EFFECT OF HIGHLY FILLED LIGHT-CURED RESIN SEALANT ON BRACKET SHEAR BOND STRENGTH IN DEMINERALIZATION CONDITIONS

A THESIS IN
Oral and Craniofacial Sciences

Presented to the Faculty of the University
Of Missouri-Kansas City in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

by
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Kansas City, Missouri
2018

EFFECT OF HIGHLY FILLED LIGHT-CURED RESIN SEALANT ON BRACKET SHEAR BOND STRENGTH IN DEMINERALIZATION CONDITIONS

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University of Missouri-Kansas City, 2018

ABSTRACT

This study examined the effect of a highly filled light-cured sealant (HFLCS) on bracket shear bond strength and subsequent adhesive remnant index (ARI) following exposure to cariogenic challenge. Specimens were divided into two groups to receive conventional primer or HFLCS prior to orthodontic bracket bonding in a simulated oral environment. After curing bracket adhesive, specimens were separated into two storage solutions, either phosphate buffered saline or a demineralization solution (pH 4.1) for 96 hours. Brackets then underwent shear bond strength testing followed by ARI scoring. Enamel surfaces were visually examined for white spot lesion (WSL) severity using a WSL scoring index.

HFLCS and exposure to demineralization conditions were not significant factors in shear bond strength or subsequent adhesive fracture pattern (ANOVA, $p > .05$). The majority of brackets were given an ARI score of 1, meaning that greater than 50% of the resin adhesive remained on the bracket. A significant difference ($p < 0.05$) was detected in WSL scores between the HFLCS and conventional primer groups when exposed to demineralization conditions. With HFLCS, no specimen developed WSLs, while all specimens with conventional primer had either minor or severe WSL formation after cariogenic challenge.   This investigation suggests that HFLCS did not have a protective effect on bracket bond strength in demineralization conditions, but HFLCS was effective in preventing enamel demineralization without compromising shear bond strength.

APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Dentistry have examined a thesis titled "Effect of Highly Filled Light-Cured Resin Sealant on Bracket Shear Bond Strength in Demineralization Conditions," presented by Brad M. Chun, candidate for the Master of Science degree, and certify that in their opinion it is worthy of acceptance.

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ACKNOWLEDGMENTS

I would like to express my sincere appreciation and give thanks to:

Dr. Mary Walker, for her guidance, expertise, and support throughout my education.at the UMKC School of Dentistry.

Dr. Yong Wang, for his expertise, feedback, and time.

Dr. Mark Johnson, for his expertise, feedback, and time.

Jim Thomas, for his expertise and assistance with photography.

Marie Thompson, for her assistance with formatting.

Jessica Moniz, for her assistance in the lab and time.

Rachel Reed, for her assistance in the lab and time.

Erin Liljequist, for her support and administrative assistance.

Jania Billups, for her support and administrative assistance.

3M Unitek, for their generous donation of brackets and bonding materials.

My family, for their love, support, and encouragement throughout my dental education.

CHAPTER 1

INTRODUCTION

Orthodontic therapy can improve esthetics, oral function and patient self-esteem. However, a common detrimental side effect of orthodontic fixed appliance therapy is increased incidence of enamel demineralization. Enamel demineralization results in an unaesthetic treatment outcome and an increased risk of cavitated lesions. Orthodontists have employed a variety of methods to provide adjunctive fluoride treatment to decrease enamel demineralization. The use of highly filled light-cured sealants (HFLCS) is one approach to preventing enamel demineralization. One reported disadvantage of HFLCS is that it may decrease shear bond strength (SBS) of orthodontic brackets *in vitro*. To date, there have been no studies which have examined the effect of HFLCS on bracket bond strength in the presence of demineralization conditions.

Enamel Demineralization around Orthodontic Brackets

Patients undergoing orthodontic fixed appliance therapy have an increased risk of enamel demineralization, which often manifests as white spot lesions (WSL). This is the most common adverse effect of orthodontic treatment, especially when associated with poor oral hygiene. WSLs are defined as "the first sign of a caries lesion on enamel that can be detected with the naked eye" (Fejerskov and Kidd 2003). Reported incidence of novel WSLs during orthodontic treatments ranges between 30%-70% of patients (Heymann and Grauer 2013).

