IDR International Dental Research

Int Dent Res 2023; 13(1): 27-34
https://doi.org/10.5577/idr.2023.vol13.no1.5

Original Article Open Access

Effect of *Streptococcus mitis* and *Streptococcus mutans* on the adhesion of *Streptococcus salivarius* to lithium disilicate glass-ceramics of varying roughnesses

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Received: 10 January 2023
Accepted: 30 March 2023

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How to cite this article:

Kavaz T, Özdemir H. Effect of *Streptococcus mitis* and *Streptococcus mutans* on the adhesion of *Streptococcus salivarius* to lithium disilicate glass-ceramics of varying roughnesses. Int Dent Res 2023;13(1):27-34. https://doi.org/10.5577/idr.2023.vol13.no1.5

Abstract

Aim: The aim of this in vitro study was to determine how different polishing kits affect the surface roughness of lithium disilicate glass-ceramic and to examine the effect of *Streptococcus mitis* and *Streptococcus mutans* on the adhesion of *Streptococcus salivarius* to the glass-ceramic.

Methodology: A total of 96 lithium disilicate glass-ceramic specimens were prepared and divided into four groups: control, rough, D+Z polished, and OptraFine polished. A total of 16 subgroups were obtained based on four combinations of three bacteria. After adding artificial saliva to the surface, all specimens were incubated in suspensions prepared for bacterial combinations. Bacterial adhesion values in the colonies formed were obtained according to the colony-forming unit (CFU) system.

Results: The highest Sa values were found for the rough group, followed by the OptraFine polished, D+Z polished, and control groups, but, except for the control group, the values were statistically similar ($p > 0.05$). The difference between *S. salivarius* and the triple bacteria combination was statistically significant for the *S. salivarius* CFU values in the control group. The difference between *S. salivarius*, *S. salivarius* + *S. mutans*, and the triple bacteria combination groups was significant for the rough group ($p < 0.05$). *S. salivarius* alone accumulated the most on all specimen surfaces. In the control group, a high level (r=0.6-0.8) of positive correlation was found between the *S + Mit* group and the *S + Mit + Mut* group. In the rough group, a high (r>0.8) positive correlation was found between the *S + Mut and S + Mit + Mut* groups in terms of *S. salivarius* adhesion.

Conclusion: For lithium disilicate glass-ceramic material, polishing systems are not sufficiently efficient after the glaze layer has been removed.

Keywords: *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius*, adhesion, lithium disilicate glass-ceramics

Introduction

More than 700 species of bacteria can be found in the human oral cavity, some of which can colonize surfaces that are covered by salivary pellicles (1). Bacteria living in the oral flora attach to the gingiva and dental hard tissues and to the surfaces of removable dentures and restorative materials (2). Bacteria that can adhere strongly to the tooth surface colonize by binding both to the surface and to each other with a secreted adhesive matrix, and these microorganisms form part of the structure of oral biofilms (3).

Streptococci are an important group of bacteria that can cause invasive infections (4) and the dorsum of the tongue of a healthy individual is colonized by a large number of *S. salivarius* (5, 6). These are organisms with a three-part enzyme alkali-producing mechanism, and considering the millimolar concentrations of urea found in saliva and gingival groove fluids, *S. salivarius* has a significant impact on oral bacterial ecology (7,8). *S. mitis* is also significant as one of the early colonizers during dental plaque formation, being the most abundant bacteria in the early stages of plaque (9) while *S. salivarius* and *S. mutans* attach to the plaque at later stages (10). *S. mutans*, because of its adhesive capacities, acid production, acid tolerance, and water-soluble glucan production, has long been recognized as one of the main oral pathogens (11), with a strong relationship between dental caries and an increased *S. mutans* count (12).

Lithium disilicate is a popular translucent dental ceramic material that can be used in the anterior region without the need for a veneering porcelain;13 it can also be used for esthetic substructure, inlays, onlays, and veneers (14, 15). In clinical practice, most ceramic restorations require adjustments before cementation, resulting in the removal of the glaze layer and roughening of the surface (16,17) and dental biofilm has been reported to accumulate more readily on such unpolished surfaces (18-22).

To address roughness, glazing has been found to provide an acceptably smooth ceramic surface (23) but repeated firing may cause deformation. Polishing with ceramic polishing kits is more straightforward and does not require additional firing. In addition, if adjustments are made after cementation, the restoration should be polished intraorally (24, 25) as, at that point, polishing is the only option (26).

