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EFFECT OF THREE DIFFERENT CONTAMINATION REMOVAL METHODS ON BOND STRENGTH OF CERAMIC TO ENAMEL CONTAMINATED WITH ALUMINUM CHLORIDE AND FERRIC SULFATE

Cesar González, D.D.S

A Thesis Presented to the Faculty of the College of Dental Medicine of Nova Southeastern University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

July 2018

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By

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Cariology and Restorative Dentistry

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DATE SUBMITED: July 2018

I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.S. degree and for this assignment.

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Cesar Gonzalez, D.D.S. Date

DEDICATION

I would like to dedicate this thesis to my mother for all her help and support throughout my life and education, showing me that with arduous work and patience, goals can be achieved.

To my wife, without her love, encouragement and generosity, I could not be where I am today. Thank you

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ABSTRACT

Effect of three different contamination removal methods on the bond strength of ceramic to enamel contaminated with aluminum chloride and ferric sulfate.

DEGREE DATE: July 11, 2018

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Background: The need to control moisture and contamination is crucial in adhesive dentistry, especially when rubber dam isolation is not feasible. Hemostatic contamination can negatively affect adhesion to tooth substrate. To achieve better outcomes, hemostatic agents should be rinsed off properly using a method that will remove the contamination and will not affect the μ-SBS. **Objective:** To evaluate and compare the effect of three different aluminum chloride and ferric sulfate contamination removal methods on the μ-SBS of lithium disilicate glass-ceramic bonded to enamel and to compare the type of fracture between samples. **Material and Methods**: Lithium disilicate blocks (IPS e.max CAD) were cut into samples of 2 mm in diameter and 3mm in height. Thirty-five human molar teeth were collected and separated into seven groups (*n=17*) **Groups: G1(control**): No contamination. **G2:** Contamination with aluminum chloride and removal by 30 seconds water-rinse. **G3**: Contamination with aluminum chloride, removal by re-etching (37.5% phosphoric acid), water-rinse. **G4:** Contamination with aluminum chloride, removal with 18% EDTA **G5:** Contamination with ferric sulfate, removal with water-rinse. **G6**: Contamination with ferric sulfate, removal by re-etching (37.5% phosphoric acid), water-rinse and dried. **G7:** Contamination with ferric sulfate, removal with 18% EDTA. The enamel surface was etched, then contaminated with aluminum chloride and ferric sulfate, cleaned using 3 different methods, previously described. Ceramic samples were etched with HF acid, silanated then bonded to enamel surface using Optibond FL, Variolink veneer cement and the Elipar S10 curing light, to avoid oxygen inhibition restoration margins were cover with a glycerin to complete polymerization of 10 -30 seconds each side. Specimens were stored in deionized water for 7 days, then subjected to μ-SBS testing, fractured specimens were examined with a stereomicroscope to determine the type of fracture, and five sample of each group were selected for SEM.

To compare differences for the outcome a general linear mode ANOVA was created, and data recorded. **Results:** There were statistically significant differences among the studied groups for the μ-SBS ($p < 0.05$). The G6 (Ferric sulfate- Re-etching) was the closest mean μ-SBS (10.75 MPa) to the G1(control group).μ-SBS (16.24 MPa), the lowest μ-SBS (6.13 MPa) for the G4 (Aluminum chloride-EDTA). The groups using ferric sulfate as a cleaning method presented higher μ-SBS MPa than the groups using aluminum chloride as a cleaning method. The type of fracture on groups with higher μ-SBS (MPa), G6 - 10.75 MPa (ferric sulfate-reetching), G5 - 9.21 μ-SBS(MPa) (Ferric sulfate-water) presented more cohesive fractures, while groups with lower μ-SBS(MPa), G4 – 6.13 MPa (Aluminum chloride- EDTA), G3 – 6.27 (aluminum chloride- re- etching) presented more mixed fractures. **Conclusions:** The present study sought to investigate the effect of three different contamination removal methods on bond strength of ceramic to enamel contaminated with aluminum chloride and ferric sulfate. Ferric sulfate hemostatic agent showed higher μ-SBS in all contamination removal methods when compare to aluminum chloride hemostatic agent. But all the contamination removal methods in both groups failed to increase the bond strength on enamel to the level of the control group. Further research is required before we can make definitive conclusions

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Chapter 1: Introduction

1.1 Adhesion:

1.1.1 Overview:

Contemporary restorative dentistry places a definitive emphasis on strong and durable adhesion to tooth substrate which is critical for clinical long term success of resin-bonded direct and indirect restorations. Adhesion to tooth substrate is based on an exchange process in which inorganic tooth materials is replaced by synthetic resin.[1] Buonocore[2] was the first to demonstrate that etching enamel with phosphoric acid enlarges the microscopic surface area for better resin penetration and increases resin-enamel bond strength. Resin treatment of acid etched enamel creates a new structure that is neither enamel nor resin but a hybridization of the two.[3] This was the first description of the hybrid layer although the term had not yet been introduced.

Adhesives are the compounds containing both hydrophilic monomers that allow bonding to tooth structure and hydrophobic monomers that contribute to coupling with restorative materials. All adhesives involve three major steps that are responsible for a durable adhesive/enamel-dentin bonding interface: 1). etching, 2). priming, 3). bonding.[1] Bond strength results obtained with one bottle two-step adhesives appear to be less consistent than those recorded for three-step adhesives. Also, three-step etch-and-rinse adhesives have demonstrated superior bonding performance compared to two-step adhesives.[4]

One-bottle two step etch-and-rinse adhesives seem more technique sensitive and provide weaker resin collagen interactions.[3] For enamel, an etch-and-rinse approach using phosphoric acid remains the preferred option. Scanning electron microscopic studies indicate that the enamel etching pattern caused by self-etch adhesive is not as deep and appears less retentive than the one achieved with phosphoric acid. The degree of enamel etching with self-etch adhesives depend of the pH of the adhesive system. Bonding of self-etch systems to enamel still remains critical, and typically separate phosphoric acid-etching of unground enamel surfaces is recommended.[5] Clinical recommendations and guidelines can be based on the individual situation and desired treatment. As a general rule, the greater the amount of remaining enamel, the more likely the use of an etch-and-rinse adhesive should be. This is especially true for indirect restorations, such as bonded laminate veneers, where the ideal preparation is preferably confined to enamel. While current trends favor fewer and simpler clinical application steps, one-step bonding systems reveal lower and less predictable bond strengths than multi-step etch-and-rinse.

Long term survival of adhesive ceramic restorations depends on the success of a reliable bond between the ceramic, the composite luting agent and the dental substrates. A number of studies[6,7] have evaluated the bond strength of the enamel/composite/ceramic joints. According with the literature, creating a porous ceramic surface texture, which is then silanated, is essential to obtain a reliable bond.[6,8] On the other hand, a reliable enamel/composite/ceramic junction has two interfaces: a composite/ceramic interface and an enamel/composite interface.[9] In clinical studies evaluating the bond strength of the enamel/composite/ceramic junction, a reliable bond can be produced by the mechanical interlocking of polymerized luting composite to acid-etch enamel.[7]

1.2 Dental Ceramic:

1.2.1 Overview:

Applications for ceramics in dentistry became increasingly popular in the 18th century, largely due to the esthetic characteristics of the material compared to other tooth substitutes.[10] Alexis Duchateau, a Parisian apothecary, integrated ceramics into dentistry when he created a complete set of dentures using porcelain ceramic material.[11] Later, in 1903, Charles Land further advanced dental ceramics by developing all-ceramic inlays, onlays, and crown restorations using fired porcelains,[12] innovations that led to the creation of porcelain jacket crowns.[13] Since then, dental ceramics have evolved with modifications to their chemical composition, esthetic properties, manufacturing processes, packaging, and indications. Highly esthetic and biocompatible results were achieved with early versions of dental ceramics, but the material's weakness in tensile and shear stresses necessitated development of ceramic materials with greater strength and durability,[14,15] especially when thicker restorations are necessary and/or cementing mainly to dentin is required. Along with CAD/CAM technology, today's pressable and millable materials enable fabrication of stronger and more minimally invasive ceramic restorations that are also esthetic.[16] This facilitates selection of the optimal metal-free ceramic material based on the specific treatment, since newer ceramic materials are stronger, easier to use, and versatile.

1.2.2 Composition, Characteristics, and Classification:

Ceramics are inorganic, nonmetallic solids produced by the heating at high temperatures and subsequent cooling of raw compounds such as nitrides, carbides, metal oxides, and borides, as well as mixtures of these materials. Therefore, a material labeled as ceramic is in fact *not* ceramic if it is created by another processing technique or has organic components. The molecules of these compounds are primarily held together by ionic and covalent bonds.

Ceramic materials may contain a crystalline or partly crystalline structure, or they may be amorphous (eg, a glass). Since most dental ceramics have at least some crystalline component, some authors limit the definition of ceramics to inorganic crystalline-containing materials, rather than including non-crystalline glasses, even though glasses are ceramics.[17] Understandably, dental ceramics are generally categorized by their microstructure,[18] which facilitates scientific understanding of the structural and chemical nature of dental ceramics but does little to aid dentists or ceramists in selecting the appropriate material for a given clinical situation. The way a ceramic is processed greatly influences its mechanical behavior and, therefore, its clinical behavior. Thus, classifying dental ceramics based on their composition and how they are processed can better provide clear clinical parameters for evaluating and appropriately choosing the most conservative ceramic for each clinical situation.[19]The categories below are presented from most conservative to least conservative in terms of healthy tooth structure preservation.

1.2.2.1 CL-I (Powder/Liquid)

Class I (CL-I) powder and liquid porcelains are created from materials primarily containing silicon dioxide and possess a glassy matrix and varying amounts of a crystalline phase within the glassy matrix (eg, Creation Porcelain, Jensen Dental; Ceramco 3, DENTSPLY International; EX-3, Kuraray Noritake Dental, Inc.). The CL-I group includes feldspathic porcelains, referred to as such because they were originally, and some continue to be made from naturally occurring feldspars (i.e., aluminosilicates composed of assorted quantities of potassium, sodium, barium, or calcium).[19] Several feldspathic material options are available on the market today (eg, VITA VM 13, VITA Zahnfabrik; Vintage Halo, Shofu). CL-I materials are fabricated by hand and can be used where tooth reduction is most conservative (enamel is still present with 0.2-0.3 mm of reduction only). It is generally the most translucent ceramic materials, but they are also the weakest.[19,20]10-The material's high translucency and esthetics create the illusion of natural teeth.[13] Powder/liquid porcelain materials are ideal for cases in which significant enamel remains and/or there is healthy tooth structure on the teeth (ie, 50% or more remaining enamel on the tooth), and 50% or more of the bonded substrate is enamel, and 70% or more of the margin is in the enamel. Feldspathic porcelain restorations that are bonded to primarily enamel substrates have proven to be highly successful long term.

Powder/liquid porcelains demonstrate high esthetics and workability, and because they can be layered very thinly and placed directly on the enamel, they are considered the most conservative of the metal-free ceramic classes.[16] CL-I porcelains require a thickness of 0.2 mm to 0.3 mm for each shade change.[21,22] This class of materials is generally indicated for anterior restorations but can also be used for the occasional bicuspid and rare molar, providing all parameters are at a very low risk level.

1.2.2.2 CL-II (Glass Ceramics)

The composition of CL-II ceramics is similar to CL-I porcelain, both possess a glassy matrix,[23] but the two classes vary in their glass-crystalline ratios and crystal types. In CL-II materials crystal types can either be added to the glass or grown into the glassy matrix. CL-II ceramics also differ from CL-I porcelains in manufacturing, as they are formed into dense industrial blocks for pressing and machining. Based on their crystal type and documented clinical behavior, CL-II pressed and machined glass ceramics can be further subdivided into two distinct groups: CL-IIa and CL-IIb.

1.2.2.3 CL-IIa

Materials in this subdivision contain low to moderate (< 50%) leucite containing feldspathic glass. Leucite (KAlSi2O6) is a potassium alumino-silicate that exhibits a tetragonal structure at room temperature and undergoes a displacive phase transformation from tetragonal to cubic at 625 °C, accompanied with a volume expansion of 1.2%. This results in a high coefficient of thermal expansion (20 to 25 × 10-6/°K). Such materials (eg, IPS Empress® CAD, Ivoclar Vivadent, Authentic®, Jenson Dental; VITABLOCS® Mark II, VITA, Zahnfabrik) contain less than 50% crystalline and perform more like a glass, which requires bonding. Like all CL-II materials, which have come to be known as glass ceramics, CL-IIa materials can be used for the same indications as CL-I materials including anterior teeth, bicuspids, and, on rare occasions, molars. Additionally, they have documented long-term clinical success in higher stress situations or when more dentin is exposed. They may be highly translucent, but traditionally they have required slightly thicker dimensions for workability and esthetics/shade matching (ie, minimum working thickness of 0.8 mm if layered with a veneering porcelain).[21,22]

Materials in this subcategory demonstrate increased material strength, primarily due to the processing technique of using a dense, industrial made block, and possibly due to the leucite and its ability to alter the coefficient of thermal expansion, inhibiting crack propagation. These dense glass and leucite-containing materials are indicated for thicker veneers, anterior crowns, and posterior inlays and onlays, but only when a long-term bond and seal can be maintained.

1.2.2.4 CL-IIb

This is a new subcategory that includes moderate-to-high (ie,> 50%) crystalline-containing glass or glass ceramics. The material’s microstructure consists of a glass matrix surrounding a second phase of individual crystals. It originates as homogeneous glass, after which a secondary treatment nucleates and grows crystals, a process that imparts improves mechanical and physical properties by maximizing the presence of crystals and the generation of compression stress around the crystals. An example of this material subcategory is lithium disilicate (eg, IPS e.max®, Ivoclar Vivadent), a glass ceramic material composed of silica, lithium dioxide, alumina, potassium oxide, and phosphorous pentoxide. After the crystalline component has reached optimal growth through the manufacturing process, it is pulverized into powder and processed through a variety of different techniques. Lithium disilicate is indicated for the same clinical situations as other glass ceramics; however, when fabricated to a full-contour monolithic restoration and seated with resin cement, it is also appropriate for higher stress situations, such as those requiring full crowns, even on molars.

New additions to this category are zirconia-reinforced lithium silicates (ZLSs) (eg, VITA Suprinity®, VITA Zahnfabrik; CELTRA™ Duo, (Dentsply-Sirona, York PA). ZLS materials comprise a lithium-silicate glass ceramic that is strengthened with approximately 10% zirconia crystals. Although these materials are still relatively new to the market, initial in vitro testing shows they have excellent optics and physical properties like lithium disilicates. Only lithium disilicates, however, have long-term clinical data to support their use as single restorations anywhere in the mouth Restorations fabricated from this material subcategory demonstrate high strength, fracture resistance, and natural-looking esthetics, yielding a versatile and strong alternative for a wider variety of indications. They are indicated when higher risks are involved (eg, less than 50% enamel remains on the tooth, less than 50% of the bonded substrate is enamel, and/or when 30% or more of the margin is in dentin). Due to the material's glass properties, adhesive bonding is recommended. However, bonding to dentin results in less predictable restorations due to dentin's flexibility; restorations bonded to enamel are much more predictable, given enamel's significant stiffness compared to dentin.[24]

1.2.2.5 CL-III (High-Strength Crystalline)

CL-III materials are high-strength crystalline ceramics with minimal or no crystalline phase and are also produced through industrial processes. They differ from glass or glass ceramics based on the way a sintered crystalline matrix of high-modulus material (85% to 100% of the volume) creates a junction with the particles in the crystalline phase.

