Short Communication

SEM Evaluation of In Situ Early Bacterial Colonization on a Y-TZP Ceramic: A Pilot Study

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This study aimed to evaluate the effect of surface glazing and polishing of yttrium-stabilized tetragonal zirconia polycrystal ceramic on early dental biofilm formation, as well as the effect of brushing on the removal of adhered bacteria. Two subjects used oral appliances with polished and glazed samples fixed to the right and left sides. After 20 minutes, 1 hour, and 6 hours, the subjects manually brushed the samples on the right side. The samples were analyzed using scanning electron microscopy. Granular material was verified on the samples, especially on irregular surfaces. After 1 hour, there was no significant difference between glazed and polished surfaces in terms of bacterial presence. However, glazed surfaces tended to accumulate more biofilm, and brushing did not completely remove the biofilm. Polished surfaces seem to present a lower tendency for biofilm formation. *Int J Prosthodont 2007;20:419–422.*

Bacterial adhesion to restorative materials is of great interest since the role of bacteria in the etiology of caries and periodontitis is well established. The bacterial adhesion to substrate involves unspecific interactions (van der Waals forces, electrostatics, and hydrophobic interactions) and specific interactions (bacterial adhesins and complementary sites in the substrate surface). Some oral bacteria can synthesize water-insoluble glucans from dietary carbohydrates.[1]

In vivo and in vitro studies have demonstrated differences in biofilm formation between dental materials.[2,3,4] These variations can be the consequence of material properties such as surface free energy and surface roughness.[1] The ceramic surface finishing and polishing method significantly affects the surface roughness and consequently the biofilm formation on the surface. However, to the authors' knowledge, no study has investigated the influence of the glazing/polishing procedures of yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramic[5] on early biofilm formation. Thus, the purpose of this pilot study was to evaluate the effect of surface type of Y-TZP ceramic on early biofilm formation, and to verify the effect of brushing in the removal of adhered bacteria on surface ceramic. The hypotheses were that glazed surfaces accumulate fewer microorganisms than polished surfaces, and that brushing reduces bacterial deposits.

Materials and Methods

Subjects

Two subjects (30 and 35 years of age) who were nonsmokers and showed appropriate oral hygiene with no signs of caries or periodontitis were selected. These subjects did not use any antibiotics during the previous 3 months. Informed consent was obtained from each participant.

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Table 1 Experimental Groups in the Study

Group/surface type†	No. of samples
Control*	
Glazed	2
Polished	2
Brushing	
Glazed	12
Polished	12
Nonbrushing	
Glazed	12
Polished	12

*SEM analysis of the topography of the ceramic surface.
†Glazing was performed with a liquid (Glaze G-4040, 3M ESPE) indicated for LAVA-Ceram ceramic (feldspar veneered ceramic); mechanical polishing was performed with a 3-step silicon rubber wheel (Silishine LC set, Odis) (10 seconds for each step).

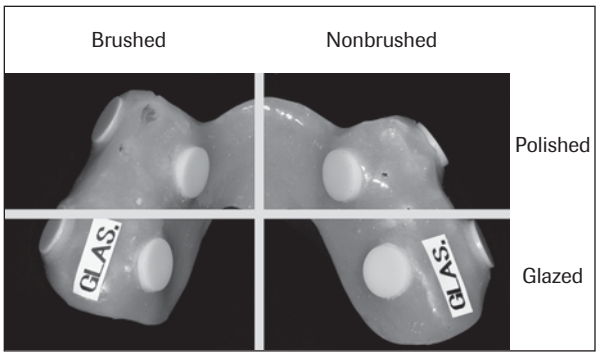


Fig 1 Study design: mesial (polished samples) and distal (glazed samples); right side (brushed samples) and left side (nonbrushed samples).

Table 2 Scores Verified Considering Brushing and Type of Ceramic Surface*

	Surface type		
Brushing	Polished	Glazed	Total
Brushed	1^{a}	1^{c}	2^{A}
Nonbrushed	$3^{a,c}$	$12^{b,c}$	15^{B}
Total	4^{A}	13^{B}	

*Different lowercase letters indicate statistical difference; same lowercase letters indicate no statistical difference. Different uppercase letters indicate statistical difference ($P = .0392$); same uppercase letters indicate no statistical difference ($P = .1019$).

Sample Preparation and Experimental Groups

Fifty-two disk-shaped samples machined from blocks of Y-TZP using the LAVA System (3M ESPE) were prepared (diameter: 5 mm; height: 1.5 mm) and divided into several experimental groups (Table 1) .

