CURRICULUM VITAE

Diane T Vo

diane.vo@gmail.com

Education:

University of Maryland, Baltimore, MD

Certificate, Residency in Prosthodontics (2014)

Master of Science, Biomedical Sciences (2015, expected)

University of the Pacific, San Francisco, CA

Doctor of Dental Surgery (2011)

University of California – Los Angeles, Los Angeles, CA

BS, Microbiology, Immunology, & Molecular Genetics (2008)

BS, Psychobiology (2008)

Research and Related Work:

Prosthodontics Residency, UMB – Carl F. Driscoll, DMD (2011-2014)

Prosthodontics Externship, UMB – Carl F. Driscoll, DMD (2010)

Researcher, UOP – Stefan Highsmith, PhD (2009-2010)

Activities:

Student Member, American College of Prosthodontics (2011-2014)

Co-President, UOP, Prosthodontics/Implants Study Club (2010-2011)

Board, UOP, Project Homeless Connect (2010-2011)

Executive Board, UOP, Jamaica Dental Mission (2009-2010)

Member, UOP, Student Community Outreach for Public Education (2008-2010)

Lectures and Presentations:

American College of Prosthodontics Maryland, Regional Chapter

May 2014 – Clinical Patient Case Presentation

American Academy of Fixed Prosthodontics, Table Clinic

February 2013 – "Restoration of the Maxillary Lateral Incisor"

American College of Prosthodontics, Table Clinic

November 2012 – "Dental Materials and Biofilm Formation"

Prosthodontics Residency, Senior Case Presentation

June 2014 – Clinical Patient Case Presentation

Lectures, University of Maryland – Residency in Prosthodontics

March 2012 – "Anatomy of a Smile"

September 2012 – "Restoration of the Class II Removable Patient"

January 2012 – "Articulators"

August 2013 – "Dental Cements"

November 2013 – "Prosthodontics by the Numbers"

Publications and Awards:

Stanley D. Tylman Grant – $4,000 (American Academy of Fixed Prosthodontics)

2013 – "Streptococcus mutans Biofilm Formation on Lithium Disilicate Porcelain Materials"

AADR Meeting Award (Research Day, UOP)

2010 – "Garlic Extract Induces Detachment of Hydroxyapatite-bound S. mutans"

Undergraduate Research Consortium in Functional Genomics (UCLA)

2007 – "Genomewide Clonal Analysis of Lethal Mutations in the Drosophila melanogaster Eye: Comparison of the X Chromosome and Autosomes."

ABSTRACT

Thesis Title: *Streptococcus mutans* Bacterial Adherence on Lithium Disilicate Porcelain Specimens

Thesis Title: Radi Masri, DDS, MS PhD; Associate Professor, Department of Endodontics, Prosthodontics, and Operative Dentistry, School of Dentistry

Diane Vo, DDS

Streptococcus mutans as it pertains to dental and oral health is significant for its role as the primary etiologic factor of caries. While primary caries results from initiation of lesions in virgin tooth structure, secondary caries is a significant contributing factor to the replacement of dental restorations. Caries formation is directly related to plaque accumulation, which is mediated by bacteria adhesion to intraoral surfaces. In the case of the restored tooth, bacteria must adhere to the restorative material, particularly along margins in order to cause recurrent pathology.

A material that has recently come into much favor is lithium disilicate, a glass based system with fillers in a homogenous glass. Lithium disilicate restorations can be either (1) pressed or (2) milled to fabricate inlays, onlays, veneers or single unit crowns. These restorations can be full-contour, or may be cut back and subsequently modified with (3) veneering fluorapatite, or (4) glazed. With respect to bacterial adhesion to restorative surfaces, the overwhelming factor is surface roughness. The threshold for this effect has previously been found to be 0.2 µm Ra value, above which there was a positive correlation between surface roughness and plaque retention.

Specimens were fabricated for each of the four preparation types per manufacturer's recommendations and incubated with *S. mutans* UA159 wild-type. Biofilms adherent to specimens were then sonicated, redispersed, and plated for quantification. Results were tested with an analysis of variance (ANOVA). Significant differences that were found were further analyzed by Tukey's Honestly Significant Difference (HSD) test. Pearson's *r* was also be used to evaluate the relationship between surface roughness and biofilm accumulation. A p-value of ≤ 0.05 was considered significant. Surface roughness, as quantified by Ra values, indicated that Press and CAD groups were not significantly different from one another, but were significantly lower than that of ZirPress/Ceram, which was lower than surface roughness of the Ceram Glaze group ($F = 513.898$, $p \leq 0.0005$). Similarly, CFUs/ml for the CAD and Press groups were significantly lower than the ZirPress/Ceram group, which were also significantly lower than those of the Ceram Glaze group ($F = 201.721$, $p \leq 0.0005$). A strong positive association was also seen between surface roughness and biofilm accumulation ($r = .95$).

Many factors, such as caries risk, presence of other restorations, and individual patient hygiene, influence whether these differences in surface roughness and biofilm accumulation become clinically relevant to the formation of caries. The present study has demonstrated that different preparations vary in their surface roughness and biofilm accumulation measurements, and that these differences in surface quality are associated with bacterial adherence.

STREPTOCOCCUS MUTANS BACTERIAL ADHERENCE ON LITHIUM DISILICATE PORCELAIN SPECIMENS

By:

Diane Vo

Thesis submitted to the Faculty of the Graduate School of the

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Diane Vo, DDS

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I. Introduction

Bacteria and dental disease

Streptococcus mutans, as it pertains to dental and oral health, is significant for its role as the primary etiologic factor of caries. A gram positive cocci and normal inhabitant of the oral flora, *S. mutans* is a nonmotile facultative anaerobic microorganism that relies principally on fermentable carbohydrates for its nutrition. Cariogenicity, the ability to produce caries, is attributed to its major virulence factors, acidogenicity (acid production), aciduricity (acid tolerance) and the resistance of the biofilm phenotype, which help to manifest in disease (Senadheera 2008).

Acidogenicity is associated with *S. mutans* metabolism of sucrose and other carbohydrates to produce such acids as lactic, formic, acetic and propionic acids, which act to demineralize tooth enamel, manifesting the clinical disease of caries. This ability to produce acid is seen in planktonic as well as biofilm bacteria. The second virulence factor is aciduricity, the ability to withstand and thrive in a low pH environment. Because many other microorganisms in the oral cavity are not as capable of withstanding such acidic conditions, *S. mutans* is able to maintain its niche successfully. Unlike the ability to produce acid, however, aciduricity as a virulence factor is expressed only in the *S. mutans* biofilm phenotype, the form which is present in the oral cavity. Resistance to environmental changes is the third major virulence factor expressed by *S. mutans* and is also only present in biofilm formation by virtue of its structure.

Aside from differential expression of specific genes, biofilm formation provides protection from transient environmental changes, facilitating growth and survival. Biofilm formation also allows for close association with other bacterial species and horizontal gene transfer of resistance factors. Characteristically, biofilm formation is directly related to the bacteria's ability to utilize carbohydrates to produce glucan polymers which aid in bacterial adhesion to the dental pellicle. Specifically, *S. mutans* biofilm is regulated by density-dependent quorum sensing, a cell-cell communication system. Once critical population densities are achieved, autoinducing chemical messengers are released and can trigger gene expression cascades that alter cell population physiology and behavior. The system utilized by *S. mutans* is comprised of the competence-stimulating peptide (CSP) and the comD/comE two component signaling system. Together, these factors mediate the typical transcriptional profile of biofilms that is markedly distinct from its planktonic counterpart, imparting aciduricity, genetic transformation and bacteriocin production in addition to the acidogenicity expressed by the planktonic form. As a result, the biofilm phenotype is more resilient, demonstrating increased genetic competence as well as becoming more highly transformable by exogenous DNA (Senadheera 2008).

In vivo, biofilm development is mediated by initial attachment of bacteria, followed by colonization and maturation. Initially, early colonizers will adhere to the proteinaceous pellicle via adhesins such as alpha amylase,

proline rich proteins, and proline-rich glycoproteins. These bacteria include members of the viridans streptococci group. Biofilms are therefore characterized by surface attachment, structural heterogeneity, complex interspecies interactions, and extracellular matrix. They contribute to high-density micro-niches that are necessary for caries formation (Hojo 2006).