1.2.2.6 CL- IIIla

CL-IIIa materials are manufactured by creating a porous matrix that is formed into a block, and then final processed to shape using CAD/CAM technology, after which a second-phase material melts and fills the pores within the material. Lanthanum aluminosilicate glass is drawn in either a liquid or molten glass form into all the pores via capillary action, creating a dense and interpenetrating material from the internal to external surfaces. The final material is an 85% crystalline mesh infused with a small amount of glass. This material is disappearing from the marketplace and being replaced entirely by 100% polycrystalline ceramics.

1.2.2.7 CL-IIIb

CL-IIIb high-strength 100% crystalline ceramics initially were alumina-based materials (eg, Procera®, Nobel Biocare); more recently they are zirconia-based (eg. LAVA™, 3M ESPE; Prettau®, Zirkonzahn). Alumina systems have proven successful for single units but are being replaced by zirconia and lithium disilicate due to the increased risk of failure in the molar region.[25,26] Zirconia can also be used when significant tooth structure is missing, when elevated risk for flexure and stress is present, for posterior full-crown and fixed partial denture situations, and when adhesive bonding is problematic, such as with subgingival margins. In cases where the bond and seal cannot be maintained (ie, high-risk bonding situations, including moisture control problems, high shear and tensile stresses on bonded interfaces, and variable bonding interfaces), high-strength CL-III ceramics or metal ceramics (CL-IV, see below) are appropriate, because they can be placed using conventional cementation techniques. A concern with full-contour zirconia, however, is wear on opposing dentition. Whether alumina or zirconia, these materials demonstrate greater strength than CL-I and CL-II materials and can be used to fabricate a core substructure to replace metal. However, they are more opaque due to their greater crystalline content, which detracts from overall esthetics. They are therefore layered with porcelain,[27] allowing these materials to offer both superior strength and improved esthetic results.[27] CL-III high-strength ceramics require a thickness of 1.2 mm to 1.5 mm, depending on the substrate color.[22,25] More translucent versions are now used in the posterior region as full contour or monolithic all-zirconia restorations. Marketed first in this category was BruxZir® (Glidewell Laboratories), with many other manufacturers subsequently entering the market.

1.2.2.8 CL-IV (Metal Ceramics)

CL-IV represents metal ceramics, which are essentially CL-I materials fused to a highly supportive substrate metal, allowing their use in high-stress clinical situations where conventional crowns and esthetics maybe required. They are ideal when minimal to no tooth structure remains. Like CL-III materials, CL-IV metal ceramics demonstrate greater strength but limited esthetic characteristics. CL-IV metal ceramics require a thickness of a t least 1.5 mm to create lifelike esthetics. These metal ceramics demonstrate similar qualities to CL-III zirconia-based restorations,[28] but the metal substructures do not have the same thermal firing sensitivity as zirconia.[28] CL-IV metal ceramics can be improved in esthetic qualities with use of a much higher gold framework material (eg, Captek™, Argen USA Inc.).

1.3 Hemostatic Agents:

1.3.1 Overview:

Tooth preparation procedures often cause gingival bleeding which may be a result of tissue trauma or gingival inflammation.[29] In addition, the need to control moisture and contamination is common in restorative dentistry, especially when rubber dam is not feasible.[30] In this cases hemostasis is of utmost importance in maintaining the ideal, contaminant-free operatory field.[31,32] Historically, techniques for soft-tissue management and control are categorized into three main methods: Mechanical, chemical or surgical.[33] Mechanical methods were the first introduced, among them gingival retraction cord is the most popular. However, plain cords not moistened with suitable medicaments generally are not able to control hemorrhage effectively.[33] The most common procedures used to control bleeding and decrease the flow of gingival fluid involve the use of a topical hemostatic agent.[31,34] These agents are based on two categories of pharmacological action: astringents (blood coagulation agents) and vasoconstrictors (adrenergic agents).[31,34]

Based on the existing information in the literature, among the widely used chemical agents for control of hemorrhage in restorative dentistry, the most common hemostatic agents are aluminum chloride (A1Cl)[35] and ferric sulfate (Fe2(SO4)3) [36] in 15-25% concentrations and 3-10 min application times. To achieve better outcomes during making impressions or using bonding agents, common hemostatic agents recommended before or during etching, should be rinsed off properly.

Hemostatic and bonding agents is a topic found in different studies at the literature, but with strong differences between them. There is no standardization between studies regarding contamination methods and how to apply them, using different hemostatic agents as, 15.5% ferric sulfate, 20% ferric sulfate, 13% ferric sulfate gel, 25 % aluminum chloride, 8% racemic epinephrine solution among others, the contamination periods range from 10 seconds to 48 hours, being water the most used cleaning method between them. Ajami and Colleagues[37] are the only ones to used water, EDTA and phosphoric acid as a cleaning method. Chaiyabutr and Kois[38] used water, phosphoric acid and aluminum oxide abrasion. Almost all these studies use permanent human extracted teeth for their in vitro studies, and just some studies use primary teeth,[39-41]using in these studies both, self-etching and total-etching systems. Between the studies in literature only two studies evaluate the influence of hemostatic agent on dental enamel.[42]

1.3.2 Chemical agents commonly used in restorative dentistry:

Chemically, active gingival retraction agents are categorized as Class I (vasoconstrictors, adrenergic) or Class II (hemostatic agents, astringents)[43]. The difference between vasoconstrictors, hemostatic agents and astringents are as follows, as described by the British Journal of Pharmaceutical Research.[44]

Vasoconstrictors like epinephrine do not coagulate, but act by constricting blood vessels and decreasing their size. There have been concerns, however, over the use of racemic epinephrine-impregnated cords due to elevation of blood pressure and increase in heart rate[45] and no benefits have been recognized over other non-impregnated cords.[46] Astringents, such as alum or aluminum potassium sulfate (KAl (SO4)^{2}),

AlCl3 and zinc chloride (ZnCl2), are substances that act by precipitating proteins on the superficial layer of mucosa and make it mechanically stronger. Styptics like ferric chloride and Fe2(SO4)3 are concentrated forms of astringents, which cause superficial and local coagulation.[44]

Hemostatic agents arrest more serious hemorrhage from cut capillaries and arterioles. AlCl3 and ferrous sulfate are preferred astringents among dentists because of minimum tissue damage[44] and also ease of use and effective results.[47] There is a wide range of products based on these two components from different manufacturers to choose from.

1.3.2.1 AlCl^{3}:

It is one of the most commonly used astringents.[32,48] It acts by constricting blood vessels and extracting fluid from tissues. The material is used in concentrations of 5-25% and has minimal systemic side-effects.[35] AlCl3 is the least irritating among hemostatic agents used with cords, but it disrupts the setting of polyvinyl siloxane impression materials. However, rinsing thoroughly with water resolves its inhibitory effect.[49]

1.3.2.2 $Fe_2(SO_4)_3$:

It does not traumatize the tissue noticeably and healing is more rapid than with AlCl3. Solutions of Fe2(SO4)3 above 15% are very acidic and can cause significant tissue irritation and post-operative root sensitivity. It coagulates blood so quickly that it must be placed directly against the cut tissue. The recommended application time is 1-3 min.[36]The resulting tissue displacement is maintained for at least 30 min.[44]The tissue is temporarily discolored for 1 or 2 days. It disrupts the setting reaction of polyvinyl siloxanes. Therefore, all traces of the medicament should be rinsed off thoroughly from the tissue before taking an impression.[32] Due to its iron content, Fe2(SO4)3 stains gingival tissues a yellow-brown to black for several days.[49]

1.3.3 Effects of hemostatic agents to tooth structure:

Hemostatic agents are acidic solutions, with pH values ranging from 0.7 to 2.0.[50-52] The use of hemostatic agents is routine in clinical procedures, understanding their effects on dentin morphology, as well as on the enamel surface and on bonding, is of unquestionable importance. Aluminum chloride and ferric sulfate are the main active ingredients in most of the hemostatic agents studied in literature. In the presence of water, these compounds undergo hydrolysis and form hydrochloric acid and sulfuric acid, respectively. Because both are strong acids, they may cause the etching effect observed on the dentin surface.[50,51]

At the same time, contaminants, such as remaining particles of the hemostatic agent, may obstruct the flow of resin monomers into the dentinal tubules. Small contaminant particles may penetrate the dentinal tubules and, ultimately, affect the development of the hybrid layer. Prabhakar and Bedi[40] pointed out that one possible explanation for the reduced shear bond strength associated with ferric sulfate could be the coagulation of plasma proteins in the dentinal fluid, which also might affect the surface architecture of the dentin. In addition, there may be discoloration of the dentin (if the tooth preparation exposes dentin) by the iron in ferric sulfate binding to the dentinal tubules and thus the restoration will look darker since the substrate is darker. For the specimens in the aluminum chloride groups in the literature, the deposition of aluminum in the form of unbound minerals on the dentin surface and the formation of a layer of residue may be responsible, in part, for the decrease in bond strength.[37]The study findings suggest that self-etching monomers may not remove these contaminants sufficiently, because their bonding ability depends on forming short resin tags and a relatively thin submicron hybrid layer. Self-etching monomers may not readily etch a more acid-resistant dentin surface that is contaminated with a hemostatic agent.[37] As a result, self-etching adhesive systems are more susceptible to reduced bond strengths after contamination because the smear layer is used as a bonding substrate. However, phosphoric acid, with a pH of 0.516 and an aggressive etching effect, seems to be able to demineralize the dentin and remove virtually all the contaminant on the dentin surface. Therefore, the phosphoric acid in etch-and-rinse systems may have acted as a cleaning agent.

The literature is limited about evaluations of the effects of the hemostatic agents to enamel, Trakyali and Oztoprak[42] reported that the bond strength is affected negatively using a based plant hemostatic on enamel. They pointed out that the μ-SBS in the contaminated dentin specimens may have been lower than that in uncontaminated specimens owing to the possible prevention of contact between the tooth enamel and the bonding agent, the obstruction of resin tags on the etched enamel surface, or both.[37]

1.3.4 Hemostatic agents and contamination removal methods:

In most cases, it appears that water alone was not sufficient to remove contamination. Researchers in only two studies assessed cleaning methods for hemostatic agents.[37,38] According to Ajami and colleagues[37], application of 10% percent ethylenediaminetetraacetic acid (EDTA) for 60 seconds followed by a 30second water spray restored the bond strength of a self-etching adhesive to dentin. However, the authors reported that phosphoric acid failed to increase the bond strength of self-etching adhesive to dentin to the level of that in the control group. They reported that a five-minute water rinse under high pressure resulted in an increase in bond strength when compared with that in the no rinsed, nonconditioned contaminated group; however, the bond strength still was much lower than that in the control group.[37] Furthermore, for some dental practices, the five-minute water rinse may be considered clinically unacceptable. Chaiyabutr and Kois[38] pointed out that after hemostatic contamination with 25 % aluminum chloride or 13% percent ferric sulfate, the cleansing protocol should include particle abrasion with low-pressure aluminum oxide or phosphoric acid etching to restore the bond strength to precontamination levels when using a self-adhesive resin cement. These authors reported that the mean bond strengths of specimens in the acid etch groups and the particle abrasion groups were not significantly different from the mean bond strength of specimens in the control group; however, the group that underwent water rinsing alone demonstrated the lowest mean bond strength.[38] Because the results reported here are limited to the materials and contamination times used in each study, a standardized study is needed in which investigators compare all cleaning methods; that is, EDTA, particle abrasion, 37 % phosphoric acid and water spray.

1.4 Purpose of the study:

The purpose of this study is to evaluate and compare the effect of three different aluminum chloride and ferric sulfate contamination removal methods on the μ-SBS of lithium disilicate glass-ceramic bonded to enamel with light cured resin cement. In addition, the study compares the type of fracture between the interface (enamel/light-cured cement and lithium disilicate glass ceramic) treated with different contamination removal methods.

1.5 Specific aims and hypothesis:

1: To evaluate the μ-SBS of three different cleaning methods (water rinse, re-etching, and EDTA) to remove aluminum chloride hemostatic agent on lithium disilicate (IPS e.max CAD) bonded to enamel using an etch-and-rinse adhesive and light cured cement.

2: To evaluate the μ-SBS of three different cleaning methods (water rinse, re-etching, and EDTA) to remove ferric sulfate hemostatic agent on lithium disilicate (IPS e.max CAD) bonded to enamel using an etch-and-rinse adhesive and light cured cement.

3: To compare the type of fracture between the interface (enamel and light-cured cement /lithium disilicate glass-ceramic) treated with different aluminum chloride removal methods.

4: To compare the type of fracture between the interface (enamel and light-cured cement /lithium disilicate glass-ceramic) treated with different aluminum chloride removal methods.

Null Hypothesis:

For aim 1- There will be no significant differences in μ-SBS of lithium disilicate (IPS e.max CAD) bonded to enamel with etch-and-rinse adhesive and light cured resin cement contaminated with aluminum chloride hemostatic agent after application of three contamination removal methods when compared to the control group.

For aim 2- There will be no significant differences in μ-SBS of lithium disilicate (IPS e.max CAD) bonded to enamel with etch-and-rinse adhesive and light cured resin cement contaminated with ferric sulfate hemostatic agent after application of three contamination removal methods when compared to the control group.

For aim 3- There will be no significant differences in type of fracture between the interface (enamel and light cured cement/lithium disilicate glass-ceramic) treated with different aluminum chloride removal methods when compared to control group.

For aim 4- There will be no significant differences in type of fracture between the interface (enamel and light cured cement/lithium disilicate glass-ceramic) treated with different ferric sulfate removal methods when compared to the control group.

1.6 Location of study:

Bioscience Research Center, Room 7356 at Nova Southeastern University, Health Professional Division, College of Dental Medicine. 3200 South University Drive Fort Lauderdale, Florida 33328-2018.

Chapter 2: Materials and methods

2.1 IRB Approval:

This research protocol was submitted to the Institutional Review Board of the Health Professions Division of Nova Southeastern University. The research was approved with the research acceptance IRB # 2018-59.

2.2 Grant:

A grant #335663 was awarded for this study after an expedited review by the Health Professions Divisions at Nova Southeastern University in the amount of $4850.98.

2.3 Sample Size Calculation:

A power analysis was performed using a G Power statistical software to compare the differences between groups. Using two-way ANOVA with effect size of 4, $\alpha<0.05$, power of 80%, resulting in 10 samples minimum per group for this study.

2.4 Groups:

Materials to be used in this study are presented in Table 1.

Thirty five caries-free human molar teeth were collected from a private dental office (Best Smile Cosmetic Dentistry, Pembroke Pines, FL) and placed in a container with 10% formalin (Henry Schein, Melville, NY) with a secure lid to prevent leaking during transport or storage and labeled with the biohazard symbol (Figure1) until sterilization is complete after two weeks in 10% Formalin. This followed the guidelines for infection control in dental health-care settings from the Centers for Disease Control and Prevention (CDC.[53] Ten % formalin is thus used to prevent dehydration of teeth as well as cross-contamination between extracted teeth and has been proven to be an appropriate storage solution for adhesion studies to enamel.[54,55] Teeth with caries, restorations, anomalous morphology or decalcifications were excluded from this study.

Samples were randomly divided into 7 groups using the buccal, lingual and interproximal surfaces of 35 extracted teeth with a total of 119 working surfaces and 17 surfaces per group. Each group was indicated with a different color of nail polish on the stone block holding the tooth. The markings were done on the surfaces facing the buccal and lingual of the tooth, G1(control) black, G2 (Aluminum chloride/ water) blue, G3 (aluminum chloride/ etch) green, G4 (Aluminum chloride/ EDTA) red, G5 (Ferric sulfate/ water) orange, G6 (Ferric sulfate/ etch) yellow, G7 (Ferric sulfate/ EDTA) purple (Figure 2).

