

Confocal Laser Microscopic Analysis of Biofilm on Newer Feldspar Ceramic

AS Brentel • KZ Kantorski • LF Valandro
SB Fúcio • RM Puppini-Rontani • MA Bottino

Clinical Relevance

When the glazed surface of glass ceramics is submitted to chairside preparation, polishing with rubber points followed by felt disks impregnated with a fine-aluminum oxide particle appears to be a good option to polish the surface of the glass ceramic, since this finishing-polishing method results in biofilm formation similar to the glazed ceramic surface.

Aline Scalone Brentel, DDS, MSD, Department of Dental Materials and Prosthodontics, São José dos Campos Dental School, São Paulo State University, São José dos Campos, Brazil

*Karla Zanini Kantorski, DDS, MSD, PhD, Division of Periodontology, Department of Stomatology, Federal University of Santa Maria, Santa Maria, Brazil

Luiz Felipe Valandro, DDS, MSD, PhD, Division of Prosthodontics, Department of Restorative Dentistry, Federal University of Santa Maria, Santa Maria, Brazil

Suzana Beatriz Fúcio, DDS, PhD, Department of Dental Materials, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

Regina Maria Puppini-Rontani, prof, DDS, PhD, Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

Marco Antonio Bottino, prof, DDS, PhD, Department of Dental Materials and Prosthodontics, São José dos Campos Dental School, São Paulo State University, São José dos Campos, Brazil

*Reprint request: Rua Marechal Floriano, 1184, 97015-372, Santa Maria, Brazil; e-mail: kzkantorski@hotmail.com

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ABSTRACT

Purpose: This study evaluated the surface roughness, hydrophobicity and *in situ* dental biofilm associated with microfilled feldspar ceramics submitted to the different finishing and polishing procedures. **Methods and Materials:** Samples were made according to the manufacturer's instructions and allocated to groups as follows: glaze (G1); glaze and diamond bur (G2); glaze, diamond bur and rubber tips (G3) and glaze, diamond bur, rubber tips and felt disks impregnated with a fine-aluminum oxide particle based paste (G4). Roughness was evaluated with a roughness analyzer (R_a). Hydrophobicity was determined by the contact angle of deionized water on samples. Biofilm was evaluated eight hours after formation in the oral environment using confocal laser-scanning microscopy (CLSM) and scanning-electron microscopy (SEM). **Results:** Significant differences were found related to roughness ($G1 < G4 < G3 < G2$). G1 presented the highest hydrophobicity. G1 and G4 presented the lowest

mean thickness and biovolume of biofilm when compared to G2 and G3, both of which were similar. Isolated cocci were verified in G1 and G4 using SEM, while G2 and G3 presented bacteria aggregates. **Conclusion:** Polishing with rubber points followed by felt disks impregnated with a fine-aluminum oxide particle resulted in biofilm formation similar to that present with a glazed ceramic surface, even though the surface is still rougher and more hydrophobic.

INTRODUCTION

Dental technicians glaze veneering feldspar ceramics in metal-free and metal-ceramic restorations for further cementation by clinicians. However, chairside preparation of the veneering ceramic before or after cementation may be necessary, modifying/removing the glaze, increasing the roughness¹ and contributing to biofilm formation.²⁻⁴ Thus, chairside finishing/polishing of a veneering ceramic should be performed using the right materials and instruments to minimize the irregularities created by chairside instrumentation.^{1,5}

Several finishing and polishing methods have been suggested to restore the surface characteristics of glazed ceramic.⁶⁻¹¹ However, there is no available information about these methods when applied to newer microfilled feldspar ceramics. Also, there is no scientific evidence to advise clinicians regarding finishing-polishing procedures to minimize the biofilm formation on glass-ceramics.