In vitro, biofilm growth must mimic what is found in nature. Biofilms grown under high shear at turbulent flow are stronger, denser and more tightly adherent, while those grown at low shear in laminar flow are fluffy (Pereira 2002). With respect to *S. mutans* biofilm, a low shear/laminar flow technique, such as on a shaker under anaerobic conditions, is most appropriate, as this allows the biofilm to be formed close to the air-liquid interface, mimicking conditions found in the oral cavity (Adams 2002).

Lithium disilicate all-ceramics in dentistry

While primary caries results from initiation of lesions in virgin tooth structure, secondary caries is a significant contributing factor to the replacement of dental restorations (Deligeorgi *et al* 2001). Caries formation is directly related to plaque accumulation, which is mediated by bacteria adhesion to intraoral surfaces. In the case of the restored tooth, bacteria must adhere to the restorative material, particularly along margins in order to cause recurrent pathology. As with natural tooth structure, surface quality can greatly affect bacterial adhesion (Bollen 1997). This can vary between direct restorative materials such as amalgam, resin composites, glass ionomer

cements and compomers as well as with cast or milled indirect materials such as gold, ceramometal margins, many different types of ceramics, and titanium or zirconia implant restorative components. For the most part, cast or milled indirect materials exhibit better marginal fit, finish and polish than direct materials (Busscher 2010).

Ceramics is one such popular indirect material. Ceramics by definition are nonmetallic inorganic materials including metal oxides, borides, carbides, and nitrides within a complex matrix. They are crystalline in structure and display a periodic arrangement of component atoms, exhibiting ionic or covalent bonding. In general, ceramics can be very strong, but also very brittle, being strong in compression but weak in tension (Ivoclar Vivadent). Ceramics can vary in translucency, which contributes to their esthetic qualities. Factors that contribute to this translucency include particle size, particle density, refractive index, and porosities, among others. A restorative dental material that has recently come into much favor is lithium disilicate, a glass-based system with fillers in a homogenous glass. Lithium disilicate glass ceramics were first introduced in 1998 by Ivoclar as Empress II (now IPS e.max pressable and machinable ceramics). The material is marketed as having a crystal content of approximately 70% with improved flexural strength over existing ceramics. The glass matrix is composed of lithium silicate with micron-size lithium-disilicate crystals interspersed, which are submicron lithium orthophosphate crystals. The projected flexural strength is 360MPa according to manufacturer literature. Esthetics is preserved as this

material can be translucent in spite of its high crystalline content due to the low refractive index of lithium disilicate crystals. Veneer porcelain of fluorapatite crystals in an aluminosilicate glass can be used to alter shade and morphology of the final restoration following initial pressing or milling. The fluorapatite consists of fluoride-containing calcium phosphate ($Ca_2(PO_4)_3F$). Lithium disilicate restorations can be either pressed or milled to fabricate inlays, onlays, veneers or single unit crowns. Indications for this material include single unit restorations as far posteriorly as second premolars. These restorations can be full-contour or may be cut back and subsequently modified with veneering fluorapatite (Ivoclar Vivadent).

Ivoclar Vivadent currently markets two types of lithium disilicate materials, IPS e.max Press and IPS e.max CAD for pressed and milled restorations, respectively. Pressed lithium disilicate restorations are made from IPS e.max Press ingots, which have been nucleated and crystallized in one heat treatment. Wax patterns of final restorations are first made with a low residue pressing wax and then invested in IPS PressVEST. IPS e.max Press ingots are then isostatically pressed at approximately 920ºC for 5-15 minutes to form 70% crystalline lithium disilicate ceramics with an approximate crystal size of 3-6 µm in length. The reaction layer that remains from slight absorption of investment material onto the lithium disilicate surface is then removed with immersion in a weak acid solution for 20-30 minutes (i.e. IPS e.max Invex Liquid). The acid solution turns the reaction layer chalky white

which is subsequently removed by blasting with 100 µm aluminum oxide at 1-2 bar (15-30 psi) pressure (Ivoclar Vivadent).

Milled lithium disilicate specimens are made from IPS e.max CAD "blue blocks" by a two-stage crystallization process. Blocks are composed of 40% volume precipitated lithium meta-silicate crystals following double nucleation, the first stage of crystallization. The "blue block" is first milled and then heat treated to approximately 840-850ºC in a porcelain furnace. This allows the final restoration to reach a fine-grain glass ceramic state with 70% crystal volume incorporated into the glass ceramic. Approximate crystal size is 1.5 µm (Ivoclar Vivadent).

IPS e.max ZirPress/Ceram, a low-fusing nano-fluorapatite glass ceramic with IPS e.max Ceram Liquid can then be used for veneering. Following veneering, discs are then polished with Proxyt pink polishing paste. Resulting nano-fluorapatite crystals are 100-300nm, and the micro-fluorapatite crystals are 1-2 µm in length (Ivoclar Vivadent).

This surface, which can be further modified with an additive IPS e.max Ceram Glaze Spray, is not polished or otherwise modified after firing. No information of resultant crystalline surface following glazing is available.

Factors that affect bacterial adhesion: surface roughness and accumulation

In considering bacterial adhesion to restorative surfaces, the overwhelming factor, as with enamel, is surface roughness. In a 1997 literature review by Bollen, it was found that a threshold for this effect was

found to be 0.2 µm Ra value, above which there was a significant positive correlation between surface roughness and plaque retention. Below 0.2 µm, there was no significant relationship. In a separate study testing plaque retention on implant abutments for overdentures of ceramic versus titanium of surface roughness of 0.2 and 0.06 µm respectively, researchers found that titanium abutments harbored supra- and sub-gingival organisms at 3 months while the ceramic abutments did not. At 12 months, however there was no difference in the presence of spirochetal and motile organisms between the two materials. Aerobic cultures indicated a higher proportion of gram-negative organisms on rougher surfaces, but this was not confirmed as significant, as no statistical test was done (Bollen 1997). In general, there is limited research available pertaining specifically to surface quality of lithium disilicate ceramics, but other ceramics have been extensively studied.

A study by Lee *et al.* in 2011 looked at initial bacterial adhesion to three different dental materials (resin, titanium, and zirconia) with respect to implant restorations in particular and considered differences in surface roughness and hydrophobicity. Scanning electron microscopy (SEM) was used to visually rank the amount of bacteria bound to one material versus another, but quantification of total biomass was done by crystal violet and measured with fluorescence intensity. Results indicated that adherence of bacteria was most closely related to surface roughness, with resins exhibiting significantly higher plaque accumulation than titanium or zirconia, whereas differences in plaque accumulation between titanium and zirconia were

nonsignificant. Samples used were commercially pure titanium cp-Ti machined into discs and 3Y-TZP (3 mol% yttria-doped tetragonal zirconia polycrystalline) powder die-pressed into discs and then isostatically pressed to 140MPA and finally polished/finished with 1µm diamond paste to a mirrorlike surface. SEM showed more abundant bacterial colonies on resin with greater total biofilm accumulation ($p<0.05$).

In a 2009 study by Al-Marzok *et al.* evaluated adhesion of oral bacteria, specifically *S. mutans*, with respect to surface roughness of ceramic restorations. Surface roughness of a feldspathic metal ceramic was varied by using four different methods of surface finishing: polishing with a diamond bur, polishing with sand paper, sandblasting and glazing. The resultant Ra values ranged from 0.005 to 0.025 µm. Samples were then inoculated with *S. mutans* isolated from human saliva. Results indicated a significant positive correlation between surface roughness and bacterial adhesion ($r= 0.813$, $p=0.003$).

Similarly, a study by Aykent *et al.* in 2010 looked at the effects of surface roughness with respect to various dental materials: indirect composite resin, direct composite resin, and ceramic. Sample preparations consisted of finishing and polishing of samples with their manufacturer recommended methods. Results indicated lower surface roughness associated with the ceramic and higher roughness associated particularly with indirect resins, possibly due to higher inorganic fillers ($p<0.05$). A positive correlation was seen between surface roughness and adhesion of *S. mutans* ($r = 0.594$).