*** Control group (G1)**

*** Water and Aluminum chloride group (G2)**

*** Re-etched and Aluminum chloride group (G3)**

*** EDTA and Aluminum chloride group (G4)**

*** Water and Ferric sulfate group (G5)**

*** Re-etched and Ferric sulfate group (G6)**

*** EDTA and Ferric sulfate group (G7)**

2.5 Sample preparation:

2.5.1 IPS e-max CAD preparation:

Lithium disilicate blocks (IPS e.max CAD, Ivoclar Vivadent, Amherst, NY) with high translucency (HT), shade A2, size C14 were used for this study (Figure 3). These blocks were cut into samples of 2mm in diameter and 3mm in height, using a low speed blade IsoMet Wafering Blades-15LC, 7in, (BUEHLER An ITW Company, USA) (Figure 4). The blocks were crystalized in a ceramic furnace (EP 600 Combi, Ivoclar Vivadent, Liechtenstein) for 25 minutes according to manufactures' instructions (Figure 5).

Samples were etched with 9.5% hydrofluoric acid (Porcelain etch, Ultradent, South Jordan, UT) for 60 seconds following manufacturer's instructions (Figure 6), washed for 10 seconds, dried and then silanated (Monobond, Ultradent, South Jordan, UT) (Figure7).

2.5.2 Enamel preparation:

Teeth were individually scaled using a hand instrument (H6/H7 scaler; Hu-Friedy, Chicago IL) (figure 8), cleaned using fluoride-free pumice (Preppies Pumice, Whip Mix, Louisville, KY) applied with a rubber cup for 10 seconds (Figure 9).[56] Teeth were then rinsed and air dried with compressed oil-free air. A planar enamel-bonding surface was created grinding down the most superficial portion of enamel on the buccal, lingual and interproximal aspect of the teeth using three low speed contouring discs (Super-snap contouring discs silicone carbide coarse, medium and soft, (Shofu Dental Corp. San Marcos, CA). The order of the disc use was first using the coarse disc to achieve the desired reduction (Figure 10), followed by the medium disc (Figure 11) to begin polishing the area and then finishing this step with the fine polishing disc (Figure 12), reducing no more than 1mm in depth to keep the surface on enamel and allowing a 2mm diameter flat surface to bond the lithium disilicate block (IPS e.max CAD, Ivoclar Vivadent, , Amherst, NY). Teeth were mounted in 25x25x25mm Type IV dental stone blocks (Resin Rock, Whip Mix, Louisville KY) such that the coronal portion was exposed, to prevent movement of the sample during testing (Figure 13).

The enamel was etched with 37.5% phosphoric acid (Ultradent, South Jordan, UT) for 20 seconds (Figure 13), then rinsed thoroughly for 15 seconds (Figure 14), air dried for 3 seconds without desiccating following manufacturer's instructions. Optibond FL (Kerr, Orange, CA) primer was applied with light brushing motion for 15 seconds and air dried for 5 seconds (Figure 15). Using the same applicator, adhesive (Kerr, Orange, CA) was applied with light brushing motion for 15 seconds and then was then air thinned for 3 seconds (Figure 16). Light curing was performed for 20 seconds using an Elipar S10(3M ESPE, Maplewood, MN) with a light intensity of 1200 Mw/cm2 +/- 10% (Figure 17).

2.5.3 Contamination procedure:

After enamel preparation buccal, lingual and interproximal surfaces of the 35 teeth were randomly selected to be divided between the control group and the other 6 groups.

Contamination of the samples was carried out following this procedure stated below, each group was marked with different colors: G1(control) black, G2 (water/ALCL) blue, G3 (etch/ ALCL) green, G4 (EDTA/ ALCL) red. G5 (water/ $Fe_2(SO_4)^3$) orange, G6 (etch/ $Fe_2(SO_4)^3$) yellow, G7 (EDTA/ $Fe_2(SO_4)^3$) purple. (Figure18)

2.5.3.1 Control group (G1): no contamination

2.5.3.2 Water and Aluminum chloride group (G2): Using a microbrush 25% aluminum chloride hemostatic agent (Viscostat Clear, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds (Figure 19), followed by the tooth surface being rinsed with water spray for 30 seconds and dried.

2.5.3.3 Re-etched and Aluminum chloride group (G3): Using a microbrush 25% aluminum chloride hemostatic agent (Viscostat Clear, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds, followed by the tooth surface being re-etched with 37.5% phosphoric acid (Ultradent, South Jordan, UT) for 20 seconds (Figure 20), rinsed with water spray for 30 seconds and dried.

2.5.3.4 EDTA and Aluminum chloride group (G4): Using a microbrush 25% aluminum chloride hemostatic agent (Viscostat Clear, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds, followed by the tooth surface being rinsed with 18% EDTA (Ultradent, South Jordan, UT) for 60 seconds and dried (Figure 21).

2.5.3.5 Water and Ferric sulfate group (G5): Using a microbrush 20% ferric sulfate hemostatic agent (Viscostat, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds (Figure 22), followed by the tooth surface being rinsed with water spray for 30 seconds and dried.

2.5.3.6 Re-etched and Ferric sulfate group (G6): Using a microbrush 20% ferric sulfate hemostatic agent (Viscostat, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds, followed by the tooth surface being re-etched with 37.5% phosphoric acid (Ultradent, South Jordan, UT) for 20 seconds (Figure 23), rinsed with water spray for 30 seconds and dried.

2.5.3.7 EDTA and Ferric sulfate group (G7): Using a microbrush 20% ferric sulfate hemostatic agent (Viscostat, Ultradent, South Jordan, UT) was applied on the selected surface for 60 seconds, followed by the tooth surface being rinsed with 18% EDTA (Ultradent, South Jordan, UT) for 60 seconds and dried (Figure 24).

2.5.4 Bonding procedure:

The lithium disilicate samples (IPS e.max CAD, Ivoclar Vivadent, Amherst, NY) were cemented perpendicular to the enamel surface of each tooth that was previously reduced

The prepared ceramic samples were randomly distributed among the groups and luted to the enamel surfaces with a uniform layer of micro filled, light-curing luting composite system (Variolink veneer Ivoclar, Vivadent, Amherst, NY) (Figure 25) maintaining light pressure on the ceramic and cured for 10 seconds following manufacturer’s instructions using a Elipar S10 (3M ESPE, Maplewood, MN) curing light with a light intensity of 1200 Mw/cm2 +/- 10% (Figure 26). To avoid oxygen inhibition restoration margins were cover with a glycerine gel (Liquid-Strip, Ivoclar Vivadent, Amherst, NY) (Figure 27) after the removal of excess, but prior to complete polymerization of 10 -30 seconds on each side.

2.6 Storage:

Following all the cementation procedures, teeth were stored in deionized water for 7 days to provide aging of the samples and to simulate mouth temperature, before any bond strength test was performed (Figure 28).

2.7 Shear bond strength test:

Shear bond strengths were determined using a universal testing machine (Istron, Canton, MA) (Figure 29). Following the protocol used by Mccarthy in 2013[57] The metal blade was oriented perpendicular to the porcelain base, (Figure 30) 1mm from the buccal surface of the tooth. An occluso-gingival force was applied at a crosshead speed of 5mm/minute until the ceramic debonded or fractured.). RStudio and R 3.2.2 software were used for all statistical analysis, and significance was accepted at $p<0.05$.

2.8 Stereomicroscopy and SEM analysis:

Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV; Olympus, Tokyo, Japan) at 40x magnification to determine the mode of failure (Figure 31).[58] Failure modes were classified as (Table 2):

Type 1: adhesive failure

Type 2: cohesive failure

Type 3: mixed failure

Five samples of each group were randomly selected from each of the seven main groups for scanning electron microscope SEM (ThermoFisher Scientific, Hillsboro, OR) (Figure 31)to analyze the interface between the veneer and cement materials (Figures 32, 33, 34, 35, 36, 37, 38).

Chapter 3: Results

Means and standard deviations were calculated for all continuous measures. For core differences for the outcome measure μ-SBS (MPa) a general linear model (ANOVA) was created. The fixed effects were grouped (*Aluminum Chloride (AC)-Re-etch vs. Ferric Sulfate (FS)-Re-etch vs *Aluminum Chloride-Water vs Ferric Sulfate-Water vs. *Aluminum Chloride-EDTA vs Ferric Sulfate-EDTA vs *Control group). RStudio and R 3.2.2 software were used for all statistical analysis, and significance was accepted at $p<0.05$.

Results in table 3 shows that there were significant differences among the studied groups for the μ-SBS. The highest mean μ-SBS (16.24 MPa) was recorded for the G1(control group), and the lowest μ-SBS (6.13 MPa) was recorded for the G4 (Aluminum chloride-EDTA). The G6 (Ferric sulfate- Re-etching) mean μ-SBS was the closest (10.75 MPA) to the G1(control group). Overall the groups using ferric sulfate as a contamination method presented higher μ-SBS (MPa) than the groups using Aluminum Chloride as a cleaning method (Table 5) (Figures 39, 40). There was a significant difference in the measurement of μ-SBS by group F[6,112) = 32.90, p < 0.001, eta-squared = 64%]. This means that 64% of the variability in μ-SBS was accounted for by the differences in treatments (Table 4).

The type of fracture presented among the groups was intimately related to the MPA in each group, groups with higher MPA, G6 - 10.75 MPA (Ferric sulfate- re etching), G5 - 9.21 MPA (Ferric sulfate-water) presented more cohesive fractures, while groups with lower MPA, G4 – 6.13 MPA (Aluminum chloride- EDTA), G3 – 6.27 (Aluminum chloride- re- etching) presented more mixed fractures.

The G1 (control group) presented 70.5% cohesive fractures, 23.5% mixed fractures and 5.8% fracture on the substrate, G2 (water/ALCL) presented 58.8% mixed fractures, 29.4% cohesive fractures, 5.8% adhesive fractures and 5.8% substrate fractures, G3 (etch/ ALCL) presented 94.1% mixed fractures and 5.8% cohesive fractures, G4 (EDTA/ ALCL) presented 58.8% mixed fractures and 41.1% cohesive fractures, G5 (water/ $Fe_2(SO_4)_{3)}$ presented 52.4% mixed fractures, 41.1% cohesive fractures and 5.8% adhesive fractures, G6 (etch/ $Fe_2(SO_4)_3$) presented 64.7% cohesive fractures, 29.4% mixed failures and 5.8% substrate fractures G7 (EDTA/ $Fe_2(SO_4)_3$) presented 58.8% cohesive fractures, 35.2% mixed fractures and 5.8% adhesive fractures.

Chapter 4: Discussion

Porcelain laminate veneers increasing popularity depends primarily on the esthetic and conservative properties of the restorations; however clinical durability is also of key importance. Bond strength may be the most crucial factor of durability.

The statistical analysis performed in this study revealed significant differences in the mean shear bond strength values between the 7 groups. The shear bond strength values in the control group were significantly higher than the remaining groups.

During the etch and rinse technique selective dissolution of hydroxyapatite crystals through etching is followed by in situ polymerization of resin that is readily absorbed by capillary attraction within the created etch pits, thereby, enveloping individually exposed hydroxyapatite crystals. Two types of resin tags interlocks within the etch pits. “Macro”-tags fill the space surrounding the enamel prism while numerous “Micro”-tags result from resin infiltration/polymerization within the tiny etch-pits at the cores of the etched enamel prism. The later are especially thought to contribute the most about retention to enamel.

The results showed that the type of hemostatic agent and contamination removal method had a significant effect on micro-shear bond strength. The highest mean μ-SBS (10.75 MPa) was observed in the group of ferric sulfate- re-etching. This result correlates with the statement that phosphoric acid reportedly breaks down and removes ferric sulfate as mentioned by Tarighi et al[33], but the fact the ferric sulfate is a viscous gel makes its removal harder. The residues of ferric sulfate may result in the inhibition of the infiltration of the resin cement in to the etched enamel surfaces as stated by Farhadpour et al[59] causing an acceptable μ-SBS but not as good as the control group that is observed in our ferric sulfate groups. In the presence of water this compound undergoes hydrolysis and forms sulfuric acid causing an etching effect that already was observed in dentin surfaces in different studies.[38,51,60]

The current study showed a relation between the higher mean of μ-SBS in the ferric sulfate groups to the type of fracture in the control group, more cohesive fractures were found in the ferric sulfate groups as well as the control group, considering this information it can be concluded that the higher the μ-SBS the highest incidence of cohesive fractures will be found as a result.

Kuphasuk[60] et al demonstrated through SEM evaluations decreased bond strength and remnants of aluminum chloride in tooth structures. This correlate with the results obtained in this study where the aluminum chloride groups reported the lowest μ-SBS when compared to the control group. In the presence of water this compound undergoes hydrolysis and forms hydrochloric acid causing an etching effect that already was observed in dentin surfaces in different studies.[38,51,60]

The current study showed a difference between the higher mean of μ-SBS in the aluminum chloride groups to the type of fractures in the control group, and more mixed fractures were found in the aluminum chloride groups than the control group, considering this information it can be concluded that the lower the μ-SBS the highest incidence of mixed fractures will be found as a result.

Chemicals including aluminum chloride and ferric sulfate which are commonly used to control bleeding and gingival fluids, are highly acidic, with Ph values of 0.7 to 3.0. It is noteworthy that all materials with and acidic pH do not have the capacity to produce a proper bond with tooth structures, because they may leave deposits on tooth structures that may interfere with bonding

Researchers have evaluated the influence of hemostatic contamination on bonding to enamel. They pointed out that the SBS in the contaminated samples is lower when compare to the no contaminated samples. Some studies have reported that contact of other astringent agents on tooth structure resulted in decreased bond strength between composite and tooth structure.[60,61]. For this study the application time of hemostatic agent was extended to 1 min, a sufficient time to achieve hemostasis. Most situations will require less time for hemostasis, and therefore teeth will have less contact time with hemostatic agents.

Chapter 5: Conclusion

Greater emphasis should be given to the moisture and contamination control prior to the bonding of ceramic restorations to enamel. The literature is limited with regard to evaluations of the effects of hemostatic agents to enamel. The present study sought to investigate the effect of three different contamination removal methods on bond strength of ceramic to enamel contaminated with aluminum chloride and ferric sulfate. In this study Ferric sulfate hemostatic agent showed higher μ-SBS in all contamination removal methods when compared to aluminum chloride hemostatic agent. But all the contamination removal methods in both groups failed to increase the bond strength on enamel to the level of the control group. Within the limitations of this study, our findings show that hemostatic agents can induce changes on the enamel surface and in bonding performed on enamel. This data suggests that the bond strength of etch-and-rinse systems is affected after contamination with a hemostatic agent.

The clinical implication from this research is that when making impressions for porcelain veneers or crowns, and using a hemostatic agent containing aluminum chloride or ferric sulfate, proper measures to remove it from the dental structures should be taken. This is recommended not only because can affect the impression material setting but also lower the bond strengths in the restorations.

From this research it can be concluded that if impregnated cord is to be used with a hemostatic agent and etch and rinse bonding systems, ferric sulfate is the agent of choice to maximize bond strength of the restoration. However, further research is required before we can make definitive conclusions.

