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Evaluation of the effect of enamel deproteinization on shear bond strength of orthodontic adhesives and resulting white spot lesion formation

Kelly Chioffe
Nova Southeastern University

AN EVALUATION OF THE EFFECT OF ENAMEL DEPROTEINIZATION ON SHEAR BOND STRENGTH OF ORTHODONTIC ADHESIVES AND RESULTING WHITE SPOT LESION FORMATION

KELLY R. CHIOFFE, D.M.D.

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

December 2014

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MASTER OF SCIENCE

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Approved as to style and content by:

Approved by: _____
Abraham B. Lifshitz, D.D.S., M.S. (Committee Chair) Date

Approved by: _____
Cristina Garcia-Godoy, D.D.S., M.P.H., C.C.R.P. Date
(Committee Member)

Approved by: _____
Mark Hall, D.M.D. (Committee Member) Date

Approved by: _____
Linda Niessen, D.M.D., M.P.H. (Dean) Date

NOVA SOUTHEASTERN UNIVERSITY

Health Professions Division
Department of Orthodontics
College of Dental Medicine

STUDENT NAME: Kelly R. Chioffe, D.M.D.

STUDENT E-MAIL ADDRESS: kellcoop@nova.edu

STUDENT TELEPHONE NUMBER: 561-271-7573

TITLE OF SUBMISSION: An Evaluation of the Effect of Enamel Deproteinization on Shear Bond Strength of Orthodontic Adhesives and Resulting White Spot Lesion Formation

DATE SUBMITTED: December 2014

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.

Student
Signature: _____

Kelly R. Chioffe, D.M.D. Date

DEDICATION

To my husband, Adam. Thank you for every minute of support you have given me throughout the years of my education. You are my biggest supporter and I am so appreciative to have you in my life. I love you.

To my daughter, Ariana. You came into my life during my writing of this manuscript and are the reason I strive for the best. May you always reach for the stars in your future endeavors. I hope I have set an example as to how hard work pays off.

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Abstract

AN EVALUATION OF THE EFFECT OF ENAMEL DEPROTEINIZATION ON SHEAR BOND STRENGTH OF ORTHODONTIC ADHESIVES AND RESULTING WHITE SPOT LESION FORMATION

DEGREE DATE: December 12, 2014

KELLY R. CHIOFFE, D.M.D.

COLLEGE OF DENTAL MEDICINE, NOVA SOUTHEASTERN UNIVERSITY

Thesis Directed By: Abraham B. Lifshitz, D.D.S, M.S.

Committee Members: Cristina Garcia-Godoy, D.D.S., M.P.H., C.C.R.P.,

Mark Hall, D.M.D.

Objective: The objectives of this study were 1) to evaluate the effect of enamel deproteinization on the shear bond strength (SBS) of orthodontic brackets bonded with a resin modified glass-ionomer (RMGI) adhesive and a composite resin, 2) to determine the mode of bond failure according to the adhesive remnant index (ARI) and 3) to evaluate the effect of these adhesives in the prevention of white spot lesions (WSLs). **Background**: WSLs are a concern for orthodontic patients. RMGI orthodontic adhesives are capable of absorbing fluoride from the oral environment and releasing it continuously over time, however, they are not frequently used as they exhibit low SBS. Techniques such as non-invasive enamel deproteinization with 5.25% sodium hypochlorite prior to acid etching rid the surface of organic components and have the potential to enhance the etching pattern. **Methods**: Eighty-eight extracted bovine incisors were randomly divided into two groups. 48 incisors in group (A) underwent SBS

testing, through debonding of brackets with the Universal Testing Machine and subsequent measurement of the ARI. 40 incisors in group (B) underwent demineralization testing by measuring the depth of WSLs formed after exposure to an acidic challenge for 96 hours. In groups A and B, the teeth were divided to have brackets bonded with GC Fuji ORTHO™ LC adhesive or Transbond™ XT adhesive and a self-etching primer. Each adhesive group had an experimental group receiving the intervention of enamel deproteinization prior to etching and bonding and a control group. **Results:** The highest mean SBS was observed in the Transbond™ XT control group (12.48 ± 6.23 MPa) and the lowest mean SBS was observed in the Fuji ORTHO™ experimental group (5.49 ± 2.97 MPa). ANOVA and Post-Hoc Tukey tests revealed statistically significant differences ($p<0.05$) in the SBS of both Fuji ORTHO™ groups compared to the Transbond™ XT control group. A significantly greater percentage of Transbond™ XT control teeth had an ARI score of 0 and a greater percentage of Fuji ORTHO™ experimental teeth had an ARI score of 3. The Transbond™ XT experimental group had the largest average demineralization lesions (62.97 ± 10.95 μm). The smallest lesion depths were found in the Fuji ORTHO™ groups, with an average of 7.74μm in the experimental group and 6.57μm in the control group. ANOVA and Post-Hoc Tukey tests revealed significant differences ($p<0.001$) in the depth of white spot lesions when comparing both composite resin groups to each other, and when each glass ionomer group was compared to each composite resin group. **Conclusions:** Enamel deproteinization did not increase the SBS of orthodontic brackets bonded with either Fuji ORTHO™ adhesive or Transbond™

XT adhesive. However, according to the ARI, more bond failures occurred at the bracket-adhesive interface in the Fuji ORTHO™ experimental group. Also, both Fuji ORTHO™ adhesive groups showed greater protection against enamel demineralization, when compared to the Transbond™ XT adhesive groups.

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Chapter 1: Introduction

1.1. Enamel Bonding

Successful bonding of fixed orthodontic attachments is one of many objectives for the orthodontist during the treatment of their patients and bond strength has been widely emphasized in the orthodontic literature over the years. Orthodontic adhesive materials are bonded to enamel for a limited time, unlike in restorative dentistry, where they are generally permanently bonded to enamel or dentin. Therefore, there are several critical requirements for orthodontic bonding that must be met, including sufficient clinical bond strength, ease of debonding, and limited risk of permanent enamel damage.[1]

Before enamel bonding became the routine procedure for placing orthodontic attachments during the late 1970s, individual banding of each tooth was the primary method used to carry out orthodontic treatment.[2-5] With Buonocore's development of the acid etch technique in 1955, one factor known to significantly influence bond strength is enamel conditioning.[6, 7]

Enamel conditioning, or acid etching, is an essential procedure prior to bonding with adhesive materials. The quality of enamel etching depends on the acid etchant's concentration, the etchant's composition and the time the etchant is in contact with the enamel.[8, 9] In 1975, Silverstone evaluated etched enamel with a Scanning Electron Microscope (SEM) and observed three different etching patterns.[9] A Type 1 pattern is classified when generalized enamel roughening is observed with the head of the enamel prism dissolved and the

peripheral material or interprismatic substance remaining intact.[9] A Type 2 pattern is classified when, oppositely, the peripheral zone of the enamel prisms is diluted, with the enamel prism head remaining intact.[9] Either of these two patterns can be produced when the enamel is exposed to similar acid concentrations for similar lengths of time.[9] A Type 3 pattern is classified when the enamel surface has no specific features of change, but exhibits some superficial dissolution that does not alter the enamel prisms.[9] Silverstone has shown that the etching patterns that are most retentive to bonding are types 1 and 2.[9, 10]

As acid etching has an effect only on the inorganic structures of the enamel, additional conditioning techniques that remove the organic components of the enamel biofilm have been shown to enhance the acid etching effect, including enamel deproteinization with 5.25% sodium hypochlorite prior to acid etching.[10, 11] Previous studies have shown that when this technique is employed, enhanced etching patterns have been observed, which can potentially increase the bond strength of the adhesive.[10-12] According to Reynolds, a minimum tensile bond strength of 5.9 to 7.8 MPa is adequate for most clinical orthodontic needs and when using certain orthodontic adhesives, such as resin-modified glass ionomers, additional enamel conditioning procedures may be indicated to achieve clinically acceptable bond strengths. [13]

Such additional procedures may include the use of sodium hypochlorite. Sodium hypochlorite is a frequently used irrigating agent in Endodontics that has shown to be non-invasive, without damaging tooth structure or healthy

keratinized tissue.[10, 14-16] It has an antimicrobial effect and the ability to rid tooth structure of the organic biofilm that instantly forms on the surface as it comes into contact with saliva.[10, 14-16] Care must still be taken during the application of sodium hypochlorite in the mouth because although it is not toxic to keratinized gingiva, it is a nonspecific agent whose action can be toxic to other cells.[14-16]

1.2. White Spot Lesions (WSLs)

Although the achievement of clinically acceptable bond strengths is important for carrying out orthodontic treatment, of more significant concern for the patient and the orthodontist is the increased incidence of white spot lesions (WSLs) seen in orthodontic patients. The incidence of orthodontic associated WSLs occurs in up to 50% of patients and this decalcification can occur as early as 4 weeks after bonding the orthodontic applicances.[17-21] The overall prevalence of WSLs in individuals undergoing fixed orthodontic treatment has been reported to range from 2-96%.[17, 22, 23]

The International Caries Detection and Assessment System II (ICDAS II) has stated that a WSL is the first visual change in enamel, and it is associated with demineralization limited to the outer half of the enamel thickness.[24] The lesion remains on the subsurface, as mineral loss at the subsurface progresses more quickly than at the surface, and the outer enamel surface layer remains intact.[25-27] The subsurface dissolution contributes to the alteration of the color, or whitening, of the enamel. [27]

As the enamel surface is usually in a state of dynamic equilibrium with saliva and the acquired pellicle, consisting of an acellular, bacteria-free, organic

film, enamel demineralization will occur when dental plaque accumulates over the pellicle and is subsequently exposed to dietary fermentable carbohydrates.[27] Since the presence of fixed orthodontic appliances makes proper oral hygiene for plaque removal more difficult, an increase in plaque accumulation with associated bacterial acid production has been found adjacent to orthodontic appliances, leaving these patients at an increased risk for enamel demineraliztion.[12, 19, 20, 28] More specifically, increased levels of *Streptococcus mutans*, a highly cariogenic bacteria, have been observed adjacent to orthodontic brackets. [29]

This presence of enamel demineralization ultimately increases the patient's risk of developing carious lesions, since the demineralized surface is a precursor of enamel caries.[30] Potentially, the lesions may warrant the future need for restorative or esthetic treatment if the demineralization process is allowed to continue without adequate remineralization.

1.3. Methods to Prevent Enamel Demineralization

Most methods shown to decrease the incidence of white spot lesions in orthodontic patients rely heavily on patient compliance and include improved oral hygiene, diet modification and topical fluoride application.[27] It has been well established in the literature that preventive fluoride has the ability to reduce the incidence of enamel demineralization. Additionally, resin-modified glass ionomer (RMGI) adhesives have since been formulated as a noncompliant alternative for use in orthodontic patients.[18-20, 30-34] Due to the increased decalcification risk seen in orthodontic patients, the need to implement a preventive continuous

application of low dose fluoride to these patients exists, as fluoride has been shown to be most effective in inhibiting the *beginning* of the carious process, and promoting remineralization of early caries.[27] The frequency of fluoride appears to be more important than its concentration in protecting the enamel.[20] The low dose fluoride is slightly less effective in inhibiting the *progression* of the carious disease state.[27]

The action of fluoride in the reduction of enamel demineralization has been shown to occur through two primary methods. The first is through its effect on the metabolism of the bacterial plaque. The presence of fluoride in the oral environment results in a reduction of bacterial acid production, due, in part, to its inhibition of the enolase enzyme, which is ultimately involved in the breakdown of sugar molecules through the process of glycolysis.[35, 36] Since the enzymatic inhibition is observed only for a short period of time after exposure to fluoride, frequent exposure of the bacterial plaque to fluoride is required in order diminish to presence of acids in the mouth, thereby reducing enamel demineralization.[35] In fact, plaque around orthodontic brackets that have been bonded with glass ionomer adhesives has been found to contain 44% greater fluoride concentration compared to those bonded with composite resin adhesive.[37] Secondly, the efficacy of fluoride in the reduction of enamel demineralization can also be contributed to its ability to form fluorapatite with the enamel surface, altering the enamel solubility and rendering it more resistant to acid dissolution.[38]

1.4. RMGI adhesives in Orthodontics

Traditional glass ionomer cements are typically composed of two components: fluoride-containing silicate glass and polyalkenoic acids.[38] An acid-base reaction between the two components allows the cement to set and a variety of ions are released during the setting reaction, the most important being fluoride.[38] With the addition of resin monomers to the polyalkenoic acids, resin-modified glass ionomer adhesives were formulated. An advantage of this material is that it can be polymerized through light curing, hastening the initial hardening process and addressing the issue of moisture sensitivity.[38, 39] In addition to the chemical bonding of RMGI adhesives to the enamel, the resin monomers can penetrate the irregularities of the enamel surface to produce a micromechanical bond.[39] Orthodontic resin-modified glass ionomer (RMGI) adhesives, such as GC Fuji ORTHO™ LC, have also been proven to be capable of absorbing fluoride from the oral environment, storing it and releasing it continuously over time.[19, 20] An initial high release of fluoride ions from the adhesive during the first 24 hours occurs due to the initial setting reaction and although the release of fluoride slowly diminishes over time, they continue to release small amounts of fluoride for up to 2.7 years.[40]

The fluoride ions released from the glass ionomer adhesives have the ability to act remotely on the enamel through its inhibition of demineralization up to a distance of 7mm away from the margin of the adhesive.[38] When compared to non-fluoride-releasing adhesives, Tantbirojn *et al.* [41] found that at a distance of 0.22mm from the glass ionomer adhesive margin, mineral loss was reduced by

80% and at 7mm, it was reduced by 37%. Pascotto *et al.*[19] also found that RMGI adhesives had the ability to reduce enamel demineralization up to 200μm away from the edge of the bracket base. If these adhesives are used for the bonding of orthodontic attachments, the benefit they can offer to the orthodontic patient who often struggles with proper oral hygiene are immeasurable.