The Sa parameter can be used as the average surface roughness value of a recorded area, as recommended by the International Organization for Standardization's (ISO) Standard 25178. Sa corresponds to the Ra used in traditional profilometers but allows for more ideal measurements (27).

The adhesion of a single strain of streptococcus to restorative materials has been studied previously (28-31) but studies examining the adhesion of combinations of bacteria to dental prosthetic materials are sparse (32). The aim of this study was to examine the effects of different polishing kits on the surface roughness of lithium disilicate glass-ceramic and the effects of the oral pathogens *S. mitis* and *S. mutans* on the adhesion of the oral flora element *S. salivarius* after the use of those kits. The null hypotheses were that the polishing kits would have an effect similar to that of glazing on the surface roughness of this material; that no correlation would be found between the adhesion of *S. salivarius* and the surface roughness of lithium disilicate glass-ceramic; and that the adhesion of *S. salivarius* to the lithium disilicate glass-ceramic surface would not be affected by the presence of *S. mitis* and *S. mutans*, either together or separately.

Materials and Methods

A total of 96 lithium disilicate glass-ceramic disks of 10 mm diameter and 1.3 mm thickness (IPS e.max CAD; Ivoclar Vivadent, Schaan, Liechtenstein) were designed digitally (Ceramill Mind CAD Design; Amann Girrbach AG, Koblach, Austria) and fabricated. No treatment was applied to 24 of the specimens (control group). The remaining 72 specimens were roughened with diamond rotary instruments (105–120 µm, 645229; D+Z GmbH, Frankfurt, Germany) at 20,000 rpm for 30 seconds under constant water cooling. Of these, 24 were left rough and 24 were polished with a D+Z kit (D+Z GmbH, Frankfurt, Germany), in accordance with the manufacturer's instructions, with rotational movements for 15 seconds at a time under water cooling for a total of 90 seconds at 10,000 rpm. The other 24 specimens were polished with an OptraFine kit (Ivoclar Vivadent AG, Schaan, Liechtenstein), in accordance with the manufacturer's instructions, with rotational movements for 15 seconds at a time under water cooling at a speed of 10,000 rpm for a total of 60 seconds. High Polishing Brush (HP) and polish paste (Ivoclar Vivadent AG, Schaan, Liechtenstein) were then applied with the same rotational movements at 6,000 rpm for 60 seconds without water cooling.

All the specimens were placed in 96% ethanol for 5 minutes and then cleaned with deionized water for 10 minutes using an ultrasonic cleaner (Elmasonic xtra ST; Elma GmbH & Co., Singen, Germany). Next, surface images of four specimens, one from each group, were recorded by means of environmental scanning electron microscopy (ESEM) and focused ion beam scanning electron microscopy (FIB/SEM) (Quanta 250 FEG; FEI Co., Hillsboro, OR, USA) at ×5,000 and ×20,000 magnifications.

The 96 lithium disilicate specimens were divided into four groups based on the bacteria to be adhered to and the utilized surface polishing system. They were then subdivided into a further four groups according to the bacterial adhesion, resulting in a total of 16 groups. The artificial saliva was formulated according to Shannon's saliva formula (33) and 200 µL of artificial saliva was added to each specimen, which were then stored for 1 hour in an incubator (redLINE Incubator; Binder GmbH, Tuttlingen, Germany) with 5% CO_2 at 37 °C for the purpose of pellicle formation.

Streptococcus salivarius (13419; ATCC), *Streptococcus mutans* (25175; ATCC), *Streptococcus mitis* (49456; ATCC), and different combinations of these strains were evaluated. Colonies grown on bacterial culture were inserted into glass tubes containing 9 mL of phosphate-buffered saline (PBS). The bacterial density in each tube was adjusted using a densitometer (DEN-1 McFarland Densitometer; Biosan, Riga, Latvia) according to the eight McFarland charts (2.4×10^9 bacteria in 1 mL) and bacterial suspensions were obtained.

