As a direct interface of the human body with the outside world, the oral cavity is a complex environment. It is characterized by an abundance of nutrients, innate host defenses, microniche, and a low-shear, laminar flow environment that hosts a diverse resident microbial community.1,2 Extrinsic factors such as fluctuation of pH with meals,3,4 regular oral hygiene and simulated mastication,5 and diet composition further enrich the complexity of this environment.5,6 These all allow for a concurrence of factors that can impact biofilm formation and oral health or disease.7-9 Biofilms are characterized by surface attachment, structural heterogeneity, complex interspecies interactions, and an extracellular matrix.10-16 Investigating the adherence of the Streptococcus mutans bacteria heavily implicated in caries etiology can therefore provide valuable information about biofilm formation and clinical caries development.

ABSTRACT

Statement of problem. Streptococcus mutans can adhere at restored tooth margins to cause recurrent caries. Limited information about surface quality and bacterial adherence is available for lithium disilicate ceramic materials.

Purpose. The purpose of this in vitro study was to investigate how bacterial adherence is influenced by commercially available preparations of lithium disilicate ceramic materials.

Material and methods. Seventeen rectangular specimens (10x10x4 mm) were fabricated for each type of lithium disilicate material: pressed (Press), milled (CAD), fluorapatite layered (ZirPress/Ceram), and glazed (Ceram Glaze). The surface roughness of each specimen was assessed before incubation with wild-type S mutans for 48 hours at 37°C with Brain Heart Infusion broth media under anaerobic conditions. Adherent bacteria were sonicated, diluted, and plated in triplicate for quantification using the plate count method to assay for colony forming units (CFUs) as an indication of bacterial viability. Statistical analysis was performed with SPSS using an analysis of variance (ANOVA) followed by the Tukey Honestly Significant Difference (HSD) test (α=0.05). The Pearson r was used to evaluate the correlation between surface roughness and adherence.

Results. The surface roughness of Ceram Glaze (1.32 ±0.19 μm) was significantly the highest, followed by ZirPress/Ceram (0.71 ±0.09 μm), which was significantly rougher than the Press (0.11 ±0.02 μm) and CAD (0.10 ±0.02 μm) groups, which were not significantly different from each other. (F=513.898, P<.001). CFUs (cells/mL) of S mutans were also significantly the highest for Ceram Glaze (61.82 ±13.76), followed by ZirPress/Ceram (28.53 ±2.40), which had significantly higher adherence than CAD (12.86 ±1.70) and Press (6.62 ±2.74), which were not significantly different from each other. (F= 201.721, P<.001). A strong positive association was found between bacterial count and surface roughness (r=.95, P<.001).

Conclusions. The surface roughness of differently prepared lithium disilicate ceramic restorations is closely related to the adherence of S mutans. (J Prosthet Dent 2015;114:696-701)

While primary caries results from the initiation of lesions in virgin tooth structure, secondary caries is a significant contributing factor to the replacement of dental
Clinical Implications

Clinicians should pay attention to the type of ceramic materials they use, because they may support bacterial adherence differently. This is crucial in the treatment of patients with a high risk of caries, inadequate oral hygiene, periodontal disease, or systemic health issues that compromise immune function.

restorations. For the restored tooth, bacteria must adhere to the restorative material, particularly at margins, in order to cause recurrent pathology. Surface quality can greatly affect adhesion. Indirect materials tend to exhibit better marginal fit, finish, and polish than direct materials.

One material that has received extensive interest in recent years is lithium disilicate ceramics. These ceramics were introduced in 1998 by Ivoclar Vivadent Inc as Empress II. Composed of a lithium silicate glass matrix with micrometer-size lithium-disilicate crystals interspersed, they have a crystal content of approximately 70% and flexural strength of 360 MPa but can be very esthetic despite their high crystalline content. Lithium disilicate ceramics are indicated for inlays, onlays, veneers, or single unit crowns as far posteriorly as the second premolars.