Although the incorporation of resin components into traditional glass ionomer adhesives allowed for an enhancement in enamel bond strength, the strength is typically lower than that of resin composites.[42] Therefore, resin-modified glass ionomer adhesives are not frequently used as bonding adhesives by orthodontic clinicians, due to their clinical disadvantage of a lower shear bond strength compared to conventional non-fluoride releasing composite resins, such as Transbond™ XT.[12, 20] One previous study has shown that when enamel is deproteinized with sodium hypochlorite prior to acid etching and bonding with RMGI orthodontic adhesives, these adhesives can produce a shear bond strength comparable to conventional orthodontic adhesives.[12]

1.5. Importance of Study

With the increased potential of orthodontic patients developing orthodontic associated white spot lesions, supplemental preventive procedures should be used with these patients whenever possible. Fluoride containing resin-modified glass ionomer adhesives have been manufactured for orthodontic bonding, but they have not shown to provide consistent clinically acceptable bond strength of orthodontic brackets.[12, 20] Since the benefit of continuous low dose fluoride in the impediment of enamel demineralization is understood, a satisfactory orthodontic

bonding protocol with the use of these adhesives needs to be tested and established. The necessity of this information is vital and should be available to clinicians in order to guide them in providing the best preventive care for their patients.

This study tested the bond strength of orthodontic brackets using a suggested bonding protocol of incorporating enamel deproteinization with sodium hypochlorite, prior to acid etching and bonding with a fluoride-releasing resin-modified glass ionomer adhesive (GC Fuji ORTHO^{TM} LC). Bonding with the use of a self-etching primer and composite resin (Transbond^{TM} XT) was used as a comparison, as today, this technique has become increasingly popular and widespread in the orthodontic field, as it decreases the chair time required for initial bonding.[43] Most importantly, it has been shown to exhibit a clinically acceptable level of bond strength.[43]

A comparison of the bond strengths and potential to decrease the occurrence and depth of enamel demineralization between this acceptable and popular bonding technique and the suggested technique, involving enamel deproteinization and RMGI adhesive, is fundamental. It will aid the clinician in choosing a clinically effective technique that fits their individual patients and will certainly be pioneering to the orthodontic field in terms of providing noncompliant preventive measures for these patients.

1.6. Purpose, Specific Aims and Hypotheses

1.6.1. Purpose

The primary purpose of this *in vitro* study was to examine the effects that enamel deproteinization with sodium hypochlorite had on the shear bond strength of orthodontic brackets bonded with two orthodontic adhesives. Furthermore, the effect of these adhesives against enamel demineralization and white spot lesion formation was also assessed.

1.6.2. Specific Aims

1. To evaluate the effects of sodium hypochlorite enamel deproteinization, prior to acid etching, on the shear bond strength of orthodontic brackets bonded with a composite resin and a fluoride-releasing resin-modified glass ionomer.
2. To determine the mode of bracket failure according to the adhesive remnant index.
3. To evaluate the effect of these bonding adhesives against enamel demineralization and white spot lesion formation.

1.6.3. Hypotheses

H_o:

1. There is no statistically significant difference in the shear bond strength of orthodontic attachments that had the enamel deproteinized prior to bonding with a resin-modified glass ionomer adhesive or a composite resin, compared to control groups, which did not receive enamel deproteinization prior to bonding with these adhesives.
2. There is no statistically significant difference in the mode of bracket failure between the four adhesive groups.

3. There is no statistically significant difference in the occurrence and depth of demineralized lesions between groups that received enamel deproteinization prior to bonding with a resin-modified glass ionomer adhesive or a composite resin, and those that did not receive enamel deproteinization prior to bonding with these adhesives.

1.7. Location of Study

The design, preparation, data collection, and analyses for this study took place at:

Bioscience Research Center

Nova Southeastern University

College of Dental Medicine

3200 South University Drive

Fort Lauderdale, FL 33328

Chapter 2: Materials and Methods

2.1. Study

Based on a power analysis, the numbers of extracted bovine teeth included in this *in vitro* study were forty-eight incisors in the first experimental group, undergoing bond strength testing and forty incisors in the second experimental group, undergoing demineralization testing.

2.1.1. Ethical Issues

No potential ethical issues were identified as part of this research study.

2.1.2. Grant

This study was funded by a grant awarded from the Health Professions Division at Nova Southeastern University.

2.2. Sample Size Estimate

A previous and similar study by Justus *et al.* in 2010[12], was used as the mock "pilot study" to determine the proper sample size that was needed for the shear bond strength testing section of the proposed research. According to G*Power 3.1, which was used to determine the appropriate sample size, it was found that a total of 48 teeth was required for testing, with 12 samples in each of the four groups.

a. Sample Size to be used = 12
b. Alpha = 0.05
c. Beta = 0.20

d. Effect Size = 1.56

e. Power = 80%

According to preliminary data in a pilot study, reported by Loucks Buren *et al.*[44] in 2008, the minimum number of specimens per group in the demineralization testing section of the proposed research needed to be 6. Due to the potential loss of specimens from sectioning of the teeth prior to viewing them under polarized light microscopy, the sample size was increased to 10 per group, for a total of 40 samples. This was confirmed to be appropriate by G*Power 3.1.

2.3. Sample Preparation

Eighty-eight extracted bovine incisors, obtained from deceased cows at a local slaughterhouse, were used in this research, as they have shown to be acceptable substitutes for human teeth in the evaluation of adhesive bond strength and white spot lesions (Fig. 1).[45-48] The incisors were obtained from cows aged 2-5 years. After extraction from the anterior mandible, the samples were stored in distilled water, for not more than 6 months, as it has been shown that changes in tooth structure occurring after extraction could influence bond strength measurements.[49-51]

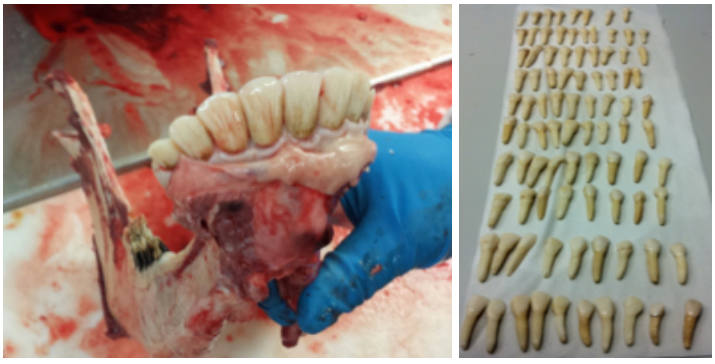

Figure 1. Bovine incisor samples

The samples were then randomly divided into two groups. The first group (named “A”; Fig 2.) underwent shear bond strength testing and the second group (named “B”; Fig 3.) underwent demineralization testing. Within groups A and B, the teeth were randomly divided to have brackets bonded with GC Fuji ORTHO™ LC adhesive (named “GI” for glass ionomer) or with Transbond™ XT adhesive and a self-etching primer (named “CR” for composite resin). Each adhesive group was then further divided into two subgroups; receiving the intervention of enamel deproteinization prior to bonding (named “E” for experimental) or not receiving this deproteinization intervention prior to bonding (named “C” for control).

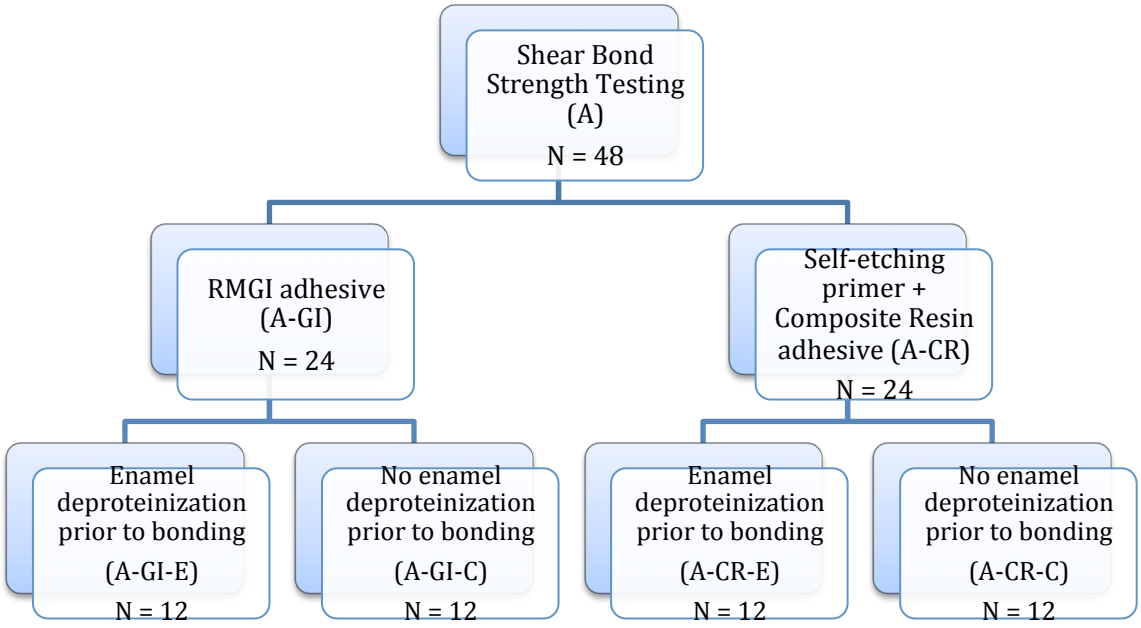

Figure 2. Flow chart of the division of the total sample in the Group (A); Shear bond strength testing

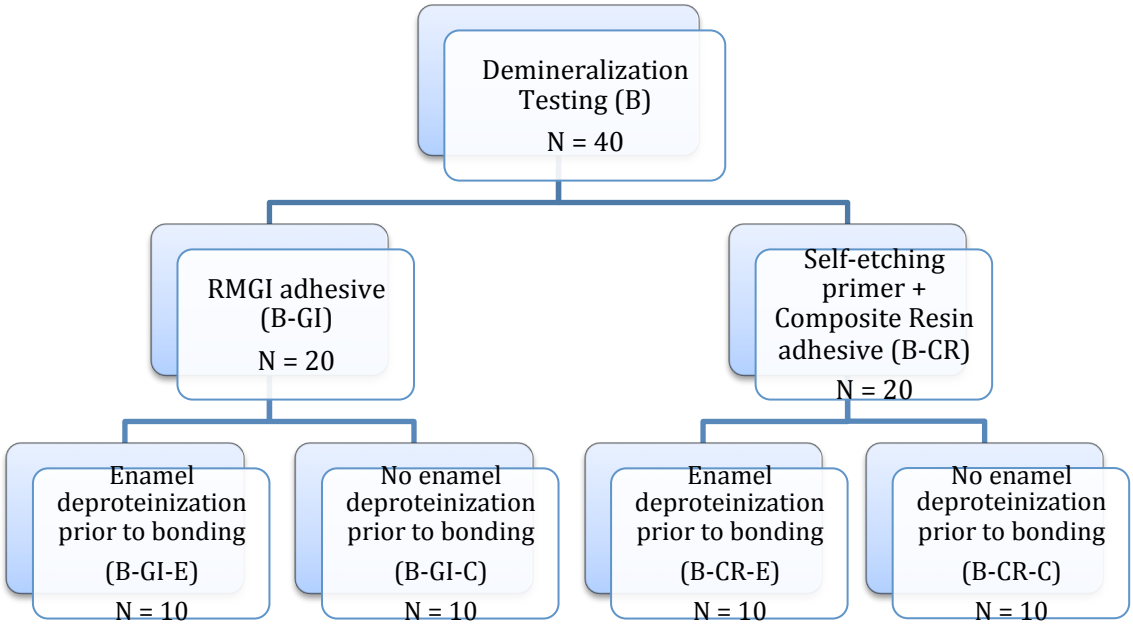

Figure 3. Flow chart of the division of the total sample in Group (B); Demineralization testing.