Four different bacterial combinations were tested with regard to adhesion (Table 1). First, 100 µL of bacterial solution was added to each specimen. The specimens were placed in Petri dishes, which were then placed in anerobic jars and incubated for 24 hours in a CO_2 incubator (redLINE Incubator; Binder). The specimens that exhibited bacterial adhesion were washed three times with 30 mL of PBS (BioShop, Ontario, Canada) to remove any non-adhesive bacteria and then separately placed in tubes containing 9 mL of PBS, where the adhered bacteria were allowed to pass into the solutions in the tubes. For the third dilution, 100 µL was separately taken for each specimen, inoculated in M17 medium, and incubated for 24 hours. In terms of the colonies, the number of colonies per milliliter was determined as the colony forming unit (CFU). Moreover, the log 10 values were recorded.

Table 1. Bacteria combinations tested

Suspensions	Bacteria Species
One-species suspension	*S. salivarius*
Two-species suspension	*S. salivarius* with *S. mitis*
Two-species suspension	*S. salivarius* with *S. mutans*
Three-species suspension	*S. salivarius* and *S. mitis* and *S. mutans*

One specimen from each of the 16 groups was fixed with 4% formaldehyde for 15 minutes and then washed with 1,000 cc of distilled water. Next, the specimens were fixed by means of incubation with 20%, 50%, 80%, and 100% ethanol for 10 minutes (29) and examined via ESEM at ×5,000 and ×20,000 magnifications. The surface roughness of the 48 specimens was evaluated using a contact profilometer (Tencor Stylus Profiler P7; KLA-Tencor, CA, USA), with three specimens being arbitrarily selected from each of the 16 groups.

Statistical analysis

Analyses were performed by using SPSS software (IBM SPSS Statistics version 23, IBM Inc., Armonk, NY, USA). Two-Way Anova test was used.

The independent samples t test was used when comparing two independent groups, whereas the one-way analysis of variance was used when comparing more than two independent groups. As the Shapiro-Wilk test revealed that the data were not normally distributed (p <0.05), the Mann-Whitney U test was used when comparing two independent groups, whereas the Kruskal-Wallis test was used when comparing more than two independent groups. The Spearman correlation coefficient was applied to determine the relationship between surface roughness and bacterial uptake and the interactions among the bacteria (α =0.05).

Results

Based on the ESEM images (Fig. 1), the rough group had a distinctly irregular surface with many pits and peaks. Moreover, the OptraFine group exhibited more microscratches than the control and D+Z groups, while the control and D+Z groups had flatter surfaces characterized by similar surface properties.

With regard to the surface roughness measurements, a significant difference was found between the control group (2.75 ± 1.52 µm) and the other groups (p < 0.001). In addition, the Sa values were highest for the rough group (7.27 ± 1.75 µm), followed by the OptraFine (6.00 ± 2.64 µm) and D+Z (5.09 ± 2 µm) groups, although these values were not statistically different (p > 0.05) (Table 2). On the basis of the three-dimensional profilometer images, the control group had a flat profile, except for a few pits, while the rough, D+Z, and OptraFine groups appeared similar, exhibiting irregular areas featuring scattered peaks and deep valleys (Fig. 2).

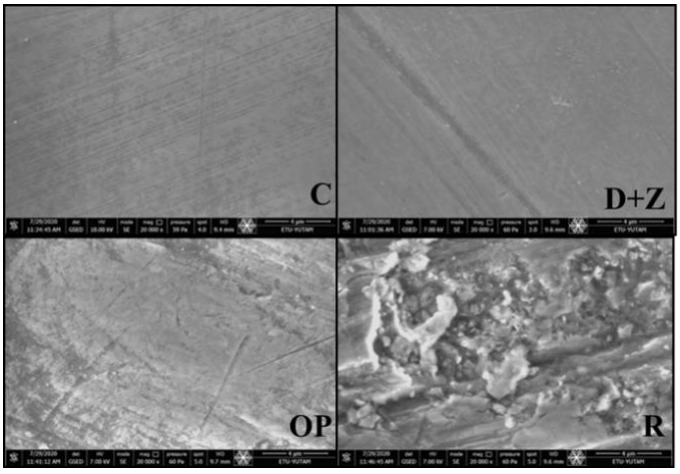

Figure 1. Environmental scanning electron microscope images of IPS e. max CAD specimens. (C): Control group. (R): Rough group. (D+Z): D+Z polished group. (OP): OptraFine polished group. Original magnification ×20,000.

Table 2. Minimums, maximums, means, and standard deviations of roughness (µm) values of the experimental groups

Group	N	Minimum	Maximum	Mean ± Standard Deviation
Control	12	1.12	5.91	2.75 ±1.52[a]
Rough	12	4.69	10.26	7.27 ±1.75[b]
D+Z	12	1.51	8.26	5.09 ±2.06[b]
OptraFine	12	2.95	10.58	6.00 ±2.64[b]

Different letters indicate statistically significant differences between groups (p < 0.05).