Chapter 6: Limitation of the study

One limitation of this research is that only one type of ceramic was included for comparison of the bond strength when the enamel was contaminated with hemostatic agents. IPS e.max blocks were selected as a material of choice for this study, since is a material used for the fabrication of ceramic veneers in dentistry, but inclusion of other types of ceramic would provide a better understanding of the interaction of ceramic with hemostatic agents.

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Tables

MATERIAL	COMPOSITION	TREATMENT	MANUFACTER
Optibond FL	48% filled light-cure adhesive		Keer Corporation
Ceramic etch	9% hydrofluoric acid solution	90 seconds	Ultradent

Enamel etch	37.5% phosphoric acid	20 seconds	Ultradent
Silane solution	Single-component material that enhances binding values	60 seconds	Ultradent
Variolink veneer	Micro filled light-curing luting composite	60 seconds	Ultradent
ViscoStat clear	25% AICI	60 seconds	Ultradent
ViscoStat	20% Fe2(SO4)3	60 seconds	Ultradent
Elipar S10	light intensity of 1200 Mw/cm2 +/- 10%	20 seconds	3M ESPE
EDTA	18% Ethylene diaminetetraacetic acid	60 seconds	Ultradent
IPS e.max CAD	Lithium disilicate glass ceramic (LDGC)	CAD/CAM block	Ivoclar vivadent

Table 1: materials composition, treatment and manufacturer.

	adhesive	cohesive	mixed	substrate
CONTROL (G1)		**12**	**4**	**1**
AC-WATER (G2)	**1**	**5**	**10**	**1**
AC- RE-ETCH (G3)		**1**	**16**	
AC-EDTA (G4)		**7**	**10**	
FS-WATER (G5)	**1**	**7**	**9**	

FS- RE-ETCH (G6)		**11**	**5**	**1**
FS-EDTA (G7)	**1**	**10**	**6**	

Table 2. Type of fractures

Groups	N	M	SD	Min	Max
AC:RE-ETCH	17	6.27	1.97	2.91	10.50
FS:RE-ETCH	17	10.75	3.26	7.11	16.76
AC:Water	17	7.82	2.49	4.60	11.61
FS:WATER	17	9.21	2.49	5.53	14.43
AC:EDTA	17	6.13	3.11	2.00	13.22
FS:EDTA	17	7.93	1.97	5.41	13.74
Control	17	16.24	1.95	13.31	19.53

Table 3. Descriptive statistics

Group		Group	Difference	Lower 95% CI	Upper 95% CI	P-Value
AC:RE-ETCH	-	FS:RE-ETCH	-4.48	-6.17	-2.79	<.0001
AC:RE-ETCH	-	AC:Water	-1.55	-3.24	0.14	0.552
AC:RE-ETCH	-	FS:WATER	-2.94	-4.63	-1.25	0.015
AC:RE-ETCH	-	AC:EDTA	0.14	-1.55	1.83	1.000
AC:RE-ETCH	-	FS:EDTA	-1.66	-3.36	0.03	0.466
AC:RE-ETCH	-	Control	-9.97	-11.66	-8.28	<.0001

FS:RE-ETCH	-	AC:Water	2.93	1.24	4.62	0.016
FS:RE-ETCH	-	FS:WATER	1.54	-0.15	3.23	0.560
FS:RE-ETCH	-	AC:EDTA	4.62	2.93	6.31	<.0001
FS:RE-ETCH	-	FS:EDTA	2.82	1.13	4.51	0.024
FS:RE-ETCH	-	Control	-5.49	-7.18	-3.79	<.0001
AC:Water	-	FS:WATER	-1.39	-3.08	0.30	0.675
AC:Water	-	AC:EDTA	1.69	-0.00	3.38	0.447
AC:Water	-	FS:EDTA	-0.11	-1.80	1.58	1.000
AC:Water	-	Control	-8.42	-10.11	-6.73	<.0001
FS:WATER	-	AC:EDTA	3.08	1.39	4.77	0.009
FS:WATER	-	FS:EDTA	1.28	-0.41	2.97	0.755
FS:WATER	-	Control	-7.03	-8.72	-5.34	<.0001
AC:EDTA	-	FS:EDTA	-1.80	-3.49	-0.11	0.366
AC:EDTA	-	Control	-10.11	-11.80	-8.42	<.0001
FS:EDTA	-	Control	-8.31	-10.00	-6.61	<.0001

Table 4. Pairwise comparisons

Figures

Figure 1. Teeth container

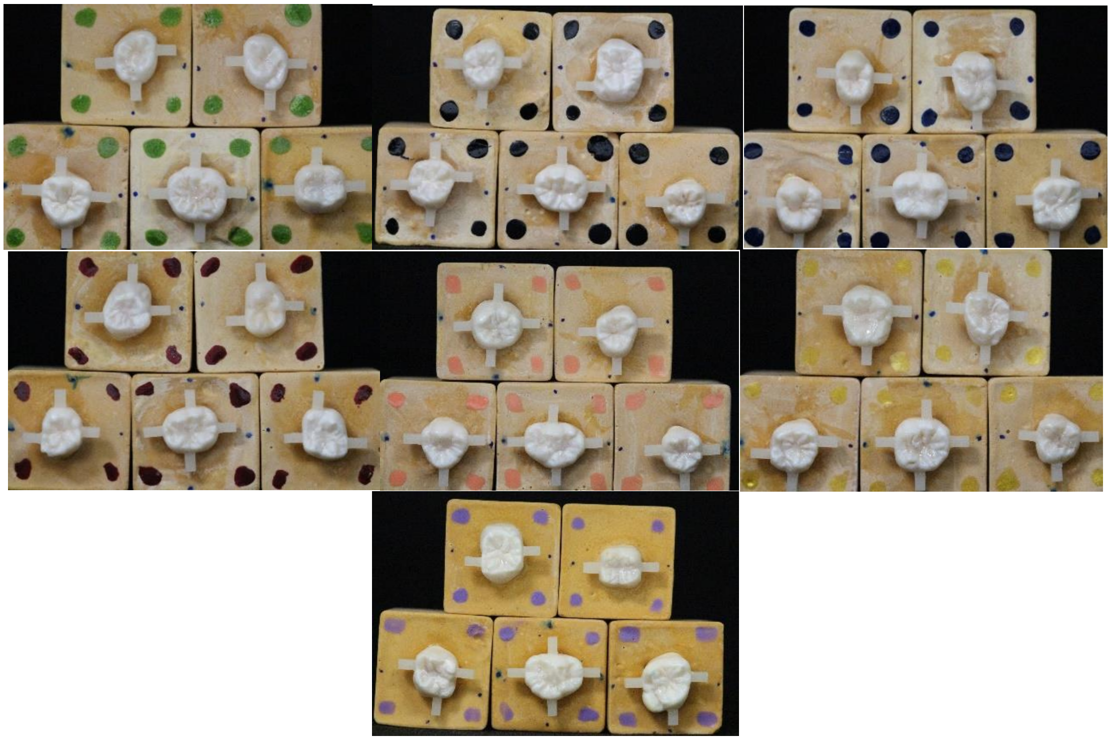

Figure 2. Groups differentiation

Figure 3. IPS e.max blocks

Figure 4. Blade IsoMet Wafering Blade-15LC, 7in

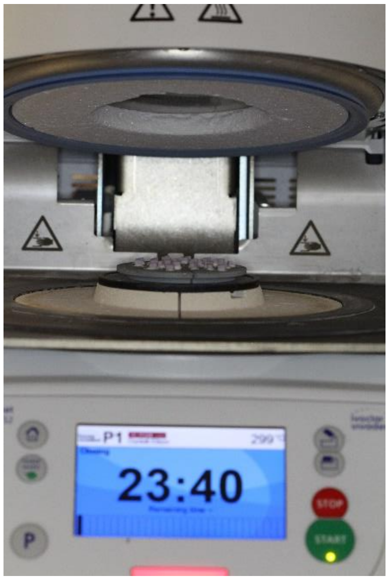

Figure 5. Ceramic furnace EP 600 Combi

Figure 6. 9.5% Hydrofluoric acid application

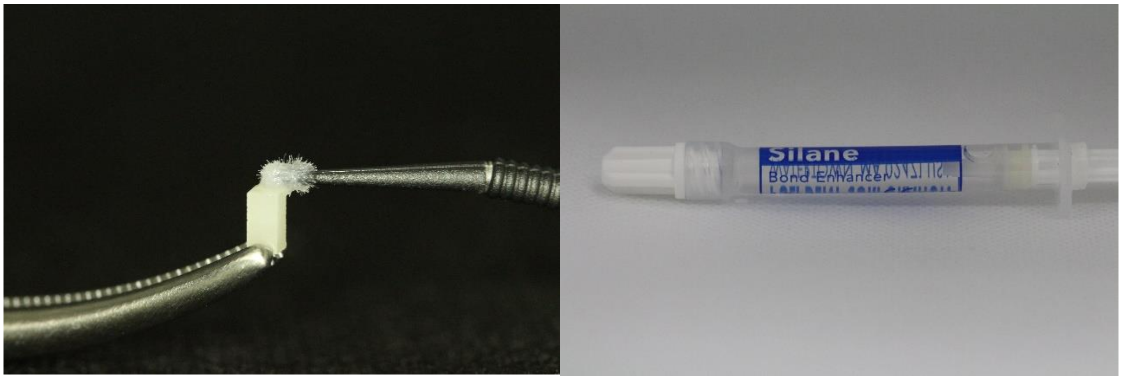

Figure 7. Silane application

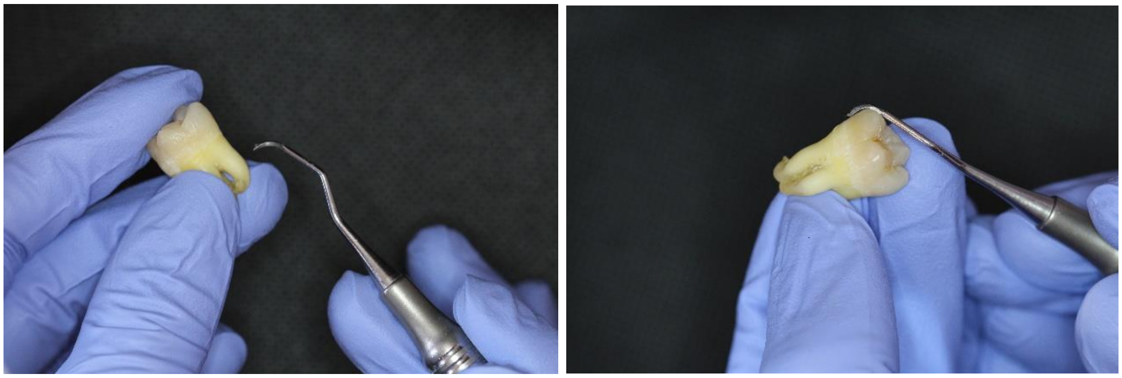

Figure 8. Teeth being scaled

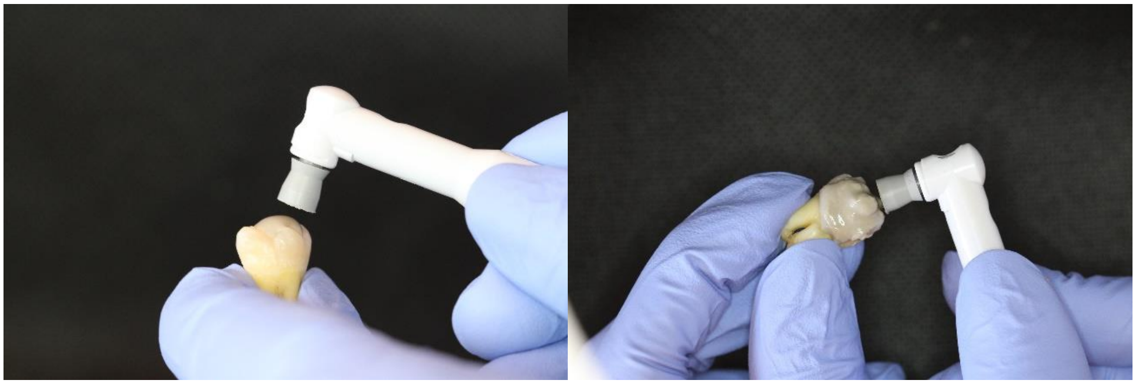

Figure 9. Teeth cleaned using fluoride-free pumice

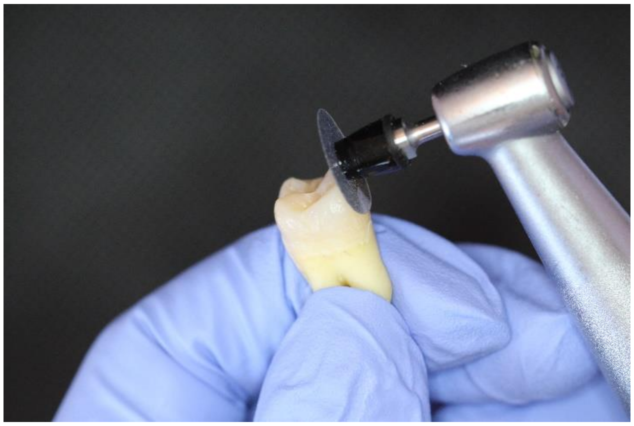

Figure 10. Reduction with Super-snap contouring discs silicone carbide coarse

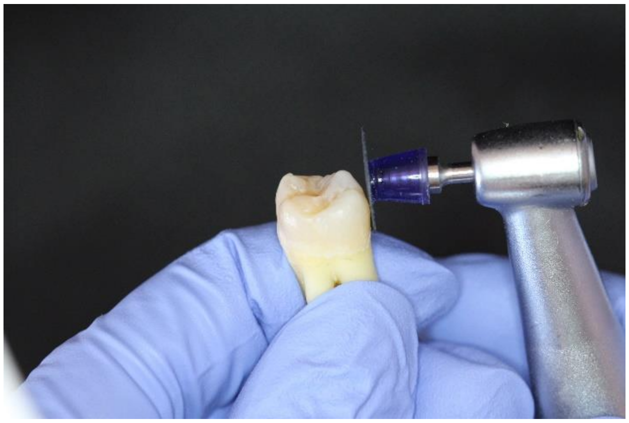

Figure 11. Reduction with Super-snap contouring discs silicone carbide medium

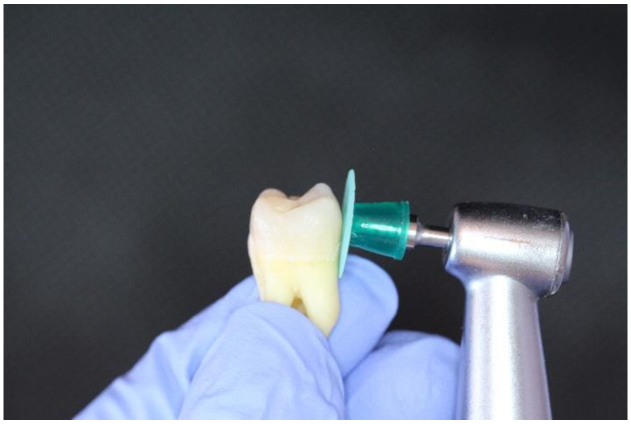

Figure 12. Polishing with Super-snap contouring discs silicone carbide fine

Figure 13. Teeth mounted in 25x25x25mm dental stone blocks

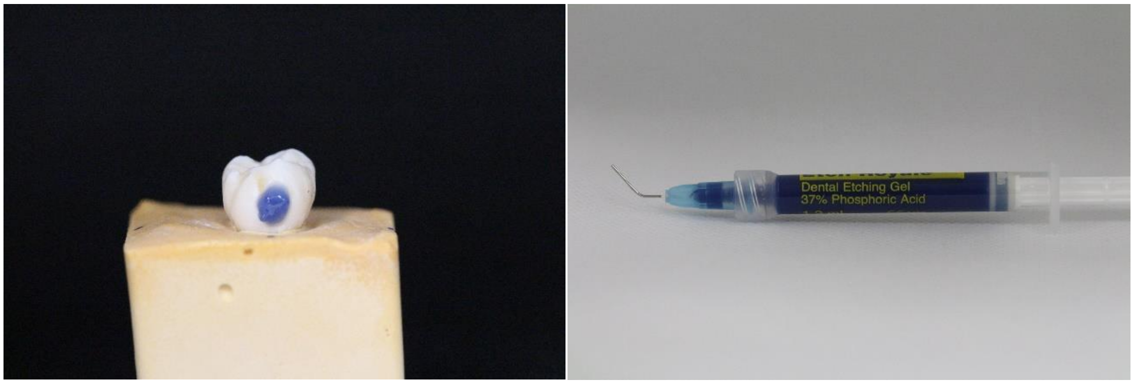

Figure 14. 37% Phosphoric acid application

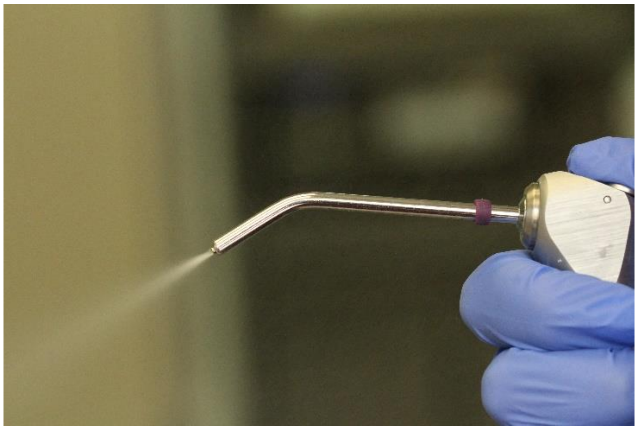

Figure 15. Rinsed with water spray and dried.