The higher incidence of enamel demineralization for orthodontic patients can be attributed to increased plaque retention due to difficulty cleaning around brackets and auxiliary attachments (Gwinnett and Ceen 1979). Lesions typically occur adjacent to the bonded orthodontic bracket on the gingival third of teeth, the area of highest plaque retention (Lowder et al. 2008). The opaque, chalky presentation of WSL is due to an optical phenomenon caused by enamel mineral loss (Heymann and Grauer 2013). This creates a poor esthetic outcome, a result particularly frustrating for orthodontic patients whose primary motivation for seeking treatment is often to improve esthetics. WSL are also a precursor to cavitated lesions, which can lead to detrimental effects on patient health, costly restorative work, and legal complications (Zabokova-Bilbilova et al. 2014).

Prevention of Enamel Demineralization around Orthodontic Brackets

The primary method of preventing enamel demineralization during orthodontic treatment is through practice of excellent oral hygiene. Therefore, patient education, oral hygiene instruction and regular dental prophylaxis visits during orthodontic treatment are essential to achieving an esthetic result. Adjunctive fluoride therapy is another approach to inhibit demineralization and promote remineralization of enamel. Orthodontists have utilized a number of methods to administer fluoride such as fluoride rinses, fluoride-releasing bonding systems, and varnishes.

Daily 0.5% sodium fluoride rinses in conjunction with fluoridated dentifrice have been shown to be effective in decreasing WSL (O'Reilly and Featherstone 1987; Geiger et al. 1988; Ogaard et al. 1988). However, fluoride rinses require patient compliance. Evidence suggests that only 15% of patients comply with daily fluoride rinses (Geiger et al. 1988). In light of these results, there was a need for compliance-free preventive systems. One such system is fluoride-releasing bonding material such as glass ionomer (GI) and resin-modified glass ionomer cement (RMGIC). There is some evidence to show that GIs reduce the prevalence of WSL. However, studies have shown that GIs create an initial burst of fluoride release which dissipates to non-therapeutic levels within a few days (Heymann and Grauer 2013). Also, GIs decrease bond strength significantly, making GIs and RMGIs not an ideal choice for orthodontic bracket bonding (Wiltshire 1994). Fluoride varnish is also a patient compliance-free preventive system that can effectively prevent WSL formation (Todd et al. 1999; Benson et al. 2005). However, varnishes require multiple applications by the orthodontist and are often only used once WSLs are detected to prevent their progression (Zabokova-Bilbilova et al. 2014).

Resin sealant application is another compliance-free means of decreasing enamel demineralization around orthodontic brackets. Sealants provide a mechanical barrier to acid to prevent demineralization. Chemically-cured sealants were shown to have a significant oxygen-inhibited layer which prevents complete polymerization and seal of the enamel surface (Joseph et al. 1994). In contrast, light-cured sealants have been shown to adequately seal enamel and to effectively prevent WSL *in vitro* (Geiger et al. 1988). When tested *in vivo*, however, unfilled light-cured sealants provided no better protection from demineralization than chemically-cured sealants (Banks and Richmond 1994). This may be partly due to unfilled and lightly-filled light-cured resin sealants' inability to withstand forces of toothbrush abrasion (Strang et al. 1986). Higher filler content is needed to provide adequate protection from abrasion throughout orthodontic treatment. Thus, highly filled resin sealants are required.

Highly Filled Light-Cured Sealants

Highly filled light-cured sealants (HFLCS) provide a promising means to prevent enamel demineralization around orthodontic brackets (Buren et al. 2008; Baysal et al. 2015;

Paschos et al. 2015). In 2004, one manufacturer released a HFLCS called LED Pro Seal[1]. The manufacturer claims that Pro Seal resists abrasion and is the "first sealant that will completely set without an oxygen inhibited layer. This creates a smooth, hard surface that prevents leakage and protects the enamel" (RelianceOrthodonticProducts 2016). LED Pro Seal contains hexafunctional urethane acrylate (30-50%), trimethylproprane triacrylate (30-50%) and nano-particles of proprietary composition (RelianceOrthodonticProducts 2012). While the exact percentages of the product components are proprietary, it has been reported that Pro Seal contains 18% filler which includes components of glass ionomer, which provides fluoride-release and resistance to abrasion (Premaraj et al. 2014).