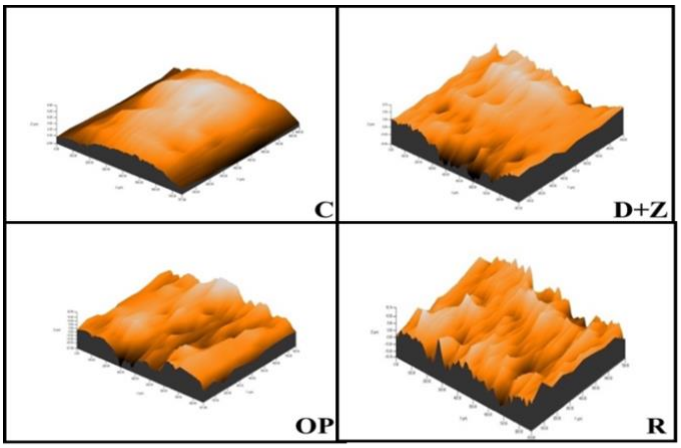

Figure 2. Three-dimensional profilometer images of IPS e.max CAD samples. (C): Control group. (R): Rough group. (D+Z): D+Z polished group. (OP): OptraFine polished group.

All the bacteria were plated on the specimens according to the McFarland charts and then diluted twice, which resulted in approximately 10^5 bacteria in each colony in the Petri dish. The *S. salivarius* adhesion values for the control, rough, OptraFine, and D+Z groups determined by means of the one-way analysis of variance are presented in Table 3. In the control group, the difference between the mean ± standard deviation of the *S. salivarius* adhesion values (7.09 ± 0.45) in the group with *S. salivarius* alone was statistically higher than that seen in relation to the combination of triple bacteria (6.56 ± 0.50) (p = 0.042). The differences between the other compared groups were not statistically significant (p > .05). In the rough group, the difference between the mean ± standard deviation adhesion values of the *S. salivarius* (7.26 ± 0.11) and both the combination of *S. salivarius* and *S. mutans* (6.31 ± 0.18) and the triple bacteria combination (6.32 ± 0.17) were statistically significantly different (p = 0.005). In the D+Z and OptraFine groups, the difference between the mean *S. salivarius* adhesion values was statistically similar (p > .05).

Table 3. Bacterial adhesion values of *S. salivarius*

	Bacteria Species	N	Minimum	Maximum	Mean ± Standard Deviation	*P*
Control	S	12	6.23	7.72	7.09 ± (0.45) [a]	<0.05
	S + Mit	14	5.85	7.64	6.76 ± (0.60) [a, b]	
	S + Mut	14	5.48	7.08	6.57 ± (0.52) [a, b]	
	S + Mit + Mut	15	5.48	7.19	6.56 ± (0.50) [b]	
Rough	S	12	6.54	7.78	7.26 ± (0.11) [a]	<0.05
	S + Mit	15	5.00	7.70	6.61 ± (0.26) [a, b]	
	S + Mut	14	5.30	7.20	6.31 ± (0.18) [b]	
	S + Mit + Mut	13	5.00	7.09	6.32 ± (0.17) [b]	
D+Z	S	12	4.95	7.52	6.49 ± (1.02)	>0.05
	S + Mit	15	5.00	7.03	6.35 ± (0.69)	
	S + Mut	12	4.95	6.74	6.14 ± (0.63)	
	S + Mit + Mut	14	4.95	7.06	6.20 ± (0.76)	
OptraFine	S	12	4.95	7.80	6.67 ± (0.84)	>0.05
	S + Mit	13	5.00	7.79	6.61 ± (0.86)	
	S + Mut	16	5.30	6.97	6.31 ± (0.46)	
	S + Mit + Mut	13	5.48	7.23	6.17 ± (0.55)	

* Different letters indicate statistically significant difference between groups (p < 0.05). *Mit, S. mitis; Mut, S. mutans; S, S. salivarius.*

The correlations of the values concerning *S. salivarius* between the control and rough groups are shown in Tables 4 and 5. In the control group, a high positive correlation (r = 0.6–0.8) was found between the S + Mit group and the S + Mit + Mut group in terms of the *S. salivarius* adhesion. In the rough group, a moderate level of positive correlation (r = 0.4–0.6) was found between the S + Mut and S + Mit groups with regard to S. salivarius adhesion, while a high level of positive correlation (r > 0.8) was found between the S + Mut and S + Mit + Mut groups. By contrast, no correlation was found between the D+Z and OptraFine groups in terms of the *S. salivarius* adhesion.