Two lithium disilicate substrates, IPS e.max Press and IPS e.max CAD for pressed and milled restorations, respectively, are currently available. Pressed lithium disilicates are made from IPS e.max Press ingots to form an approximate crystal size of 3 to 6 μm. IPS e.max Press can be veneered with low-fusing fluorapatite IPS e.max Ceram composed of nanofluorapatite (100 to 300 nm) and microfluorapatite (1 to 2 μm) crystals for greater esthetics. IPS e.max Ceram surfaces can be further treated with an additive IPS e.max Ceram Glaze Spray. No information on the resultant crystalline surface of IPS e.max Ceram surfaces was obtained. Lithium disilicates also can be milled from IPS e.max CAD “blue blocks,” then heat treated, reaching 70% crystal volume and 1.5 μm approximate crystal size. Pressed and milled forms differ primarily by fabrication technique and crystal size. Fluorapatite layering and glazing are additional surface modifications commonly applied to lithium disilicate ceramics.

In considering bacterial adhesion to these ceramics, the overwhelming factor, as with enamel, is surface roughness, most commonly quantified as Ra value. Bollen et al found a threshold for this effect to be 0.2 μm Ra value, above which a significant positive correlation was found between surface roughness and plaque retention. Similarly, this was seen for feldspatic specimens treated with 4 different polishing methods. Kawai et al demonstrated similar findings for refinished surfaces but found that un-refinished glazed surfaces, the smoothest, actually had the most plaque, possibly due to undulating rough surfaces with irregularities. Similar studies examined this relationship in the presence and absence of initial coating with saliva and found correlations for both uncoated and saliva-coated ceramic surfaces for surface roughness and bacterial adherence. Uncoated surfaces had higher variability, but all surfaces with saliva were approximately equal.

Little is known about bacterial adherence to lithium disilicate ceramics. Because of differences in surface crystal size, post-fabrication modification, and surface roughness effects on adherence, the following 3 hypotheses were tested: IPS e.max Ceram Glaze, which is not polished after firing, was expected to have the highest surface roughness, followed by IPS e.max Press, IPS e.max CAD, and IPS e.max ZirPress/Ceram in order of decreasing surface crystal size; adherence was also expected to vary with crystal size; and surface roughness and bacterial adherence were expected to be correlated.

**MATERIAL AND METHODS**

Four different groups of lithium disilicate ceramic specimens (17 per group, each measuring 10×10×4 mm) were tested (Table 1). These 4 groups were as follows: lithium disilicate pressed (Press), lithium disilicate milled (CAD), lithium disilicate fluorapatite veneered (ZirPress/Ceram), and lithium disilicate glazed (Ceram Glaze). Press specimens were made from IPS e.max Press ingots. Wax patterns were created of baseplate wax. Patterns were sprued, invested with IPS e.max PressVEST, wax-eliminated, and ingots were pressed to create specimens as directed by the Ivoclar Vivadent protocol. Wax elimination and pressing was done off site by a single operator (D.V.) using equipment at Ivoclar Vivadent Inc (Jelenko Accu-Term III 6000, Programat EP 5000). CAD specimens were made by sectioning IPS e.max CAD blue blocks into squares. Specimens were then heat treated to between 840°C and 850°C for crystallization (Programat P500). ZirPress/Ceram specimens were made from IPS e.max ZirPress ingots and minimally cut back with a fine diamond rotary cutting instrument after pressing, veneered with IPS e.max Ceram, and fired. Ceram Glaze specimens were made from IPS e.max Press ingots with the same protocol as the Press specimens. Specimens were finished, polished, sprayed with IPS e.max Ceram

<table>
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<th>Table 1. Lithium disilicate ceramics tested</th>
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<tr>
<td><strong>Material</strong></td>
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<td>IPS e.max Press</td>
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<td>IPS e.max CAD</td>
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<td>IPS e.max ZirPress/Ceram</td>
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<td>IPS e.max Press + Ceram Glaze Spray</td>
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*Ivoclar Vivadent Inc.*
Glaze Spray, and heat treated to approximately 405°C in a porcelain furnace (Programat P500) for glaze firing as directed by the Ivoclar Vivadent protocol.

