Analysis of Biofilm Adhesion on CAD/CAM Lithium Disilicates Under Different Types of Intraoral Polishing

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ABSTRACT

Purpose: To evaluate the adhesion of Streptococcus mutans (S. mutans) on lithium disilicate ceramics, submitted to different intraoral polishing protocols, and the degree of surface smoothness obtained. Materials and Methods: Fifty lithium disilicate specimens were divided into 5 groups (n=10): G1-Glaze Group (positive control); G2-Glaze Group + Wear + Glaze; G3-Wear Group (negative control); G4-Ceramisté Wear Group; G5-Optrafine Wear Group. Surface roughness (Ra - µm) was evaluated and the surface characteristics were assessed using a scanning electron microscope (SEM); to assess S. mutans biofilm, the number of cultured cells was evaluated by counting colony-forming units (CFU/mL). The data underwent one-way ANOVA followed by Tukey's test (P<.05). Results: There was a significant difference in the surface roughness of all groups compared with G3. There was no significant difference between the G4 and G5 groups that received polishing. G1 group had the lowest mean roughness values. There was a difference in Log values (CFU/mL) between the G3 group and the groups that received glaze (G1 and G2). The G3 group had the highest adhesion of S. mutans (4.53 Log). Conclusion: The most effective polishing protocol after wear is glazing, presenting the lowest roughness and CFU/mL values.

INTRODUCTION

Lithium disilicate reinforced ceramic restorations are widely used, especially in aesthetic regions. This material is a high strength glass ceramic, available in both CAD/CAM blocks and pressed ceramic inserts.[1,2] This results in a wide variety of applications in anterior and posterior teeth, with good longevity.[3,4,5] Performing clinical adjustments on ceramic restorations is often necessary to ensure adequate contact with antagonist and adjacent teeth and the gingival margin. The professional is responsible for choosing the most effective technique for polishing the ceramic surface after functional adjustments, whether cervical and/or occlusal. The material can achieve a high gloss and smoothness after being treated with polisher kits.[6] However, ceramics generally have high brittleness during the finishing and polishing procedures can induce crack propagation through the restoration, resulting in fracture.[7,8]

Another important factor to consider is the surface roughness caused by polishing instruments, since surface smoothness of the ceramic cannot always return on clinical procedures. Thus, the surface roughness of the material will directly influence the adhesion of microorganisms, as the

presence of irregularities acts as a microbial reservoir, providing a greater chance for microorganisms to stay on surfaces even after conventional hygiene procedures.[9,10] The literature recommends roughness values close to or less than 0.2 µm to hinder microbial adhesion.[10] The degree of roughness interferes on the formation and quantity of biofilm on the surface of the materials.[11,12,13]

Dental biofilm is a complex microbial community composed by a diversity of bacterial species interposed between them and with hosted components, extracellular polysaccharide products able to adhere to dental surfaces and the most varied restorative substrate.[14] Biofilm formation on the surface of restorative materials is important when considering the possibility of secondary caries formation.[15] This lesion can be defined as the appearance of a new caries lesion on the margin of an existing restoration and represent the main indication for replacement restoration.[16] *Streptococcus* from group *mutans* are implicated in the etiology of secondary caries.[17] As the surfaces of ceramic restorations must be smooth,[18,19,20,21] investigating intraoral finishing and polishing protocols that can be performed on clinical practice is extremely important to find the one that promotes the lowest microbial adhesion and formation of disease precursor of biofilms, such as caries. Thus, this study aimed to evaluate the influence of different intraoral polishing protocols of CAD/CAM lithium disilicate ceramic on *Streptococcus mutans* biofilm adhesion and the surface smoothness obtained using *in vitro* procedures.

The null hypotheses of this study were that the polishing protocols used would not influence *S. mutans* biofilm adhesion and there would be no difference in surface roughness promoted by the different ceramic polishing protocols.