Table 4. Pearson correlation coefficients of *S. salivarius* in the control group

	S	S + Mit	S + Mut
S + Mit	0.078		
S + Mut	0.131	-0.288	
S + Mit + Mut	0.249	0.618*	0.069

Mit, S. mitis; Mut, S. mutans; S, S. salivarius. *:P<0.05

Table 5. Pearson correlation coefficients of *S. salivarius* in the rough group

	S	S + Mit	S + Mut
S + Mit	0.562		
S + Mut	0.020	0.595*	
S + Mit + Mut	0.159	0.413	0.881**

Mit, S. mitis; Mut, S. mutans; S, S. salivarius. *:$p < 0.05$, **:$p < 0.001$

ESEM images of the specimens' surfaces following bacterial fixation are shown in Figures 3-6. The highest adhesion was observed in the case of *S. salivarius* alone. Yet, similar numbers of bacterial adhesions were also observed in the rough group with the highest surface roughness and in the control group with the lowest surface roughness. Moreover, similar numbers of colony adhesions were observed in the D+Z and OptraFine groups.

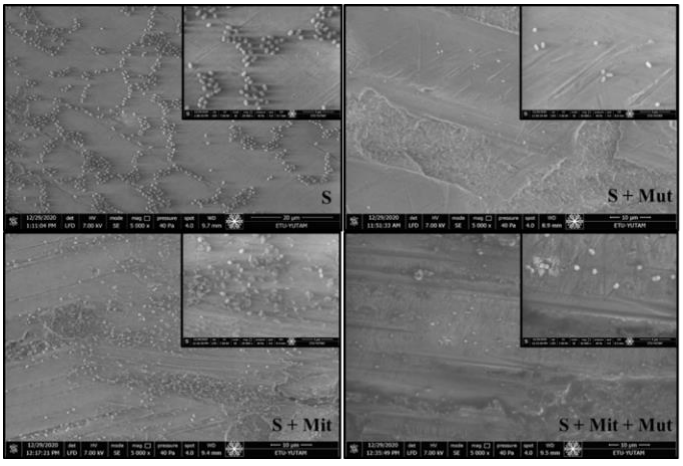

Figure 3. Environmental scanning electron microscope images after bacterial adhesion in the control group. *Mit, S. mitis; Mut, S. mutans; S, S. salivarius*. Original magnifications ×5,000 and ×20,000.

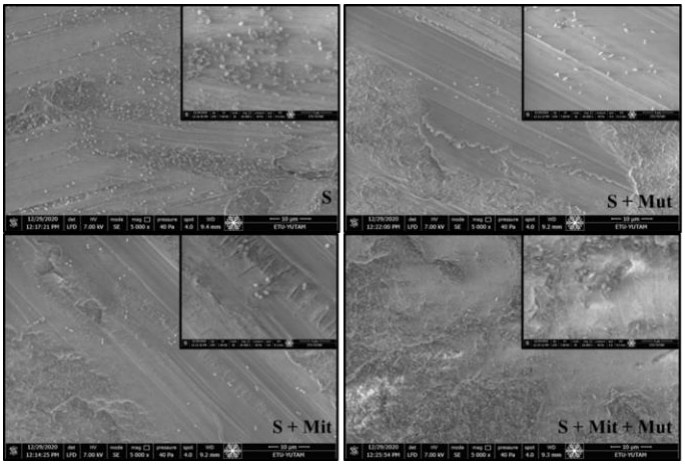

Figure 4. Environmental scanning electron microscope images after bacterial adhesion in the rough group. *Mit, S. mitis; Mut, S. mutans; S, S. salivarius*. Original magnifications ×5,000 and ×20,000.