Surface roughness²⁻³ and surface-free energy¹²⁻¹³ of restorative materials could influence the formation of biofilm. These two properties could perhaps be altered by finishing and polishing procedures, thereby promoting or inhibiting the formation of biofilm. Rough surfaces contribute to the adhesion of bacteria, since the roughness provides niches where bacteria can adhere and grow protected from brushing, muscular action and salivary flow.² High surface-free energy (hydrophilic) substrates present more biofilm than low surface-free energy (hydrophobic) substrates.^{2,12-13} This fact is not due to the promotion of bacterial adhesion on hydrophilic materials, but to the higher retention of biofilm on these substrates.^{2,12-13}

Some studies have evaluated the formation of *in situ* biofilm in restorative materials by using scanning electron microscopy^{3-4,14-16} and transmission electron microscopy.¹⁷⁻¹⁸ Recently, confocal laser microscopy has been employed for the same purpose.¹⁹⁻²³

Thus, the current study evaluated the effect of different finishing and polishing systems on the surface roughness and hydrophobicity of a microfilled feldspar ceramic, while also evaluating the *in situ* formation of biofilm. The clinical relevance of this study is to determine what chairside finishing and polishing method

can result in similar performance to the glazed surface in terms of biofilm formation.

METHODS AND MATERIALS

Preparation of Samples

Ninety-six standardized cylindrical samples of feldspar ceramic (VM7, Vita Zahnfabrik, Bad Sackingen, Germany) (5 mm in diameter and 2.5 mm thick) were made according to the manufacturer's specifications. The samples were produced using a metallic plate having calibrated holes with a 5.3 mm diameter to compensate for the sintering shrinkage of the ceramic (nearly 12%). The powder and liquid of the ceramic were mixed and placed into the receptacle. The samples were sintered in the Vacumat furnace (VITA Zahnfabrik), standardized with increasing grits of silicon-carbide abrasive paper (360, 600, 1200) (3M ESPE, St Paul, MN, USA) and ultra-sonically cleaned in distilled water for five minutes. The samples were then glazed using Vita Akzent 25 (VITA Zahnfabrik).

Experimental Groups

Samples were randomly divided into four groups according to finishing and polishing procedures: G1: no finishing was done and the glazed surfaces remained unaltered (control); G2: finishing with a #4138 coarse diamond bur (KG Sorensen, Barueri, SP, Brazil); G3: finishing with a #4138 coarse diamond bur and polishing with silicon rubber tips (KG Sorensen; batch #9232PM) and G4: finishing with a #4138 coarse diamond bur and polishing with silicon rubber tips and a felt disk impregnated with a fine-diamond particle based paste (Diamond Excel, KG Sorensen). One trained operator conducted each step of the finishing and polishing procedures using standardized circular movements for 10 seconds under distilled water.

After finishing and polishing, all of the samples were ultra-sonically cleaned for five minutes (Vitasonic, VITA Zahnfabrik) in distilled water.

Analysis of the Surface Roughness

Surface roughness quantitative analysis was performed for 10 specimens of each group using a roughness analyzer (Mitutoyo SJ-400, Tokyo, Japan). Four measurements were carried out for each specimen with the R_a (μm) parameter recorded for each measurement: 2 parallel and 2 perpendicular measurements (1 mm of distance between each measurement, cutoff value = 3 mm). A mean value was obtained from the four measurements. The R_a value indicates an average surface roughness value, based on the mean profile height above (peak) and below (hill) the central line.

Contact Angle Analysis

Three samples from each group were used to evaluate the contact angle measurement of deionized water.

One drop of deionized water was deposited on the smooth and flat surface of the ceramic samples using a syringe attached to a goniometer (Ramé-Hart-DROPimage, Advanced, Ramé-Hart Instrument Co, Netcong, NJ, USA). Contact angle (degree) was calculated with the Advanced Drop Shape Analysis software provided with the equipment. The software calculated mean and standard deviation from three measurements of contact angle for each sample.

Assessment of Early Dental Biofilm

Ten dental students with good oral health (without signs of dental caries or periodontal diseases) were selected. Exclusion criteria included the use of antibiotics or chlorhexidine three months prior to the study, tobacco use and xerostomia. Informed consent was obtained from the subjects.

For each participant, oral appliances were made using a light-cured resin (Elite LC Tray, Zhermack, Rovigo, Italy). These appliances covered the crowns of the maxillary premolars and molars. One sample from each group was mechanically fixed to the buccal surface of the right and left sides of the appliance. Samples attached to the right side were used for biofilm analysis with SEM, while samples attached to the left side were employed for biofilm analysis with CLSM. Biofilm analysis with CLSM was carried out with 10 specimens from each group, while SEM analysis was performed with five specimens per group. Prior to the experimental trials, each participant performed oral hygiene procedures using a toothbrush and dental floss without toothpaste. During the intraoral exposure of the samples (eight hours), no food or beverages were consumed.