Pro Seal is intended to be applied to the facial surface of teeth prior to orthodontic bracket bonding, in lieu of a conventional primer. The practitioner should pumice, etch, and dry teeth before applying Pro Seal in a thin layer. After light curing Pro Seal, the orthodontic bracket may be bonded without the use of conventional primers. The manufacturer claims that Pro Seal is compatible with any light cure, chemical cure or dual cure orthodontic bracket adhesive system.

A number of *in vitro* studies verified the efficacy of Pro Seal in preventing enamel demineralization with or without orthodontic brackets (Hu and Featherstone 2005; Buren et al. 2008; Knosel et al. 2012). Hu and Featherstone applied Pro Seal to the buccal surfaces of extracted teeth and placed the sealed teeth in demineralization solution. Sealed teeth were then sectioned and evaluated quantitatively by microhardness testing. Teeth sealed with Pro Seal exhibited 30% less demineralization than controls (Hu and Featherstone 2005; Buren et

[1] Pro Seal® and LED Pro Seal®, Reliance Orthodontic Products, 1540 West Thorndale Ave, Itasca, IL 60143

al. 2008). Another study utilized a similar protocol of applying Pro Seal then subjecting sealed teeth to acidic challenge, but examined sections of sealed teeth via polarized light microscopy to determine average lesion depth (Buren et al. 2008). Teeth sealed with Pro Seal exhibited 97% decrease in average lesion depth compared to controls. These studies also showed that Pro Seal can withstand toothbrush abrasion by subjecting the sealed teeth to 15,000 toothbrush strokes prior to acidic challenge to simulate two years of brushing. Other studies have bonded orthodontic brackets onto teeth sealed with Pro Seal prior to acidic challenge (Buren et al. 2008; Baysal et al. 2015; Paschos et al. 2015). These studies demonstrated that Pro Seal effectively inhibits demineralization around orthodontic brackets *in vitro*.

Orthodontic Bracket Shear Bond Strength

Bracket bond failure is an inconvenient and costly problem during orthodontic treatment. Bond failure negatively impacts both patients and orthodontists, as it leads to delayed treatment time, increased overhead costs, and can affect the integrity of orthodontic appliances (Powers et al. 1997; Finnema et al. 2010). Therefore, it is imperative that bonding systems have adequate bond strength to minimize bond failure during treatment. In order to overcome the 40-120N of force sustained by brackets during mastication, it has been speculated that 6-8 MPa is the minimum clinically acceptable bond strength of orthodontic adhesives (Reynolds 1975). While low bond strengths cause premature bond failure, excessive bond strengths can also be detrimental. During the debonding procedure at the termination of treatment, bond strengths greater than 13.5 MPa may damage enamel by causing enamel fracture (Pickett et al. 2001; Verma et al. 2013). Thus, using bonding systems that exhibit appropriate bond strengths is paramount for successful treatment outcomes and patient satisfaction.

There are no standardized protocols for measuring bond strength *in vivo* other than recording incidence of bond failure (Powers et al. 1997). There are two means of recording bond strength *in vitro*: measuring tensile forces or shear forces during debonding. While both tests are valid, shear bond strength testing is more commonly cited in the literature. One meta-analysis reported *in vitro* bracket bond strengths ranging from 3.5-27.8 MPa (Finnema et al. 2010). The considerable heterogeneity in reported strengths can be linked to a variety of factors affecting bond strength. These factors include storage medium, storage temperature, adhesive type, etching time, quality of enamel, and site of adhesive failure, among others (Fox et al. 1994; Finnema et al. 2010).

It has been reported that quality of enamel is a significant factor affecting bond strength (Finnema et al. 2010). Deviations from sound, non-carious enamel are detrimental to bond strength. For example, bonding to fluorosed enamel has been shown to significantly decrease the bond strength of light-cure resin adhesives (Gungor et al. 2009). There are also a number of studies which show that bonding to demineralized or carious enamel decreases bond strength significantly (Attin et al. 2012; Ekizer et al. 2012; Shahabi et al. 2012; Tedesco et al. 2014). Resin infiltration or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) are accepted approaches of remineralizing enamel. Pre-treating demineralized enamel with resin infiltration or CPP-ACP prior to bonding provides higher debonding forces than untreated demineralized surfaces (Attin et al. 2012; Ekizer et al. 2012). However, pre-treatment with adjunctive fluoride such as fluoride varnishes or acidulated phosphate fluoride solution did not increase SBS of demineralized enamel (Attin et al. 2012; Ekizer et al. 2012).