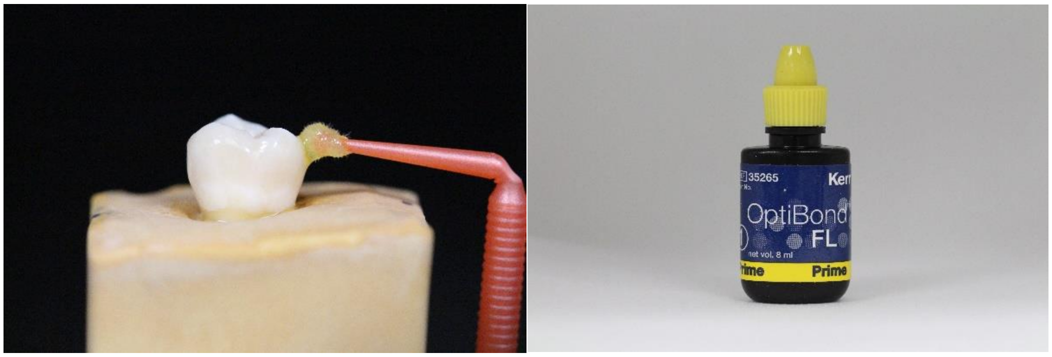

Figure 16. Optibond FL primer application with light brushing motion

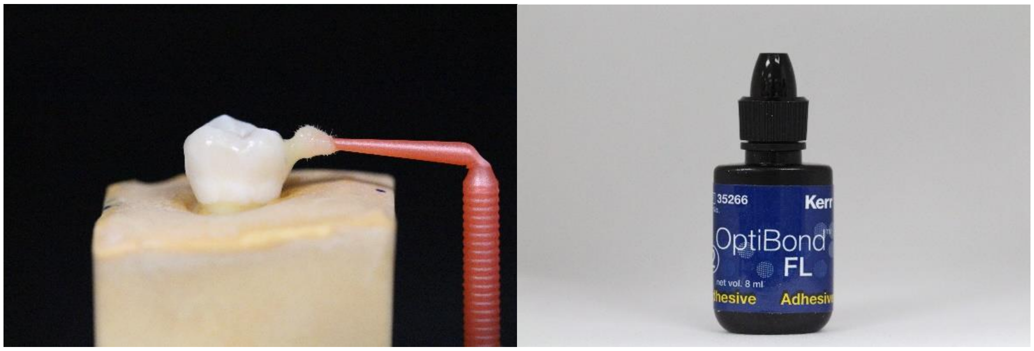

Figure 17. Optibond FL adhesive application with light brushing motion

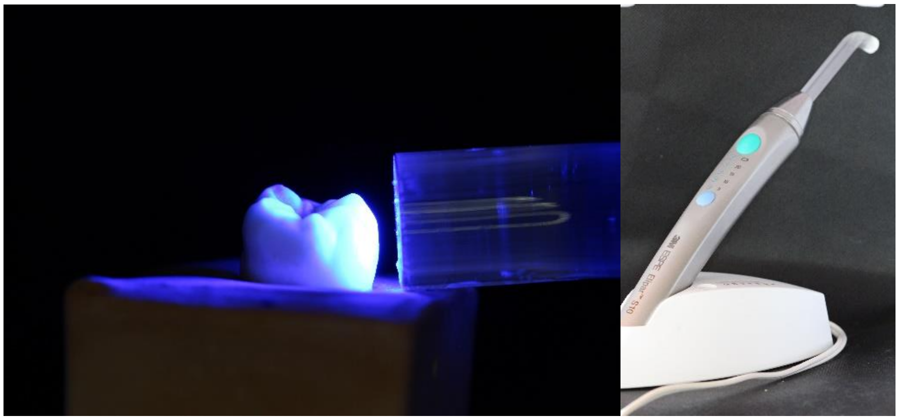

Figure 18. Light cured for 20 seconds using an Elipar S10

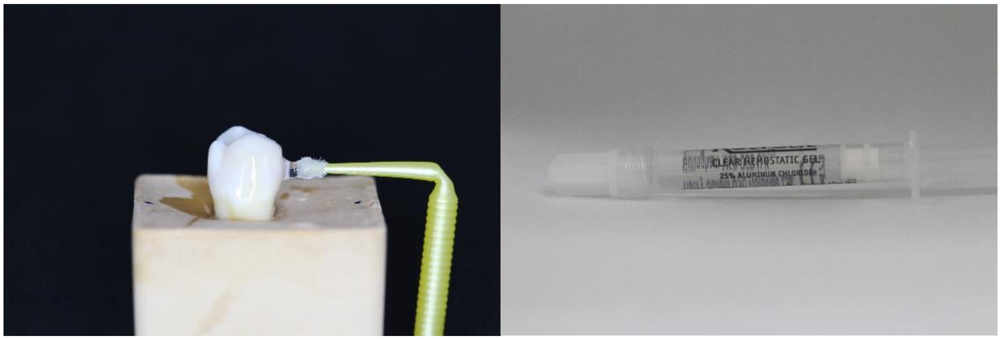

Figure 19. 25% aluminum chloride hemostatic agent application for 60 seconds

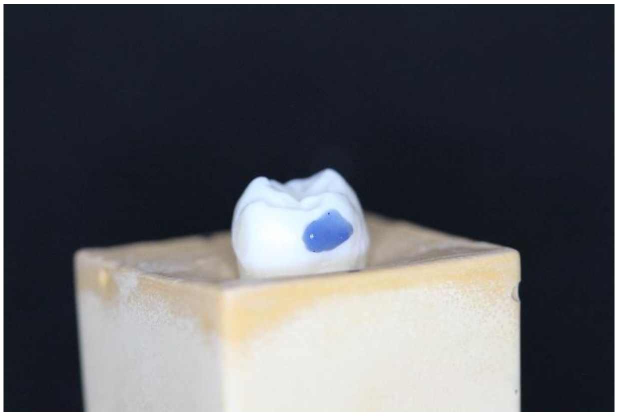

Figure 20. Tooth surface re-etched with 37.5% phosphoric acid for 20 seconds after aluminum chloride application

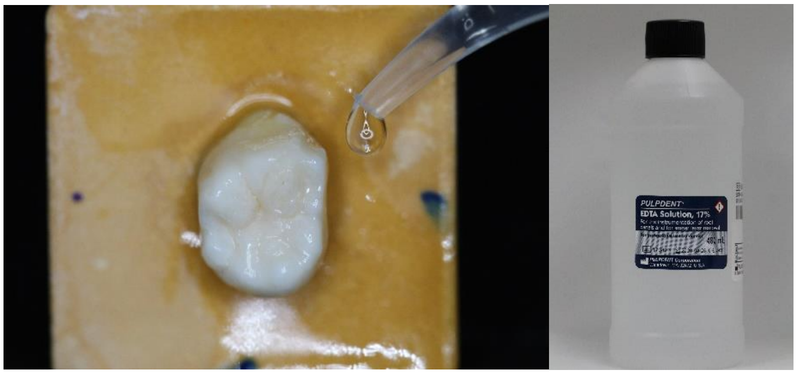

Figure 21. Tooth surface rinsed with 17% EDTA for 60 seconds and dried.

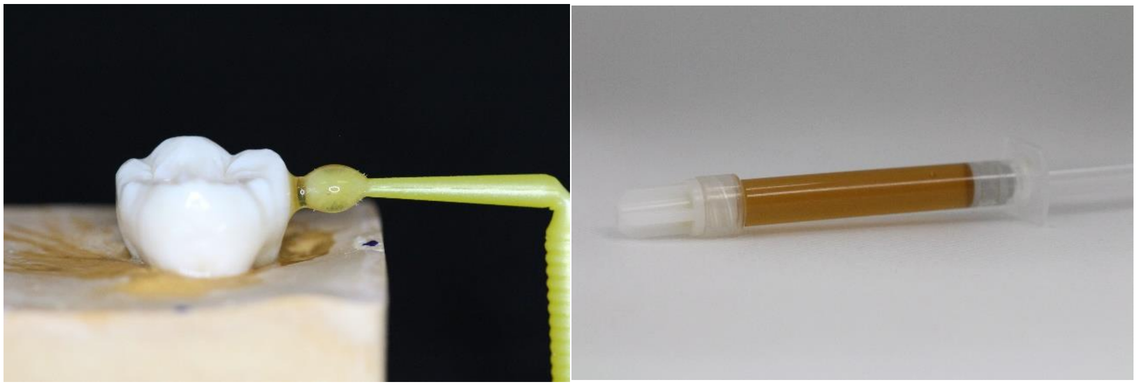

Figure 22. 20% ferric sulfate application for 60 seconds

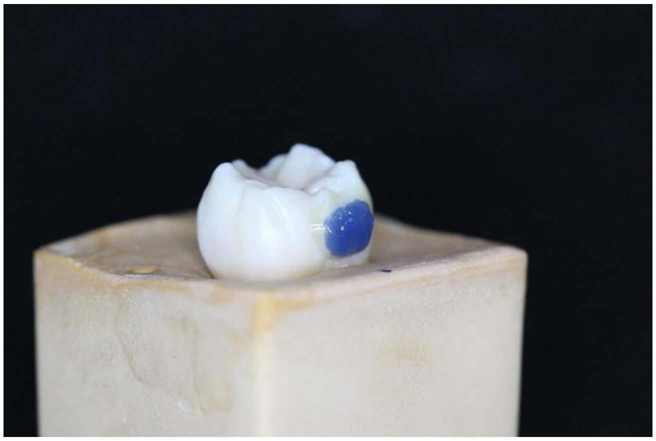

Figure 23. Tooth surface re-etched with 37.5% phosphoric acid for 20 seconds

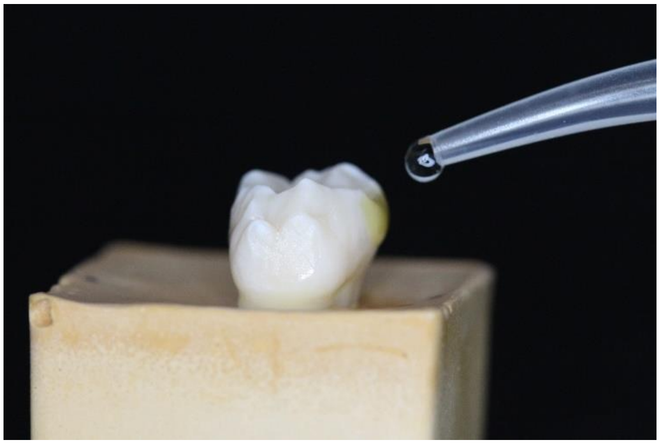

Figure 24. Tooth surface rinsed with 17% EDTA for 60 seconds and dried.

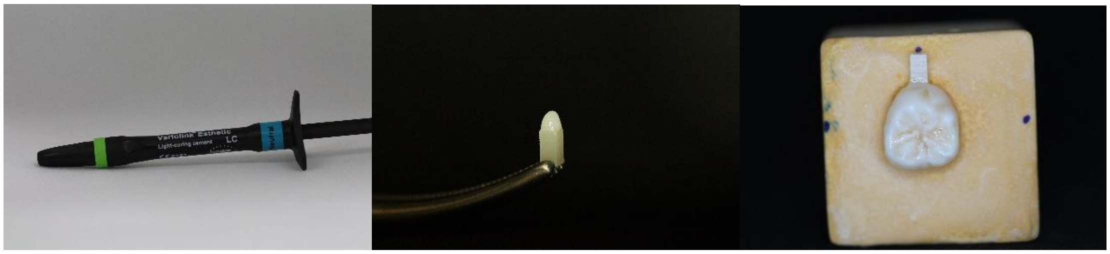

Figure 25. Lithium disilicate sample luted to the enamel surfaces with a uniform layer of micro filled, light-curing luting composite system

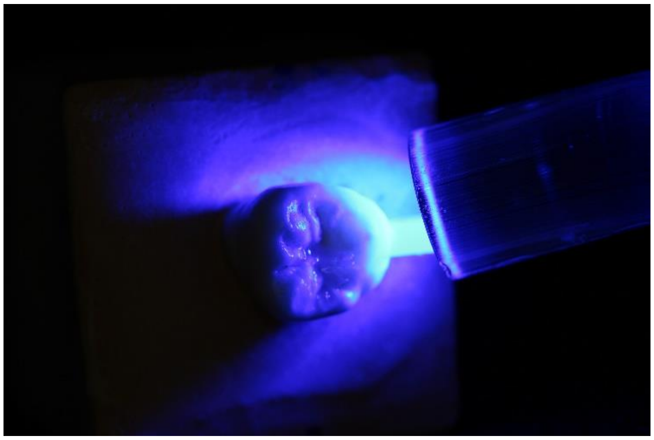

Figure 26. Light cured for 10 seconds using an Elipar S10

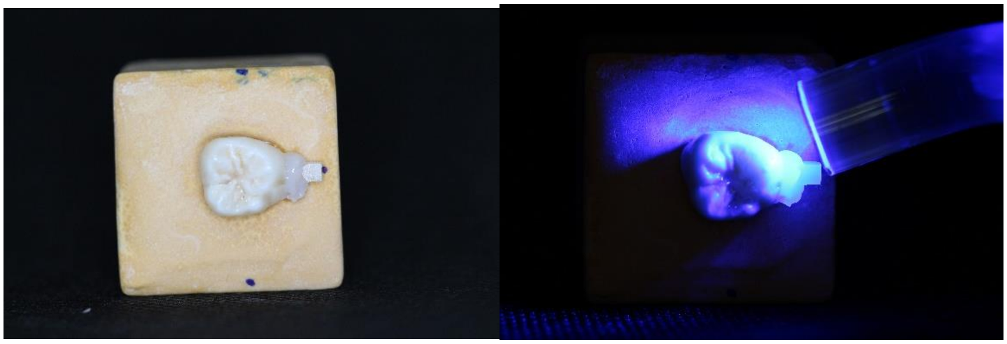

Figure 27. Margins covered with a glycerine gel after the removal of excess, but prior to complete polymerization of 10 -30 seconds each side.