Confocal Laser-Scanning Microscopy Evaluation (CLSM)

After eight hours, the samples were removed from the intra-oral device and stored in a sterile container with humid sterile cotton on its bottom (NaCl 0.9%). The containers were stored in a thermal box with ice (to maintain the temperature so that denaturation of bacterial cells was avoided) and taken to the microscope after no longer than one hour.

The samples were placed in sterile petri dishes and stained with the Live/Dead Bac Light Bacterial Viability and Counting kit (Molecular Probes, Eugene, OR, USA). This kit is composed of two nucleic acid-based fluorescent dyes: SYTO 9 and propidium iodide. SYTO 9 penetrates all bacterial membranes and stains the cells green, while propidium iodide only penetrates cells with damaged membranes, with the combination of the two stains producing red fluorescing cells. As recommended by the manufacturer, the dyes were diluted separately in sterile saline solution (NaCl 0.9%) in a plastic container at a proportion of 10 ml of saline solution to 4 µl of dye.²⁴

Using automatic pipettes (Pipetman P-Gilson SAS, Villiers le Bel, France) pre-calibrated to 10 ml and with a sterile point, one drop of each dye was applied to the surface of the biofilm formed on the sample. Pipette points were changed after each application. After application, each petri dish was closed and wrapped with aluminum foil to incubate the dyes for 15 minutes at room temperature under light protection.²⁵

The samples were then positioned on a glass slide, leaving the surface in contact with the slide. The CLSM (LSM 510 META, Carl Zeiss AG, Stuttgart, Germany) delivered light, with a wavelength of 488 nm to excite the dyes. Optical lenses were utilized with magnifications of 4x/0.13 to evaluate the mean thickness of biofilm and biovolume and 10x/0.3 to evaluate the biofilm structure.

COMSTAT²⁶ was used to quantify the biovolume and mean thickness of the biofilm. This is a program for quantification of three-dimensional biofilm structures. It analyzes stacks of images acquired with confocal laser-scanning microscopy. The program is menu controlled, user-friendly and requires no prior knowledge in programming or images analyses.²⁶

Biovolume was defined as the number of biomass pixels in all images of a stack, multiplied by the voxel size $\{(\text{pixel size})_x \times (\text{pixel size})_y \times (\text{pixel size})_z \}$ and divided by the substratum area of the image stack. The resulting value was biomass volume divided by substratum area ($\mu\text{m}^3/\mu\text{m}^2$).²⁶ The mean thickness of biofilm provided a measure of the space size of the biofilm and is the most common variable in biofilm literature.²⁶

COMSTAT evaluated the tridimensional images obtained by CLSM. An optical section with a magnification of 4x formed each analyzed image. This magnification was selected to cover the highest surface area of a sample to obtain representative values from each one. The number of optical sections varied between samples, according to the thickness of the formed biofilm.

Before COMSTAT analysis, an operator established a threshold value to each set of optical sections (or rather, to each sample). The threshold helped the program identify the presence or absence of biomass in each pixel from every image. Thus, the operator analyzed the sections of all samples and selected a threshold where only the biofilm could be quantified by the program, making the easily identified background (ceramic sample surface) turn into a black base.

Two tools of the COMSTAT program were selected to quantify the properties of the biofilm: mean thickness (μm) and biovolume ($\mu\text{m}^3/\mu\text{m}^2$). These tools are quantitative indicators of adaptation and maturation of biofilm on different surfaces.²⁶

SEM Evaluation

After the experimental procedures, all of the samples were removed and immediately fixed in a 4% glutaraldehyde solution in a 0.2 M sodium cacodylate buffer for 24 hours. The samples were then rinsed in 0.1 M buffered sodium cacodylate and dehydrated by serial transfers in various concentrations of ethylic alcohol (50%, 70%, 80%, 90%, 95%, and 100% for 30 minutes each). After 24 hours at room temperature, the specimens were mounted on metal stubs, coated with a layer of gold-palladium (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK) under continuous tilting and rotation of the vacuum coating unit. The scanning electron microscope (JEOL 5400, JEOL Ltd, Tokyo, Japan) was operated at 10 and 15 kV.^{4,16}

Scanning was accomplished on the whole surface of the specimen to obtain a descriptive analysis of the materials, such as the presence of isolated bacteria and/or bacterial aggregates, the bacterial morphology and the characteristics of the non-cellular material.