Effect of HFLCS on Bracket Shear Bond Strength

Questions have been raised about the effect of HFLCS on bond strength when used under orthodontic brackets. The literature provides mixed results. Some studies have reported HFLCS do not have a significant effect on bond strength or method of bond failure for metal or ceramic brackets (Bishara et al. 2005; Varlik and Ulusoy 2009; Mahajan 2013). In contrast, according to Tarvade et al., the use of HFLCS significantly decreased mean SBS compared to conventional resin bonding agents when SBS testing occurs five minutes after bonding (2014). Lowder et al. reported similar results when SBS testing was conducted 30 days after bonding. Bond strengths of HFLCS used under four different resin bonding agents were significantly lower than bond strengths of the conventional resin agents alone. HFLCS and resin bonding agent combinations had mean SBS values ranging from 10.1-13.1 MPa, all higher than Reynold's minimum clinically acceptable bond strength (2008). These studies examined bracket SBS with and without HFLCS in storage medium over time. However, no studies to date have examined SBS of brackets bonded with HFLCS following exposure of the bonded bracket to demineralization conditions.

Adhesive Remnant Index

At termination of orthodontic treatment, brackets are debonded from teeth. The process of debracketing leaves residual bonding resin on the tooth or bracket, depending on the mode of adhesive bond failure. The adhesive bond failure fracture pattern is measured using the Adhesive Remnant Index (ARI) visual assessment (Artun and Bergland 1984) and is often included in studies measuring bracket bond strength to evaluate the quality of the bond (Fox et al. 1994; Montasser and Drummond 2009). Bond failure may occur between the adhesive and bracket interface, between the adhesive and tooth interface, or within the adhesive itself. Orthodontists must remove residual adhesive on teeth following debonding, and this is typically accomplished with a bur and high-speed handpiece. This process is time consuming for orthodontists and can result in irreversible damage to enamel, ranging from 30 to 60μm of enamel loss (Thompson and Way 1981; Bishara and Fehr 1997). To minimize the need for adhesive removal and subsequent enamel damage, it is beneficial for adhesives to fail between the tooth and adhesive interface, leaving little to no residual adhesive on the tooth.

The ARI was originally developed using a 4-point scale. The scale is defined as follows: 0 = all adhesive remained on the bracket base; 1 = >50% of the adhesive remained on the bracket base; 2 = <50% of the adhesive remained on the bracket base; and 3 = no adhesive is present on the bracket base (Artun and Bergland 1984). Bishara and Trulove later developed a modified ARI which uses a 5-point scale. Modified ARI scores are defined as: 1 = no adhesive is present on the bracket base; 2 = <10% of the adhesive remained on the bracket base; 3 = >10% but <90% of the adhesive remained on the bracket base; 4 = >90% of the adhesive remained on the bracket base; and 5 = all of the adhesive is present on the bracket base (1990). The ARI scoring system provides a quick and simple method of evaluating method of bond failure.

Problem Statement

It is known that bonding to demineralized enamel decreases bond strength. It has also been reported that HFLCS may decrease bond strength, but is effective in preventing demineralization. To date, there have been no known studies that have examined the effect of HFLCS (Pro Seal) on shear bond strength when subjected to cariogenic challenge. The purpose of this study is to examine whether the demineralization inhibition properties of

HFLCS may have a protective effect on bond strength and subsequent adhesive fracture pattern following exposure to demineralization conditions.

Hypotheses

1. There were significant difference in shear bond strength for brackets bonded with or without HFLCS following bonded bracket exposure to demineralization conditions or saline.
2. The adhesive fracture pattern measured via the Adhesive Remnant Index will vary as a function of HFLCS use and storage medium.