Figure 28. Teeth maintained in deionized water for 7 days

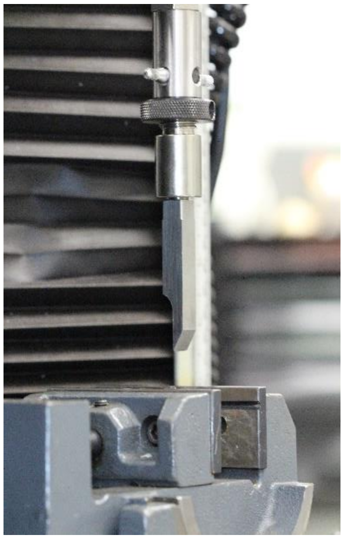

Figure 29.   Shear bond strengths determined using a universal testing machine

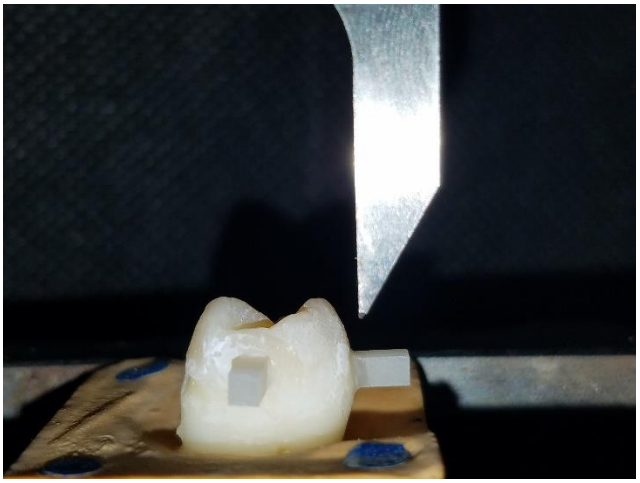

Figure 30. The metal blade oriented perpendicular to the porcelain base 1mm from the buccal surface of the tooth

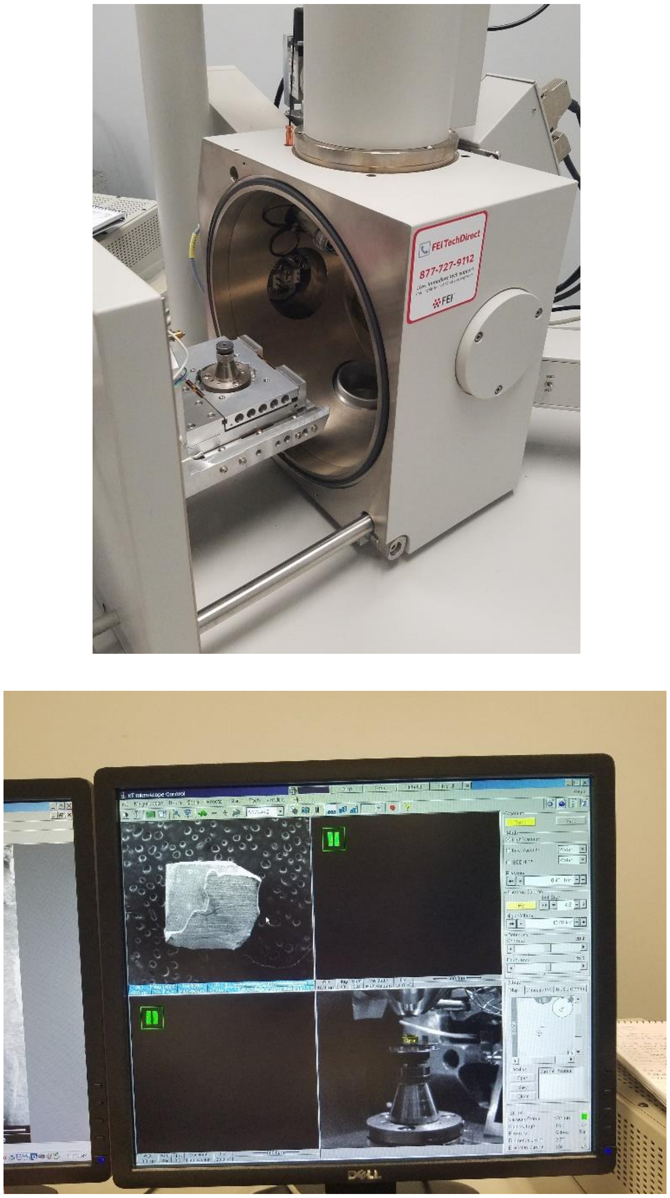

Figure 31. Scanning electron microscope

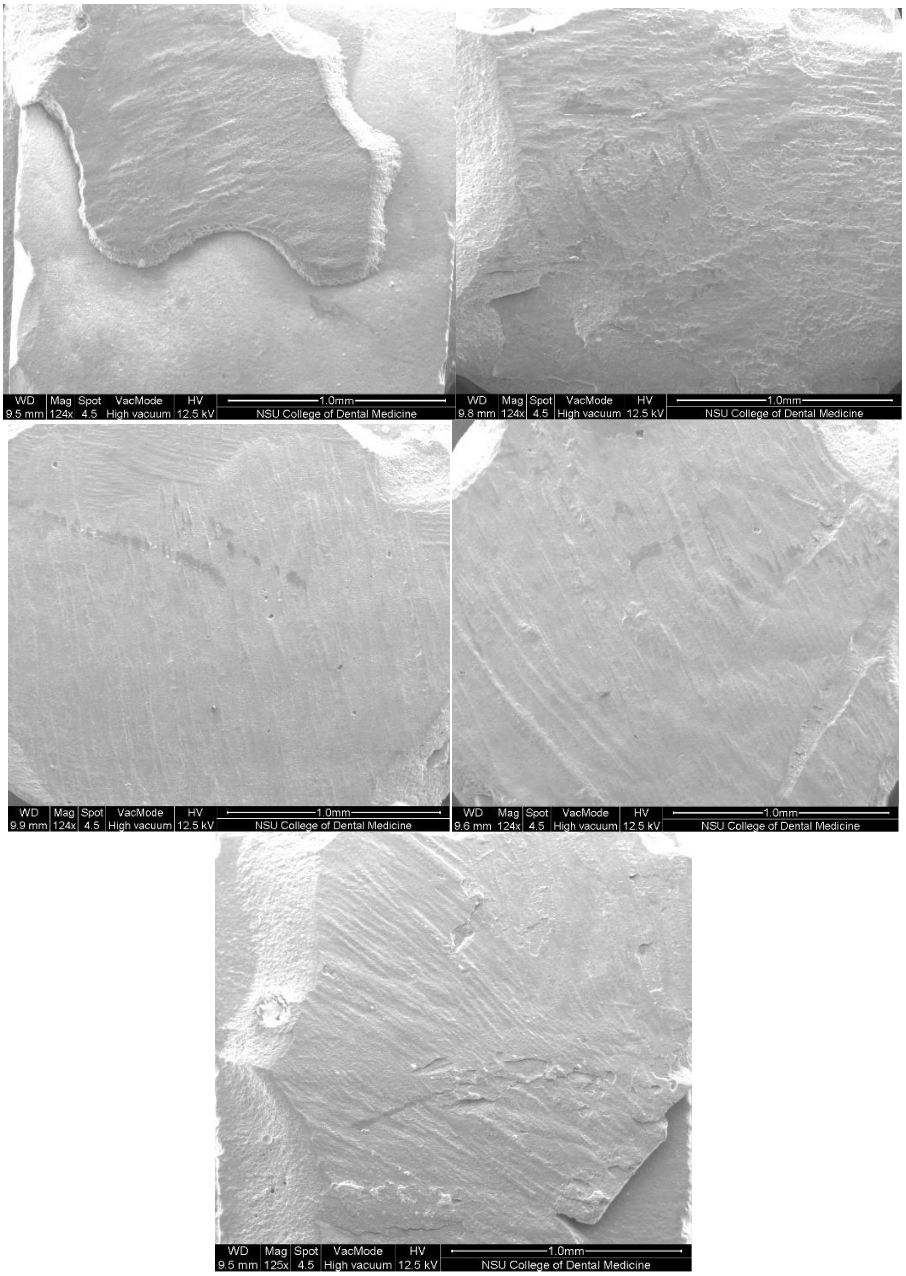

Figure 32. SEM images from control group (G1)

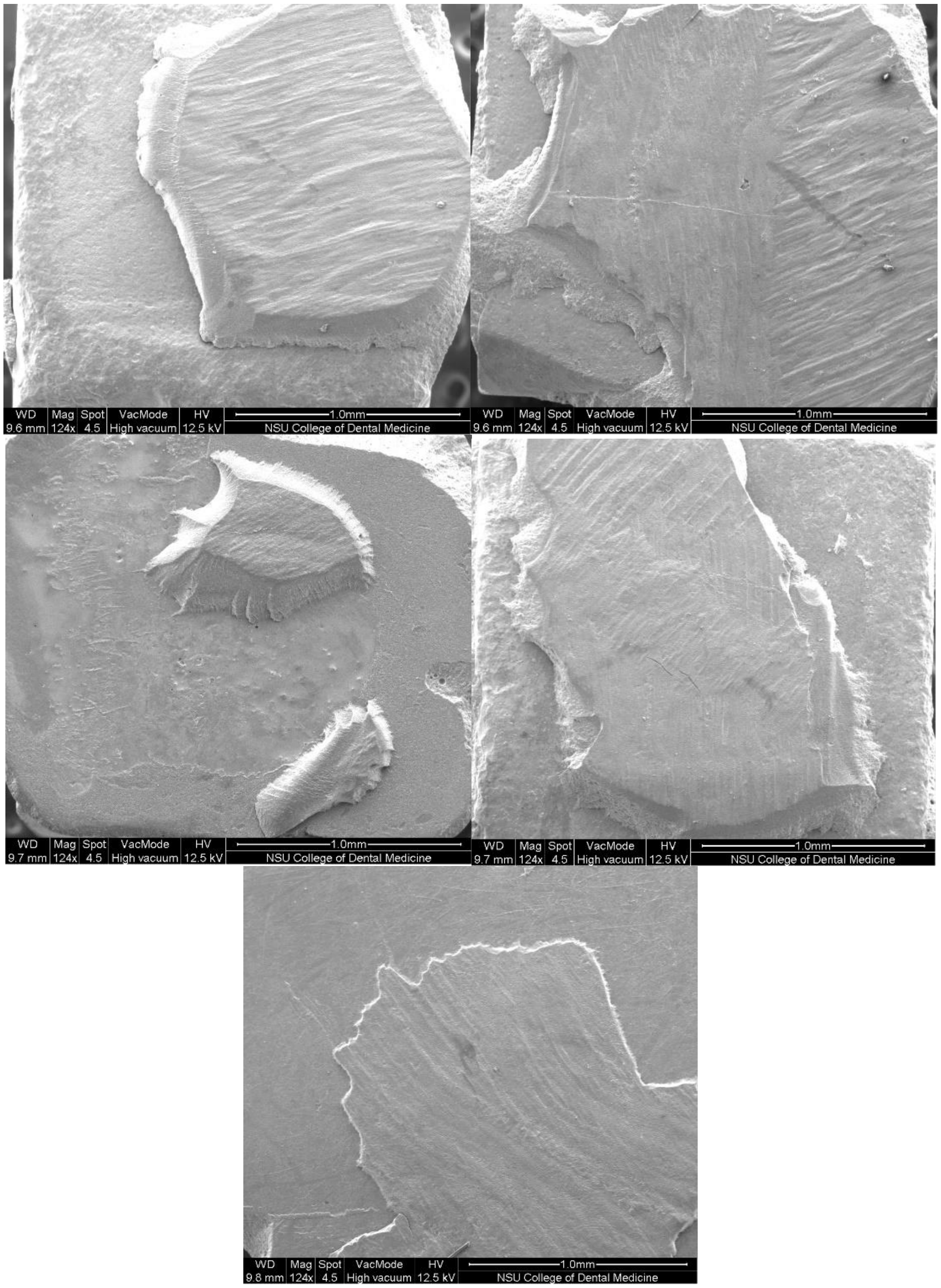

Figure 33. SEM images from Water and Aluminum chloride group (G2)

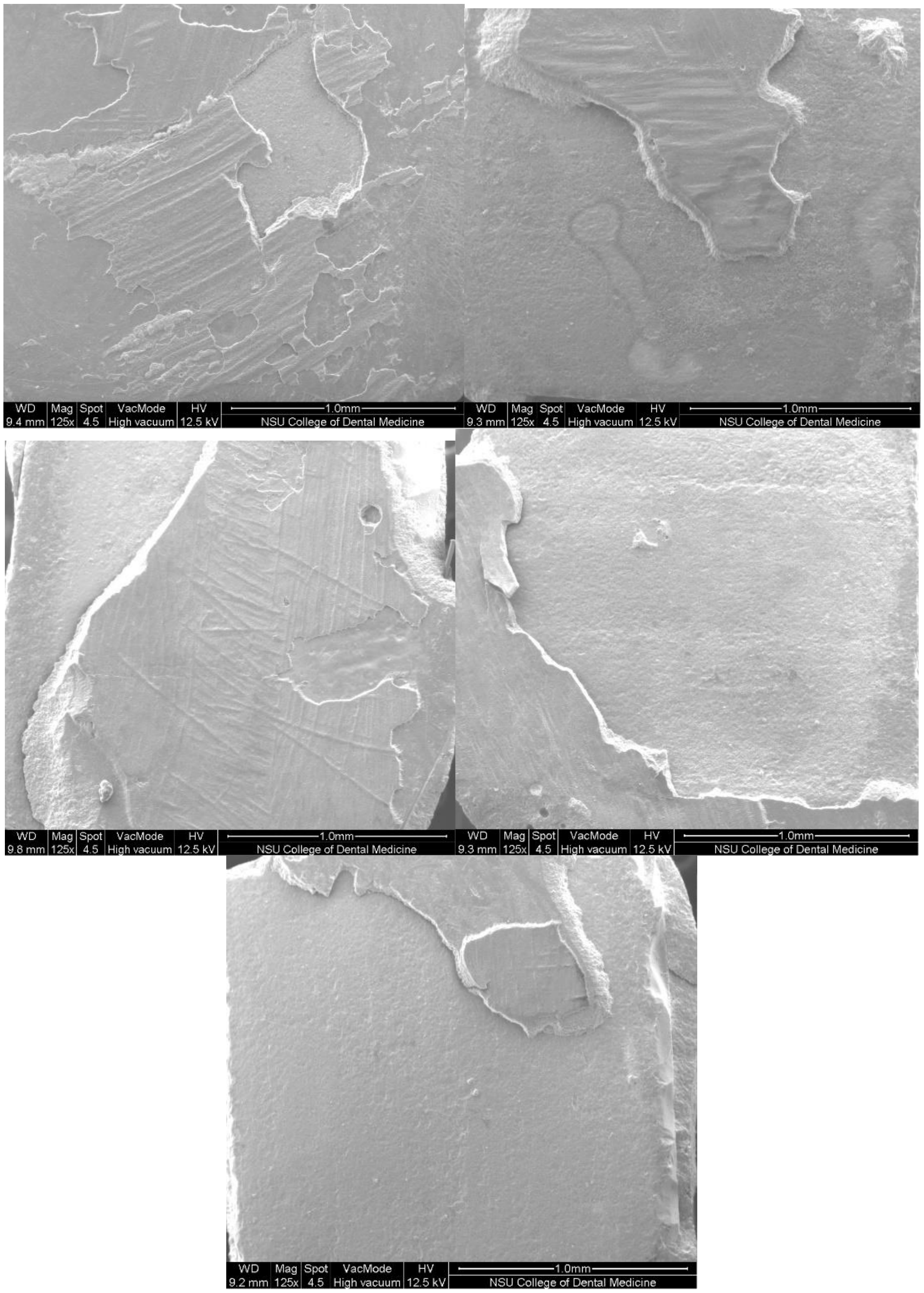

Figure 34. SEM images from Re-etched and Aluminum chloride group (G3)