Morphology of Ceramic Surface

The surface morphology was evaluated in SEM with five specimens from each group that were not exposed to the oral environment.

Statistical Analysis

MINITAB (Minitab, version 14.12, 2004), STATISTICA (StatSoft, version 5.5, 2000) and STATISTIX (Analytical Software, version 8.0, 2003) were used for the statistical analysis.

The evaluated factors were polishing and finishing types at four levels according to groups G1, G2, G3 and G4.

Data from the surface roughness (μm) and contact angle (degree) results were submitted to descriptive statistics, one-way variance analysis and Tukey's test. Biovolume data ($\mu\text{m}^3/\mu\text{m}^2$) and mean thickness (μm) of the biofilm were submitted to descriptive statistics, RM variance analysis and the Tukey's test. The Pearson linear coefficient was used to verify if there was any correlation between the surface roughness

Figure 1: Representative images of formed biofilm (4x).

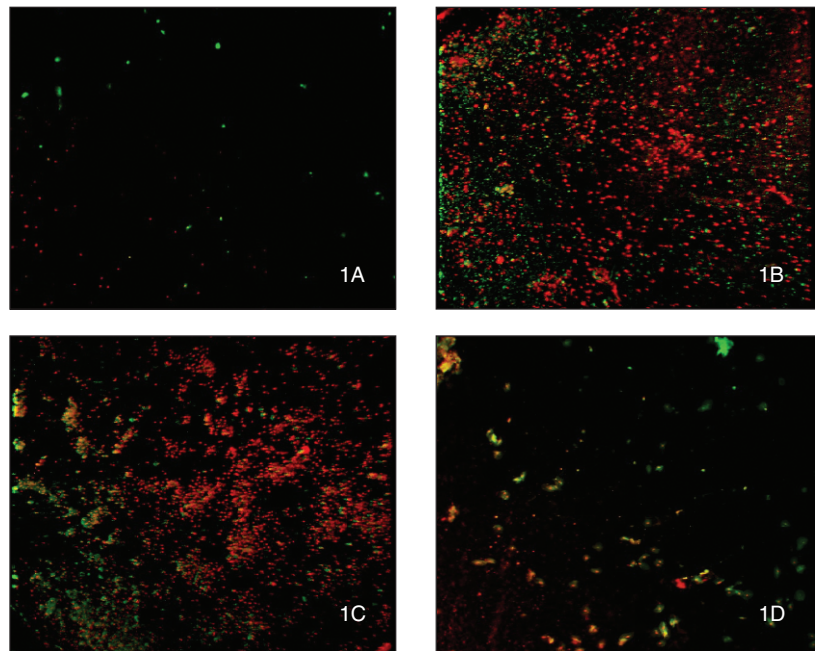


Figure 1A- Glaze (G1); Figure 1B- glaze + diamond bur (G2); Figure 1C- glaze + diamond bur + rubber tips (G3); Figure 1D- glaze + diamond bur + rubber tips + felt disks impregnated with a fine-aluminum oxide particle-based paste (G4).

(μm) and biovolume ($\mu\text{m}^3/\mu\text{m}^2$) and between the surface roughness (μm) and mean thickness (μm) of biofilm.

A significance level of 5% was used in all the statistical tests.

RESULTS

Surface Roughness and Hydrophobicity

One-way ANOVA demonstrated that finishing and polishing procedures significantly influenced the surface roughness (μm) ($p < 0.0001$). The Tukey's test (Table 1) demonstrated that the group submitted to only glaze treatment (G1) presented less surface roughness, while the group submitted to glaze and diamond bur (G2) presented the greatest roughness. All groups were different from each other.

One-way ANOVA observed that the contact angle was influenced by finishing and polishing procedures ($p < 0.0001$). The Tukey's test (Table 1) showed that the glazed ceramic surface (G1) presented a significantly higher contact angle than the other groups, all of which presented more hydrophobic surfaces.