Before bonding of the orthodontic bracket, the incisors in group A were individually mounted in mounting stone (WhipMix, Louisville, KY) in order to provide a base for the sample during debonding. The crown was left exposed and kept moist with damp gauze as the stone set. The mounted samples were then stored in distilled water until bonding was performed. The samples in group B were not mounted in mounting stone, as the brackets were not debonded with the Universal Testing Machine.

Immediately before bonding, the entire enamel surface of each incisor was cleaned with a non-fluoridated prophylaxis paste (Preppies, WhipMix, Louisville, KY) and rubber cup for 10 seconds and thoroughly rinsed with distilled water.

2.3.1. Bonding and Storage of Samples in Group A

In group A-GI, all orthodontic brackets were bonded by the principal investigator in the following manner, according to the manufacturer's instructions of GC Fuji ORTHO™ LC Automix adhesive (GC America, Alsip, IL):

The buccal surface of the incisors, in group A-GI-E only, were deproteinized with 5.25% sodium hypochlorite with a microbrush for one minute and thoroughly rinsed and dried. The bleach was manufactured as 6% sodium hypochlorite (NaOCl; Great Value Bleach, KIK Custom Products, Concord, Ontario) and was diluted to a concentration of 5.25% by mixing 220mL of 6% sodium hypochlorite with 30mL of distilled water. Group A-GI-C did not receive the intervention of enamel deproteinization prior to acid etching. Ortho Conditioner™ (GC America, Alsip, IL), a 10% polyacrylic acid solution, was applied to the buccal surface using a microbrush for 10 seconds. The conditioner was then thoroughly rinsed away and the enamel surface remained moist. This was followed by immediate placement of the orthodontic bracket to the enamel by applying GC Fuji ORTHO™ LC adhesive to the bracket base and a firm, consistent pressure to the enamel of 300 grams for 10 seconds, as measured by a Dontrix gauge (Ortho-Pli, Philadelphia, PA). [52, 53] The adhesive was expelled from an Automix Paste Pak onto a mixing pad before it was applied to the bracket base. Excess composite was removed with a sharp explorer. Light curing was then performed for 4 seconds on three sides of the orthodontic bracket, with the VALO® cordless curing light (Ultradent Products, South Jordan, UT) (Fig. 4).

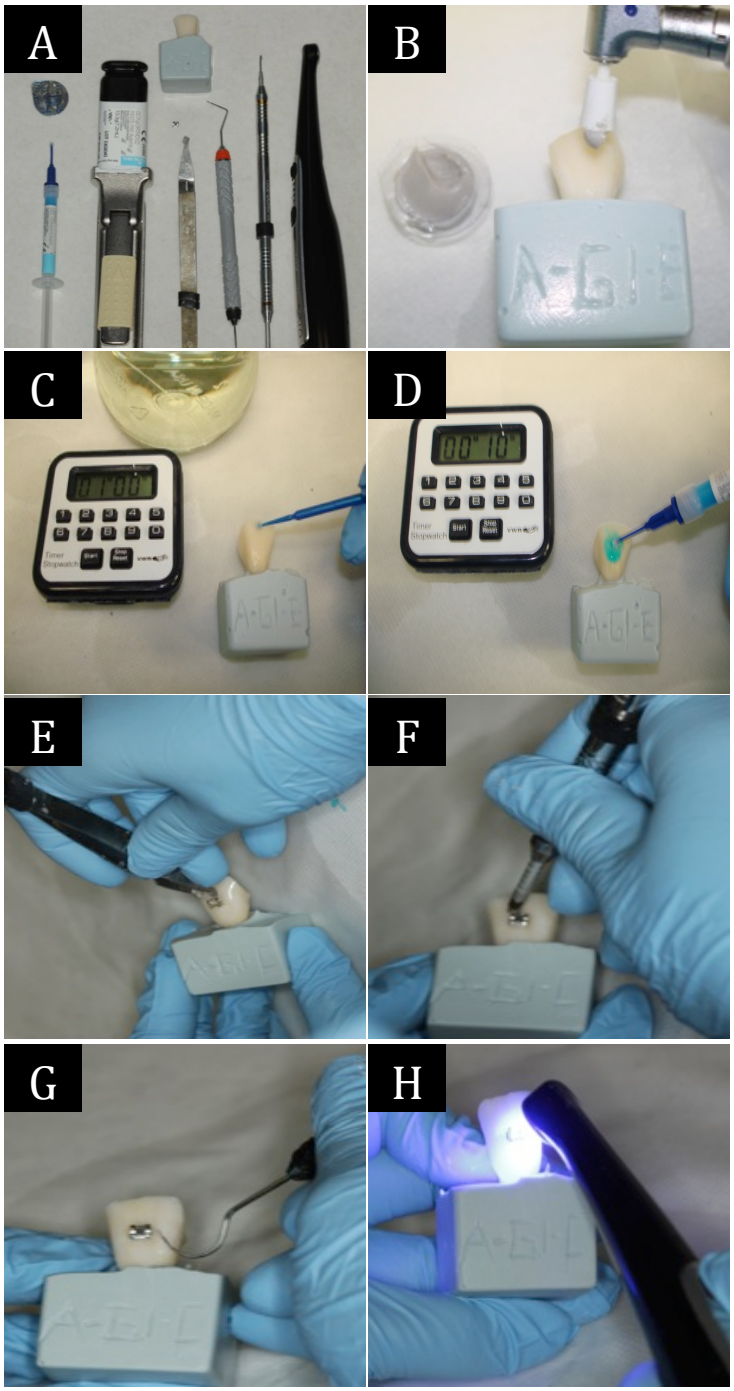

Figure 4. A. Materials set up for bonding in Group A-GI-E B. Enamel surface cleaned with non-fluoridated prophylaxis paste and rubber cup C. Application of 5.25% sodium hypochlorite with a microbrush for one minute D. Application of Ortho Conditioner™ for 10 seconds E. Placement of orthodontic bracket with GC Fuji ORTHO™ LC Automix adhesive F. Bracket placement with use of Dontrix gauge and 300g of pressure for 10 seconds G. Removal of excess composite with sharp explorer H. Light curing of adhesive with VALO® cordless curing light

During light curing, the VALO® cordless curing light was placed in 'High Power Mode' and frequently checked with a Demetron® L.E.D. Radiometer (Kerr Corporation, Orange, CA) to ensure a constant output of 1000mW/cm^2 (Fig. 5).

Figure 5. Valo® cordless curing light and Demetron® L.E.D. Radiometer

In group A-CR, all orthodontic brackets were bonded by the principal investigator in the following manner, according to the manufacturer's instructions of Transbond™ XT adhesive (3M Unitek, Monrovia, CA):

The buccal surface of the incisors, in group A-CR-E only, were deproteinized with 5.25% sodium hypochlorite with a microbrush for one minute and thoroughly rinsed and dried. Group A-CR-C did not receive the intervention of enamel deproteinization prior to acid etching. Transbond™ Plus Self Etching Primer (3M Unitek, Monrovia, CA), containing methacrylated phosphoric acid esters, was lightly rubbed onto the buccal surface for a minimum of 3-5 seconds, followed by a burst of moisture free air for 1-2 seconds. This was followed by immediate placement of the orthodontic bracket to the enamel by applying Transbond™ XT adhesive to the bracket base and a firm, consistent pressure to the enamel of 300 grams for 10 seconds, as measured by a Dontrix gauge

(Ortho-Pli, Philadelphia, PA). [52, 53] Excess composite was removed with a sharp explorer. Light curing was performed for 4 seconds on three sides of the orthodontic bracket with the VALO® cordless curing light (Ultradent Products, South Jordan, UT, USA) (Fig. 6).

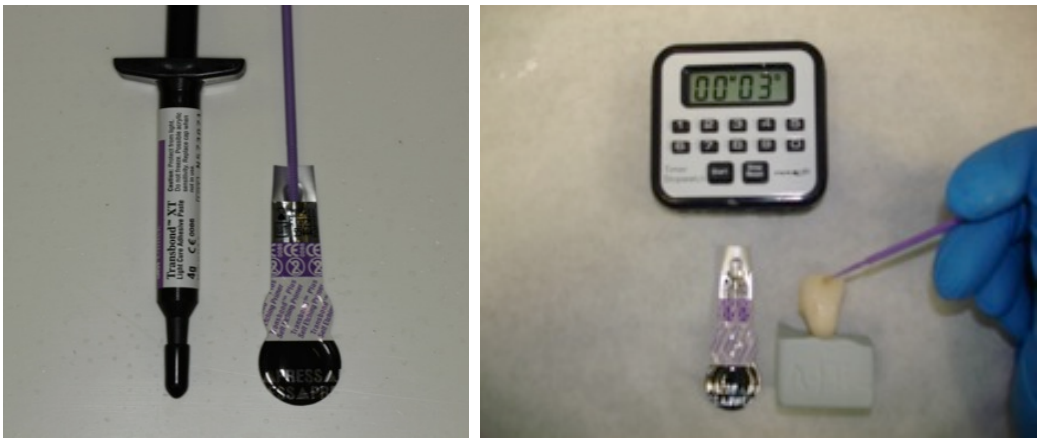

Figure 6. A. Transbond™ XT adhesive and Transbond™ Plus Self Etching Primer B. Application of Transbond™ Plus Self Etching Primer for 3 seconds.

According to the ISO Standards for testing of adhesion to tooth structure, after bonding of orthodontic brackets to the enamel surface in all four subgroups, the samples in group A were stored in 37°C distilled water, in order to discriminate between those materials that can and those that cannot withstand a wet environment (Fig. 7).[51] The samples were then thermocycled to simulate oral conditions (Fig. 8). The ISO Standards states that this is a Test type 2, consisting of "Thermocycling of 500 cycles in water between 5°C and 55°C, starting after a minimum of 20 hours to 24 hours storage in water at 37°C, where the exposure to each bath should be at least 20 seconds, and the transfer time between baths should be 5 seconds to 10 seconds." [51] After thermocycling and before debonding of orthodontic brackets using the Universal Testing Machine, the

samples were stored in 37°C distilled water for an additional 20 hours (Figs. 9 and 10).