All specimens were measured with a digital micrometer caliper to confirm appropriate dimensions. IPS e.max Press CAD, and ZirPress/Ceram specimen testing surfaces were finished with a fine diamond wheel as necessary and polished with diamond-impregnated wheels (Dialite LD Lithium Disilicate Extra-Oral Adjustment/Polishing Wheels, medium and fine grit; Brasseler USA). Specimens were further polished with stiff bristle brushes impregnated with a diamond polishing paste (DiaShine Fine Polish; VH Technologies) until a gloss finish was attained. IPS e.max Ceram Glaze specimens were polished with medium and fine grit wheels, steam-cleaned, and glazed but were not polished after heat treatment.

Surface roughness for all specimens was measured with a profilometer located at the University of Maryland, Baltimore County (Model T8000; Hommelwerke). The profilometer was calibrated with a standard reference specimen and set to travel at 0.1 mm/s with a range of 600 μm during testing and an amplitude transmittance set at 50%. Specimens were subjected to a 10-μm tip radius diamond stylus under a constant measuring force of 3.9 mN. Each specimen was analyzed with 3 passes of the profilometer, with profiles obtained at 2.5 mm, 5 mm, and 7.5 mm along 1 dimension on the surface of the specimen. The mean Ra value was used for statistical analysis.

The specimens were autoclaved with a conventional glassware protocol at 121°C for 1 hour prior to bacterial growth. Wild-type S mutans UA159 (S mutans Clarke) was retrieved from deep-freeze at −80°C and maintained on Brain Heart Infusion (BHI) agar plates (Sigma Aldrich). Bacteria were grown in BHI broth (Teknova) to stationary phase of growth for inoculation of specimens. Cells were harvested by centrifugation (8000 rpm, 18°C, 3 min), washed 3 times with 10 mM Dulbecco’s Phosphate Buffered Saline (PBS; Sigma Aldrich) and resuspended in 1 mL PBS. The optical density of the suspension was adjusted to 0.13 at 550 nm (BioMate 3S UV-Visible Spectrophotometer; Thermo Scientific), which corresponds to a concentration of 1.3×10^8 cells/mL.

Lithium disilicate specimens were placed into the wells of a 24-well microtiter plate, with 1 specimen per well. Cell density was adjusted to a final concentration of 1×10^8 cells/mL BHI for inoculation of specimen surfaces.28 Two mL of this bacterial solution was then added to each well. The specimens were then incubated for 48 hours with a CO₂ pack for anaerobic conditions on a shaker at 37°C.

To assay for viability, the specimens were removed and washed serially 3 times with PBS and placed in 50-mL centrifuge tubes with 1.5 mL PBS. Adherent bacteria were then dissociated from the specimens by using a sonicator (Model 120 Sonic Dismembrator; Fisher Scientific). The specimens were removed and a series of dilutions were then made. Suspensions were dotted in 10-μL aliquots on BHI agar in triplicate for colony forming units (cells/mL) to assess bacterial recovery as a quantification of adherence to specimen surfaces.

Statistical software (SPSS v22; IBM Corp) was used to perform a 1-way ANOVA to test differences in surface roughness and bacterial adherence. Significant differences were further analyzed by the Tukey Honestly Significant Difference (HSD) test. The Pearson r was used to evaluate the relationship between surface roughness and bacterial adherence for the data from all the groups (α=.05).

RESULTS

With respect to surface roughness, statistical analysis revealed significant differences among the groups (F=513.9, P<.001, ANOVA, Fig. 1A). Ra values for CAD and Press ceramics were significantly lower than for ZirPress/Ceram (P<.05), which were also significantly lower than those of Ceram Glaze (P<.05).

With respect to bacterial adherence, statistical analysis also revealed significant differences among the groups (F=201.7, P<.001, ANOVA, Fig. 1B). Recovered CFUs of CAD and Press ceramics were significantly lower than those of ZirPress/Ceram (P<.05), which were also significantly lower than those of the Ceram Glaze group (P<.05).