Study Design

The split-mouth study design is shown in Fig 1. The samples were fixed on buccal and palatal surfaces of individual oral appliances made of a light-cured resin (Elite LC Tray, Zhermack). Prior to the experimental trial, each participant performed oral hygiene maintenance of his own teeth using a toothbrush and dental floss without toothpaste. No food intake was permitted during the experimental period. After 20 minutes, the appliances were removed and the participants manually brushed the right side of the appliances (buccal and palatal surfaces). This procedure was carried out with a toothbrush (Meridol, GABA International) without toothpaste for 15 seconds with normal pressure to simulate conventional hygiene procedures. The samples were then removed and prepared[2] for scanning electron microscopy (SEM) (Jeol 5400). The same procedures were repeated at 1 hour and 6 hours.

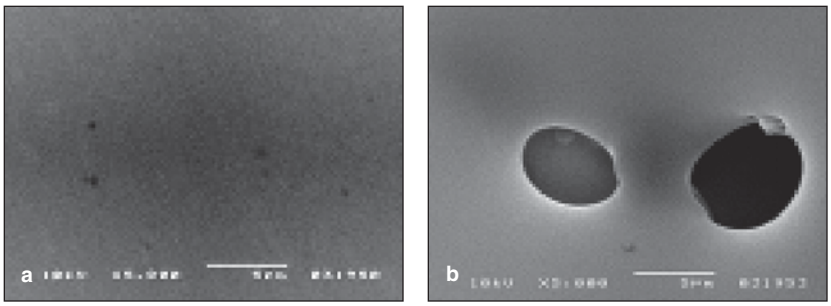
SEM Analysis

For the samples evaluated at 1 hour, 2 analyses were carried out: *(1)* the whole ceramic surface was scanned to perform a descriptive analysis of the coated materials, and *(2)* an area measuring 100 × 125 μm was examined for each sample. Five fields measuring 20 × 25 μm each were selected. A central point was established (field 1), and from this point, 4 fields were defined at a distance of approximately 1.5 mm from this central point. For each field, the presence or absence of bacteria was recorded (presence = 1 and absence = 0).[2] The values computed for each total area represented the cumulative sum of each field.[2] With this methodology the same relative positions were used for analyzing the different samples. At 20 minutes and 6 hours, only a descriptive analysis of the coated materials was carried out.