MATERIALS AND METHOD

Specimens (n=50) were assigned into 5 groups according to the treatments performed on their surfaces *(Table 1)*: G1-positive control (Glaze Group) – glaze treatment only; G2 (Glaze Group + Wear + Glaze) – wear simulating occlusal adjustment with diamond bur and new glaze applied; G3-negative control (Wear Group) – only received wear; G4 (Ceramisté Wear Group) – wear + polishing with Ceramisté Polishing Kit; G5 (Optrafine Wear Group) – wear + polishing with Optrafine Polishing Kit.

Fifty specimens of pre-sintered lithium disilicate (IPS e.max CAD) sized 5x5x1.2 mm were fabricated and milled with low speed diamond disc (Isomet) under water cooling. The IPS e.max CAD pre-sintered blocks, from which specimens were obtained, were in an intermediate crystalline stage and were taken to a ceramic furnace, where firing was performed at 840ºC – 850ºC (1544-1562 F) for 20 to 25 minutes following the manufacturer's guidelines. This heating process changed the microstructure of the lithium disilicate crystals. After sintering, the specimens were ultrasonically bathed in alcohol for 3 minutes and then air dried. Then, all 50 specimens received glaze treatment (Glaze IPS e.max Ceram Paste), following the manufacturer's instructions (850ºC, Programat CS2).[6] The glaze layer was applied and fired at a pre-drying temperature for 6 minutes at 403°C at a heat rate of 45°C/min. Vacuum start and stop temperatures were 450ºC and 769ºC, respectively, with a hold time of 1 minute. G1, which received only glaze treatment, was considered the positive control, as it is the most commonly used treatment.

Table 1. Materials and kits of polishing used in the study

Materials	Composition	Manufacturer
IPS e.max CAD	Components: SiO_2 Additional Content: Al_2O_3, ZnO_2, Na_2O, K_2O, ZrO, CaO, P_2O_5, fluoride and pigments	Ivoclar Vivadent AG, Schaan, Liechtenstein
Glaze IPS e.Max Ceram Paste	Methane, Argon e HMDSO	Ivoclar Vivadent AG, Schaan, Liechtenstein
OptraFine Polishing Kit	1-OptraFine F, finishing (light blue), 2-OptraFine P, polishing (dark blue), 3-OptraFine HP polishing brush (nylon) and diamond paste	Ivoclar Vivadent AG, Schaan, Liechtenstein
Ceramisté Polishing Kit	Ceramisté Standard Ceramisté Ultra Ceramisté Ultra II	SHOFU, Kyoto, Japan

After glazing, specimens from groups G2 to G5 received a 0.3 mm wear on one side, to simulate clinical procedures of occlusal adjustments, performed with a diamond point (#852F, JOTA, Florianópolis, SC, Brazil) fitted to a high-speed handpiece (Kavo of Brazil Ind. Com. LTDA, Joinville, SC, Brazil). The wear was performed by the same operator, using gentle movements for 10 s with air/water cooling. The same diamond point was used for 06 test specimens and then discarded.8 After wear, the specimens were ultrasonically bathed in alcohol for 3 minutes and then air dried. G2 received a new glaze layer (re-glaze) after wear, and fired at a pre-drying temperature for 6 minutes at 403°C at a 45°C/min heat rate, following the exact glaze protocol mentioned above. G4, after glaze and wear, was polished with the Ceramisté Polishing Kit formed by 3 tips: Ceramisté Standard for pre-polishing; Ceramisté Ultra for polishing; Ceramisté Ultra II for high gloss polishing. G5 received polish by OptraFine Polishing Kit, consisting of 3 tips and a diamond paste: OptraFine F for finishing; OptraFine P for polishing; OptraFine HP for polishing with nylon brush and diamond paste (2-4 µm). Polishing was performed by the same researcher and standardized: 15 seconds in one direction and 15 seconds at 90 degrees from the first direction,

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with a low rotation counter-angle at 8000 rpm[3], conducted on all faces of the specimens respecting the polishing sequence in the manufacturer's instructions.