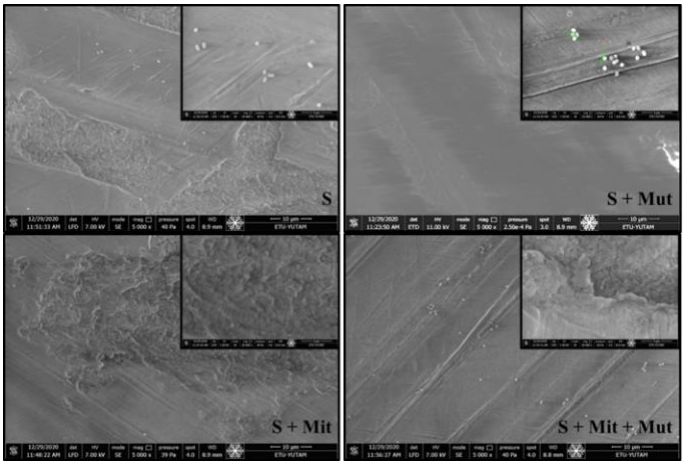

Figure 5. Environmental scanning electron microscope images after bacterial adhesion in the D+Z group. *Mit, S. mitis; Mut, S. mutans; S, S. salivarius*. Original magnifications ×5,000 and ×20,000.

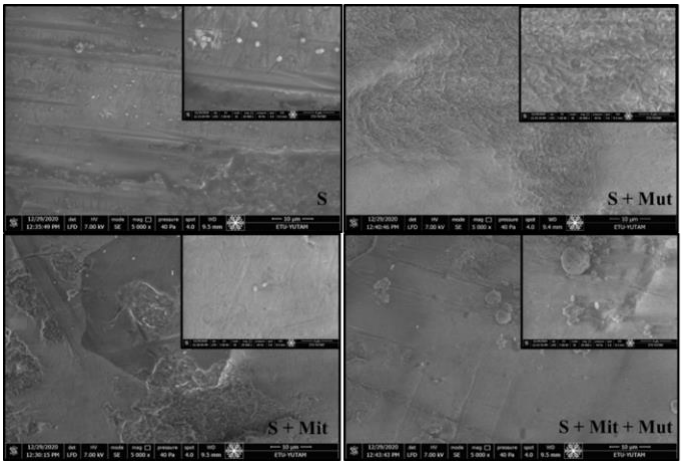

Figure 6. Environmental scanning electron microscope images after bacterial adhesion in the OptraFine group. *Mit, S. mitis; Mut, S. mutans; S, S. salivarius*. Original magnifications ×5,000 and ×20,000.

Discussion

The purpose of this study was to compare the efficacy of the glazing and mechanical polishing of lithium disilicate glass-ceramic to evaluate the effectiveness of mechanical polishing when it is necessary to repolish a roughened lithium disilicate surface and to investigate the effects of *S. mutans*, the main pathogen of dental caries, and *S. mitis*, an opportunistic pathogen of oral flora, on the proliferation and adhesion capacity of *S. salivarius*. *S. salivarius* has beneficial effects on oral flora by identifying the relationship between surface roughness and bacterial adhesion. The polishing systems tested were not as effective as glazing in reducing the surface roughness of this material; therefore, the null hypothesis was rejected. However, no correlation was found between surface roughness and bacterial adhesion in terms of *S. salivarius* adhesion; therefore, the null hypothesis was accepted. Although the total *S. salivarius*

count decreased in the presence of *S. mitis* and *S. mutans*, *S. salivarius* adhesion values in the control and rough groups showed a positive correlation between different combinations; therefore, the null hypothesis was rejected.

A consensus on the comparative effectiveness of glazing and polishing is lacking (17). In the present study, the control group showed significantly less surface roughness. However, different polishing techniques and systems can lead to different results.

The human oral cavity is the main habitat of *S. salivarius* (8) and was used in the present study because it is the main element of the oral flora. However, few studies have investigated the adhesion of *S. salivarius* to restorative or tooth surfaces (10, 34). Some strains (K12 and M18) of *S. salivarius* have been reported to have inhibitory effects on *S. mutans* with lantibiotic activity, but these probiotic bacteria are not as common in the oral cavity as the *S. salivarius* used in this study (ATCC 13419) and are produced exclusively by genetic manipulation for commercial purposes (35). Therefore, the presence of *S. salivarius* in some groups was not significantly affected by the presence of *S. mutans*.