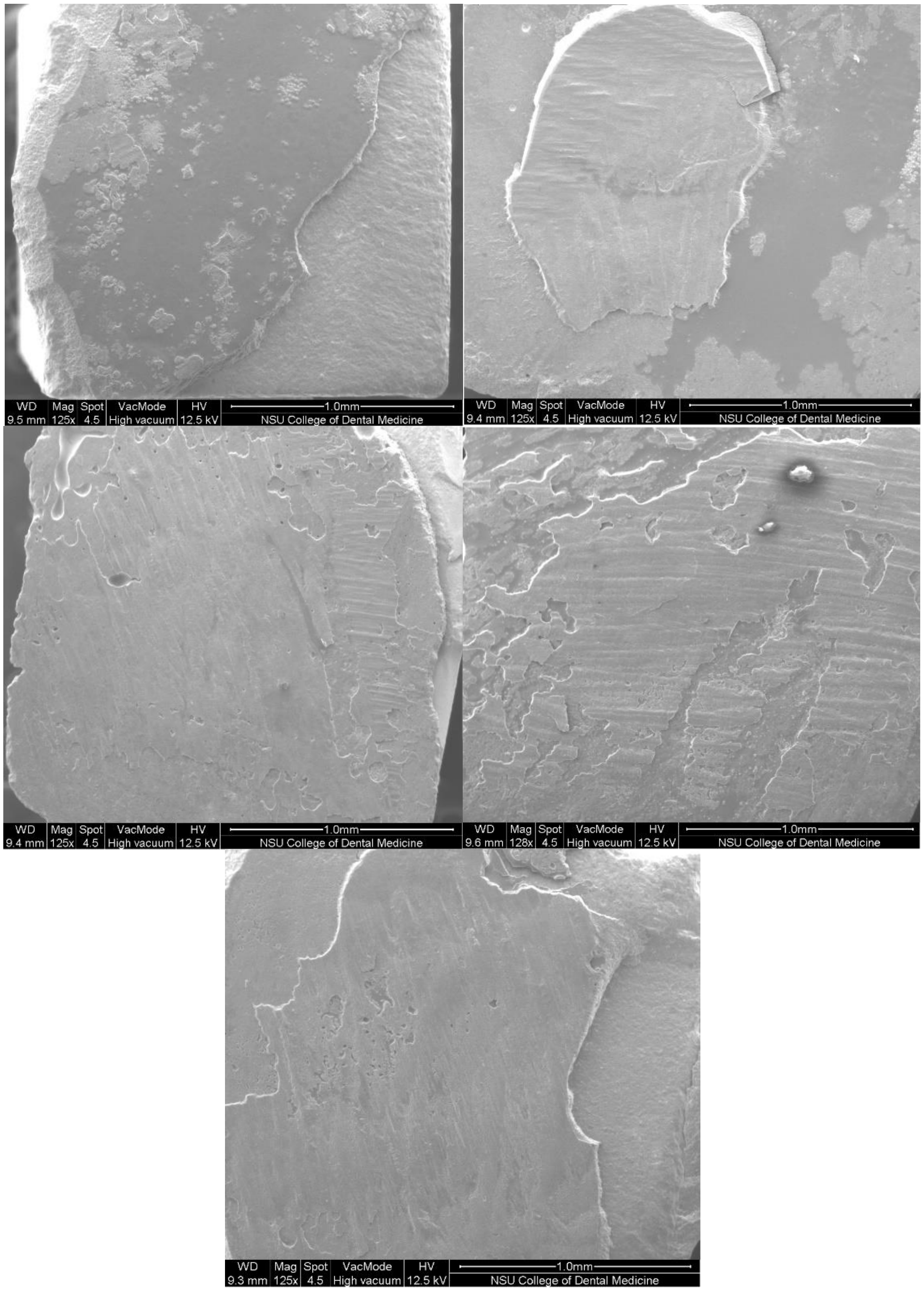

Figure 35. SEM images from group EDTA and Aluminum chloride group (G4)

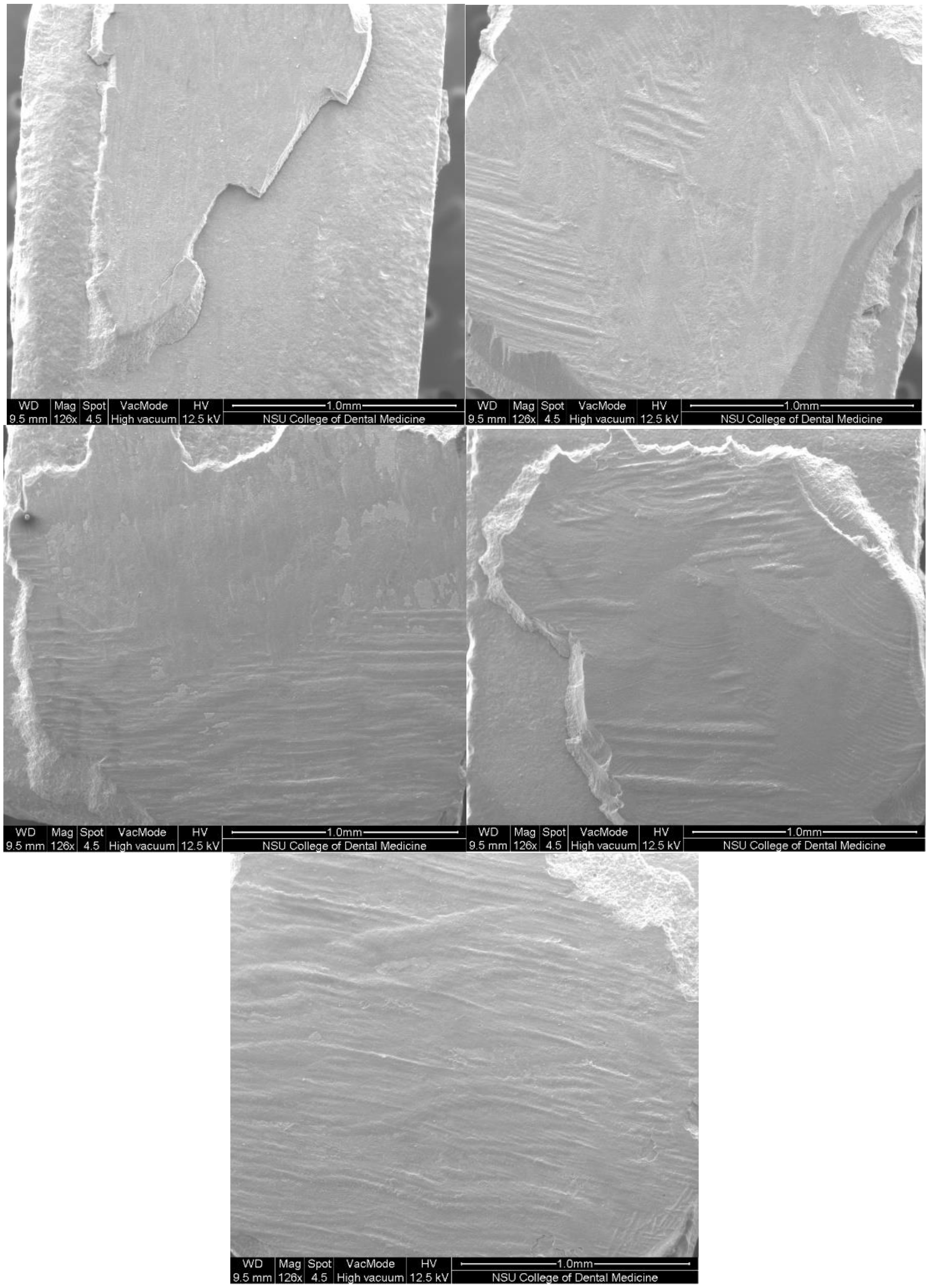

Figure 36. SEM images from group Water and Ferric sulfate group (G5)

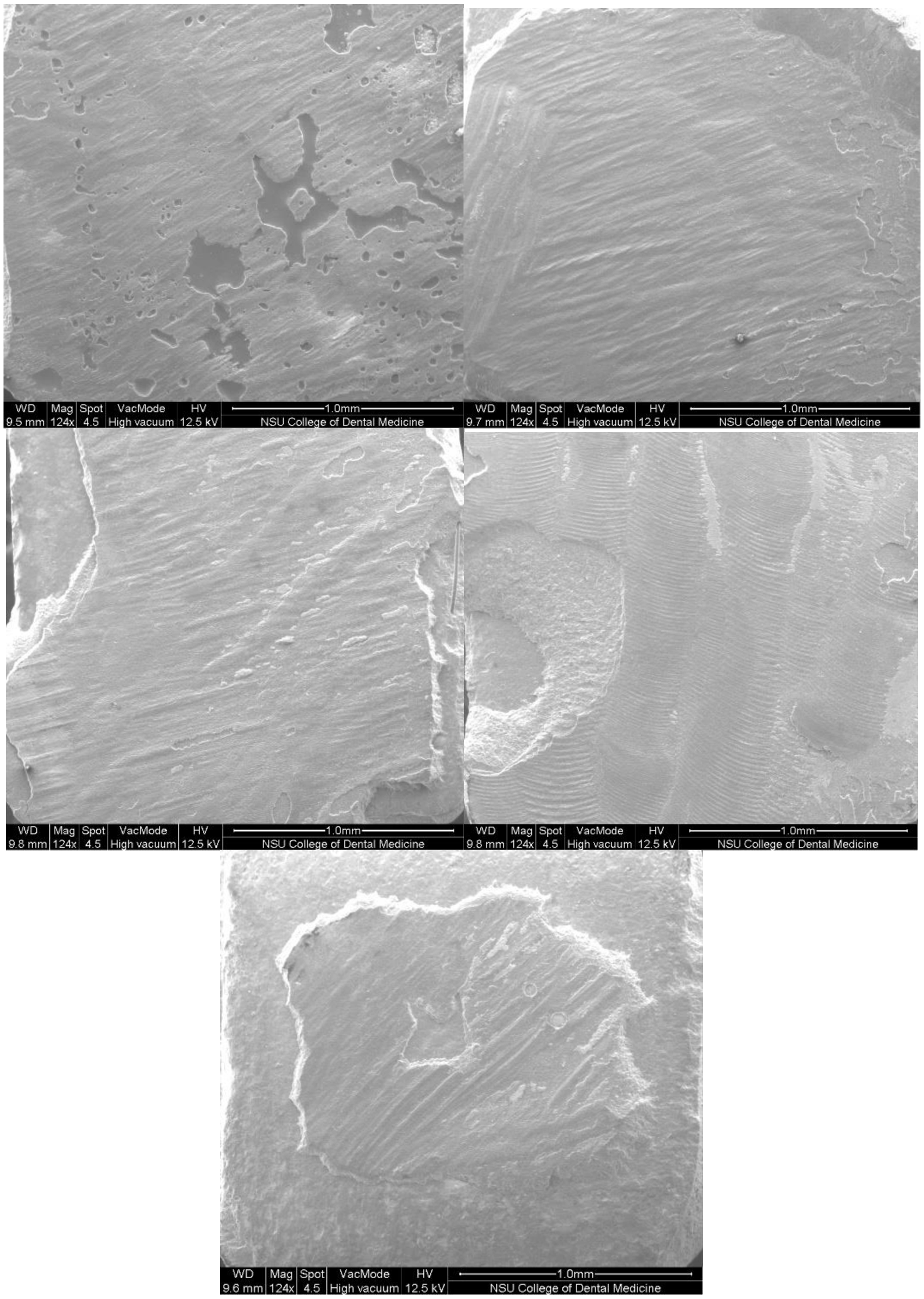

Figure 37. SEM images from group Re-etched and Ferric sulfate group (G6)

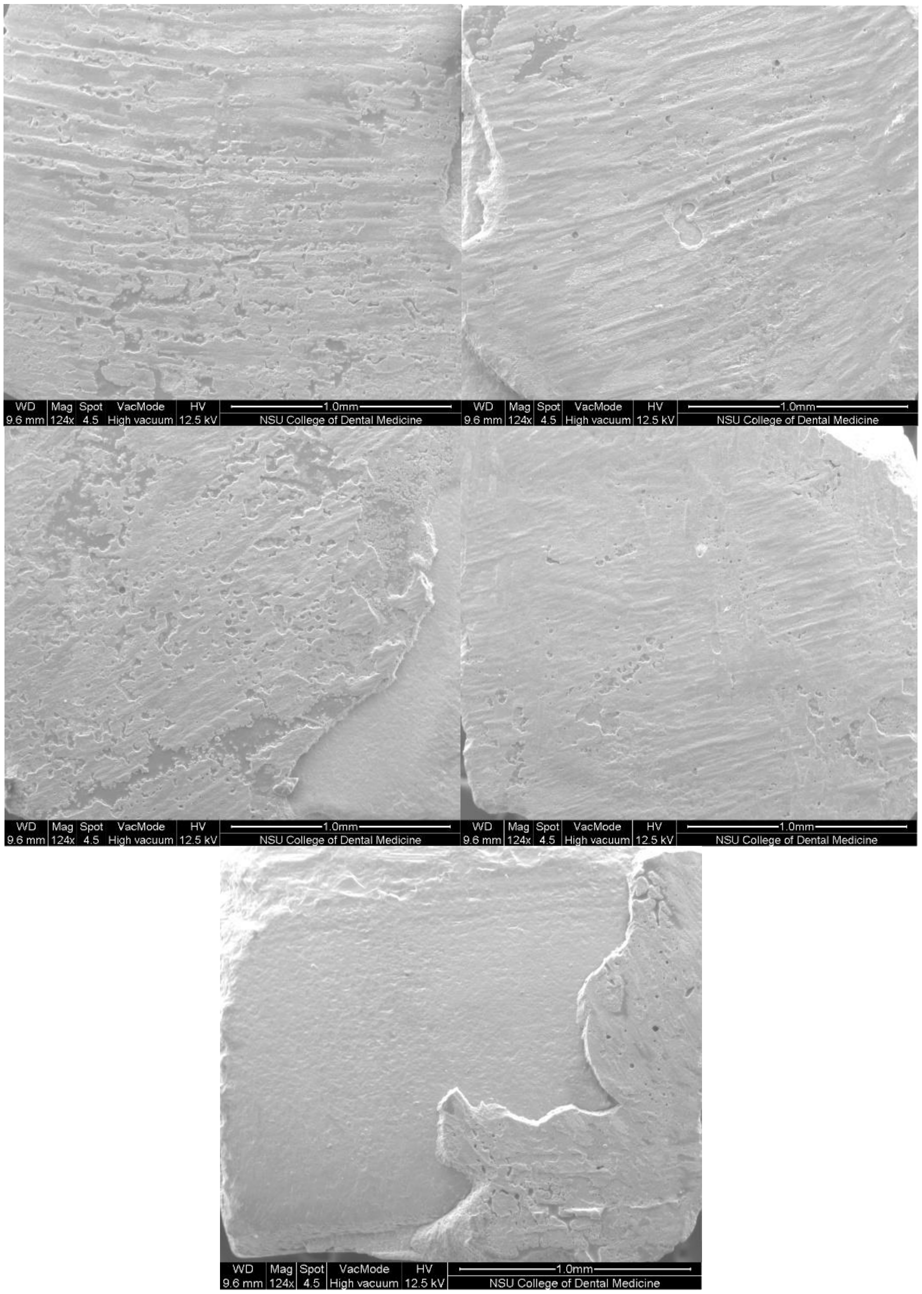

Figure 38. SEM images from EDTA and Ferric sulfate group (G7)