This effect was further studied by de Fucio *et al.* in 2009 using different restorative materials (ceramic, resin composite, conventional glass ionomer, and resin modified glass ionomer). Fifteen disks of each material were incubated with *S. mutans* for 30 days. No significant difference between the materials was found for bio-volume of biofilm, roughness coefficient, and surface to volume ratio. Biofilm thickness of ceramics and resin composites significantly exceeded that of glass ionomer cement ($p<0.05$).

Kantorski *et al.* (2009) conducted similar experiments in which surface roughness was evaluated for adherence of *S. mutans* on microparticulate feldspathic porcelain, leucite-reinforced feldspathic porcelain, microhybrid resin composite, and microfilled resin composite. Greater bacterial adherence and surface roughness was found on leucite reinforced feldspathic ceramic relative to microparticulate feldspathic ceramic. No significant differences were found between composite types or between each type of ceramic and composite groups ($p<0.0001$).

In another sudy in 2008, Kantorski again looked at adherence of *S. mutans* to different natural and restorative surfaces, enamel, resin composite, leucite/feldspathic ceramic, and feldspathic ceramic. SEM revealed that plaque accumulation began with protein pellicle formation and bacterial adsorption. Next, granular aggregates of organic material collected and developed into globular, granular and fibrillar aggregates of salivary proteins and polysaccharides. The SEM also indicated that biofilm formation was much denser on enamel and composite surfaces than on ceramic. Results

indicated that adherence was closely associated with surface roughness both in the uncoated and saliva-coated applications. Enamel was significantly rougher than leucite/feldspathic ceramic, which was significantly rougher than feldspathic ceramic ($p<0.0001$). The authors explained their results by indicating that enamel might had surface irregularities that could have contributed to these findings. In addition, leucite –reinforced feldspathic ceramic contained several sizes of quartz and leucite crystals that might have influenced roughness while regular feldspathic ceramic was more homogenous. SEM and TEM showed that bacterial adherence was initiated in surface irregularities and then expanded.

Finishing of porcelain surfaces and resultant bacterial adhesion was addressed in a 2000 article by Kawai *et al.* For all refinished porcelain surfaces, a direct correlation was found between surface roughness and bacterial plaque accumulation ($r = 0.990$, $p<0.05$). Diamond paste polished surfaces had the lowest Ra values, followed by No. 600 polished surfaces, then No. 120 polished surfaces, with corresponding increasing bacterial accumulation. In contrast, nonrefinished glazed surfaces, which were the smoothest, actually demonstrated the greatest amount of plaque accumulation. This was hypothesized to be due to undulating rough surfaces with irregularities that would induce greater adhesion of bacteria.

In their 2009 study, Bremer *et al.* expanded on the study of plaque accumulation on different types of dental ceramics *in vivo*. The materials used were veneering glass ceramic, lithium disilicate glass ceramic, yttrium-

stabilized zirconia, hot isostatically pressed Y-TZP ceramic, and HIP Y-TZP ceramic with 25% alumina. Samples were attached to acrylic splints which were worn by subjects for 24 hours. Coating and biofilm thickness were measured by confocal microscopy. The highest surface coating and biofilm thickness was associated with lithium disilicate glass ceramic and was statistically significantly different from other materials tested ($p<0.001$).

Factors that affect bacterial pathogenicity: biofilm viability

As noted previously, surface roughness is a significant contributing factor to bacterial adhesion and biofilm accumulation. However, in addition to sheer bio-volume, it is necessary to examine other qualities of biofilms that adhere to these surfaces, as these materials can exhibit other surface properties that affect biofilm formation, distribution, and pathogenicity. Some studies have examined bacterial viability as a more accurate quantification of bacterial pathogenicity.

In their 2011 paper, Hahnel *et al.* looked at two materials, glass-ionomer cement and ceramic with respect to biofilm accumulation. In terms of surface roughness, glass ionomer cements had a significantly higher Ra than ceramics ($p<0.001$). *S. mutans* was found to have significantly higher absorbance levels on ceramics than on glass ionomer cement, indicating significantly higher levels of cell viability ($p<0.001$). The paper further differentiated biofilm induction of caries in terms of viable biomass and metabolic activity. Metabolic activity, which was used to infer bacterial

viability, was measured by assaying for lactate production, which has been shown to demineralize tooth structure, producing caries. It was shown that the biofilm between ceramic and glass ionomer cement did not demonstrate a significant stratum-dependent difference in lactate production ($p=0.067$), although their data may have approached significance with a larger sample size: However, the authors did not look at the fluoride release of the glass ionomer cement but assumed that leaving samples in solution allowed the majority of the fluoride to dissipate to minimal levels.

Meier *et al.*, in 2008, also investigated adhesion of oral streptococci to all-ceramics, specifically at adhesion of initial colonizers after an incubation time of one hour. The samples used were different types of ceramics (glass, feldspathic, glass-infiltrated alumina, zirconia reinforced glass infiltrated alumina, tetragonal stabilized zirconia). 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. Surface roughness and contact angles of uncoated and saliva-coated surfaces were measured for reference. Uncoated surfaces exhibited higher variability, but all surfaces were approximately equal after coating with saliva. Surface hydrophobicity of bacteria was quantified by partitioning to hexadecane in PBS and to human saliva. Again, surface quality was equalized with introduction of human saliva. Samples were incubated in a shaker with bacteria. Viability was

quantified with the Baclight Live/Dead assay with fluorescent dyes and then fluorescence was measured.

Auschill *et al.* in 2002 took an *in vivo* approach to the question of surface coating, biofilm vitality, and biofilm height. Three volunteers each wore six different specimens (amalgam, gold, ceramic, resin composite, compomer, and glass ionomer cement) for five days. Samples were removed only during meals and oral hygiene, with brushing done using only tap water. After testing, samples were removed and placed in saline. Adhering biofilm was vital-stained with fluorescein diacetate (FDA) and ethidium bromide (EB) to visualize live and dead bacteria. Confocal scanning laser microscopy was used to assay bacterial viability and accumulation. Among the three subjects, bacterial surface coating varied between 27.9% and 92% after an incubation time of 120 hours each. After five days, biofilms on these materials used in the Auschill study were thick (11-17 μm) and completely covered the surfaces, but were barely viable (<8% compared to 41/56% for normal enamel). For gold in particular, which is biocompatible and inert, low viability was hypothesized to be due to the sheer biomass of the biofilm such that nutrients cannot permeate rather than due to the quality of the material itself. For ceramics, biofilms were relatively thin (1-6 μm) but highly viable (34-86%). This suggests that thin biofilms are more viable than thick ones. Results for the other five sample types were not discussed here as they were not germane to the current discussion.

Results of this Auschill study and others were summarized in Busscher's 2010 review of Biofilm Formation on Dental Restorative and Implant Materials. Surface qualities that were thought to affect this difference in surface coating and viability were hypothesized to be related to the fact that most surfaces were negatively charged and electron-donating with a small hydrogen-donating component. *In vitro* relationships are governed primarily by substratum hydrophobicity, surface free-energy and charge, depth of DLVO-interaction energy minima or surface roughness. A hydrophobic surface harvests less biofilm than a hydrophilic one due to fluctuating sheer in the oral cavity, but will still attract bacteria subgingivally under constant sheer. In general, smooth surfaces attract less biofilm than rough ones. For metallic biomaterials, bacterial adherence is mediated by electron-transfer, where a negatively charged bacterium and oppositely charged material have a strong electrostatic attractive force.

Given the available information regarding differences in surface crystal size, post-fabrication modification, and surface roughness effects on bacterial accumulation, several outcomes might be anticipated. IPS e.max Ceram Glaze, which is not polished after firing, would be 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. Crystal size would also be expected to be directly correlated to bacterial adherence.

II. Purpose

The purpose of this study was to investigate how biofilm accumulation is affected by surface quality of differently prepared lithium disilicate all-ceramic materials. A correlation between surface roughness and bacterial adherence was also evaluated. Microscopy was used to visualize differences in biofilm accumulation.

The different types of preparations of lithium disilicate tested were:

1. IPS e.max Press
2. IPS e.max CAD
3. IPS e.max ZirPress/Ceram
4. IPS e.max Press with IPS e.max Ceram Glaze Spray

III. Hypothesis

Null Hypothesis:

1. There is no significant difference in surface roughness on the four differently prepared lithium disilicate materials.
2. When treated *in vitro* with a simple oral bacterial culture, there is no significant difference in adherence on the four differently prepared lithium disilicate materials.
3. There are no significant correlations between surface roughness and adherence on specimens of all four groups when considered together or on each of the differently prepared lithium disilicate materials, separately.