Analysis of Initial *In Situ* Dental Biofilm in Confocal Laser Microscopy

Representative images of biofilm formed on the specimens are presented in Figure 1. In a descriptive analysis, the biofilm in G1 and G4 seemed to present

Table 1: Mean \pm Standard Deviation (SD) for Surface Roughness (μm) and Contact Angle (degrees)

Groups	Surface Roughness (Mean \pm SD)	Contact Angle (Mean \pm SD)
G1	0.53 \pm 0.11 ^a	107.66 \pm 1.25 ^a
G2	2.02 \pm 0.12 ^b	64.54 \pm 0.53 ^b
G3	1.27 \pm 0.14 ^c	73.74 \pm 3.74 ^b
G4	0.88 \pm 0.11 ^d	74.80 \pm 3.71 ^b

*Representation of the heterogeneous groups observed by the Tukey test ($\alpha = 0.05$). Different letters mean statistical difference. (Capital letters for surface roughness; lower letters for contact angle).

a lower quantity of bacterial cells, as it was possible to observe the ceramic surface. In G2, the biofilm seemed to completely cover the ceramic surface.

ANOVA verified that the finishing/polishing procedures significantly influenced the mean thickness ($p<0.0001$) and biovolume ($p<0.0001$). The Tukey's test (Table 2) demonstrated that groups 1 and 4 were similar to each other for mean thickness and biovolume, and groups 2 and 3 were similar to each other.

Correlation Between Surface Roughness Versus Biovolume and Mean Thickness

Pearson linear correlation showed a statistically significant positive correlation between the surface roughness and biovolume ($p<0.0001$) (Figure 2) and between the surface roughness and mean thickness of biofilm ($p<0.0001$) (Figure 3).

Analysis of *In Situ* Initial Dental Biofilm in SEM

Isolated cocci and diplococci with low acellular material were observed in G1 (Figure 4A). Cocci in chain and rods were observed in G2 and G3 (Figures 4B and 4C). Isolated cocci were verified in G4 (Figure 4D). The acellular material in the biofilm presented with granular characteristics in some samples and with fibrillar characteristics in other samples.

Surface Morphology

The glazed ceramic surface (G1) presented a uniform, smooth aspect. An irregular surface, characterized by peaks and hills, was observed in G2. G3 resulted in a more regular surface than was found in G2, while G4 was similar to G1 in some areas but with the persistence of some little irregularities (Figure 5).

DISCUSSION

Studies involving the adhesion of microorganisms to restorative materials are important, due to the role of biofilm in the pathogenesis of dental caries and periodontal disease. Studies applying different methods have shown differences in biofilm formed on different restorative materials.⁴ These variations can occur due to differences in the properties of materials, such as surface-free energy and surface roughness.^{2-3,13}

Analysis of the biofilm formed on different materials has been carried out through SEM.^{3-4,16} However, SEM analysis requires biofilm fixation and dehydration, factors that could alter its characteristics. With the advent of CLSM, biofilm can be evaluated without the necessity of fixation and dehydration, allowing the biofilm to keep its original architecture.²²⁻²³ CLSM pres-

Table 2: Mean \pm Standard Deviation (SD) and Coefficient of Variation (CV) for Mean Thickness (μm) and Bio-volume ($\mu\text{m}^3/\mu\text{m}^2$)

Groups	Mean Thickness (μm)		Bio-volume ($\mu\text{m}^3/\mu\text{m}^2$)	
	Mean \pm SD	CV (%)	Mean \pm SD	CV (%)
G1	20.3 \pm 20.6 ^A	101.5	25.1 \pm 30.5 ^a	121.7
G2	175.7 \pm 87.3 ^B	49.7	231.2 \pm 131.2 ^b	56.8
G3	166.0 \pm 80.3 ^B	48.4	223.1 \pm 126.2 ^b	56.8
G4	50.5 \pm 37.8 ^A	74.8	68.0 \pm 52.5 ^a	77.2

*Representation of the heterogeneous groups observed by the Tukey test ($\alpha=0.05$).

Different letters mean statistical differences. (Capital letters for mean thickness; lower letters represent bio-volume).