To observe the smoothness pattern obtained after polishing protocols, the surface roughness (Ra-µm) was analyzed. The initial readings of the specimens were measured with a roughness meter (Surftest SJ-401) with 3 parallel readings (length 2.4 mm, cut-off 0.8 mm, speed 0.5 mm/s).[22,23] The Ra values were obtained by the arithmetic mean. After this process, the specimens were glued on the walls of the wells of the polystyrene plates (24-well) using a double-sided tape and sterilized with ethylene oxide (OXIMED), a dominant sterilization agent used due to its effectiveness and compatibility with most materials.[24,25] Further readings of surface roughness were recorded after the interaction of the specimens' surface with the biofilms to verify if biofilm by-products modify the surface of CAD/CAM lithium disilicate ceramic. These substances are acidic and can modify some physical properties of the materials, such as roughness.[26]

The American type of culture collection (ATCC) strain used in this study, *Streptococcus mutans* (ATCC 25175), was supplied by Instituto Oswaldo Cruz-FIOCRUZ (Rio de Janeiro, Brazil). *S. mutans* was reactivated in Mitis Agar Salivarius Agar culture with 0.2 µ/mL bacitracin and the plates were incubated under anaerobic conditions at 37°C for 48 hours. After growth, 5 colonies were collected and inserted in Brain Heart Infusion broth supplemented with sucrose and grown under the same conditions from 18 to 24 hours. A biofilm assay was conducted using *S. mutans* cultures with Optical Density 0.5 (108 cells/mL).[27]

Biofilms were formed on the surfaces of the specimens previously glued inside wells of 24-well polystyrene plates. One milliliter of the inoculum (10^8 cells/mL) was added to the wells containing the specimens. The plates were then kept in CO^2 incubator (Forma Direct Heat) at 37°C for 48 hours for biofilm formation.[28] The number of viable cells in the biofilms formed was evaluated by counting colony-forming units (CFUs) using a manual colony counter. For recovering bacteria from the surface of the specimens, each specimen was removed from the well using sterile forceps and transferred to a test tube containing 1 mL of PBS (pH 7, 0.1 mol L^{-1}) and was subjected to ultrasonication for 10 minutes, followed by vortexing (AP 56) for 1 minute.[29] Biofilm suspensions were then vigorously shaken for 90 seconds and serial decimal dilutions in PBS (pH 7, 0.1 mol L^{-1}) were plated on BHI agar for counting colonies of *S. mutans*. After 48 hours of incubation at 37°C, the number of CFUs/mL was counted, expressed as Log^{10} (CFU/mL). The experiments were performed in two independent replicates.

To assess the surface morphology and the influence of different polishing protocols on surface roughness and biofilm formation, one specimen per group were evaluated by SEM (Vega3) using specimens with adhered biofilm. The specimens were washed with PBS and immersed on 2.5% glutaraldehyde for 4 h to fix the biofilm on the lithium disilicate surface. Following, they were washed with PBS, three times for 10 minutes each time, and then dehydrated in growing ethanol washes (50%, 60%, 70%, 80%, 90%, and 100%) for 10 minutes in each solution and the concentration of 100% performed three times. Subsequently, the specimens were placed in a desiccator for 24 h.[30] The specimens were then evaluated by SEM, and the images were obtained with magnification 10.0× and 20.0×.

The data obtained in CFU/mL underwent normal curve adherence tests to determine whether or not they came from a normal distribution. As the data distribution was parametric, the data underwent one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test (α=.05); data obtained in the roughness test, presenting a normal distribution, were analyzed by variance (ANOVA), followed by the Tukey-Kramer test (α=.05), to compare the mean roughness values of the specimens studied. Considering each group separately, regarding time (initial and final), the Student's t-test was applied. All statistical analyses were performed using IBM SPSS Statistics.