Figure 7. Storage of samples in 37°C distilled water after bonding

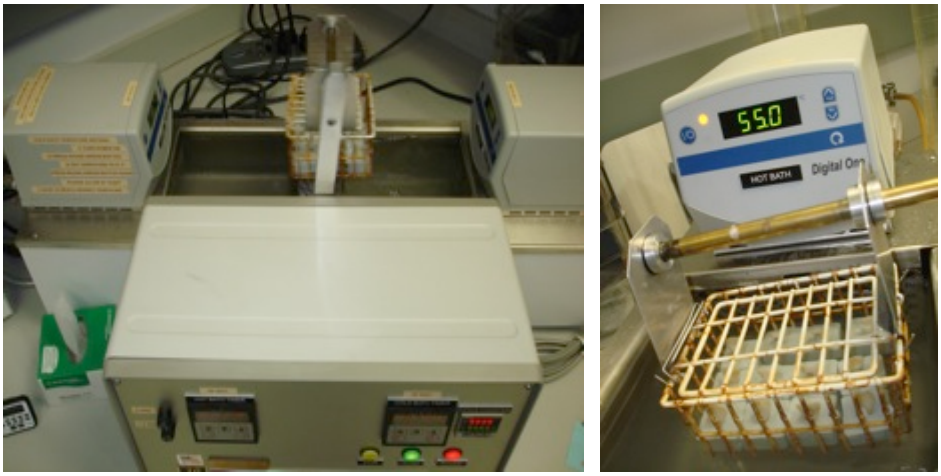

Figure 8. Thermocycling of samples for 500 cycles between 5°C and 55°C

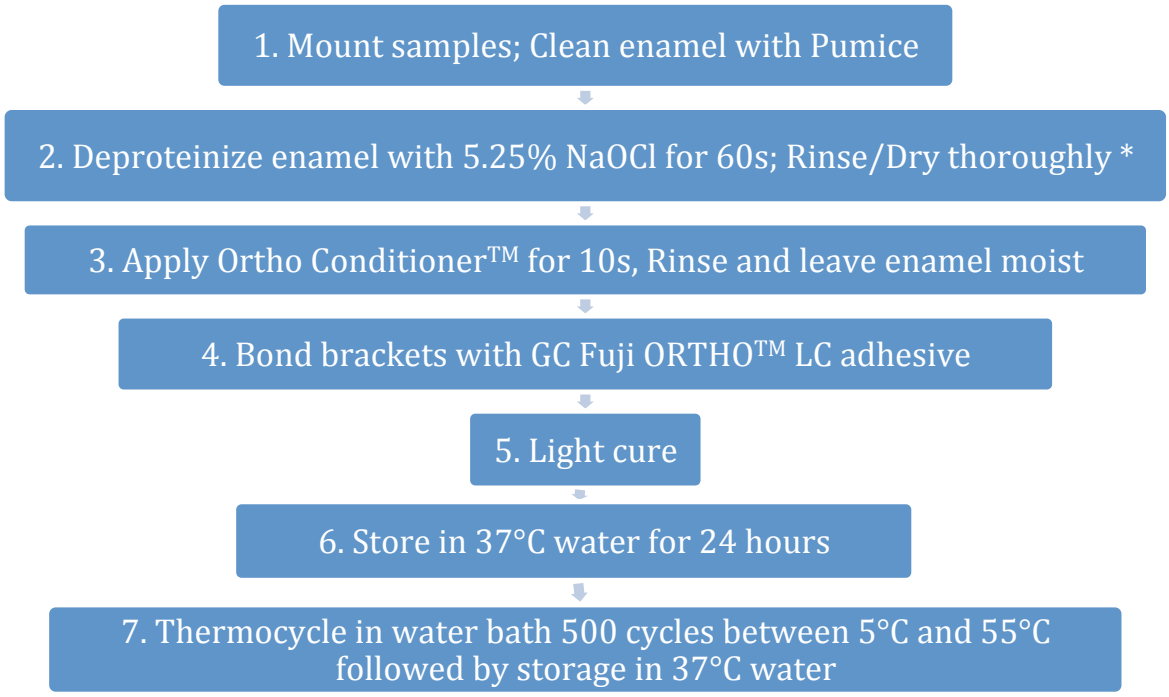

Figure 9. Flow chart of sample preparation of group A-GI. (* in group A-GI-E only)

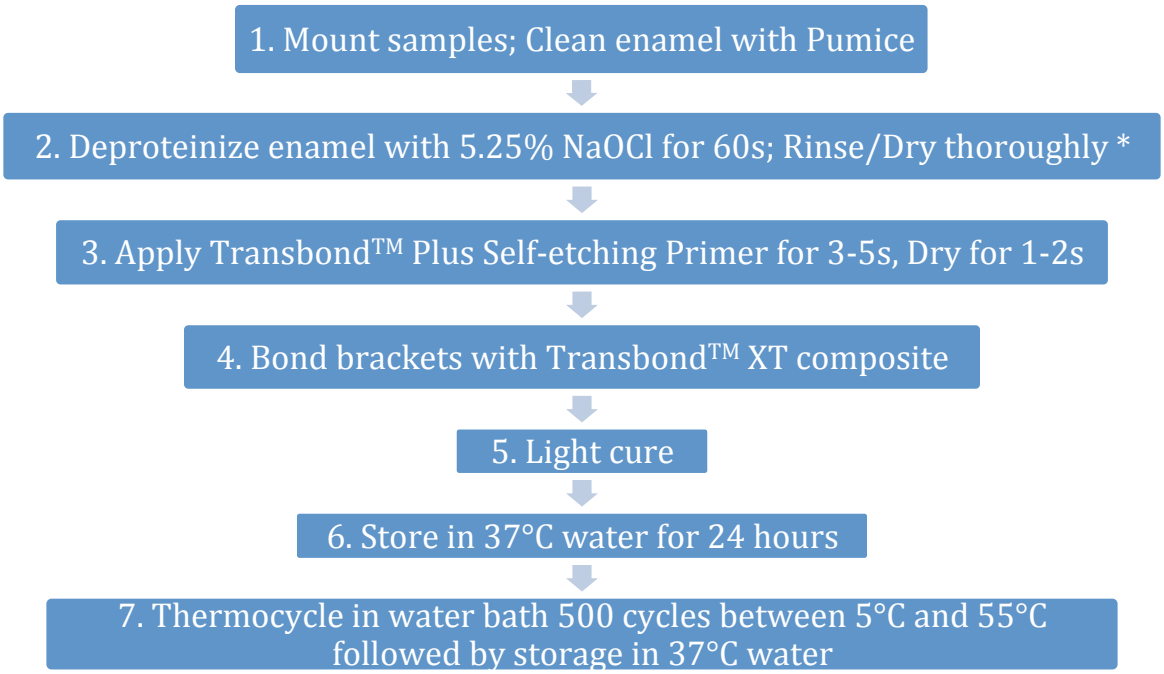

Figure 10. Flow chart of sample preparation of group A-CR. (* in group A-CR-E only)

2.3.2. Bonding and Storage of Samples in Group B

The samples in the four subgroups of Group B were prepared, bonded, thermocycled and stored using the same procedures in group A.

2.3.3. Orthodontic brackets

The orthodontic brackets used in this study were 0.022" slot size central incisor metal brackets (Victory Series, 3M Unitek, Monrovia, CA) with the average surface area of the bracket base determined to be 11.7 mm^2. The dimensions of the bracket base were obtained using an electric digital caliper (Ortho-Pli, Philadelphia, PA).

2.4. Experiment

2.4.1. Group A (Shear Bond Strength Measurement)

After removal from the incubated water storage, the bonded and mounted teeth in each of the four subgroups of Group A were tested for shear strength in the Universal Testing Machine (Instron Corp, Canton, Mass), set at a cross-head speed of 5.0 mm/min.[54] Before debonding, the samples were oriented so the debonding arm was parallel to the long axis of the tooth crown and bracket base, allowing the arm to produce a shear force at the bracket-tooth interface (Fig. 11).

Figure 11. Debonding in the Universal Testing Machine

During debonding, the shear force required to debond each bracket was recorded in Newtons (N) by a computer that was electronically connected to the Universal Testing Machine. After debonding, all samples were observed by the principal investigator under 10x magnification light microscopy and were visually graded, according to the amount of composite remaining on the enamel surface using the Adhesive Remnant Index (ARI). The ARI, developed by Årtun and Bergland in 1984, was used to determine the site of bond failure, in regards to the enamel, adhesive and bracket base.[55] It consists of a 4-point scale of 0 to 3: a score of 0 was used to describe an enamel surface having no composite remaining on the tooth; a score of 1 represented less than 50% of the composite remaining; a score of 2 represented more than 50% of the composite remaining; and a score of 3 represented all of the composite remaining with a distinct impression of the bracket mesh (Fig.12). The principal investigator scored all teeth again, one week later, in order to confirm consistency of grading.

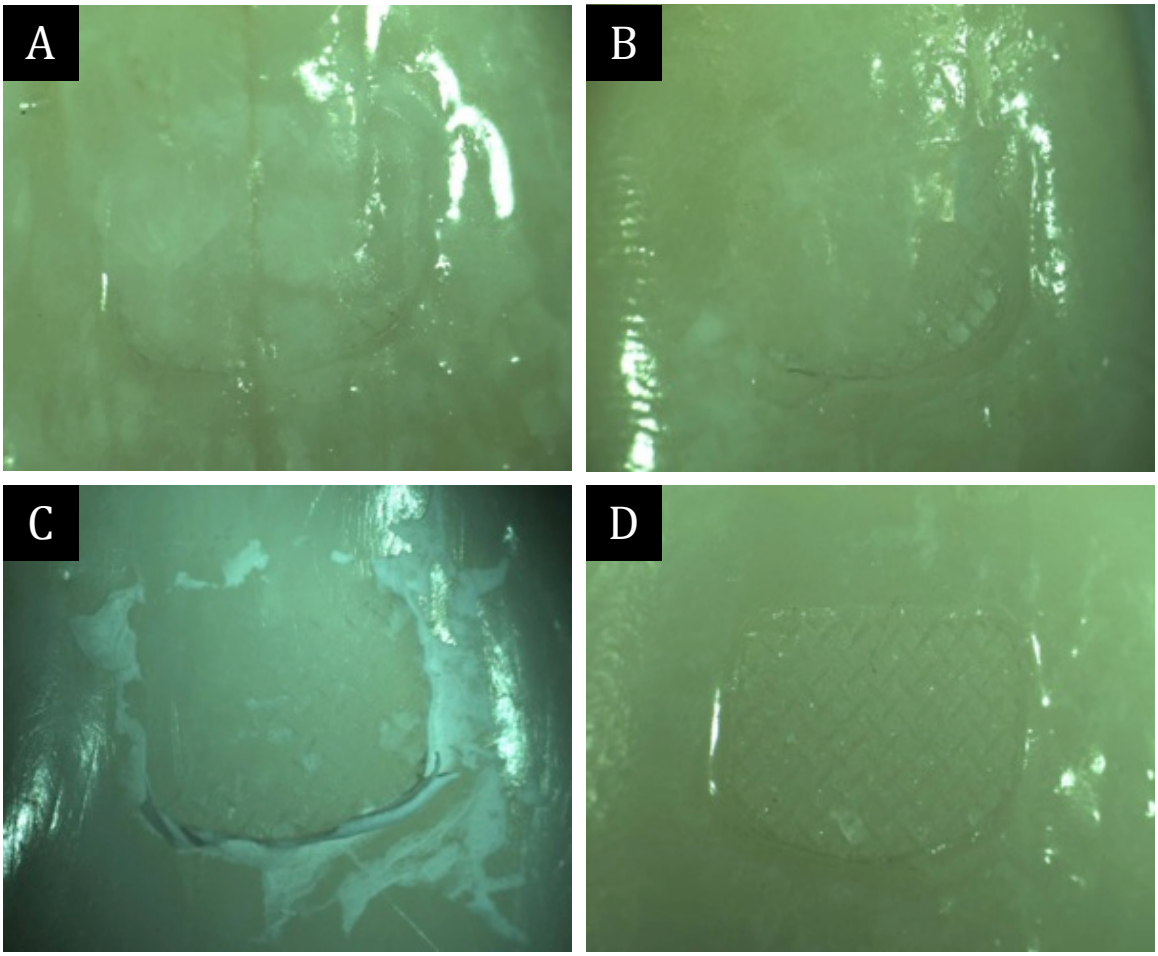

Figure 12. Samples viewed under 10x light microscopy
A. ARI Score 0 **B.** ARI score 1 **C.** ARI score 2 **D.** ARI score 3

2.4.2. Group B (White Spot Lesion Measurement)

After removal from the incubated water storage, the bonded teeth in each of the four subgroups of Group B were painted with acid resistant nail varnish (Revlon, New York, NT), leaving a 1.0mm window of exposed enamel around all four sides of the orthodontic bracket. 1.0mm of enamel was left exposed around the bracket, as the effective zone of the fluoride in RMGI has been estimated to be about 1.0mm.[41] After the varnish was applied, the orthodontic brackets were carefully removed from each sample by hand with a debonding plier (Ortho-Pli,

Philadelphia, PA). In each sample, all of the composite, with a direct impression of the bracket mesh, remained on the enamel surface (Fig. 13).