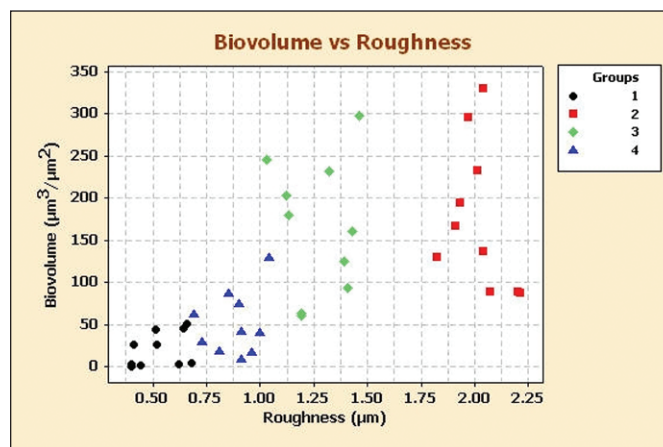


Figure 2. Graphic of the correlation between ceramic surface roughness (μm) and biovolume of the biofilm ($\mu\text{m}^3/\mu\text{m}^2$) for different groups.

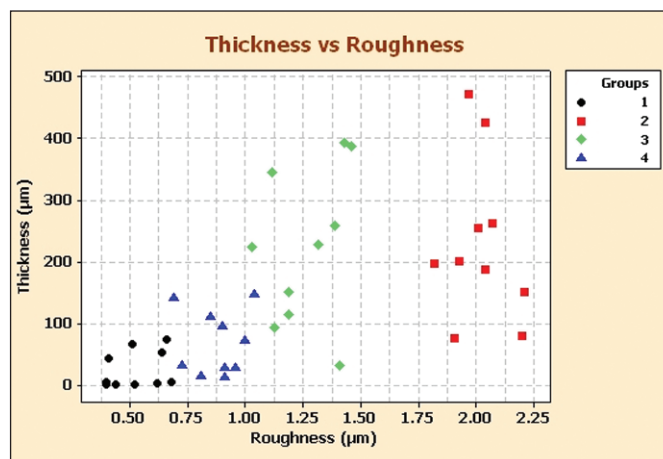


Figure 3. Graphic of the correlation between ceramic surface roughness (μm) and mean thickness of the biofilm (μm) for different groups.

ents non-invasive and non-destructive characteristics and is actually considered the tool of choice for study of *in situ* biofilm.²⁷⁻²⁸ Fluorescent dyes, such as the LIVE/DEAD kit (Bac light), have been used together with CLSM to describe the architecture of biofilm,²⁹ quantify the viability of microorganisms^{19,21,25} and evaluate the mean thickness^{23,26,29-30} and biovolume of biofilm.^{26,30-31} Quantitative data calculated using the COMSTAT program represents a way to obtain

Figure 4: Representative micrographs from the different groups.

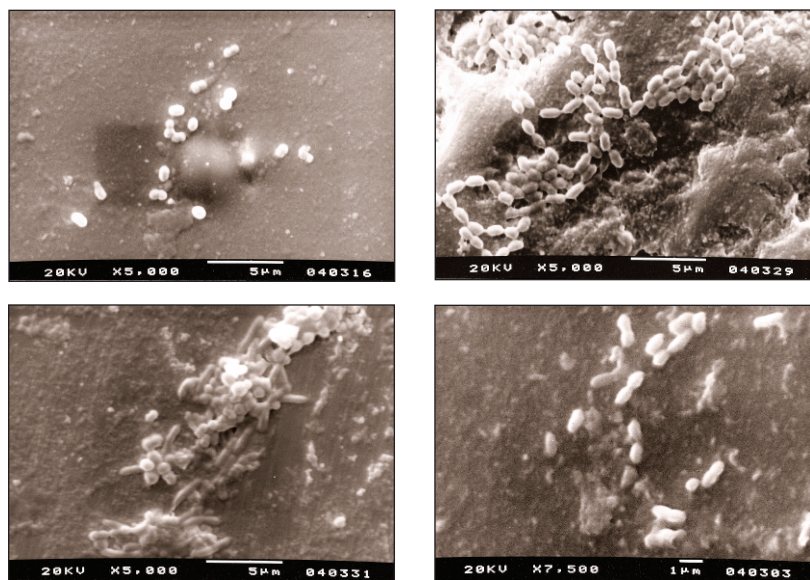


Figure 4A G1: presence of cocci; Figure 4B G2: presence of streptococci; Figure 4C G3: presence of cocci and aggregated rods; Figure 4D G4: presence of cocci.

Figure 5: Representative micrographs of the ceramic surface (VM7) (750x).