Data were also analyzed to test for correlations among specimens examined across all 4 groups. Results demonstrated that a strong positive correlation was present when all specimens were considered (r = .95, P<.001, Fig. 2). This implies a relationship between the 2 variables, such that greater surface roughness was strongly associated with higher bacterial adherence. This also suggests an association between bacterial adherence and surface roughness.

DISCUSSION

Four groups of lithium disilicate specimens (Press, CAD, ZirPress/Ceram, and Ceram Glaze) were measured for surface roughness and evaluated for bacterial adherence. Data from all specimens were included in the statistical analysis. The results indicated that different lithium disilicate types exhibited significant differences for both surface roughness and bacterial adherence. When specimens were considered across all four preparation types, a significant correlation was found between surface roughness and bacterial adherence.

Ra values varied such that machined, minimally modified, and subsequently polished surfaces (CAD and
Press) exhibited significantly lower Ra values, while ZirPress/Ceram surfaces composed of ZirPress with Ceram modification followed by polishing demonstrated intermediate Ra values. Ra values for the Ceram Glaze group were significantly higher. The surfaces of the Ceram Glaze group specimens were not polished after an additive glazing and firing, as specified by manufacturer protocol.

Initially, differences were hypothesized to be related to surface crystal size, with Ceram Glaze expected to be the roughest due to the absence of post-fabrication polishing, followed by Press (3 to 6 µm), CAD (1.5 µm), and ZirPress/Ceram (1 to 2 µm). However, the results appear to be better explained by surface treatment during and after fabrication. Ceram Glaze specimens were visually the roughest, as predicted. The surface roughness of ZirPress/Ceram, however, significantly exceeded that of Press and CAD despite smaller crystal size. ZirPress specimens were modified with a technique-sensitive addition of fluorapatite veneering porcelain, an addition that may account for nonuniform surface characteristics and the introduction of additional surface roughness not accounted for purely by surface crystal size. CAD and Press groups, despite the significantly different crystal sizes, had nonsignificant differences in surface roughness. Ra value measurements therefore appeared to reflect macroscopic physical differences achieved by the method of fabrication and subsequent modification by polishing rather than corresponding to surface crystal size.

Bacterial adherence varied such that Press and CAD ceramics did not demonstrate significant differences but were significantly lower than ZirPress/Ceram, which was significantly lower than Ceram Glaze specimens. Likewise, differences in adherence appeared to follow the variations in Ra values as well as the fabrication and surface modification factors rather than differences in crystal size.

A significant relationship between surface roughness and bacterial adherence was also demonstrated, with a correlation coefficient r=.95, indicating a strong positive association. Intuitively, it follows that macroscopically higher surface roughness would increase the propensity of a material to accumulate bacteria and biofilm. Assuming that surface roughness is a critical factor in S. mutans adherence and has a causal relationship, lower surface roughness with additional polishing may be expected to reduce bacterial adherence and the clinical appearance of caries.

As seen with bacteria-dental interactions typical of enamel or cementum surfaces, in vivo biofilm formation on restorative surfaces has been shown to rely on a combination of physiochemical and biochemical interactions. Physiochemical mechanisms include Lifshtiz-Van der Waals, acid-base, and electrostatic forces, which contribute to interfacial free energies of liquids and interacting solids (DLVO theory). Biochemical interactions additionally include more specific interactions such as receptor-ligand recognition. Furthermore, biofilms are complex and diverse, encompassing thousands of microbial species, nutrients, and molecules that may contribute to adherence. In depth characterization of these interactions and disparities as compared with
interactions with natural tooth structures is therefore integral to the understanding of this system. Adherence and biofilm formation can therefore be regulated by surface qualities, salivary pellicle attachment, nutrient or structural binding, and coaggregation with other microorganisms. Additionally, the presence of different microorganisms may also affect the strength of adhesive forces.15

For biofilms, which typically exist as an air-liquid on fixed surfaces, these microorganisms and substrates are further bathed in an aqueous environment, in this case, saliva, which suspends proteins, nutrients, and other organic molecules. These molecules can quickly adsorb to the restorative material surface to form a salivary pellicle to further modify its physiochemical and biochemical properties, and, therefore, its interaction with bacteria.20,27