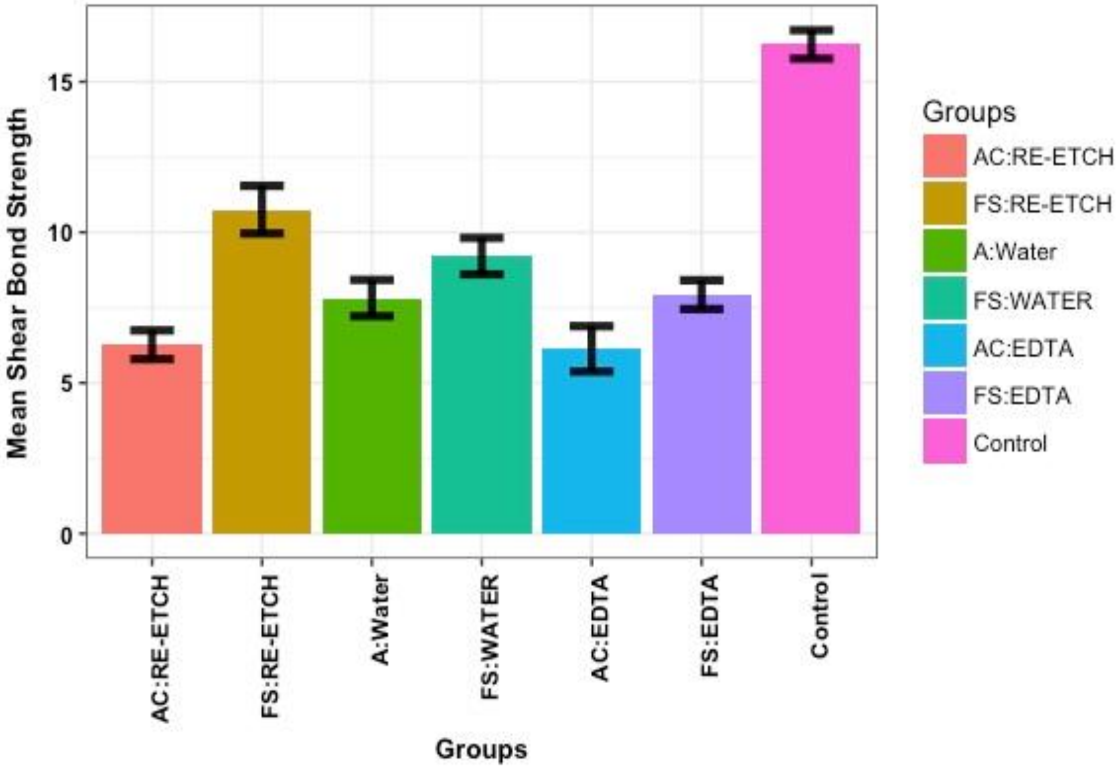

Figure 39. shear bond strength graph

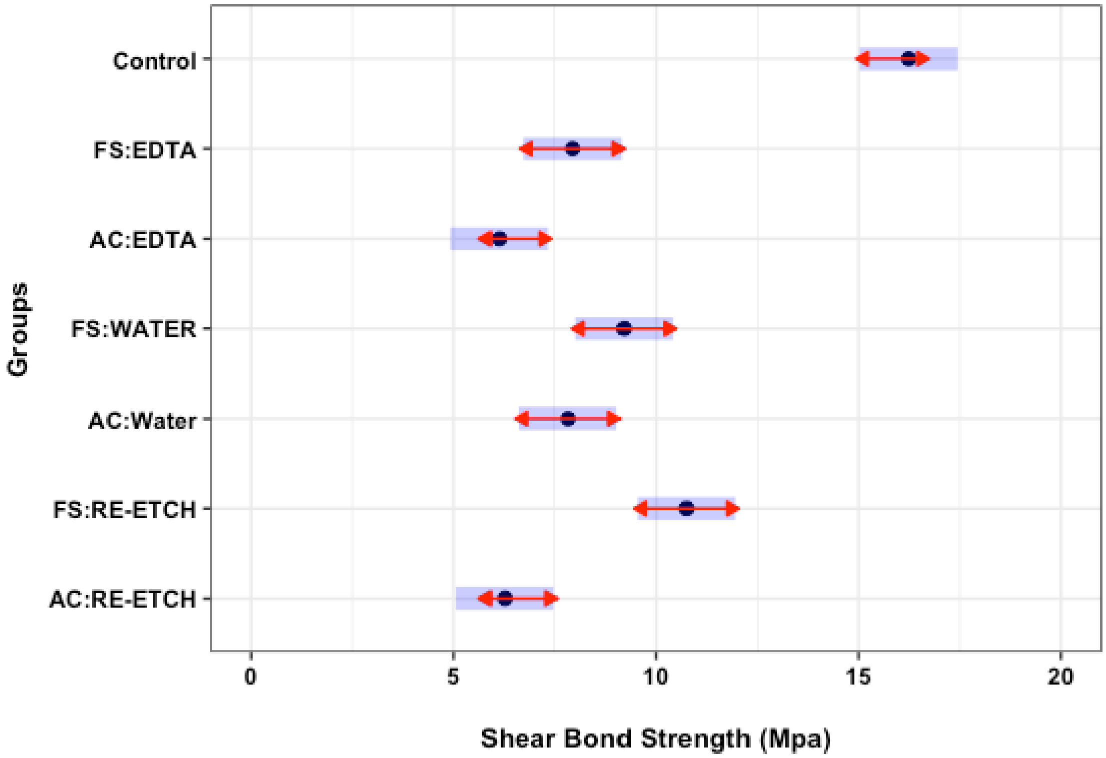

Figure 40. Plot of Shear Bond Strength by Treatment Group. The blue bars are confidence intervals for the means, and the red arrows are for the comparisons among them. If an arrow from one mean overlaps an arrow from another group, the difference is not significant

Appendices

Appendix A: Measurements from Shear Bond Test, Row Data

	load (N)	shear bond (MPa)
control 1	95.37	15.89
control 2	116.59	19.43
control 3	97.7	16.28
control 4	73.32	17.65
control 5	99.21	16.53
control 6	108.94	18.16
control 7	79.88	13.31
control 8	85.7	14.28
control 9	84.32	14.05
control 10	117.16	19.53
control 11	105.68	17.61
control 12	99.56	16.59
control 13	83.07	13.84
control 14	98.05	16.34
control 15	82.59	13.76
control 16	91.5	15.25
control 17	105.2	17.53
AC- water 1	69.64	11.61
AC- water 2	55.44	9.24
AC- water 3	50.01	8.33
AC- water 4	68.29	11.38
AC- water 5	28.03	4.67
AC- water 6	33.53	5.59
AC- water 7	43.79	7.3
AC- water 8	50.61	8.43
AC- water 9	29.35	4.89
AC- water 10	48.66	8.11
AC- water 11	62.98	10.5
AC- water 12	63.74	10.62
AC- water 13	28.35	4.73
AC- water 14	56.68	9.45
AC-water 15	15.59	4.6
AC-water 16	30.25	5.04
AC-water 17	50.63	8.44

AC- etch 1	17.43	2.91
AC- RE-ETCH 2	51.4	8.57
AC- RE-ETCH 3	60.01	10
AC- RE-ETCH 4	31.64	5.27
AC- RE-ETCH 5	34.74	5.79
AC- RE-ETCH 6	62.99	10.5
AC- RE-ETCH 7	38.64	6.44
AC- RE-ETCH 8	36.13	6.02
AC- RE-ETCH 9	30.61	5.1
AC- RE-ETCH 10	34.32	5.72
AC- RE-ETCH11	36.14	6.02
AC- RE-ETCH 12	41	6.83
AC- RE-ETCH 13	28.24	4.71
AC- RE-ETCH 14	26.43	4.41
AC- RE-ETCH 15	26.53	4.42
AC- RE-ETCH 16	42.22	7.04
AC- RE-ETCH 17	41.33	6.8
AC-EDTA 1	12.02	2
AC-EDTA 2	42.03	7
AC-EDTA 3	15.93	2.66
AC-EDTA 4	14.17	2.36
AC-EDTA 5	65.73	10.96
AC-EDTA 6	35.68	5.95
AC-EDTA 7	20.55	3.96
AC-EDTA 8	79.31	13.22
AC-EDTA 9	19.88	3.31
AC-EDTA 10	25.17	4.19
AC-EDTA 11	55.32	9.22
AC-EDTA 12	35.65	6.43
AC-EDTA 13	34.78	5.8
AC-EDTA 14	28	4.67
AC-EDTA 15	37.31	6.22
AC-EDTA 16	45.3	7.55
AC-EDTA 17	52.21	8.7
FS-WATER 1	86.58	14.43
FS-WATER 2	66.78	11.13
FS-WATER 3	37.93	6.32
FS-WATER 4	72.8	12.13
FS-WATER 5	51.9	8.65
FS-WATER 6	52.68	8.78
FS-WATER 7	61.87	10.31
FS-WATER 8	42.38	7.06
FS-WATER 9	64.5	10.75

FS-WATER 10	33.18	5.53
FS-WATER 11	74.25	12.38
FS-WATER 12	61.1	10.18
FS-WATER 13	44.89	7.48
FS-WATER 14	42.77	7.13
FS-WATER 15	52.28	8.71
FS-WATER 16	57.95	9.66
FS-WATER 17	35.64	5.94
FS- RE-ETCH 1	66.69	11.12
FS- RE-ETCH 2	47.17	7.86
FS- RE-ETCH 3	83.74	13.96
FS- RE-ETCH 4	61.56	10.26
FS- RE-ETCH 5	46.15	7.69
FS- RE-ETCH 6	88.36	14.73
FS- RE-ETCH 7	47.56	7.93
FS- RE-ETCH 8	42.65	7.11
FS- RE-ETCH 9	67.61	11.27
FS- RE-ETCH 10	49.93	8.32
FS- RE-ETCH11	64.06	10.68
FS- RE-ETCH 12	99.17	16.76
FS- RE-ETCH 13	96.36	16.06
FS- RE-ETCH 14	55.32	9.22
FS- RE-ETCH 15	46.94	7.82
FS- RE-ETCH 16	86.43	14.41
FS- RE-ETCH 17	45.93	7.57
FS-EDTA 1	40.05	6.67
FS-EDTA 2	51.63	8.61
FS-EDTA 3	46.79	7.8
FS-EDTA 4	45.2	7.53
FS-EDTA 5	50.66	8.44
FS-EDTA 6	55.32	9.22
FS-EDTA 7	82.45	13.74
FS-EDTA 8	54.15	9.03
FS-EDTA 9	36.57	6.1
FS-EDTA 10	42.73	7.12
FS-EDTA 11	49.25	8.21
FS-EDTA 12	32.45	5.41
FS-EDTA 13	43.44	7.24
FS-EDTA 14	35.66	5.94
FS-EDTA 15	33.69	5.61
FS-EDTA 16	52.65	8.77
FS-EDTA 17	56.62	9.4

Appendix B: Observations from Stereomicroscopy, Row Data

	adhesive	**cohesive**	**mixed**	**substrate**
control 1		*		
control 2			*	
control 3			*	
control 4			*	
control 5			*	
control 6		*		
control7		*		
control 8				*
control 9		*		
control 10		*		
control 11		*		
control 12		*		
control 13		*		
control 14		*		
control 15		*		
control 16		*		
control 17		*		
AC- water 1				
AC- water 2			*	
AC- water 3			*	
AC- water 4			*	
AC- water 5			*	
AC- water 6		*		
AC- water 7			*	
AC- water 8		*		
AC- water 9		*		
AC- water 10		*		
AC- water 11			*	
AC- water 12		*		
AC- water 13	*			
AC- water 14			*	
AC-water 15			*	
AC-water 16			*	
AC-water 17				*
AC- etch 1			*	
AC-ETCH 2			*	
AC-ETCH 3			*	

AC-ETCH 4			*	
AC-ETCH 5			*	
AC-ETCH 6			*	
AC- ETCH 7		*		
AC-ETCH 8			*	
AC-ETCH 9			*	
AC-ETCH 10			*	
AC-ETCH11			*	
AC-ETCH 12			*	
AC-ETCH 13			*	
AC-ETCH 14			*	
AC-ETCH 15			*	
AC-ETCH 16			*	
AC-ETCH 17			*	
AC-EDTA 1			*	
AC-EDTA 2		*		
AC-EDTA 3			*	
AC-EDTA 4		*		
AC-EDTA 5			*	
AC-EDTA 6			*	
AC-EDTA 7		*		
AC-EDTA 8			*	
AC-EDTA 9		*		
AC-EDTA 10		*		
AC-EDTA 11			*	
AC-EDTA 12		*		
AC-EDTA 13			*	
AC-EDTA 14			*	
AC-EDTA 15		*		
AC-EDTA 16			*	
AC-EDTA 17			*	
FS-WATER 1			*	
FS-WATER 2		*		
FS-WATER 3		*		
FS-WATER 4			*	
FS-WATER 5			*	
FS-WATER 6			*	
FS-WATER 7	*			
FS-WATER 8			*	
FS-WATER 9			*	
FS-WATER 10		*		
FS-WATER 11		*		
FS-WATER 12			*	

FS-WATER 13		*		
FS-WATER 14		*		
FS-WATER 15			*	
FS-WATER 16		*		
FS-WATER 17			*	
FS- ETCH 1				*
FS-ETCH 2		*		
FS-ETCH 3			*	
FS-ETCH 4		*		
FS-ETCH 5		*		
FS-ETCH 6		*		
FS- ETCH 7		*		
FS-ETCH 8			*	
FS-ETCH 9		*		
FS-ETCH 10		*		
FS-ETCH11		*		
FS-ETCH 12			*	
FS-ETCH 13		*		
FS-ETCH 14		*		
FS-ETCH 15			*	
FS-ETCH 16		*		
FS-ETCH 17			*	
FS-EDTA 1			*	
FS-EDTA 2	*			
FS-EDTA 3		*		
FS-EDTA 4		*		
FS-EDTA 5		*		
FS-EDTA 6		*		
FS-EDTA 7		*		
FS-EDTA 8			*	
FS-EDTA 9			*	
FS-EDTA 10			*	
FS-EDTA 11			*	
FS-EDTA 12		*		
FS-EDTA 13		*		
FS-EDTA 14		*		
FS-EDTA 15		*		
FS-EDTA 16			*	
FS-EDTA 17		*		