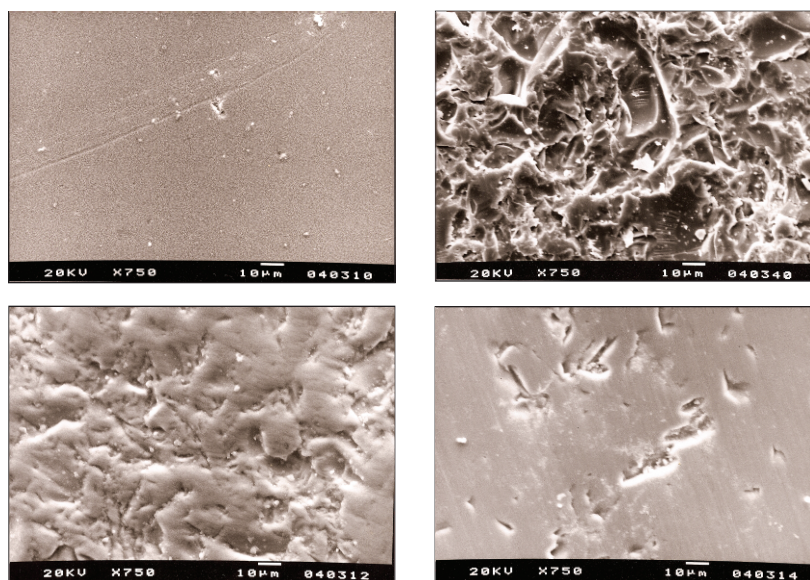


Figure 5A G1; Figure 5B G2; Figure 5C G3; Figure 5D G4.

numeric information from biofilm, allowing for comparisons.²⁶ Among the tools offered by COMSTAT, the current study used mean thickness and volume of bacterial biofilm, the most commonly used tools for evaluating biofilm according to the literature.^{26,31}

In the current study, the glazed ceramic surface submitted to wear with a diamond bur (G2) presented the highest surface roughness values. When the ceramic surface was treated with a bur and polished with rubber tips (G3), roughness decreased significantly. This

reduction was even more significant after polishing with a rubber tip and felt disk (G4). These results indicate that, when the adjustment of feldspar ceramics after cementation is necessary, it is best to perform polishing to reduce the roughness caused by bur preparation.

Previous studies mentioned that the use of only a diamond bur to polish ceramics was not enough to restore the surface smoothness initially obtained with glaze.^{6,9} Other authors demonstrated that feldspar ceramics prepared with a diamond bur (60 μm of granulation), and subsequent polishing with a diamond bur (1 μm to 4 μm of granulation) and diamond paste, reached surface roughnesses similar to a glazed ceramic.¹⁰

In alumina reinforced feldspar ceramic, finishing with a diamond bur and polishing with rubber tips and felt disks impregnated with a fine-aluminum oxide particle-based paste resulted in a surface roughness similar to that obtained with glaze.¹¹ However, the current study indicated that the microfilled feldspar ceramic surface that received the same procedures of finishing and polishing was rougher than a glazed surface. The manufacturer of the ceramic evaluated in the current study indicated that the microstructure of the ceramic presents a more homogeneous distribution of the glassy phase, allowing for a smooth and homogeneous surface. The microstructural characteristics, such as size and shape of crystals,³² could explain the differences between the two types of ceramics.

In the surface morphology analysis using SEM, the current study indicated that polishing with a rubber tip after using a diamond bur was not enough to reproduce surface characteristics similar to glaze. However, adding the use of a felt disk impregnated with a fine-aluminum oxide particle-based paste promoted a more uniform surface, although this smoother surface did not reach the smoothness of the glaze, due to the permanence of some irregularities produced by the diamond bur.

The current study observed interesting findings related to the influence of differing roughness in the formation of biofilm. There was a significant difference between the glazed ceramic + treatment with a bur (G2) and ceramic treated with a bur + polished with a rubber tip (G3) related to surface roughness. However, the extent of this difference did not influence the formation of biofilm, since no differences were verified in biovolume and mean thickness of biofilm. The same relation was verified between glazed ceramic (G1) and

glazed ceramic + bur treatment + polishing with rubber tips and felt disks (G4). There was a significant difference in surface roughness, but no difference in biofilm. Therefore, it is possible to consider that, when treatment of glazed ceramic after cementation is necessary, polishing with rubber tips + felt disks impregnated with fine-aluminum oxide particle-based paste is suggested to make the polished ceramic surface similar to a glazed ceramic surface with regards to biofilm formation. These finishing/polishing procedures have clinical relevance and can have a negative clinical impact when clinicians make these procedures close to the cervical area of indirect restorations, where the biofilm formation can contribute to dental caries and periodontal disease due to proximity to gingival tissues.