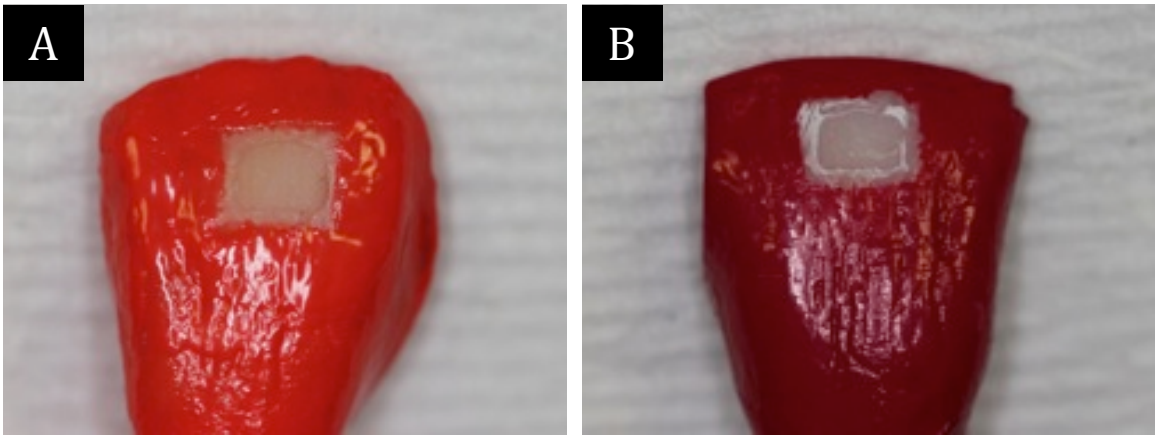

Figure 13. Samples after manual removal of orthodontic brackets
A. Group B-CR **B.** Group B-GI

After manual removal of the brackets, the samples were then soaked in demineralization solution for 96 hours, representing 3 months of real time, while being stored in a 37°C incubator to simulate oral conditions.[56] The demineralization solution, as described by Kumar, consisted of 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4 and 0.05M acetic acid, with the pH adjusted to 4.4 with 1 M KOH.[56] In order to create a subsurface white spot lesion, with the surface layer remaining intact, 0.5ppm fluoride as NaF was added to the demineralization solution, as suggested by Mukai *et al*.[57]

Following removal of the samples from the demineralization solution, the samples were subsequently stored in distilled water, incubated in 37°C, until they were individually sectioned with the hard tissue microtome (Series 1000 Deluxe Silverstone-Taylor, Scientific Fabrications, Littleton, CO, USA) (Fig. 14). The microtome bucco-lingually sectioned the exposed enamel of each tooth into 100-140μm wide sections (Fig. 15). Three random sections from each tooth were

selected for viewing under polarized light microscopy. Those sections with visual white spots were preferentially selected for microscopic evaluation.

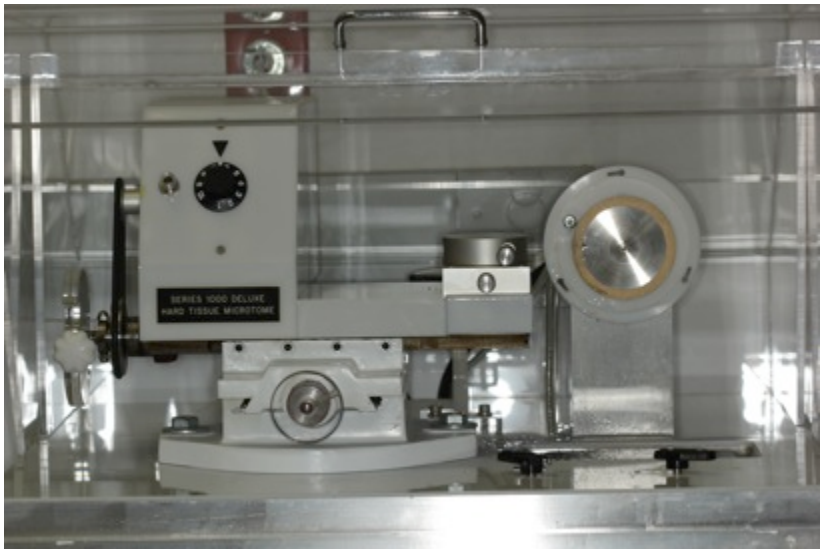

Figure 14. Hard Tissue Microtome

Figure 15. Bucco-lingual sections created by hard tissue microtome

Each of the three sections were placed on a microscope slide, imbibed in distilled water, covered with a slide cover and viewed under polarized light microscopy at maximum illumination and 10x magnification (Olympus CX41, Melville, NY, USA). During evaluation of the slices, no lesion, one lesion or two lesions were visible on each slice (Fig. 16). When lesions were visualized on the slices, they were divided into three equal sections if two lesions were present on the slice or four equal sections, if only one lesion was present on the slice, in

order to provide more measurements for those slices.[21] The sections were subsequently measured, from the enamel surface to the bottom of the lesion, using analySIS imaging software (Olympus Soft Imaging Solutions, Munster, Germany). [27] The average lesion depth for the tooth was calculated from the measurements obtained from the three slices. If no lesion was present, the depth was recorded at 0 μm.

Images were captured with Olympus MicroSuite™ Basic imaging software (Olympus, Melville, NY, USA and Soft Imaging System Corp., Lakewood, CO, USA). In order to confirm consistency of measurements, the principal investigator remeasured lesion depths from three teeth in each of the four groups one week later.

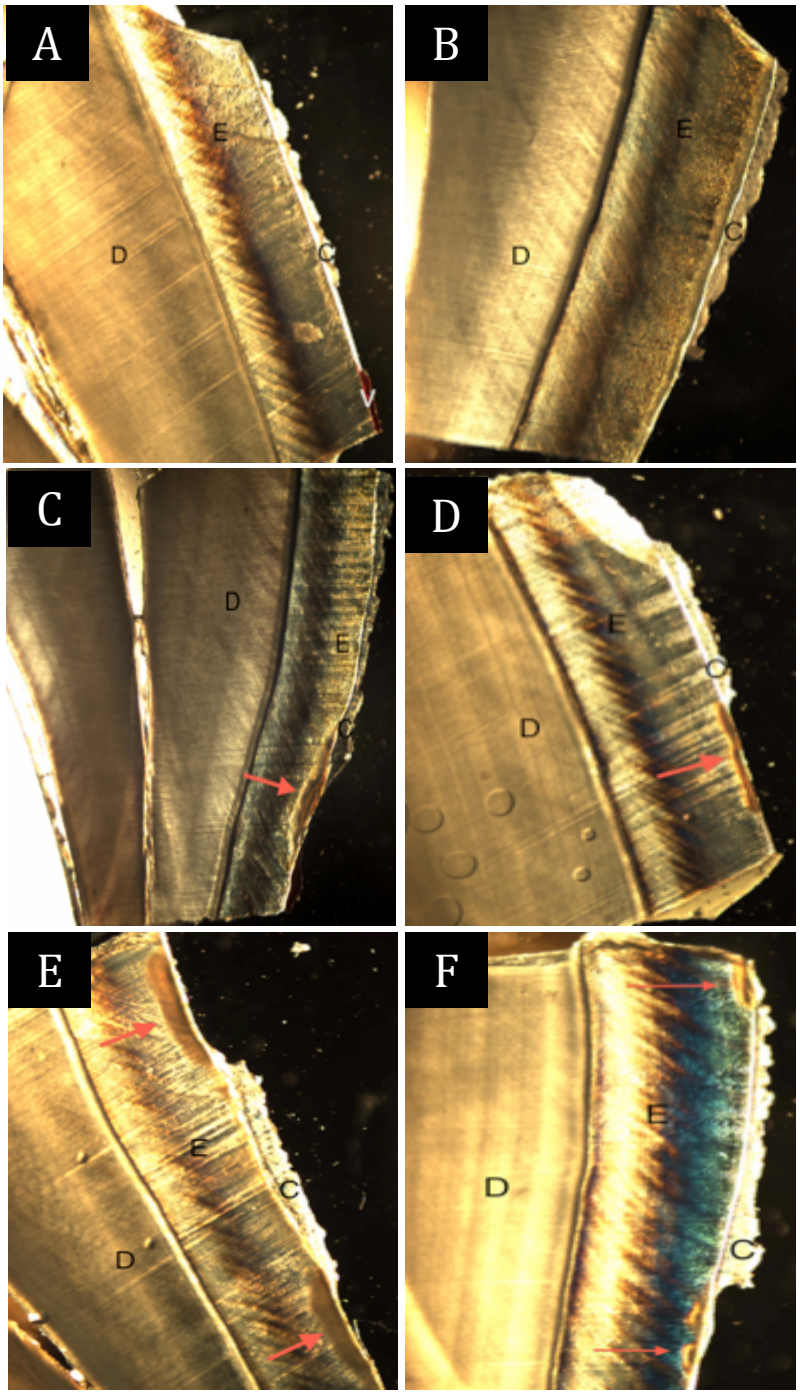

Figure 16. Tooth sections at 10x magnification under polarized light microscopy **A-B.** No white spots visible **C-D.** One white spot visible **E-F.** Two white spots visible *D*, Dentin; *E*, Enamel; *C*, Composite; *V*, Varnish; Arrows point to lesions

2.5. Data Storage

The data was entered and stored on excel spreadsheets on a password protected computer.

2.6. Statistical Analysis

Descriptive statistics, including means, standard deviations, minimums and maximums were used to describe the shear bond strength in each of the four subgroups in Group A. Descriptive statistics were also used to describe the frequency and depth of white spot lesions in each of the four subgroups in Group B.

In Group A, an analysis of variance was used to determine if there were statistically significant differences in the shear bond strength of the orthodontic brackets in the four subgroups. In order to determine between which groups the statistically significant differences existed, a Post-Hoc Tukey test was performed. Statistical significance was predetermined at $p \leq 0.05$. In order to determine if there were statistically significant differences between groups in regards to ARI scores, a Fisher's Exact test was performed.

In Group B, a random-effects general linear model ANOVA was used to determine if there were statistically significant differences in the depth of demineralization white spot lesions in the four subgroups. In order to determine between which groups the statistically significant differences existed, a Post-Hoc Tukey test was performed. Statistical significance was predetermined at $p \leq 0.05$.

Chapter 3: Results

3.1 Assessment of Shear Bond Strength in Group A

The shear force recorded on the Universal Testing Machine for each sample in Group A was subsequently converted into megapascals (MPa) by the principal investigator, which is a ratio of Newtons to surface area of the bracket base ($N/11.7mm^2$). The descriptive statistics of these force levels are reported in Table 1.

The composite resin control group, A-CR-C, was found to have the highest mean shear bond strength (12.48 ± 6.23 MPa), followed by the composite resin experimental group, A-CR-E (8.96 ± 6.57 MPa). The glass ionomer control group, A-GI-C, had a mean shear bond strength of 6.13 ± 2.97 MPa and the glass ionomer experimental group, A-GI-E had the lowest mean shear bond strength (5.49 ± 2.97 MPa). The statistical analysis, ANOVA, revealed statistically significant differences between groups in regards to shear bond strength at the $p<0.05$ level [$F(3,44 = 4.90$].

A Post-Hoc Tukey test (Table 2 & Fig. 17) showed significant differences ($p<0.05$) in the shear bond strength between the glass ionomer control group, A-GI-C, and the composite resin control group, A-CR-C. Statistically significant differences were also found between the glass ionomer experimental group, A-GI-E, and the composite resin control group, A-CR-C. No statistically significant differences in shear bond strength were observed when comparing all other groups.