RESULTS

Mean (SD) roughness (Ra) are shown in Figure 1 and Table 2. Results showed a significant difference in the surface roughness of all groups in relation to G3 (Negative Control; $P<.05$), showing 1.68 µm mean Ra. There was no difference ($P=.878$) between the two groups that received mechanical polishing (G4 – 1.31 µm and G5 – 1.06 µm), but it was observed a significant difference between the groups that received the glaze ($P<.05$). Group G1 (positive control) had the lowest mean roughness values (0.4 µm), followed by G2 (0.73 µm). There was no statistically significant difference ($P>.05$), considering each group separately, regarding the initial and final time, that is, prior and after exposure to biofilm.

Table 3 shows the results of counting colony-forming units – Log (CFU/mL), where it can be observed a significant difference in Log values (CFU/mL) only between the groups that received glaze (G1 and G2, $P<.05$) when compared with group G3 (Negative Control), which presented the highest adhesion of *S. mutans* (4.53 Log). Note that there was no difference in bacterial adhesion between the two groups that received mechanical polishing (G4 - 4.28 Log and G5 - 4.29 Log), as observed in the groups that received glaze (G1 - 4.10 Log and G2 - 4.05 Log).

The SEM images (*Figure 2*) qualitatively revealed a clear difference in the biofilm formation of *S. mutans* on surface of the specimens that received glaze and polishing, compared to the G3 group, that showed a greater amount of biofilm than other groups, especially when comparing it to surfaces that were treated with glaze (G1 and G2). The groups that received polishing (G4 and G5) showed slight surface irregularities with some adhesion of bacteria to their surface. G3 had the most surface irregularities with multiple scratch marks and increased bacterial adhesion, whereas G1 and G2 showed a fine and smooth surface with reduced bacterial adhesion on their surfaces.