Research Hypotheses:

1. Press with Ceram Glaze lithium disilicate will exhibit significantly higher surface roughness than Press, CAD, or ZirPress/Ceram lithium disilicate substrates; Press substrates will have the next highest surface roughness, with CAD and ZirPress/Ceram surfaces significantly lower.
2. When treated *in vitro* with a simple oral bacterial culture, Press with Ceram Glaze lithium disilicate substrates will exhibit significantly greater adherence than Press, CAD, or ZirPress/Ceram lithium disilicate substrates: Press substrates will have the next greatest adherence, with CAD and ZirPress/Ceram surfaces significantly lower.
3. There is a positive correlation between surface roughness and adherence on specimens of all four groups when considered together and on each of the differently prepared lithium disilicate materials, separately.

IV. Materials and Methods

Table 1. Materials for specimens

Material	Composition	Preparation
IPS e.max Press	Lithium disilicate	Pressed
IPS e.max CAD	Lithium disilicate	Milled
IPS e.max ZirPress/Ceram	Fluorapatite	Pressed
IPS e.max Press + Ceram Glaze Spray	Fluorapatite + Glaze	Pressed

* Ivoclar Vivadent Inc. (175 Pineview Drive, Amherst, NY 14228)

A. Preparation of Samples

Four different groups of lithium disilicate all-ceramic specimens (17 in each group) were tested (Table I). These four groups were: (1) lithium disilicate pressed, (2) lithium disilicate milled, (3) lithium disilicate fluorapatite veneer, and (4) lithium disilicate glazed.

Pressed lithium disilicate specimens were made from IPS e.max Press ingots. Wax patterns were created as squares of baseplate wax 10mm x 10mm and 4mm in thickness. This was standardized using a mold of putty vinyl polysiloxane material and a micrometer digital caliper. Patterns were then sprued, invested, and using the lost wax technique, burned out and ingots were pressed to create specimens, as directed by the Ivoclar Vivadent protocol. Burning out and pressing of specimens was done off site, by a single operator, utilizing equipment at Ivoclar Vivadent Inc (Jelenko Accu-Term III 6000, Programat EP 5000). Resultant specimens were restandardized using sandpaper wheels and measured with micrometer digital caliper to confirm appropriate dimensions. Testing surfaces were then polished per recommendations utilizing Dialite LD

Lithium Disilicate Extra-Oral Adjustment/Polishing Wheels (Brasseler USA). Specimens were otherwise unaltered prior to testing. (Ivoclar Vivadent Inc).

Milled lithium disilicate specimens were made from IPS e.max CAD “blue blocks.” “Blue blocks” were sectioned to squares 10mm x 10mm and 4mm in thickness. Discs were then heat treated to approximately 840-850ºC in a porcelain furnace for crystallization, as directed by the Ivoclar Vivadent protocol (Programat P500). Resultant specimens were restandardized using sandpaper wheels and measured with micrometer digital caliper to confirm appropriate dimensions. Testing surfaces were then polished per recommendations utilizing Dialite LD Lithium Disilicate Extra-Oral Adjustment/Polishing Wheels (Brasseler USA). Specimens were otherwise unaltered prior to testing. (Ivoclar Vivadent Inc).

Lithium disilicate fluorapatite veneer specimens were made from IPS e.max ZirPress ingots and later cut back and veneered with IPS e.max Ceram . Wax patterns were created as squares of baseplate wax 10mm x 10mm and 4mm in thickness. This was standardized using a mold of putty vinyl polysiloxane material and a micrometer digital caliper. Patterns were then sprued, invested, and, using the lost wax technique, burned out and ingots were pressed to create specimens, as directed by the Ivoclar Vivadent protocol. Burning out and pressing of specimens was done off site by a single operator utilizing equipment at Ivoclar Vivadent Inc

(Jelenko Accu-Term III 6000, Programat EP 5000). Resultant specimens were minimally cut back and IPS e.max Ceram was layered and fired. Specimens were then restandardized using sandpaper wheels and measured with micrometer digital caliper to confirm appropriate dimensions. Testing surfaces were then polished per recommendations utilizing Dialite LD Lithium Disilicate Extra-Oral Adjustment/Polishing Wheels (Brasseler USA). Specimens were otherwise unaltered prior to testing. (Ivoclar Vivadent Inc).

Lithium disilicate glazed specimens were made from IPS e.max Press ingots. Wax patterns were created as squares of baseplate wax 10mm x 10mm and 4mm in thickness. This was standardized using a mold of putty vinyl polysiloxane material and a micrometer digital caliper. Patterns were then sprued, invested, and using the lost wax technique, burned out and ingots were pressed to create specimens, as directed by the Ivoclar Vivadent protocol. Burning out and pressing of specimens was done off site by a single operator utilizing equipment at Ivoclar Vivadent Inc (Jelenko Accu-Term III 6000, Programat EP 5000). Specimens were then sprayed with IPS e.max Ceram Glaze Spray and heat treated to approximately 405ºC in a porcelain furnace for glaze firing, as directed by Ivoclar Vivadent protocol (Programat P500). Resultant specimens were restandardized using sandpaper wheels and measured with micrometer digital caliper to confirm appropriate dimensions. Testing surfaces were

then polished per recommendations utilizing Dialite LD Lithium Disilicate Extra-Oral Adjustment/Polishing Wheels (Brasseler USA).

B. Assessment of Surface roughness – (Ra)

Surface roughness for all specimens was measured using a profilometer located at the University of Maryland, Baltimore County (Model T8000 Hommelwerke, Rochester Hills, MI). The profilometer was previously calibrated with a standard reference specimen and set to travel at 0.100 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 by three passes of the profilometer, with profiles obtained at 2.5mm, 5mm, and 7.5mm along one dimension on the surface of the specimen. Mean Ra was used for statistical analysis.

C. Bacterial adherence

Specimens were stored in Dulbecco's phosphate buffered saline (PBS) (Sigma Aldrich, St. Louis, MO) after fabrication. Specimens were washed with PBS and autoclaved prior to biofilm growth.

Wild-type *S. mutans* UA159 (*S. mutans* Clarke, Manassas, VA) was retrieved from deep-freeze at -80 ºC, maintained on Brain Heart Infusion (BHI) agar plates (Sigma Aldrich, St. Louis, MO) and grown in BHI broth (Teknova, Hollister, CA) anaerobically in an anaerobic jar at 37ºC until the early-stationary phase of growth. Cells were then harvested by centrifugation (8000 rpm, 18ºC, 3 min), washed three times with

10mM Dulbecco's Phosphate Buffered Saline (Sigma Aldrich, St. Louis, MO) and resuspended in PBS. Optical density of the suspensions was adjusted to 0.13 at 550nm (BioMate 3S UV-Visible Spectrophotometer, Thermo Scientific, Waltham, MA) which corresponds to a microbial concentration of 1.3×10^8 cells/mL. Microbial suspension was confirmed by using spectrophotometry against predetermined absorbance values for bacterial density.

Lithium disilicate specimens were placed into wells, with one specimen per well. Cell density was adjusted to a final concentration of 1×10^8 cells/mL BHI for inoculation of specimen surfaces (Satau et al 1988). Two ml of bacterial solution was added to each well. Specimens were then incubated for 48 hours with a CO_2 pack in anaerobic conditions on a shaker at 37ºC.

D. Assessment of Biofilm Accumulation

Viability Assay: Following incubation, specimens were removed and washed serially three times with PBS. Specimens were placed in 50ml centrifuge tubes with 2ml PBS. Biofilms were then dissociated from specimens using a sonicator. A series of dilutions were then made and suspensions were dotted in 10µl aliquots on BHI agar in triplicate for colony forming units (CFU/ml) to assay for bacterial viability as a quantification of biofilm accumulation.

