) harbored significantly higher total bacterial cell counts than the titanium/abutment ($Ra = 0.16 \mu m$) for both early and mature biofilms.\textsuperscript{17} It has been reported that the chemical composition and surface characteristics of the different substrates used for abutment and implant components directly affect microorganism adhesion and oral biofilm maturity.\textsuperscript{28,29}

Both titanium and zirconia abutments exhibit surface scratches and pits created during manufacturing. For titanium, polishing with burs has been shown to remove these defects and provide an excellent finish.\textsuperscript{18} For ceramics, and zirconia in particular, there are two options to reach a smooth surface: glazing or polishing. The limitation with glazing is that it has been shown to wear out after a few months.\textsuperscript{30} Additionally, if intraoperative adjustments are required, those will inadvertently remove the glaze and leave a rough surface. Therefore, the correct polishing technique and sequence is valuable, as it can provide long-lasting results as a replacement for glazing\textsuperscript{9} or when adjustments are made.\textsuperscript{31,32}

When comparing the two groups with the finest finish within each material, the present study showed that there is no significant difference between the Supergreenie and CeraMaster sequential polishes for the titanium group. This suggests that either protocol is adequate for clinical use when titanium abutments are placed. However, in the zirconia groups, the CeraMaster protocol showed significantly less bacterial adhesion than the Supergreenie protocol. Therefore, it is recommended to use the CeraMaster burs, sequentially, for zirconia implant abutments.

The present study provides additional information on simple polishing procedures that seem to reduce initial adhesion of bacteria to surfaces of implant abutment materials. The limitations of this study include the small number of samples in each study group and the in vitro conditions that do not account for the impact of a real oral environment. Therefore, the results of the study cannot be generalized without any interaction with other microorganisms of the oral cavity. An additional limitation of the study is the use of casted titanium, which was selected to consistently achieve a specific specimen size in order to precisely count the CFUs. Future studies are recommended to further analyze topographic differences between various abutment materials and to correlate their findings to differences in biofilm formation. In addition, clinical studies are needed to correlate the effects of biofilm formation on abutment materials treated in the laboratory on inhibiting peri-implantitis in vivo.
Conclusions

Inadequate polishing roughens surfaces and promotes microbial adhesion to titanium and zirconia. Sequential polishing to the finest-finish polishing tips minimizes bacterial adherence to abutment surfaces. Zirconia displays less bacterial adhesion than titanium. However, this in vitro study did not simulate the real conditions of the oral environment, including interactions with other microorganisms. Therefore, more clinical studies are needed with respect to other oral bacteria and their effects on the results.

Acknowledgments

The authors declare no conflicts of interest.

References