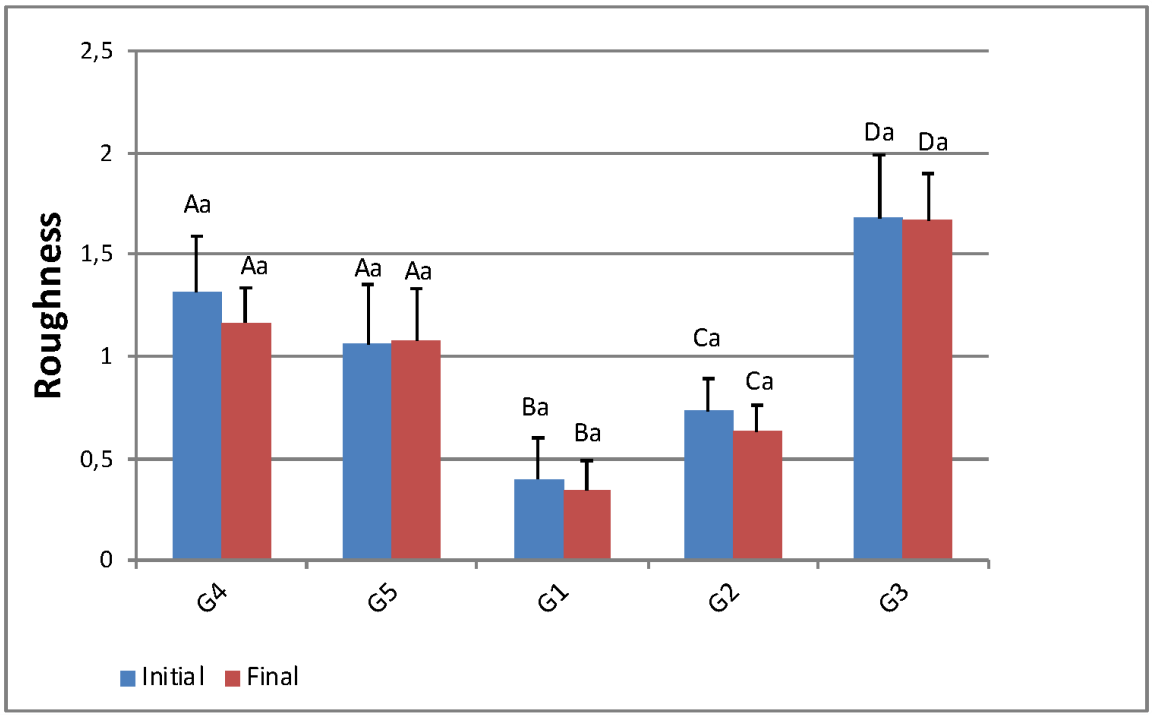

Figure 1: Mean values of surface roughness of the specimens, varying the polishing protocols.
*Different capital letters show difference between groups according to the Tukey test (P≤.05). Different lowercase letters show statistical difference between the times, considering each group separately, according to the Student t test.

Table 2. Mean values (± SD) of ceramic roughness (Ra - µm), exposed to different polishing protocols

Groups	Before the exposition to biofilm	After the exposition to biofilm
G1- Positive Control (Glaze Group)	0.399 ± 0.20 ^{Da}	0.343 ± 0.14 ^{Da}
G2- Glaze Group + Wear + Glaze	0.729 ± 0.16 ^{Ca}	0.632 ± 0.13 ^{Ca}
G3- Negative Control (Wear Group)	1.676 ± 0.31 ^{Aa}	1.667 ± 0.23 ^{Aa}
G4- Ceramisté Group (SHOFU)	1.315 ± 0.27 ^{Ba}	1.166 ± 0.17 ^{Ba}
G5- Optrafine Group (IVOCLAR)	1.057 ± 0.29 ^{Ba}	1.079 ± 0.25 ^{Ba}

*Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ at the 5% significance level (P<.05) by the Tukey test.

Table 3. Mean values (± SD) of Log (CFU/mL) according to different polishing protocols

Groups	Log (CFU/mL)
G1- Positive Control (Glaze Group)	4.10 ± 0.45 ^{a}
G2- Glaze Group + Wear + Glaze	4.05 ± 0.36 ^{a}
G3- Negative Control (Wear Group)	4.53 ± 0.30 ^{b}
G4- Ceramisté Group (SHOFU)	4.28 ± 0.29 ^{a,b}
G5- Optrafine Group (IVOCLAR)	4.29 ± 0.20 ^{a,b}

*Different lowercase letters show difference between groups according to the Tukey test (P≤.05).