Table 1. Descriptive Statistics Continuous Measures – MPa

Statistic	N	Mean	St. Dev.	Min	Max
Group = A-CR-C					
mPA	12	12.48	6.23	4.70	21.97
Group = A-CR-E					
mPA	12	8.96	6.57	2.55	19.83
Group = A-GI-C					
mPA	12	6.13	2.97	1.27	9.27
Group = A-GI-E					
mPA	12	5.49	2.97	2.70	10.68

Table 2. Tukey Post-Hoc Comparisons – MPa

mPA	Difference	Lower 95% CI	Upper 95% CI	P-Value
A-CR-E vs. A-CR-C	-3.52	-8.96	1.92	0.32
A-GI-C vs. A-CR-C	-6.36	-11.79	-0.92	0.02*
A-GI-E vs. A-CR-C	-6.99	-12.43	-1.55	0.01*
A-GI-C vs. A-CR-E	-2.83	-8.27	2.61	0.51
A-GI-E vs. A-CR-E	-3.47	-8.91	1.97	0.33
A-GI-E vs. A-GI-C	-0.64	-6.07	4.80	0.99

Figure 17. Tukey HSD Plot; Groups not connected by the same letter are significantly different ($p < 0.05$).

3.2 Assessment of Adhesive Remnant Index in Group A

The descriptive statistics of ARI scores in Group A are reported in Table 3. The percentages of ARI scores for each subgroup in Group A are shown in Table 4. A Fisher's Exact test revealed statistically significant differences between the groups in regards to ARI scores at the $p<0.05$ level ($c^2(9, N = 48) = 27.03$) (Fig. 18). It revealed that a greater percentage of teeth in the A-CR-C group possessed an ARI score of 0, which was statistically significant ($p<0.05$). It was also found that a greater percentage of teeth in the A-GI-E group possessed an ARI score of 3, which was statistically significant ($p<0.05$). This indicates that when enamel deproteinization was performed prior to bonding orthodontic brackets with a RMGI adhesive, more adhesive remained on the tooth. All other

groups possessed similar responses for ARI scores, with no statistically significant differences between groups.

Table 3. Descriptive Statistics Continuous Measures – ARI Score

Statistic	N	Mean	St. Dev.	Min	Max
Group = A-CR-E					
ARI	12	1.67	0.89	1	3
Group = A-CR-C					
ARI	12	1.00	0.74	0	3
Group = A-GI-C					
ARI	12	1.08	0.29	1	3
Group = A-GI-E					
ARI	12	2.50	0.80	1	3

Table 4. Descriptive Statistics Categorical Measures – ARI Score

		ARI Score				
		0	1	2	3	Total
A-CR-E	Count	0	7	2	3	12
	Percent	0.0%	58.3%	16.7%	25.0%	25.0%
A-CR-C	Count	**2**	9	0	1	12
	Percent	**16.7%**	75.0%	0.0%	8.3%	25.0%
A-GI-C	Count	0	11	1	0	12
	Percent	0.0%	91.7%	8.3%	0.0%	25.0%
A-GI-E	Count	0	2	2	**8**	12
	Percent	0.0%	16.7%	16.7%	**66.7%**	25.0%
Total		2	29	5	12	48

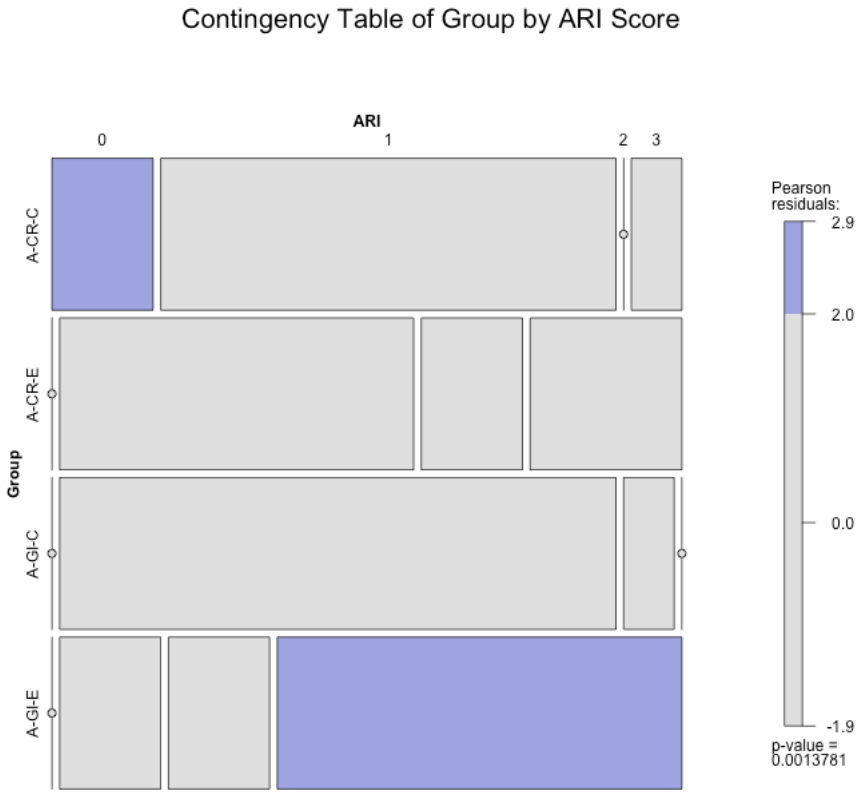

Figure 18. Mosiac Plot of Fisher's Exact Test; Colored regions (blue) are statistically significant ($p < 0.05$).

3.3 Measurements of Demineralization in Group B

The mean depth, standard deviation, minimum and maximum measurements of demineralization lesions for Group B are presented in Table 5. A random-effects general linear model ANOVA was conducted, which takes into account variability between teeth, since there were a different number of measurements nested within a tooth. The fixed effect was group and the random effect was tooth. The ANOVA revealed statistically significant differences between the groups in regards to depth of demineralization lesions at the $p<0.01$ level [$F(3,24 = 900.80$].

The largest (62.97 ± 10.95 μm) average demineralization lesions were found in group B-CR-E, composite resin with enamel deproteinization. The second largest (48.20 ± 11.35 μm) average lesions were found in group B-CR-C, composite resin without enamel deproteinization. The smallest lesion depths were found in groups B-GI-E (7.74 ± 8.73 μm) and B-GI-C (6.57 ± 10.83 μm), glass ionomer with and without enamel deproteinization, respectively.

A Post-Hoc Tukey test (Table 6 & Fig. 19) showed significant differences ($p<0.001$) in the depth of white spot lesions when comparing both composite resin groups to each other, and when each glass ionomer group was compared to each composite resin group. No statistically significant differences in white spot lesion depth were observed when comparing the two glass ionomer groups, B-GI-E and B-GI-C, to each other.

Table 5. Descriptive Statistics Continuous Measures - White Spot Lesion Depth (μm)

Statistic	N	Mean	St. Dev.	Min	Max
B-GI-E	120	7.74	8.73	0.00	26.63
B-GI-C	120	6.57	10.83	0.00	33.80
B-CR-C	134	48.30	11.35	25.50	75.49
B-CR-E	146	62.93	10.95	40.56	94.00

Table 6. Tukey Pair-Wise Comparisons – White Spot Lesion Depth (μm)

Group		Group	Difference	Lower 95% CI	Upper 95% CI	P-Value
B-CR-E	-	B-CR-C	15.42	12.03	18.81	<0.001*
B-GI-C	-	B-CR-C	-41.57	-44.96	-38.18	<0.001*
B-GI-E	-	B-CR-C	-40.40	-43.79	-37.01	<0.001*
B-GI-C	-	B-CR-E	-56.99	-60.38	-53.60	<0.001*
B-GI-E	-	B-CR-E	-55.82	-59.21	-52.43	<0.001*
B-GI-E	-	B-GI-C	1.17	-2.22	4.56	0.811

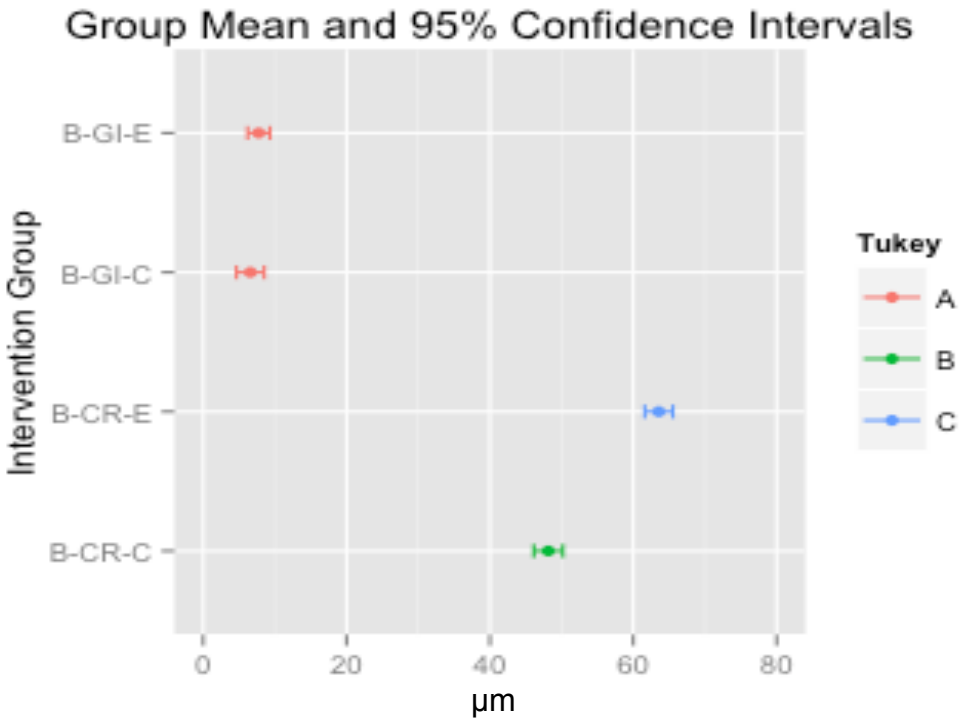

Figure 19. Tukey Plot; Groups not connected by the same letter are significantly different ($p < 0.01$).

Chapter 4: Discussion

The purpose of this *in vitro* study was to evaluate the effects that enamel deproteinization, with sodium hypochlorite, had on the shear bond strength of orthodontic brackets bonded with a glass ionomer adhesive and a composite resin adhesive. Both adhesives were tested for shear bond strength against control groups, which did not receive the enamel deproteinization intervention. The effectiveness of these adhesives against enamel demineralization and white spot lesion formation was also assessed through exposure of the samples to simulated intraoral conditions, including an acidic challenge. This research was conducted with its focus on the search for a suitable orthodontic bonding protocol for a resin-modified glass ionomer adhesive, GC Fuji ORTHO™ LC Automix adhesive (GC America, Alsip, IL), which claims to provide preventative care to the enamel against formation of white spot lesions, however it is not frequently used by clinicians due to its observed lowered clinical shear bond strength.[12, 20, 42]

Two previous studies have been conducted to test the effectiveness of enamel deproteinization on the shear bond strength of brackets bonded with orthodontic adhesives. In 2010, Justus *et al*.[12] compared the bond strength of orthodontic brackets bonded to human premolars in four groups. Group 1 had the enamel prepared using sodium hypochlorite, acid etching with 37% phosphoric acid and application of primer, followed by brackets bonded with Transbond™ XT adhesive. Group 3 had the enamel prepared using sodium hypochlorite, acid

etching with 37% phosphoric acid and remoistening of enamel, followed by brackets bonded with Fuji ORTHO^{TM} LC adhesive. Groups 2 and 4 were similar to groups 1 and 3, respectively; except sodium hypochlorite deproteinization was not performed prior to etching. In 2013, Pereira *et al*.[58] compared the bond strength of orthodontic brackets bonded to human premolars in five groups. Group 1 had the enamel prepared using acid etching with 37% phosphoric acid and application of primer, followed by brackets bonded with Transbond^{TM} XT adhesive. Group 2 had the enamel prepared using 10% polyacrylic acid conditioning and remoistening of enamel, followed by brackets bonded with conventional glass ionomer cement. Group 4 had the enamel prepared using 10% polyacrylic acid conditioner and remoistening of enamel, followed by brackets bonded with Fuji ORTHO^{TM} LC adhesive. Groups 3 and 5 were similar to groups 2 and 4, respectively; except sodium hypochlorite deproteinization was performed prior to conditioning.