E. Statistical Analysis

Since this research was new in focus, no data was available to use to conduct a power analysis. Therefore, a pilot study of n = 3 was conducted and the data was used to establish the exact n for the current study. For surface roughness, the results of the power analysis showed that, with an n = 3, a one-way ANOVA, a one-tailed test, four ceramic preparation types, and an effect size of .51, power was equal to .94. This is considered a "very large" effect size. For biofilm accumulation, with an n = 3, a one-way ANOVA, a one-tailed test, four ceramic preparation types, and an effect size of 24.26, power was equal to 1.00. This is also considered a "very large" effect size. While an n = 3 in each group would have been sufficient according to the power analysis, an actual n = 17 was utilized.

A one-way analysis of variance (ANOVA) was used to test the first two hypotheses. Significant differences were further analyzed by Tukey's Honestly Significant Difference (HSD) test. Pearson's *r* was used to evaluate the relationship between surface roughness and biofilm accumulation for data from all the groups together and each of the groups separately. A p value of ≤ 0.05 was considered significant.

V. Results

Four differently prepared lithium disilicate surfaces were measured for surface roughness and tested for differences in bacterial adherence (see Appendix). All specimens were included in the statistical analysis. Results indicated that these different surfaces exhibit differences for both surface roughness and bacterial adherence and that there is a positive relationship between the two variables.

Surface Roughness of IPS e.max Specimens

With respect to surface roughness, ANOVA results demonstrated a significant difference. Ra values for the CAD and Press groups were significantly lower than the ZirPress/Ceram group, which were also significantly lower than those of the Ceram Glaze group ($F = 513.898$, $p \leq 0.0005$, see Table 2, Figure 1).

Table 2. ANOVA Table Comparing Surface Roughness – Ra (µm)

Surface Type	N p	Mean ± SD	F
CAD .0005**	17	$0.10 \pm 0.02^{a*}$	513.898
Press	17	0.11 ± 0.02^{a}	
ZirPress/Ceram	17	0.71 ± 0.09^{b}	
Ceram Glaze	17	1.32 ± 0.20^{c}	

*Groups with the same letter are not significantly different.
**Significant.

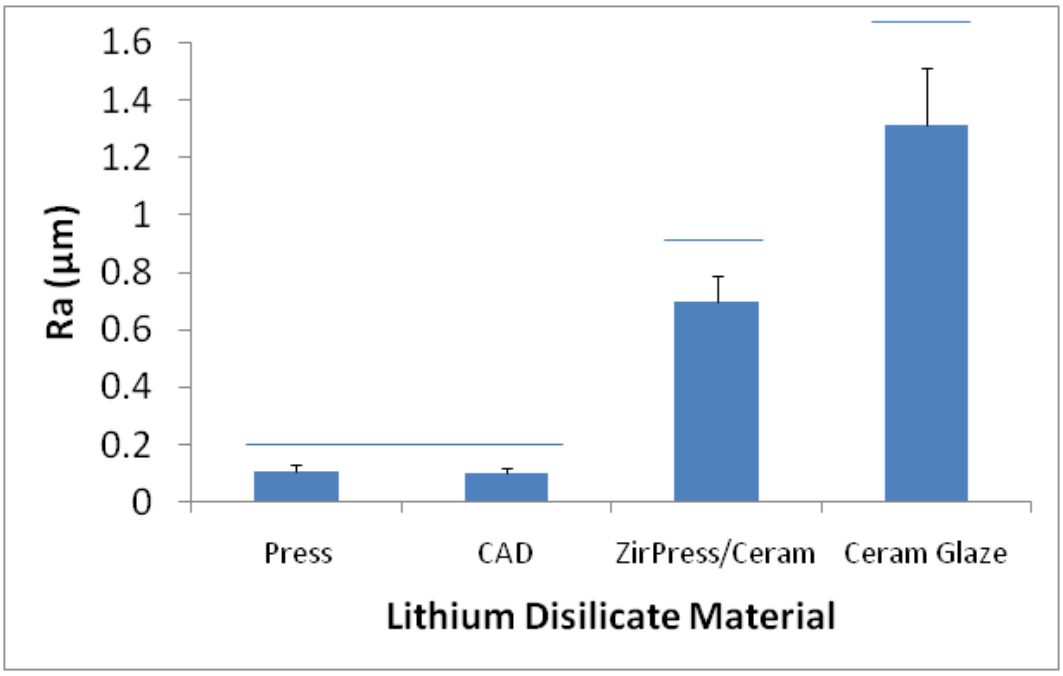

Figure 1. Surface Roughness across Four Lithium Disilicate Surface Types. Horizontal bars connecting groups denote results that are not significantly different. Error bars denote standard deviation within each data group ($F = 513.898$, $p \leq 0.0005$).

Bacterial Adherence of IPS e.max Specimens

With respect to biofilm accumulation, ANOVA results also demonstrated a significant difference. CFUs/ml for the CAD and Press groups were significantly lower than the ZirPress/Ceram group, which were also significantly lower than those of the Ceram Glaze group ($F = 201.721$, $p \leq 0.0005$, see Table 3, Figure 2).

Table 3. ANOVA Table Comparing Bacterial Adherence – CFUs/ml ($x10^4$)

Surface Type	N p	Mean ± SD	F
Press .0005**	17	6.64 ± 2.79[a*]	201.721
CAD	17	12.86 ± 1.70[a]	
ZirPress/Ceram	17	28.53 ± 2.40[b]	
Ceram Glaze	17	61.82 ± 13.76[c]	

*Groups with the same letter are not significantly different.
**Significant.

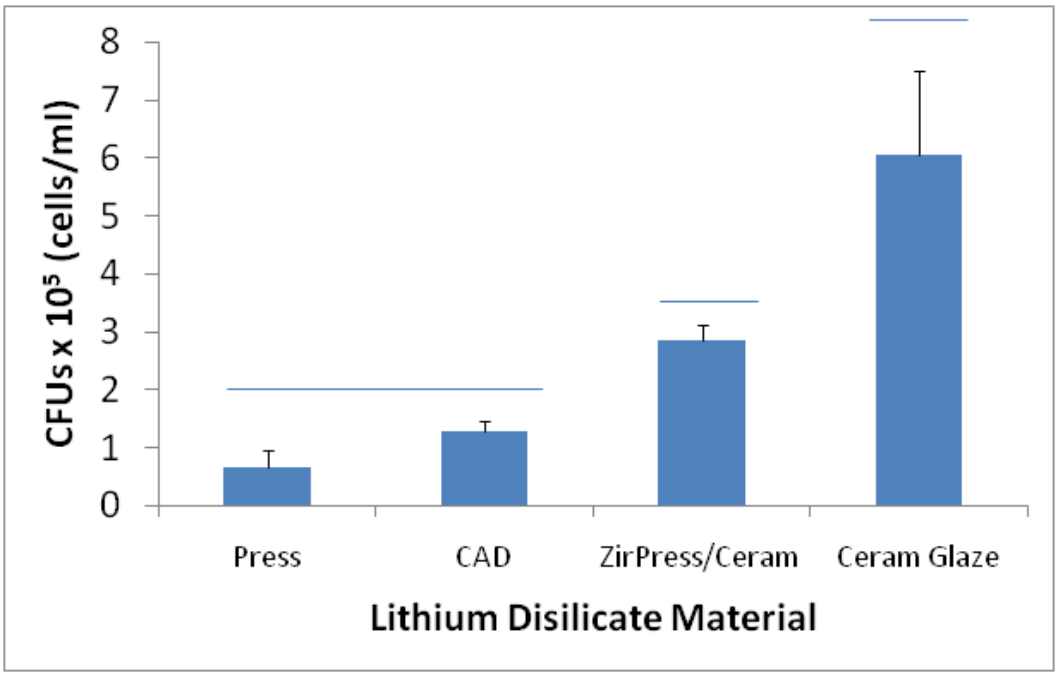

Figure 2. Bacterial Adherence across Four Lithium Disilicate Surface Types. Horizontal bars connecting groups denote results that are not significantly different. Error bars denote standard deviation within each data group ($F = 201.721$, $p \leq 0.0005$).

Relationship Between Surface Roughness and Bacterial Adherence

Data was also analyzed for correlations among all specimens examined together as well as within each preparation group separately. Results demonstrated that a strong positive correlation was present when all specimens were considered ($r = .95$, $p \leq .0005$, see Figure 3).

For the Press, CAD, and ZirPress/Ceram groups separately (within groups), significant correlations were not found between surface roughness and biofilm accumulation. For the Ceram Glaze group, a significant positive correlation was found between surface roughness and biofilm accumulation ($r = .62$ $p=.007$, see Figure 4).