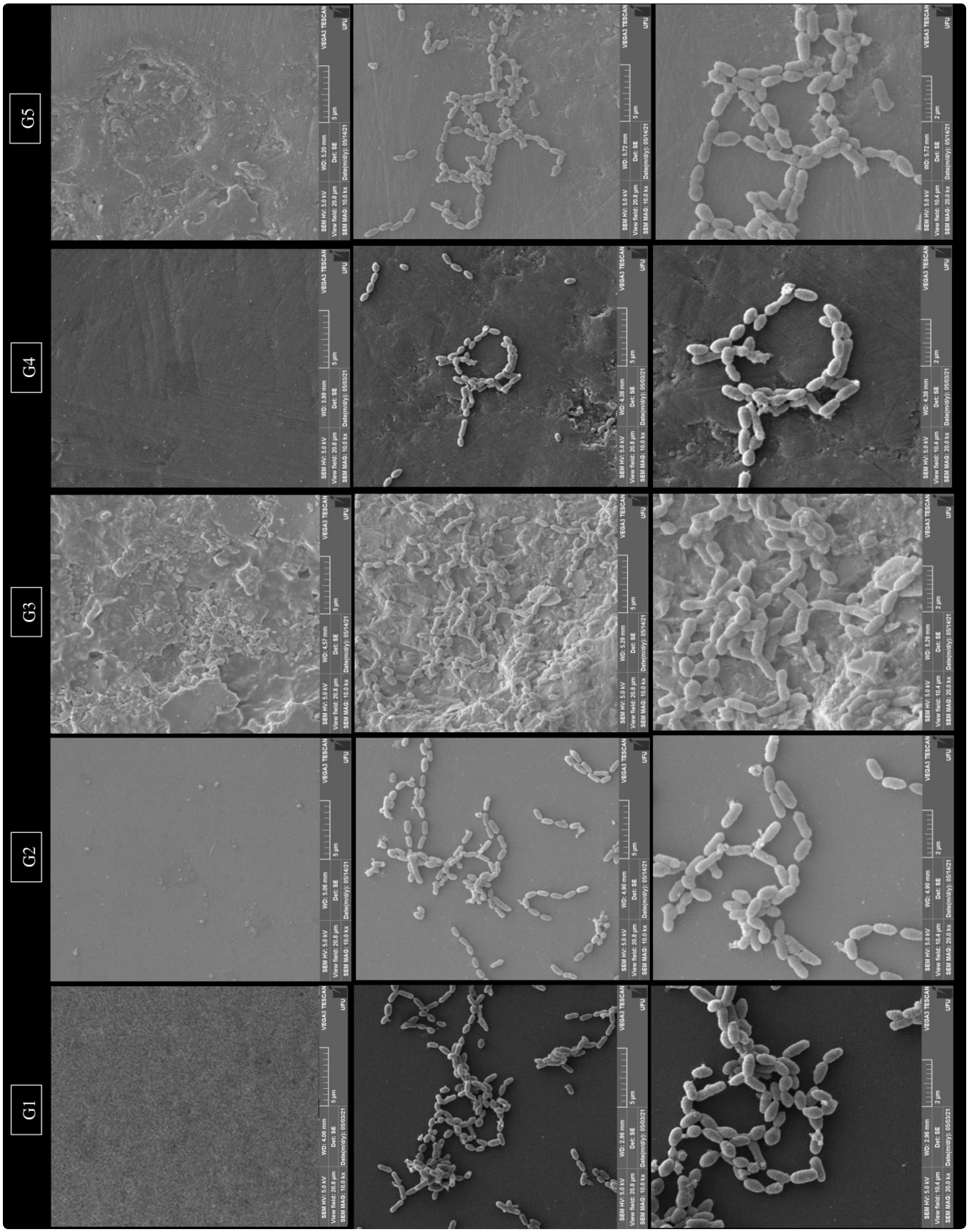

Figure 2: Scanning electron microscope (images) of roughness and the adhesion of *Streptococcus mutans* on lithium disilicate specimens, according to the polishing protocols, at 10.0× and 20.0× magnification.

DISCUSSION

The use of CAD/CAM technologies is growing in the dental clinical scenario, with promising materials from a microbiological point of view.[31] The preferred surface coating procedure for ceramic restorations is the glaze technique, defined by the use of a thermally compatible low melting glass layer on the ceramic surface.[32] Adjustment procedures on ceramic pieces, usually performed with fine-grained diamond drills, remove the glaze layer, resulting in rough surfaces and color change.[33,34] The surface roughness of restorative materials directly influences biofilm accumulation, thus leading to gingivitis, periodontitis and dental caries.[34-36] Willers *et al*[37] found a significant correlation between the surface roughness and biofilm deposition on the glaze layer. In addition, this surface roughness can generate stress concentration areas, adversely affecting the strength of the material.[35-40] Therefore, a smooth surface in dental ceramics is required both for aesthetics and biological and mechanical reasons, aiming to improve restorative strength and reduce wear on antagonist teeth, thereby increasing treatment longevity.[35,36,41,42]

Several polishing protocols exist to eliminate or at least reduce the grooves that arise during the adjustment of ceramic restorations, thus obtaining a smooth surface. However, results concerning the action of polishing systems are discordant due to different measurement parameters and different associations of polishing methods and ceramic materials.[36,38,43] Surface roughness can be measured using a roughness meter, allowing different measurement standards to be obtained. The average surface roughness (Ra) is one of the most used parameters in studies evaluating the effect of ceramic polishing protocols.[43,44] The null hypotheses that the polishing protocols used would not influence the adhesion of *S. mutans* biofilm and that there would be no difference in surface roughness promoted by the different ceramic polishing protocols were rejected. The different types of lithium disilicate polishing protocols showed significant differences for both surface roughness and bacterial adhesion values.