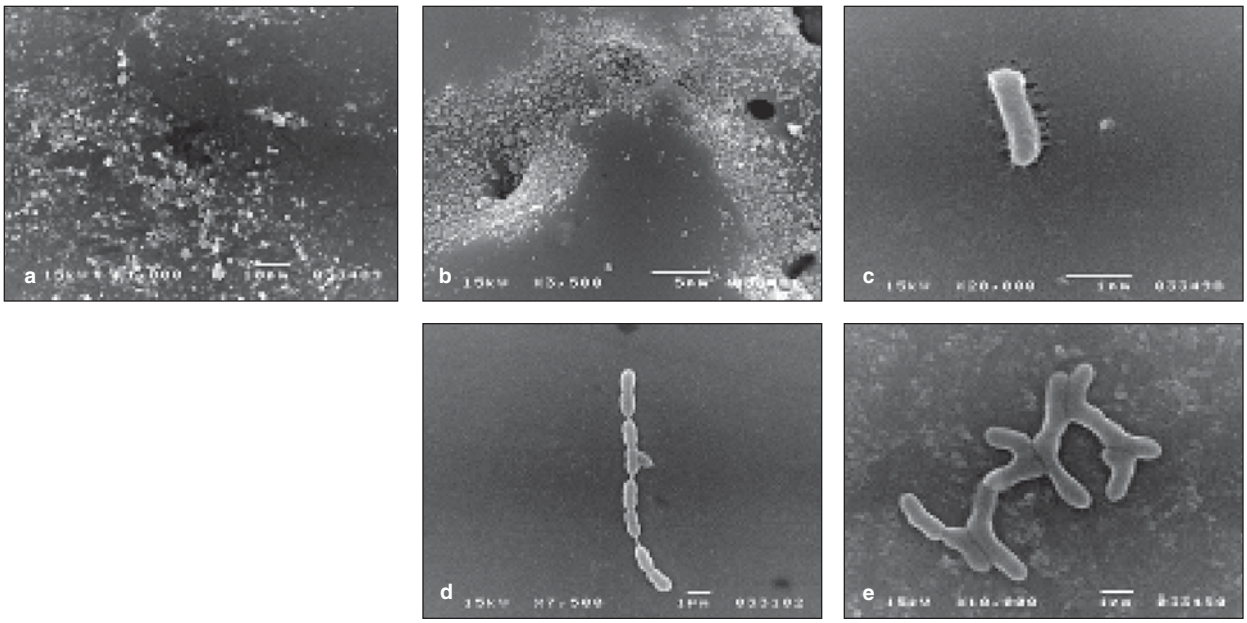
Results

Statistical Analysis at 1 Hour

Kruskal-Wallis analysis of variance was used ($\alpha = .05$) (Table 2). Brushing significantly decreased the bacterial deposits compared to the nonbrushed samples ($P = .0392$). No significant difference was observed between the brushed and nonbrushed samples ($P = .8665$) when considering only the polished surface. On the glazed surface, brushing significantly decreased the presence of adhered bacteria ($P = .0084$). The surface type was not statistically significant ($P = .1019$). There was no significant difference between the glazed and polished surfaces in the brushed ($P = 1.0$) and nonbrushed groups ($P = .0686$). Thus, the hypotheses were partially accepted.



Figs 2a and 2b Topographic analysis (×5,000). **(a)** Polished Y-TZP ceramic shows a densely compacted surface without biofilm; **(b)** glazed Y-TZP ceramic shows a porous ceramic surface without biofilm.



Figs 3a to 3e SEM analysis of bacterial adherence. **(a)** Polished sample after 20 minutes (×1,000): uniform distribution of the granular material. **(b)** Glazed sample after 20 minutes (×3,500): intense deposition of the granular material around the irregularities. **(c)** Glazed sample after 1 hour (×20,000): rod-shaped adherence through fimbriae. **(d)** Polished sample after 1 hour (×7,500): presence of *Streptococcus*. **(e)** Polished sample after 6 hours (×10,000): aggregates of the rod-shaped organisms.

Descriptive SEM Analysis

The glazed samples presented irregularities similar to pores, indicating a rough surface. The polished samples presented a smoother surface (Figs 2a and 2b).

At 20 minutes, a granular material was distributed evenly on the polished surfaces (Fig 3a). However, on the glazed surfaces, this granular material was deposited more intensely around the irregularities (Fig 3b). The deposition was similar on the buccal and palatal sides. Some isolated bacteria were visualized. Brushing seemed to reduce the materials deposited.

The distribution of the granular material was similarly observed after 1 hour. The thickness of the deposited material was greater on the buccal sides. With brushing, the deposits from the smoother areas were removed; however, these deposits were not removed in the irregular areas.

At 6 hours, the entirety of each surface was densely coated with granular aggregates. However, these aggregates were thicker on the buccal sides. Brushing removed only part of these deposits, even in the smoother areas.

Intense bacterial colonization was not observed after 20 minutes, 1 hour, or 6 hours. Morphologically isolated aggregates of cocci and rods were observed (Figs 3c to 3e). Isolated spirochetes were verified in 1 subject. No difference in bacterial morphology was observed between the surfaces at the evaluated periods.

Discussion

No significant difference was found in the bacteria presence between glazed and polished samples. However, the glazed samples showed a tendency to accumulate more bacteria, probably because of the

irregularities, which contributed to quicker biofilm formation. Roughness favors adhesion because it increases the available area and promotes niches were bacteria are protected from shear forces.[1] This may explain why brushing was not as effective on the irregular surfaces. Brushing reduced but did not totally remove the biofilm. However, brushing remains essential in the prevention of caries and periodontal disease, because it impedes the biofilm maturation characterized by the presence of more virulent microorganisms.

Some studies have indicated that bacteria retention seems to depend on the substrate surface free energy, and that bacteria detachment seems to be the consequence of cohesive failure in the acquired pellicle, whose features are dependent on the type of substratum.[6,7] There is doubt whether this property can influence the bacterial retention when intense forces (brushing) are considered. Only a dental ceramic (Y-TZP) was evaluated in the present study. Theoretically, all samples presented the same surface free energy and bacterial retention, considering that no significant difference was observed as to the bacteria presence between the glazed and polished brushed samples of the same material.

Conclusions

1. The glazed and polished surfaces accumulated bacteria similarly. However, polished surfaces seemed to accumulate less biofilm than glazed surfaces.
2. Brushing significantly decreased the bacterial deposits but did not completely remove the biofilm.
3. Further clinical studies on this subject should be conducted.

References

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