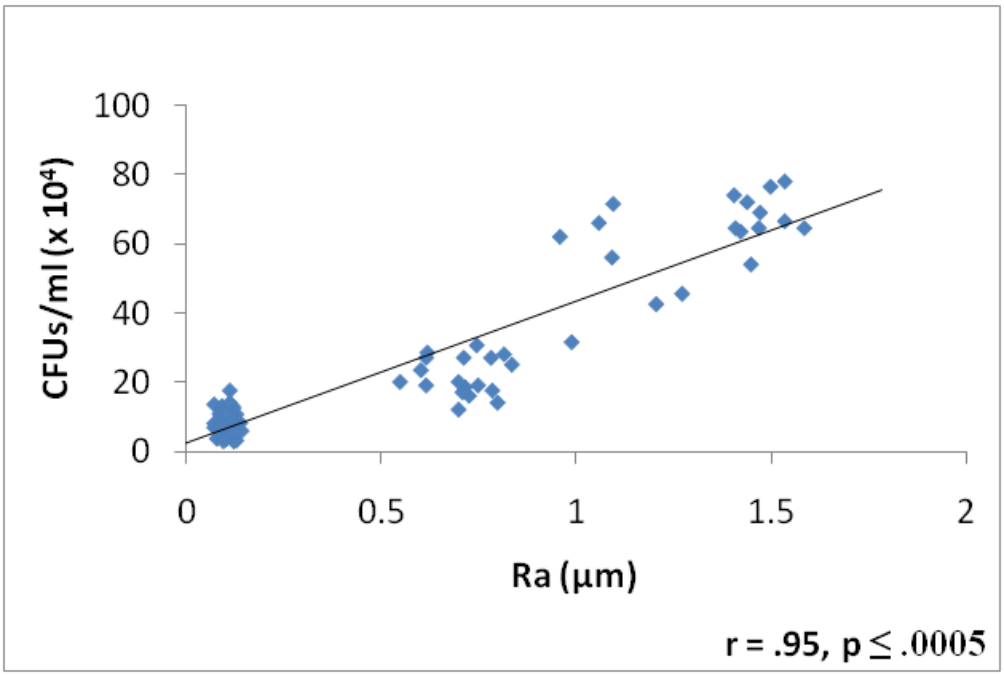

Figure 3. Correlation between Surface Roughness and Bacterial Adherence (Total)

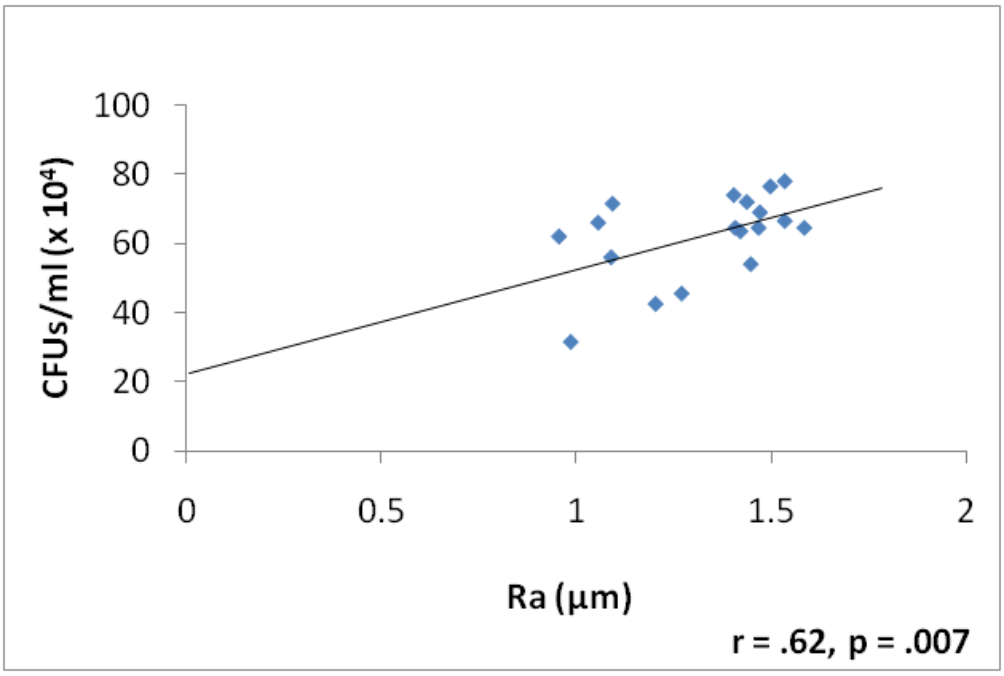

Figure 4. Correlation between Surface Roughness and Bacterial Adherence (IPS e.max Ceram Glaze)

VI. Discussion

In the present study, differences in surface roughness (as quantified by Ra values) and bacterial adherence (as quantified by CFUs/ml) were examined across four different lithium disilicate groups. Based on the results of this study, the following null hypotheses were rejected:

1. There is no significant difference in surface roughness on the four differently prepared lithium disilicate materials.
2. When treated *in vitro* with a simple oral bacterial culture, there is no significant difference in bacterial adherence on the four differently prepared lithium disilicate materials.
3. There are no significant correlations between surface roughness and bacterial adherence on specimens of all four groups when considered together or on each of the four differently prepared lithium disilicate materials, separately.

The results of the present study rejected the first two hypotheses. Some, but not all, correlations were significant; therefore the results also rejected portions of the third hypothesis.

Surface Roughness of IPS e.max Specimens

As predicted, Ra values varied as expected, with machined, minimally modified, and subsequently polished surfaces such as Press and CAD exhibiting significantly lower Ra values, while ZirPress/Ceram surfaces composed of ZirPress with Ceram modification followed by polishing demonstrated intermediate Ra values. Following the same trend, Ra values for

the Ceram Glaze group were significantly higher than the other three groups. The surface of the Ceram Glaze group specimens were subsequently sprayed with an additive glaze prior to final firing and were not polished after glazing as specified by the manufacturer. However, this may explain why they exhibited the greatest surface roughness.

Initially, differences were hypothesized to be related to surface crystal size, with Ceram Glaze expected to be the roughest, followed by Press (3-6μm), and then CAD (1.5μm) and ZirPress/Ceram (1-2μm). However, the results appear to be better explained by surface treatment during and after fabrication. Ceram Glaze specimens were roughest, as predicted, but ZirPress significantly exceeded Press and CAD in surface roughness. Ra value measurements reflected macroscopic physical differences achieved by fabrication method and subsequent polishing rather than corresponding to surface crystal sizes presented in the manufacturer's literature. Clinically, these differences can be minimized by the clinician or dental laboratory by additional supplemental polishing following glazing. The polishing protocol utilized for the present study was limited to specific finishing and polishing instruments advocated by one manufacturer. As lithium disilicate materials are relatively new to the market, as are the instruments to be used with them, new methods of surface modification that can be used to achieve a more optimal final polish may be introduced.

Bacterial Adherence of IPS e.max Specimens

Bacterial adherence measurements also demonstrated significant differences. As with surface roughness, biofilm accumulation varied such that Press and CAD groups did not demonstrate significant differences, but were both significantly lower than ZirPress/Ceram, which was significantly lower than Ceram Glaze specimens. Differences in biofilm accumulation appeared to follow variations in Ra values. *In vivo*, oral biofilms are typically an aggregate of many different microorganisms, with *S. mutans* as the etiologic agent of caries. As the accepted etiologic agent for caries formation, this is a logical choice. However, because attachment in biofilm is a complex process, actual *in vivo* biofilm formation would not be expected to mimic these results exactly. For the clinical manifestation of caries, more complex biofilms with the presence of additional host factors and saliva may show greater relevance.

Relationship Between Surface Roughness and Bacterial Adherence

In addition, a direct correlation between surface roughness and biofilm accumulation was also demonstrated when all specimens across all surface types were considered together. Significance was seen between surface roughness and biofilm with a correlation coefficient $r = 0.95$, indicating a strong positive association. Within groups, correlations ranged from non-significant to significant and moderate, to significant and strong. As was seen in previous studies, differences in Ra values greater than 0.2µm (Bollen 1997) did significantly correlate with biofilm accumulation. This is

seen with Press and CAD groups. The ZirPress/Ceram group, which exceeded the 0.2µm threshold for Ra values, did not demonstrate a correlation.