Rough surfaces facilitate the adhesion of bacteria, since the roughness provides niches where bacteria can adhere and grow protected from brushing, muscular action and salivary flow.² *In situ* studies that use scanning electron microscopy have revealed that the initial adhesion of microorganisms begins in irregularities, then expands to the remainder of the surface.¹⁴⁻¹⁶ Previous studies demonstrated an association between the quantity of biofilm and surface roughness in different dental materials, such as ceramic, resin composite, titanium and acrylic resin.^{3-4,33-35}

When biofilm was evaluated using SEM, similarities were observed related to bacterial morphology between G1 and G4, with a predominance of isolated cocci and diplococci; and between G2 and G3, with a predominance of cocci in chain form (streptococcus) and aggregated rods. Biofilm formation begins with the adhesion of cocci and short rods, proceeded by filamentous microorganisms. With the maturation of biofilm, filamentous and spirochete microorganisms are found.³⁶ Faster maturation of biofilm has been observed in irregularities, most likely due to bacterial growth with little or no disturbances, indicating that more pathogenic microorganisms will be present earlier.³ This could explain the differences in predominant bacterial morphotypes among groups with higher roughness (G2, G3) when compared to groups with lower roughness (G1, G4).

In addition to roughness, the surface-free energy of restorative materials can influence the formation of biofilm.^{2,13} The great majority of microorganisms found in the oral environment present high free energy and adhere preferentially to substrates with high energy (hydrophilic).³⁷ *In vivo*^{2,12-13} and *in vitro* studies³⁸ have demonstrated that significantly less biofilm was formed on materials with low surface-free energy (hydrophobic materials), due to the lower retention of biofilm formed on hydrophobic surfaces. This free energy can influence the type of salivary protein adsorbed during the formation of pellicle and/or the

conformation and quantity of adsorbed molecules.¹²⁻¹³ Some authors have suggested that the removal of biofilm seems to happen in the salivary protein/surface interface or in the mass of salivary proteins adsorbed, indicating a cohesive strength that is lower in acquired pellicle formed on materials with a low free energy.³⁹

In the current study, glazed ceramic (G1) was the most hydrophobic. Additionally, there was no difference between the other groups (Table 1). This result indicated that the same material can present alterations in surface-free energy according to applied finishing and polishing procedures. Other authors mentioned that glaze resulted in a smoother, more hydrophobic surface when compared to surfaces that received other polishing techniques.⁴⁰

Glazed ceramic (G1) was more hydrophobic and smooth when compared to glazed ceramic finished with a bur and polished with rubber tips and felt disks (G4). Nevertheless, G1 and G4 presented similar mean thicknesses and biovolumes of biofilm. Thus, the differences in roughness and hydrophobicity between these groups were not enough to alter the formation of biofilm.

Quirynen and others² performed an *in vivo* evaluation of formed biofilm on two materials with differing surface-free energy. Samples of each material presented a smooth and a rough section. Those authors verified a lower amount of biofilm on the smooth part of material with a low surface-free energy, but did not find differences between the two materials on the rough part of the samples, suggesting that roughness is more important than surface-free energy with regards to the formation of biofilm.

Therefore, differences related to biovolume and mean thickness of biofilm formed *in situ* after eight hours between groups in the current study can be attributed to differences in surface roughness.

This study evaluated a microfilled feldspar ceramic submitted to different finishing and polishing procedures. The results cannot be applied to other dental ceramics with other structural characteristics. Thus, more studies are necessary to verify which is the best finishing and polishing system for other ceramics with regards to dental biofilm formation.

CONCLUSIONS

In situations that require the intraoral adjustment of microfilled feldspar ceramics, polishing with rubber tips + felt disk impregnated with fine-aluminum oxide particle-based paste provides a surface with similar characteristics to a glazed ceramic surface with regards to the formation of biofilm, even though the surface is still rougher and more hydrophilic than a glazed ceramic surface.

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