There was a difference in the roughness values of all groups compared with the non-polished group (negative control – G3), that is, any polishing protocol significantly decreased surface roughness (Ra). IPS e.max is a partially crystalline glass ceramic containing 40% lithium metasilicate crystals with 0.2 to 1 nm grain size, which converts to up to 70% lithium disilicate crystals once crystallized.[45] The silica content varies among ceramics, which could influence their polish ability, since a higher content of the crystalline phase decreases the smoothening and polishing capability of ceramic restorations.[46] Ceramics containing higher glass matrix or silica content and lower hardness may be easier to polish by with polishing systems that use diamonds. Another variable may be the amount of diamond contained within the impregnated polishing cups.[47] Silva *et al*[48] conducted a study with different polishing protocols on surface roughness and morphology of lithium disilicate ceramics (IPS e.max CAD) and found that all tested protocols were effective in reducing surface roughness of this material. Abdalla *et al*[13] reported that roughened ceramic surfaces contributed to biofilm adhesion, and despite polishing, the surfaces still facilitated biofilm development, so care should be taken while adjusting such ceramics in order to minimize the risk of bacterial adhesion and recurrent caries.

The present study found better results for the groups that received the glaze layer, corroborating other literature results.[49] The mean roughness values for the glazed and reglazed groups were approximately 0.4 and 0.7 µm, similar to that observed in the study by Mores *et al*[49], where the means obtained for the glaze groups were 0.43 µm. These values also corroborate the range reported in previous studies - between 0.2 to 0.7 µm.[50-52] Similarly, results showed no difference between the groups that received mechanical polishing (mean values between 1 and 1.3 µm). Again, these results agree with the study by Mores *et al*[49], where the groups that were only polished presented higher average roughness than the glazed groups, ranging from 0.85 to 0.94 µm. These results also align with Vieira *et al*[8], who concluded that mechanical finishing and polishing methods were unable to provide a smoother surface than using glaze on the tested ceramics, corroborating previous studies that indicate that intraoral polishing with diamond paste can result in a superior surface finish but although not to that of reglazing.[53]

Although the polishing protocols decreased the roughness (Ra) values of the ceramic tested in the present study, the values were higher than 0.2 µm, which according to the literature would facilitate microbial adhesion on the surface of materials.[10] None of the protocols tested were able to achieve a roughness of less than 0.2 µm. However, it is important to note that the intact human enamel Ra values are generally between 0.45 and 0.65 µm.[54,55] Only the glaze groups presented Ra values similar to those reported for the enamel, thus being considered the most adequate procedure to smooth the ceramic surface to a clinically adequate level. Poole *et al*[56] revealed that grinding with diamond burs led to greater roughness on ceramic surfaces, and the rugosity of the ceramic material surface seemed to favor susceptibility to microbial adhesion. Nevertheless, other factors than roughness may also influence microbial adhesion, such as chemical composition, surface hydrophobicity, surface free energy, etc.[14,57,58]

As expected, the study revealed a significant difference in the number of CFU of the negative control group (G3) and the glazed groups (G1 and G2), indicating that glaze on the material surface provided better results, both in lower surface roughness values and lower biofilm adhesion, as shown in the SEM images. These results agree with other studies, such as Vo *et al*[59] who verified bacterial adhesion in different commercial types of lithium disilicate ceramic materials and found a strong positive association between bacterial count and surface roughness. Kim *et al*[60] investigated the surface roughness (Ra) of four indirect restorative materials (Vita Enamic, Lava Ultimate, Vitablocs Mark II and Wieland Reflex) after adjustments and simulated intraoral polishing methods and their

relationship with biofilm development, noting that the roughness values were higher in all materials after the polishing methods, resulting in greater biofilm accumulation. Irregular topography and rough surfaces provide favorable interfaces for bacterial colonization, protecting bacteria against shear forces during their initial reversible binding and biofilm formation, but it is not a prime factor for this to occur, as glazed groups also promoted bacterial adhesion.