These findings can be explained by the fact that IPS e.max Ceram modification on the ZirPress surface requires the addition of fluorapatite material via a layering technique that can result in a greatly variable surface, depending on the skill of the technician. This is contrasted to a purely milled or pressed surface that does not show nearly the same degree of surface variation. Because the measurements made for Ra take into account the trajectory of a stylus across only three different paths on the surface of each specimen, this may be a skewed interpretation of the surface's true characteristics and may account for the differences in surface roughness. In practice, IPS e.max Ceram can improve esthetics by allowing intrinsic and extrinsic color or contour modification. In the present study, the addition of this material appears to induce greater surface roughness. The limitations of using Ra measurements in the characterization of the ZirPress/Ceram surface are in contrast to the quantification of bacterial adherence, which is more comprehensive as it takes into account all surfaces of the specimens and is better able to detect variations over the surface area of the specimens examined.

The Ceram Glaze group, which was well above this threshold, did show significant correlations between the two variables. The stronger positive association found for the total group is most likely due to the large range of values between the four groups and the higher sample size. While the CAD

and Press groups exhibited low variability, they demonstrated low values for both surface roughness and biofilm accumulation. When contrasted to the high variability and high values of the Ceram Glaze group across the same two variables, a significant positive correlation became likely.

While the results demonstrated a correlation, this does not necessarily indicate causation. Logically, it follows that macroscopically higher surface roughness would increase the propensity of a material to accumulate bacteria and biofilm: However, this may also be affected by other surface characteristics. Surface charge, zeta potential, structural mechanical properties are some of these factors. Assuming that surface roughness is a critical factor in biofilm accumulation and has a causal relationship, lower surface roughness with additional polishing may be expected to reduce biofilm accumulation and the clinical appearance of caries.

Limitations

The ceramic used, lithium disilicate, is a relatively new material and has not been subjected to the rigorous studies that its predecessors have. The specimens used for this study were fabricated with lithium disilicate from a single manufacturer and followed this manufacturer's recommendations for preparation. Finishing and polishing steps were also limited to a very specific protocol design for use with lithium disilicates, although other instruments and materials may have provided different results.

Clinically, these differences can be minimized by the clinician or dental laboratory by additional supplemental polishing following glazing. The

polishing protocol utilized for the present study was limited to specific finishing and polishing instruments advocated by one manufacturer. As lithium disilicate materials are relatively new to the market, as are the instruments to be used with them, new methods of surface modification that can be used to achieve a more optimal final polish may be introduced.

Surface roughness, Ra, was used to quantify surface characteristics, but other variables may also be addressed 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, as it takes into account surface variability without normalization.

Likewise, with biofilm accumulation, as discussed before, the biofilm utilized for this study was composed of a single microorganism type, *S. mutans*, while true biofilm is much more heterogeneic, with more varied modes of attachment to intraoral restorative surfaces. These modes may also depend on the presence of additional modifying factors such as salivary proteins and polymers, as well as innate surface properties that mediate attachment. These factors were not addressed in the present study to minimize the confusion of potential confounding variables.

Further Research

With the limitations of the present study, it follows that there may be potential avenues for further research. With the introduction of different finishing and polishing methods and materials, surface roughness may be improved. An in-depth characterization of the lithium disilicate surface may

also be beneficial in elucidating the attachment of *S. mutans*, and biofilm in general, to such a surface. This may include evaluation of the zeta potential in the presence of saliva or the surface landscape with quantification via scanning electron microscopy. With respect to the interaction that this surface may have with biofilm, the complex nature of the environment in which these materials are used may also be considered. Variations may include fluctuation of pH with meals, mechanical debridement simulation, regular oral hygiene, or the introduction of the flux characteristic of such a habitat.

Further research is possible and necessary to explore the intricacies of the function of these lithium disilicate ceramic materials for dental restoration. As more variables are elucidated and studied, it will be easier to understand how they may impact the greater clinical manifestation of caries.

VII. Conclusion

In the present study, differences in surface roughness as quantified by Ra values and biofilm accumulation as quantified by CFUs/ml were examined across four different lithium disilicate fabrication groups. For both surface roughness and biofilm accumulation, CAD and Press groups were significantly lower than the ZirPress/Ceram group, which was also significantly lower than the Ceram Glaze group. These differences appear to be directly related. Furthermore, these variables have a strong positive correlation to one another.

Clinical Relevance

As demonstrated by the present study, surface roughness of various types of lithium disilicate has been shown to vary, and that these differences can be positively associated with biofilm accumulation. This has significant implications on oral and systemic health. Firstly, lithium disilicate in dentistry is used as an indirect restorative material. In general, the presence of dental restorations is associated with higher caries risk due to the existence of margins that may potentially trap bacteria, in this case demonstrated for *S. mutans*, the etiologic agent of caries.

When indirect dental restorations interface with tooth structure at margins on cleansable buccal or lingual surfaces, this is less of a concern, as patient hygiene adequately address bacterial removal. However, when these margins are interproximal or along the gingival crest, other concerns arise. Periodontal bacteria, which may also form biofilm can be directly affected by

roughness of these restorations. This may result in periodontal disease. This in turn may translate to systemic diseases, as research has demonstrated the presence of periodontal pathogens in remote areas of the body, presumably passed through blood.

The scope of the current research is deliberately narrow in focus to elucidate the exact relationships that these lithium disilicate restorations may have in the oral environment. However, the potential implications and impact on health that this may have are great and should be considered carefully as more advances are made in the field of dentistry.

VIII. Appendix

	IPS e.max Press							
	Surface Roughness (Ra)			Ave	CFU/ml (1/15000 dilution)			Undiluted (10^4)
1	0.12	0.13	0.10	0.12	4.3	3.5	4.4	6.1
2	0.08	0.07	0.06	0.07	4.5	4.7	4.4	6.8
3	0.16	0.13	0.12	0.14	4.4	5.6	6.3	8.2
4	0.09	0.09	0.09	0.09	6.0	4.6	6.2	8.4
5	0.12	0.11	0.13	0.12	5.2	3.3	8.0	8.3
6	0.12	0.09	0.09	0.10	5.4	5.2	5.3	8.0
7	0.10	0.11	0.10	0.10	6.1	6.2	5.2	8.8
8	0.07	0.08	0.10	0.08	5.9	5.3	5.3	8.3
9	0.12	0.11	0.11	0.11	5.0	5.6	4.6	7.6
10	0.15	0.14	0.13	0.14	4.2	4.2	3.2	5.8
11	0.12	0.10	0.09	0.10	4.6	4.0	4.0	6.3
12	0.13	0.13	0.12	0.13	7.5	6.8	6.9	10.6
13	0.10	0.09	0.14	0.11	7.5	7.0	7.4	11.0
14	0.12	0.10	0.12	0.11	6.2	5.2	6.4	8.9
15	0.13	0.12	0.13	0.13	2.1	2.1	1.9	3.1
16	0.06	0.08	0.09	0.08	2.4	2.4	2.4	3.6
17	0.12	0.12	0.12	0.12	1.6	1.5	2.4	2.8
18	0.09	0.10	0.09	0.09	1.9	2.3	1.5	2.9
19	0.12	0.08	0.10	0.10	2.4	2.2	2.1	3.4
20	0.12	0.12	0.10	0.11	2.5	3.5	3.3	4.7