No statistically significant difference in roughness was found between the OptraFine group and the rough group. The specimens were abraded only with 180-grit silicon carbide abrasives before glazing. Mohammadibassir et al.(25) tested polished IPS e.max CAD by examining the effect of OptraFine Assortment and D+Z polishing kits on the surface roughness and topography of monolithic lithium disilicate glass-ceramic. The group that was roughened and then polished with OptraFine showed the lowest Ra value. The roughened Ra values from the highest to the lowest belonged to the group polished with D+Z, the control group, and the nonroughened glaze group, in that order. The ceramic specimens were abraded with 600-, 800-, and 1200-grit silicon carbide in a finishing and polishing machine. Differences from the present study are attributed to the use of finer grit abrasives.

No correlation was found between surface roughness and bacterial adhesion. Abdalla et al. (22) roughened each of the lithium disilicate ceramics, feldspathic ceramics, and zirconia-reinforced lithium silicate ceramics and then polished them with their own polishing kits. Contrary to the present study, a positive correlation was found between surface roughness and bacterial adhesion. The reason for this may be that bacterial adhesion is facilitated above a certain roughness threshold (21).

The average surface roughness value of the IPS e.max CAD group was 2.75 ± 1.52 µm, possibly because of incomplete flattening before the glazing process. In addition, when the rough group was polished with two different polishing systems, surface roughness was not significantly reduced compared with that of the control group. Increasing the polishing time can change the surface roughness values as well as the bacterial adhesion values. In addition, different polishing systems and surface roughness measurement methods may change roughness values (18, 23).

Studies examining the adhesion of bacteria to dental prosthetic materials in combinations are sparse (32). Since microbial dental plaque can vary between different teeth in the same individual, standardization can be difficult in clinical studies (36). For this reason, this study was carried out in vitro.

No negative correlation was found between *S. salivarius* and the pathogenic environmental bacteria *S. mitis* and *S. mutans*. Only the CFU value of *S. salivarius* was found to be higher in the control, rough, D+Z, and OptraFine polished groups compared with the combinations, possibly because of the tendency of microorganisms to reduce their colony-forming capacity to live in the same environment (37) In addition, the presence of *S. mitis* and *S. mutans* did not significantly reduce the rate of *S. salivarius* in either the control or rough groups. *S. salivarius* had no inhibitory effect on the proliferation of *S. mutans* because the two bacteria were able to grow on top of each other in the medium. Since the effect of polishing methods on the physicochemical properties of the specimens was not investigated in the present study, further large-scale studies analyzing surfaces are needed.

Limitations of the present study include the fact that, as oral biofilm formation is a complex process, the results of in vitro studies may not match those of the clinical situation (2). Different systems stimulate bacterial growth conditions and could have been used to stimulate biofilm deposition. Open systems, such as the drip flow biofilm reactor and flow reactor, allow low shear stress and low biofilm detection in the presence of a continuous fresh medium. Continuous circulation in the mouth brings open systems closer to in vivo conditions in biofilm mass measurements (38). In this study, a closed microplate system was used.

Lithium disilicate glass-ceramic material can act as a reservoir for biofilm deposition. Developments in the design and quality of prosthetic materials in the oral environment and other parts of the body have greatly increased life expectancy and quality of life. However, many problems still arise regarding the control of microbial settlements and their spread on these surfaces. Therefore, it is essential to improve these materials to reduce complications and the recurrence of oral and other disease (39).

Conclusion

Based on the findings of this in vitro study, the following conclusions were drawn:

1. None of the commercially available ceramic polishing kits provided a smoother ceramic surface than glazing.
2. The presence of *S. mutans* affected *S. salivarius* more than did *S. mitis*.
3. *S. salivarius* was less affected by the presence of *S. mitis* and *S. mutans* on the rough lithium disilicate surface than on the glazed surface.

Disclosures

Acknowledgments: Authors thank to Sevda Urçar Gelen for her help in microbiological tests and Memiş Özdemir for his assistance with statistical analysis.

Ethical Approval: Ethics committee approval was received for this study from Atatürk University, Faculty of Dentistry, Research Ethics Committee, in accordance with the World Medical Association Declaration of Helsinki, with the approval number: 2019/08-60).

Peer-review: Externally peer-reviewed.

Author Contributions: Conception – T.K., H.Ö.; Design – T.K., H.Ö.; Supervision – H.Ö.; Materials – T.K., H.Ö.; Data Collection and/or Processing – T.K., H.Ö.; Analysis and/or Interpretation – T.K., H.Ö.; Literature Review – T.K., H.Ö.; Writer –T.K.; Critical Review – H.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Funding: This study was supported by Atatürk University Scientific Research Projects Coordinatorship (Project number: TDH 2020-8390).

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