In the present study, specimens were fabricated using lithium disilicate from a single manufacturer and followed this manufacturer’s recommendations. Finishing and polishing steps were also limited to a specific protocol. New instruments and methods of surface modification for lithium disilicate that can achieve a more optimal final polish may be introduced with more pervasive use of lithium disilicates and a dental restorative material. Surface roughness has been demonstrated to vary with different polishing techniques for feldspathic surfaces and amongst different modes of measurement; also finishing and polishing significantly changes feldspathic surface roughness characteristics.10,20,23-25 In addition, although the bacterial adherence among 3 different groups of ceramics was assessed, a comparison of bacterial adherence against enamel or cementum would have been valuable.

Furthermore, in the present study, surface roughness, Ra value, was used to quantify surface characteristics, but other variables may also be used to give a more comprehensive description of surface characteristics. Rz, for instance, may have given a more reliable macroscopic description of the surfaces in question, in that it takes into account surface variability without normalization and includes extremes of surface variation.21,23 Considering these other values for surface roughness analysis and the effects that different polishing techniques may have on these lithium disilicates as they are developed and implemented for use may therefore be informative.

Bacteria used for the present study were composed of a single microorganism type, S mutans, while true biofilms are much more heterogeneous, with more varied modes of attachment and a significant degree of interspecies interactions.12-14 S mutans has been demonstrated as the primary etiologic agent in caries initiation, and a reductionist approach can elucidate vital information regarding its interaction with lithium disilicate surfaces; however, additional studies may also use a more holistic approach.14 Pathogenic communities involving Bifidobacterium dentium, Scardovia wiggsiae, Bifidobacterium longum, Bifidobacterium adolescentis, Prevotella spp, Selenomonas spp, and Lactobacilli spp have also been demonstrated to be coincident in the etiology of dental caries.13 By considering S mutans in a multispecies complex oral biofilm with in vivo modifying factors, a more accurate model and understanding of this interaction may be achieved.10

Furthermore, the introduction of modifying factors such as salivary proteins and polymers and the innate surface properties that mediate attachment may also be included in further study. In the oral environment, the adsorption of salivary proteins to the tooth or restorative surface precedes and promotes bacterial adherence. They can form an acquired salivary pellicle to which bacteria and structural substrates may bind.10,15 This affects adhesion strength and may alter surface free energies and zeta potentials formed by surface charge modification that can impact subsequent bacterial binding.16,22 Meier et al in 200826 combined many of these factors in their investigation of adhesion of oral streptococci to ceramics. Results indicated that plaque accumulation was more influenced by the presence of a salivary pellicle than by material type. Viability, however, was influenced by material composition, in this case, differentiated by glass content. Uncoated surfaces exhibited higher variability, but all surfaces were approximately equal after coating with saliva.26,27

Likewise, growth conditions can also be manipulated to better recreate the typical oral environment. Instruments such as a drip flow reactor, which allows low-shear, laminar flow conditions close to the air-liquid interface can be used to simulate such an environment.1,2

An in-depth characterization of the lithium disilicate surface may also be beneficial in elucidating the attachment of S mutans, and biofilm in general. This may include an evaluation of the surface free energy or zeta potential in the presence of saliva or the surface landscape with quantification via scanning electron microscopy.27 Macroscopic variables such as the fluctuation of pH with meals,3,4 mechanical debridement or stimulation of salivary flow with regular oral hygiene or simulated mastication,5 or diet composition of nutrients of different sugar content or textures are other modifications that may also be considered.5,6 Furthermore, these factors may be manipulated in vivo by the patient or the clinician and juxtaposed with natural dental surfaces of enamel or cementum to achieve greater control and understanding of bacterial adherence, biofilm development and caries formation.27

CONCLUSION

The results of this study demonstrate that the surface roughness of differently prepared lithium disilicate ceramic restorations and bacterial adherence have a
strong positive correlation. CAD and Press lithium disilicate ceramics were significantly smoother and harbored fewer bacteria than the ZirPress/Ceram group, which was also smoother and exhibited less bacterial adherence than the Ceram Glaze group.

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