	IPS e.max CAD							
	Surface Roughness (Ra)			Ave	CFU/ml (1/15000 diluti			Undiluted (10^4)
1	0.09	0.06	0.10	0.08	8.1	7.0	6.3	10.70
2	0.06	0.08	0.07	0.07	11.0	8.0	8.0	13.50
3	0.13	0.13	0.09	0.12	8.3	8.9	8.8	13.00
4	0.10	0.12	0.11	0.11	6.0	5.0	10.0	10.50
5	0.09	0.13	0.12	0.11	12.0	6.0	7.0	12.50
6	0.13	0.07	0.07	0.09	8.3	8.9	8.8	13.00
7	0.10	0.09	0.07	0.09	7.5	7.6	7.1	11.10
8	0.10	0.12	0.11	0.11	12.0	10.0	13.0	17.50
9	0.11	0.11	0.14	0.12	8.1	7.9	8.9	12.45
10	0.09	0.06	0.10	0.08	7.0	2.0	2.0	5.50
11	0.06	0.08	0.07	0.07	5.0	5.0	6.0	8.00
12	0.13	0.13	0.09	0.12	6.0	7.0	5.0	9.00
13	0.10	0.12	0.11	0.11	10.0	11.0	7.0	14.00
14	0.09	0.12	0.13	0.11	7.2	6.8	8.1	11.05
15	0.13	0.07	0.07	0.09	4.9	5.6	5.9	8.20
16	0.08	0.09	0.07	0.08	3.0	3.0	6.0	6.00
17	0.10	0.12	0.11	0.11	7.0	7.0	6.0	10.00
18	0.11	0.11	0.14	0.12	4.0	3.0	4.0	5.50
19	0.08	0.07	0.11	0.09	4.0	7.0	3.0	7.00
20	0.10	0.08	0.12	0.10	7.0	7.0	8.0	11.00

	IPS e.max ZirPress							
	Surface Roughness (Ra)			Ave	CFU/ml (1/15000 dilution)			Undiluted (10^4)
1	0.67	0.73	0.83	0.74	20.2	20.4	20.6	30.6
2	0.83	0.74	0.93	0.83	17.6	16.6	15.8	25.0
3	0.72	0.51	0.57	0.60	15.2	16.0	15.6	23.4
4	0.75	0.53	0.56	0.61	12.0	12.0	14.0	19.0
5	0.63	0.91	0.55	0.70	7.0	12.0	5.0	12.0
6	0.73	0.75	0.65	0.71	17.0	17.0	20.0	27.0
7	0.85	0.65	0.89	0.80	9.0	8.0	11.0	14.0
8	0.67	0.88	0.62	0.72	7.0	12.0	13.0	16.0
9	0.79	0.60	0.75	0.71	13.0	10.0	14.0	18.5
10	0.66	0.94	0.75	0.78	12.0	14.0	9.0	17.5
11	0.64	0.45	0.55	0.55	8.0	15.0	17.0	20.0
12	0.51	0.62	0.72	0.62	23.0	13.0	21.0	28.5
13	0.86	0.74	0.49	0.70	15.0	12.0	13.0	20.0
14	0.65	0.56	0.63	0.61	19.0	15.0	20.0	27.0
15	0.71	0.91	0.82	0.81	20.0	17.0	19.0	28.0
16	0.85	0.64	0.75	0.75	12.0	12.0	14.0	19.0
17	0.67	0.83	0.62	0.71	7.0	12.0	15.0	17.0
18	0.75	0.73	0.86	0.78	17.0	17.0	20.0	27.0
19	0.83	0.76	0.74	0.78	16.0	16.0	13.0	22.5
20	0.68	0.88	0.83	0.80	15.0	14.0	12.0	20.5

	IPS e.max Press with IPS e.max Ceram Glaze Spray							
	Surface Roughness (Ra)			Ave	CFU/ml (1/15000 diluti			Undiluted (10^4)
1	1.48	1.41	1.52	1.47	44.0	43.0	51.0	69.00
2	1.66	1.48	1.61	1.58	41.0	52.0	36.0	64.50
3	1.28	0.77	0.82	0.96	48.0	30.0	46.0	62.00
4	1.08	1.16	1.04	1.09	49.0	45.0	49.0	71.50
5	1.44	1.39	1.48	1.44	47.0	51.0	46.0	72.00
6	1.49	1.56	1.55	1.53	56.0	53.0	47.0	78.00
7	1.49	1.43	1.48	1.47	41.0	44.0	44.0	64.50
8	1.42	1.42	1.37	1.40	61.0	43.0	44.0	74.00
9	1.05	1.06	1.06	1.06	40.0	47.0	45.0	66.00
10	1.62	1.24	1.40	1.42	45.0	41.0	41.0	63.50
11	1.51	1.26	1.83	1.53	45.0	43.0	45.0	66.50
12	1.40	1.44	1.50	1.45	39.0	31.0	38.0	54.00
13	1.26	1.29	1.26	1.27	30.0	34.0	27.0	45.50
14	1.22	1.10	1.29	1.20	26.0	28.0	31.0	42.50
15	0.98	0.99	0.99	0.99	19.0	20.0	24.0	31.50
16	1.43	1.39	1.40	1.41	42.0	45.0	42.0	64.50
17	1.06	1.16	1.05	1.09	30.0	39.0	43.0	56.00
18	1.49	1.49	1.51	1.50	54.0	53.0	46.0	76.50
19	1.37	1.44	1.49	1.43	44.0	50.0	46.0	70.00
20	1.48	1.40	1.48	1.45	51.0	48.0	43.0	71.00

IX. References

1. Al-Marzok, MI, Al-Azzawi HJ. The effect of surface roughness of porcelain on the adhesion of oral Streptococcus mutans. J Contemp Dent Pract, 2009. 10(6):E017-24.
2. Auschill TM, et al. The effect of dental restorative materials on dental biofilm. Eur J Oral Sci 2002; 110:48-53.
3. Aykent F. Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. J Prosthet Dent. 2010 Apr; 103(4):221-7
4. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface for bacterial plaque retention: a review of the literature. Dent Mater, 1997. 13(4): 258-69.
5. Bremer F, Kohorst P, Stiesch M. *In vivo* biofilm formation on different dental ceramics. Quintessence International. 42(7):565-74.
6. Busscher HJ, et al. Biofilm Formation on Dental Restorative and Implant Materials. J Dent Res 89(7): 657-665, 2010.
7. Conrad HJ, Seong WJ, Pesun IJ. Current ceramic materials and systems with clinical recommendations: a systematic review. J Prosthet Dent. 2007 Nov; 98(5): 389-404
8. De Fucio SB, et al. Analyses of biofilms accumulated on dental restorative materials. Am J Dent, 2009. 22(3):131-6

9. Goeres D, et al. A method for growing a biofilm under low shear at the air-liquid interface using the drip flow biofilm reactor. Nature Protocols. 4(5); 2009: 783-788
10. Hahnel S, et al. Streptococcus mutans and streptococcus sobrinus biofilm formation and metabolic activity on dental materials. Acta Odontologica Scandinavia. 2011 Jul 25.
11. Hojo, K, et al. Bacterial Interactions in Dental Biofilm Development. J Dent Res 88(11): 982-990, 2009.
12. Ivoclar Vivadent. "IPS e.max lithium disilicate: The Future of All-Ceramic Dentistry" http://www.glidewelldental.com/downloads/dentist/services/e.max-lithium-disilicate-scientific.pdf
13. Kantorski KZ, et al. Adherence of Streptococcus mutans to uncoated and saliva-coated glass-ceramics and composites. Gen Dent. 2008 Nov-Dec; 56(7): 740-7
14. Kantorski KZ, et al. Surface roughness and bacterial adherence to resin composites and ceramics. Oral Health Prev Dent, 2009. 7(1): 29-32
15. Kawai K, Urbano M, Ebisu, S. Effect of surface roughness of porcelain on adhesion of bacteria and their synthesizing glucans. J prosthet Dent, 200. 83(6):664-7
16. Kolenbrander PE, et al. Bacterial interactions and successions during plaque development. Periodontol. 2006; 42:47-79.
17. Lee BC, et al. Initial bacterial adhesion on resin, titanium and zirconia in vitro. J Adv Prosthodont 2011;3:81-4.

18. Li M, Yang S, Wang Z. [The influence of surface roughness on bacteria adhesion on titanium]. Zhonghua Kou Qiang Yi Xue Za Zhi, 2001. 36(6):431-3.
19. Meier R, et al. Adhesion of oral streptococci to all-ceramics dental restorative materials *in vivo*. J Mater Sci: Mater Med, 2008. 19:3249-3253.
20. Satau J, Fukunaga A, Satau N, Shintani H, Okuda K Streptococcal adherence on various restorative materials. J Dent Res 1988; 67: 588-91
21. Senadheera D, Cvitkovitch DG. Quorum sensing and biofilm formation by Streptococcus mutans. Adv Exp Med Biol. 2008; 631:178-88