Contrary to the results of the study by Justus *et al.,*[12] which found that enamel deproteinization prior to bonding significantly increased the shear bond strength of orthodontic brackets bonded with Fuji ORTHO^{TM} LC adhesive, this study did not find statistically significant differences between the two Fuji ORTHO^{TM} LC adhesive groups. Adding the additional step of enamel deproteinization prior to bonding did not increase the average shear bond strength of the brackets bonded with RMGI adhesive. The results of this study were more consistent with those found by Pereira *et al.*[58] This finding is likely due to the fact that a weaker acid etchant/conditioner, 10% polyacrylic acid, was used

prior to bonding with the RMGI adhesive in these two studies (as recommended by the manufacturer's instructions), whereas 37% phosphoric acid was used in the study by Justus *et al*.[12]

The stronger acid etchant used by Justus, in combination with enamel deproteinization to rid the surface of organic components, may have contributed to the observed greater shear bond strength of the RMGI adhesive in that study. During his study, he also evaluated, under Scanning Electron Microscopy (SEM), five samples that received enamel deproteinization with sodium hypochlorite, followed by acid etching with 37% phosphoric acid, and five samples that only received acid etching. The results of his study, along with previous studies by Espinosa *et al*.,[10, 11] found that a deeper etch and a rougher enamel surface were produced when sodium hypochlorite deproteinization was combined with acid etching, and these surfaces were more consistent with superior quality etching patterns types 1 and 2.[12] Silverstone has previously shown that types 1 and 2 etching patterns are the most retentive to bonding.[9] Although the samples in the current study were not evaluated under SEM after deproteinization and conditioning with 10% polyacrylic acid, it has been shown that conditioning the tooth surface with polyacrylic acid does not cause as much damage to the enamel as etching with 37% phosphoric acid, meaning that type 1 and 2 etching patterns were most likely not produced.[59] This most likely contributed to the lower bond strengths of the RMGI groups in the current study. It is imperative, however, for the clinician to follow the recommended manufacturer's instructions, in order to obtain appropriate clinical results.

The observed differences between the studies may also relate to possible adhesive variability, as the Fuji Ortho LC adhesive that used in the study by Justus *et al*.[12] was hand-mixed, rather than being dispensed from Automix Paste Pak, as in the current study. There may be a slightly increased margin of error in adhesive quality and uniformity when numerous batches are mixed by hand. The mixing of the RMGI adhesive was not specified in the study by Pereira *et al*.[58]

An interesting fact to note was that although there were no statistically significant differences between the Fuji Ortho LC adhesive groups, the minimum shear bond strength observed in the control group, without enamel deproteinization (A-GI-C), was actually 1.43 MPa lower than the minimum strength in the experimental group, with enamel deproteinization (A-GI-E). Also, the maximum shear bond strength observed in the experimental group was 1.41 MPa higher than the maximum strength in the control group, implying that enamel deproteinization may have some potential to increase the bond strength of the GC Fuji ORTHO^{TM} LC adhesive.

In accordance with the study by Justus *et al.,*[12] there were no statistically significant differences in the shear bond strengths of the two Transbond^{TM} XT adhesive groups in the current study. Adding the additional step of enamel deproteinization prior to bonding did not increase the shear bond strength of the composite resin adhesive. One important difference between this study and the study by Justus *et al*., [12] in regards to the composite resin groups, was that a self-etching primer was used in this study prior to bonding with the Transbond^{TM} XT adhesive, rather than separate 37% phosphoric acid etching and priming

steps. The current study revealed that clinically acceptable levels of bond strength, defined by Reynolds as ranging between 5.9-7.8 MPa, can be achieved when orthodontic brackets are bonded with Transbond™ XT adhesive and a self etching primer.[13] Supplementary steps, including enamel deproteinization, are therefore not required in order to obtain satisfactory bond strengths with this adhesive and bonding protocol.

Although the average shear bond strengths of the samples in both adhesive groups that received enamel deproteinization (A-GI-E and A-CR-E) were actually lower than the samples which did not receive enamel deproteinization (A-GI-C and A-CR-C, respectively), they were not different enough to be statistically significant. These observed differences were not consistent with previous studies and may be due to the smaller sample sizes that were used in this study.[12, 58]

Justus *el al.*[12] also found that when the RMGI groups were compared with the composite resin groups, those samples that had the enamel deproteinized prior to bonding with the RMGI adhesive, achieved shear bond strength comparable to the composite resin groups. This was not what was found in the current study, as the shear bond strength of both GC Fuji ORTHO™ LC adhesive groups showed a statistically significant difference from the values found in the Transbond™ XT adhesive control group (A-CR-C). The bond strength was lower in both GC Fuji ORTHO™ LC adhesive groups and again, is most likely due to the fact that a weaker enamel conditioner, 10% polyacrylic acid, was used in the

current study, which may not be capable of achieving superior quality etching patterns.

In the current study, statistically significant differences were observed with regard to ARI scores and treatment groups, meaning the different bonding techniques demonstrated different modes of bond failure. When the enamel was deproteinized prior to bonding orthodontic attachments with GC Fuji ORTHO^{TM} LC adhesive (A-GI-E), most of the cement remained on the enamel surface after debonding (ARI score of 3) and bond failure occurred at the bracket-adhesive interface. This differed significantly from other groups and these results were consistent with those found by Justus et al.[12] and Pereira *et al.* [58] When the enamel was not deproteinized prior to bonding orthodontic attachments with the Transbond^{TM} XT adhesive (A-CR-C), significantly more bond failures occurred at the enamel-adhesive interface (ARI score of 0). These results demonstrate that with the addition enamel deproteinization prior to bonding, the bond failure was more likely to occur at bracket-adhesive interface. According to Bishara *et al*.[52] this is an advantageous mode of failure because it leaves the enamel surface intact, but conversely, it requires more chair time to remove residual adhesive during debonding.

Furthermore, numerous studies have been conducted to evaluate the effectiveness of fluoride-containing RMGI adhesives on preventing or diminishing the depth of white spot lesions around orthodontic attachments. Schmit *et al*.[27] found that when Fuji ORTHO^{TM} LC adhesive was used as a bonding agent, there was a greater than 50% reduction in lesion depth compared to when a non-

fluoride releasing composite resin adhesive was used. In a 30-day *in vitro* study performed by Vorhies *et al.,*[60] it was also found that teeth bonded with Fuji ORTHO™ LC adhesive had significantly smaller demineralization lesions around the bracket base, compared to those that were bonded with a composite resin adhesive. Similarly, a 30-day *in vivo* study performed by Pascotto *et al*.[19] found that Fuji ORTHO™ LC was statistically more efficient than composite resin in reducing enamel demineralization around orthodontic brackets.

The current study specifically evaluated the effectiveness of four different bonding techniques against white spot lesion formation, after exposure of the samples to an acidic challenge. The results found that the samples in both Fuji ORTHO™ LC adhesive groups had a statistically significant decrease in the depth of white spot lesions, compared to the samples in both Transbond™ XT groups. In fact, when the average lesion depth from both Fuji ORTHO™ LC adhesive groups was compared to the average lesion depth from both Transbond™ XT adhesive groups, an 87% reduction in lesion depth was observed. These results were in agreement with the previous studies mentioned earlier and prove the effectiveness of the fluoride released from RMGI orthodontic adhesives in diminishing or preventing enamel demineralization around orthodontic brackets.

Enamel deproteinization, with the use of RMGI adhesive, did not affect the depth of white spot lesions, as there were no statistically significant differences between GC Fuji ORTHO™ LC adhesive groups. However, when the enamel was deproteinized prior to bonding orthodontic attachments with self-etching

primer and Transbond™ XT, the largest demineralization lesions were found. This result is evidently understood, as the deproteinization of the enamel surface with sodium hypochlorite allowed the etchant to establish a deeper etching pattern, which subsequently resulted in more damage to the enamel. This left the bare enamel more prone to demineralization when it was exposed to the acidic challenge and consequently, larger lesions were formed in this group. The samples in this study had exposed enamel surfaces outside the area of the bracket base that had been etched, as additional steps to block out the enamel surface around the brackets was not performed. It was specifically chosen to eliminate this procedure in this study, as this would not be something that would be performed in a real clinical setting.

4.1. Limitations, Implications and Future Studies

The foremost limitation of this research study acknowledges the fact that it is an *in vitro* laboratory study, which may or may not directly represent an actual clinical setting. One must keep this in mind when interpreting the results in relation to clinical orthodontic patients.

Another significant limitation is appreciating the fact that bovine teeth samples, rather than human teeth samples, were used to conduct the current research study. Although bovine teeth have been shown in previous literature to be suitable substitutes for human teeth in the evaluation of adhesive bond strength and white spot lesions, slight differences in morphology, compared to human enamel, were observed when the samples in this study were examined with the untrained eye and light microscopy.[45-48]

The bovine enamel topography appeared variable, with some enamel surfaces being roughened with ridges more markedly than others. This observed rougher bovine enamel surface was consistent with what was observed under Scanning Electron Microscopy (SEM) by Nakamichi *et al.*[46] and may be associated with the differing results obtained in this study, compared to those studies that used human teeth samples.[12, 58] A previous study by Oesterle *et al.,*[45] found that when orthodontic adhesives were used, the bond strength to bovine enamel was significantly lower compared to that of human enamel, with the bovine teeth showing strengths 35% below that of human enamel. Therefore, one must be guarded in generalizing these results to clinical orthodontic situations.

Due to the conservative trend of dentistry and the increasing limitation of human teeth samples, a future study could use the bonding protocols described in this study to compare the bond strengths and lesion depths of bovine teeth versus human teeth. The results could confirm or disprove the reliability of using bovine teeth as substitutes for human teeth in these types of *in vitro* studies, in order to determine if they can be associated with clinical orthodontic situations.

Chapter 5: Conclusions

Enamel deproteinization with sodium hypochlorite did not increase the shear bond strength of orthodontic brackets bonded with Fuji ORTHO™ LC adhesive or self-etching primer and Transbond™ XT adhesive. Our results revealed statistically significant differences between both Fuji ORTHO™ LC adhesive groups and the Transbond™ XT control group, with the bond strengths of the Fuji groups being lower. When the enamel was deproteinized prior to bonding orthodontic attachments with Fuji ORTHO™ LC adhesive, significantly more adhesive remained on the enamel surface after debonding, with most bond failures occurring at the bracket-adhesive interface. Both Fuji ORTHO™ LC adhesive groups provided significant reductions in enamel demineralization compared to both Transbond™ XT groups. When searching for the paramount bonding protocol for bonding orthodontic brackets, adding the additional step of enamel deproteinization prior to bonding is not necessary, as clinically acceptable levels of bond strength could be achieved in both adhesive control groups. However, in order to provide compliance free protection against demineralization to the orthodontic patient, a RMGI adhesive is the preferred bonding adhesive.