As discussed in the present study, bacterial adhesion and biofilm formation may be strongly influenced by surface roughness, but other factors are as or more important, such as the chemical composition of the material, surface free energy and ion release.[58] Importantly, some clinical factors are also involved in microbial adhesion, such as marginal integrity, emergence profile, gingival contour and periodontal health of tissues adjacent to the sites receiving restorative materials and the patient's oral hygiene condition.[61]

Glazing showed the most effective results in this study, however, clinical adjustments in cemented ceramic restorations are usually necessary to return the patient to an adequate occlusion, making mechanical polishing the only viable option. Chairside polishing after adjustment leads to surfaces as smooth as lab side polishing after milling and grinding.[62] Brescansin *et al*[63] observed that polishing is necessary to revert optical impairments, and that both glaze and polishing can reestablish surface roughness and smoothness. The ceramic restorations should be submitted to posterior specifically treatments after clinical adjustment with diamond burs in order to reestablish adequate surface. Thus, it is up to professional to select the most appropriate chairside finishing and polishing procedures for intraoral adjustment of ceramic restorations, minimizing future clinical problems, such as colonization by *S. mutans* and, consequently, the development of secondary caries.

This *in vitro* study used simple biofilm, while oral biofilms are complex microbial communities. Other factors were also not evaluated, such as the influence of saliva, temperature and pH changes on biofilm growth. Despite these limitations, this study shows that intraoral polishing methods with polishers result in greater roughness in the investigated material, but without statistical difference in biofilm growth among the glaze and polished groups. This shows that using polishers is an important resource when a prosthetic crown is already cemented. Other polishing protocols may be proposed and used in further investigations.

CONCLUSION

Within the limitations of this study, the following conclusions were drawn:

1. The most effective polishing protocol of lithium disilicate ceramics after wear is glazing, presenting the lowest roughness and CFU values.
2. Intraoral polishing systems are viable, since the microbial adherence was similar to the glazed groups.

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MANUFACTURERS' DETAILS

- Ceramisté Polishing Kit - Shofu Inc., Kyoto, Japan
- Optrafine Polishing Kit - Ivoclar Vivadent, Schaan, Liechtenstein
- IPS e.max CAD - Ivoclar Vivadent AG, Schaan, Liechtenstein
- Diamond Disc - Isomet; Buehler, Lake Bluff, Illinois
- Ceramic furnace - Ivoclar Vivadent AG, Schaan, Liechtenstein
- Glaze IPS e.max Ceram Paste - Ivoclar Vivadent AG, Schaan, Liechtenstein
- Programat CS2 - Ivoclar Vivadent AG, Schaan, Liechtenstein
- Diamond Bur #852F - Jota AG, Rüthi, Switzerland
- Ceramisté Polishing Kit - Shofu Inc., Kyoto, Japan
- OptraFine Polishing Kit - Ivoclar Vivadent AG, Schaan, Liechtenstein
- Ethylene oxide - Oximed, São José do Rio Preto, Brazil
- Mitis Agar Salivarius Agar culture - Difco Laboratories, Kansas City, MO, USA
- Brain Heart Infusion broth - Difco Laboratories, Kansas City, MO, USA
- CO^2 incubator - Forma Direct Heat; Thermo Fisher Scientific, MA, USA
- Vortex - AP 56 Phoenix Luferco, Araraquara, Brazil
- BHI agar - Difco Laboratories, Kansas City, MO, USA
- Roughness meter - Mitutoyo SJ-401; Mitutoyo Corp., Suzano, Brazil
- IBM SPSS Statistics- v20.0; IBM Corp
- Vega3 - Tescan

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