Appendix A: Raw Data - Group A

Group A-CR-C	Bond Strength - Newtons	Bond Strength - mPA	Adhesive Remnant Index
Tooth 1	90.14	7.7	1
Tooth 2	207.21	17.71	1
Tooth 3	92.95	7.94	1
Tooth 4	257	21.97	0
Tooth 5	105.6	9.03	1
Tooth 6	253.24	21.64	1
Tooth 7	133.26	11.39	1
Tooth 8	69.88	5.97	3
Tooth 9	54.99	4.7	1
Tooth 10	140.17	11.98	1
Tooth 11	235.07	20.09	0
Tooth 12	113.2	9.68	1
Sum	1752.71	149.8	12
Average	146.059167	12.4833333	1
Minimum	54.99	4.7	0
Maximum	257	21.97	3
ST DEV	72.874436	6.22881622	0.73854895

Group A-CR-E	Bond Strength - Newtons	Bond Strength - mPA	Adhesive Remnant Index
Tooth 1	54.05	4.62	1
Tooth 2	53.08	4.54	1
Tooth 3	71.87	6.14	2
Tooth 4	231.91	19.83	1
Tooth 5	53.39	4.56	2
Tooth 6	131.34	11.23	3
Tooth 7	29.86	2.55	1
Tooth 8	228.28	19.51	1
Tooth 9	73.6	6.29	3
Tooth 10	217.07	18.55	1
Tooth 11	53.56	4.58	1
Tooth 12	59.92	5.12	3
Sum	1257.93	107.52	20
Average	104.8275	8.96	1.66666667
Minimum	29.86	2.55	1
Maximum	231.91	19.83	3
ST DEV	76.8234502	6.56721748	0.88762536

Group A-GI-C	Bond Strength - Newtons	Bond Strength - mPA	Adhesive Remnant Index
Tooth 1	14.88	1.27	1
Tooth 2	85.88	7.34	1
Tooth 3	49.05	4.19	1
Tooth 4	105.19	8.99	1
Tooth 5	105.41	9.01	1
Tooth 6	80.79	6.91	1
Tooth 7	23.38	2	1
Tooth 8	100.59	8.6	1
Tooth 9	55.58	4.75	1
Tooth 10	98.19	8.39	2
Tooth 11	33	2.82	1
Tooth 12	108.44	9.27	1
Sum	860.38	73.54	13
Average	71.6983333	6.12833333	1.08333333
Minimum	14.88	1.27	1
Maximum	108.44	9.27	2
ST DEV	34.7219163	2.96824354	0.28867513

Group A-GI-E	Bond Strength - Newtons	Bond Strength - mPA	Adhesive Remnant Index
Tooth 1	62.94	5.38	3
Tooth 2	92.55	7.91	3
Tooth 3	125.01	10.68	2
Tooth 4	32.48	2.78	3
Tooth 5	31.63	2.7	3
Tooth 6	32.36	2.77	3
Tooth 7	58.79	5.02	1
Tooth 8	39.26	3.36	3
Tooth 9	35.65	3.05	3
Tooth 10	50.87	4.35	3
Tooth 11	125	10.68	2
Tooth 12	84.63	7.23	1
Sum	771.17	65.91	30
Average	64.2641667	5.4925	2.5
Minimum	31.63	2.7	1
Maximum	125.01	10.68	3
ST DEV	34.826784	2.97421991	0.79772404

Appendix B: Raw Data - Group B

Group B-CR-C

Slice 1

Tooth 1	45.89	41.36	39.91	32.53	36.93	36.12
Tooth 2	53.18	47.27	41.39	39.91		
Tooth 3	30.74	34.86	40.56	35.76	42.61	40.78
Tooth 4	57.02	63.1	66.65	59.47		
Tooth 5	37.98	41.7	39.77	42.53		
Tooth 6	48.39	46.71	37.98	37.66		
Tooth 7	38.66	53.26	54.97	53.26		
Tooth 8	41.15	47.2	57.47	50.24		
Tooth 9	59.54	53.77	51.38	51.87		
Tooth 10	60.56	51.95	58.81	62.7		

Slice 2

Tooth 1	32.53	32.4	33.49	33.42		
Tooth 2	49.1	48.29	40.64	41.62	41.7	34.48
Tooth 3	53.77	41.7	49.98	49.52	46.36	49.12
Tooth 4	49.52	55.6	59.82	58.86		
Tooth 5	40.34	38.32	31.82	29.43		
Tooth 6	54.81	62	59.12	64.94		
Tooth 7	66.4	56.54	61.44	52.45		
Tooth 8	68.71	66.93	71.59	62.11		
Tooth 9	56.67	59.07	49.98	52.94		
Tooth 10	56.72	59.2	51.94	55.27		

Slice 3

Tooth 1	43.06	40.18	75.49	38.52	73.35	55.74
Tooth 2	28.53	31.82	37.37	37.98		
Tooth 3	33.68	34.77	27.24	30.45	33.33	42.3
Tooth 4	38.86	35.48	34.1	39.99	41.78	36.7
Tooth 5	43.47	25.5	29.69	43.06		
Tooth 6	50.52	54.67	41.07	51.7		
Tooth 7	74.16	65.39	66.75	66.75		
Tooth 8	36.93	48.09	55.84	55.6		
Tooth 9	48.92	46.22	51.92	55.68		
Tooth 10	50.87	48.9	46.94	43.64		

	Sum	Average	Minimum	Max	STDEV
Tooth 1	690.92	43.1825	32.4	75.49	13.6262372
Tooth 2	615.58	41.0387	28.53	53.18	6.62792929
Tooth 3	717.53	39.8628	27.24	53.77	7.72692864
Tooth 4	696.95	49.7821	34.1	66.65	11.5227290
Tooth 5	443.61	36.9675	25.5	43.47	6.19822140
Tooth 6	609.57	50.7975	37.66	64.94	8.94817617
Tooth 7	710.03	59.1692	38.66	74.16	9.51534162
Tooth 8	661.86	55.155	36.93	71.59	10.9234547
Tooth 9	637.96	53.1633333	46.22	59.54	4.01527839
Tooth 10	647.5	53.9583333	43.64	62.7	5.87224880

Group B-CR-E

Slice 1

Tooth 1	78.51	72.62	62.06	58.08		
Tooth 2	67.64	59.82	61.47	44.66		
Tooth 3	91.2	72.44	63.2	85.58	66.93	68.86
Tooth 4	54.47	93.15	57.93	64.94	88.49	78.95
Tooth 5	57.64	54.83	61.01	52.22		
Tooth 6	56.4	53.28	64.89	58.64	51.27	48.23
Tooth 7	59.96	58.86	54.81	55.31		
Tooth 8	53.54	71.35	69.36	65.13		
Tooth 9	71.35	68.42	62.11	54.89		
Tooth 10	49.3	57.68	61.44	66.06		

Slice 2

Tooth 1	64.13	63.1	62.84	78.75	48.9	56.84
Tooth 2	63.43	50.22	60.29	70.79	64.74	65.43
Tooth 3	94	91	87.6	81.9		
Tooth 4	76.82	65.88	71.3	52.27	55.37	48.09
Tooth 5	63.37	65.39	55.42	56.72		
Tooth 6	66.8	73.34	60.72	60.72	60.9	55.5
Tooth 7	53.18	51.7	56.21	57.42		
Tooth 8	49.54	43.74	40.56	46.64		
Tooth 9	58.38	57.68	53.03	48.39		
Tooth 10	67.96	70.9	64.99	67.95		

Slice 3

Tooth 1	57.68	48.23	47.11	81.94	63.22	59.98
Tooth 2	72.98	73.86	65.88	84.66	62.7	68.28
Tooth 3	76.82	70.05	78.4	57.53	61.7	72.26
Tooth 4	77.54	73.46	56.25	86.06	81.47	75.69
Tooth 5	65.81	67.51	59.52	71.88	62.48	63.48
Tooth 6	68.09	64.13	64.94	55.68	52.2	55.31
Tooth 7	69.44	57.68	57.91	53.52		
Tooth 8	65.41	64.08	67.38	70.15		
Tooth 9	56.15	54.67	50.24	54.67		
Tooth 10	66.06	59.45	44.92	58.83		

	Sum	Average	Minimum	Max	STDEV
Tooth 1	1003.99	62.749375	47.11	81.94	10.6775774
Tooth 2	1036.85	64.803125	44.66	84.66	9.24391960
Tooth 3	1303.3	76.216906	57.53	94	11.4065638
Tooth 4	1258.13	69.89611	48.09	93.15	13.5549887
Tooth 5	980.18	61.23435	52.22	71.88	5.50834472
Tooth 6	1071.04	59.502222	48.23	73.34	6.63708525
Tooth 7	686	57.166667	51.7	69.44	4.58433565
Tooth 8	706.88	58.906667	40.56	71.35	11.2965798
Tooth 9	689.98	57.498333	48.39	71.35	6.83214838
Tooth 10	735.54	61.295	44.92	70.9	7.8312945

Group B-GI-C

Slice 1

Tooth 1	0	0	0	0
Tooth 2	26.75	23.68	30.73	24.68
Tooth 3	0	0	0	0
Tooth 4	0	0	0	0
Tooth 5	0	0	0	0
Tooth 6	0	0	0	0
Tooth 7	0	0	0	0
Tooth 8	0	0	0	0
Tooth 9	13.42	12.81	14.6	14.22
Tooth 10	0	0	0	0

Slice 2

Tooth 1	0	0	0	0
Tooth 2	33.75	31.3	33.82	32.26
Tooth 3	0	0	0	0
Tooth 4	0	0	0	0
Tooth 5	19.87	25.15	22.16	21.98
Tooth 6	0	0	0	0
Tooth 7	0	0	0	0
Tooth 8	0	0	0	0
Tooth 9	9.89	10.61	10.04	11.68
Tooth 10	0	0	0	0

Slice 3

Tooth 1	0	0	0	0
Tooth 2	30.84	31.58	29.87	29.02
Tooth 3	0	0	0	0
Tooth 4	0	0	0	0
Tooth 5	0	0	0	0
Tooth 6	0	0	0	0
Tooth 7	25.5	24.05	25.65	25.79
Tooth 8	17.6	19.2	16.45	16.98
Tooth 9	18.79	17.92	16.48	19.89
Tooth 10	0	0	0	0

	Sum	Average	Minimum	Max	STDEV
Tooth 1	0	0	0	0	0
Tooth 2	358.28	29.8566667	23.68	33.82	3.28530428
Tooth 3	0	0	0	0	0
Tooth 4	0	0	0	0	0
Tooth 5	89.16	7.43	0	25.15	11.0332827
Tooth 6	0	0	0	0	0
Tooth 7	100.99	8.41583333	0	25.79	12.4381527
Tooth 8	70.23	5.8525	0	19.2	8.66708415
Tooth 9	170.35	14.1958333	9.89	19.89	3.44174759
Tooth 10	0	0	0	0	0

Group B-GI-E

Slice 1

Tooth 1	0	0	0	0
Tooth 2	13.93	11.25	13.93	12.71
Tooth 3	0	0	0	0
Tooth 4	17.79	18.03	17.79	21.98
Tooth 5	13.38	11.91	15.06	15.06
Tooth 6	0	0	0	0
Tooth 7	0	0	0	0
Tooth 8	0	0	0	0
Tooth 9	16.48	18.49	17.01	14.98
Tooth 10	0	0	0	0

Slice 2

Tooth 1	14.55	15.21	17.85	21.15
Tooth 2	21.81	13.62	19.26	17.23
Tooth 3	17.85	16.84	18.68	16.51
Tooth 4	19.26	18.03	21.96	22.55
Tooth 5	9.34	9.34	8.98	9.36
Tooth 6	0	0	0	0
Tooth 7	0	0	0	0
Tooth 8	17.79	18.92	16.71	21.3
Tooth 9	0	0	0	0
Tooth 10	0	0	0	0

Slice 3

Tooth 1	16.25	14.77	17.73	10.34
Tooth 2	13.62	12.71	15.21	9.91
Tooth 3	0	0	0	0
Tooth 4	26.63	21.96	22.98	17.79
Tooth 5	18.27	17.85	19.2	20.57
Tooth 6	0	0	0	0
Tooth 7	0	0	0	0
Tooth 8	0	0	0	0
Tooth 9	0	0	0	0
Tooth 10	0	0	0	0

	Sum	Average	Minimum	Max	STDEV
Tooth 1	127.85	10.6541667	0	21.15	8.25910127
Tooth 2	175.19	14.5991667	9.91	21.81	3.35979019
Tooth 3	69.88	5.82333333	0	18.68	8.61708697
Tooth 4	246.75	20.5625	17.79	26.63	2.86210706
Tooth 5	168.32	14.0266667	8.98	20.57	4.26841110
Tooth 6	0	0	0	0	0
Tooth 7	0	0	0	0	0
Tooth 8	74.72	6.22666667	0	21.3	9.25452256
Tooth 9	66.96	5.58	0	18.49	8.27688018
Tooth 10	0	0	0	0	0

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