CHAPTER 2

MATERIALS AND METHODS

Tooth Specimen Collection

Human premolar teeth are frequently used for *in vitro* orthodontic bonding studies, as they are routinely extracted for orthodontic treatment. However, due to high demand and limited availability of extracted premolars at UMKC School of Dentistry, this study utilized extracted human third molars. It has been shown that there is no difference in SBS when using maxillary premolar brackets on premolars or third molars (Ries 2010). Forty intact, maxillary third molar teeth were collected from various departments in the UMKC School of Dentistry and from oral surgery private practice offices in Honolulu, Hawaii. Teeth were collected without patient identifiers and stored in individual containers with 0.9% phosphate buffered saline[2] (PBS). Only teeth with sound enamel, as detected visually and tactically with a dental explorer, were included in this study. Teeth with cavitated lesions, fluorosis, restorations, cracks, buccal surface damage, or abnormal morphology were discarded. Teeth meeting the inclusion criteria teeth were cleaned of all soft tissue debris and stored in PBS with 0.002% sodium azide at 4°C to inhibit microbial growth for up to four months prior to testing.

Orthodontic Bracket

Twin-wing maxillary universal premolar metal brackets with 0.018-inch wire slot[3] were utilized in this study. The brackets do not contain hooks or adhesive pre-coating. The

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[2] Dulbecco's Phosphate Buffered Saline, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103
[3] Victory Series™ Low Profile MBT Metal Brackets, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016

universal maxillary premolar brackets have a concave bracket base which is manufactured to adapt to the both the left and right maxillary premolar buccal surfaces. These brackets were used on maxillary third molar teeth because there are no brackets manufactured specifically for third molar teeth. It has been shown that using a premolar bracket on a third molar teeth yields no differences in bond strength (Ries 2010).

Light-Cured Resin Primer and Adhesive

This study used commercially available orthodontic conventional light-cured diacrylic resin primer[4] and adhesive[5]. Prior to application, the primer requires enamel to be etched with 34% phosphoric acid, per the manufacturer's instruction. The primer is composed of 45-55% bisphenol a diglycidyl ether dimethacrylate, 45-55% triethylene glycol dimethacrylate, and less than 1% of triphenylantimony, 4-(dimethylamino)-benzeneethanol, and DL-camphorquinone (Unitek 2016).

The diacryl resin adhesive contains 70-80% silane-treated quartz filler, 10-20% Bis-GMA, 5-10% Bisphenol A Bis (2-hydroxyethyl ether) dimethacrylate (Bis-EDMA), and 2% silane-treated silica (Unitek 2016).

Highly Filled Light-Cured Sealant

For the experimental groups, a highly filled light-cured resin sealant[6] (HFLCS) was used instead of the conventional primer. This sealant contains a UV fluorescing agent to allow for monitoring of sealant coverage throughout orthodontic treatment. The HFLCS contains hexafunctional urethane acrylate (30-50%), trimethylproprane triacrylate (30-50%)

[4] Transbond XT Primer, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016
[5] Transbond XT Light Cure Adhesive, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016
[6] L.E.D. Pro Seal®, Reliance Orthodontic Products, 1540 West Thorndale Ave, Itasca, IL 60143

and 18% filler content of glass ionomer and nano-particles of proprietary composition. (RelianceOrthodonticProducts 2012). The exact percentages of the HFLCS composition are also proprietary. Per manufacturer instructions, the sealant should be applied in a thin layer to enamel that has been pumiced, etched, and dried. The HFLCS should be light-cured for 20 seconds and can be used prior to bracket bonding, replacing the use of a conventional primer.

Bracket Bonding Protocol

In order to accommodate SBS testing, teeth were individually mounted in self-curing acrylic resin[7] using a mounting jig and plastic mounting ring[8] with the tooth submerged to approximately 2 mm below the cemento-enamel junction (fig. 1). The flattest surface of the mesio-buccal crown surface was positioned perpendicular to the mounting ring. A leveling device[9] as utilized to confirm this orientation to ensure that vertical shear force was applied during shear bond strength testing.

Once mounted, the buccal surfaces of all teeth were polished with a fluoride-free pumice, then rinsed with de-ionized water and dried with oil and moisture-free air. All exposed crown and root surfaces were painted with an acid-resistant varnish[10], except for a window of enamel on the middle third of the mesio-buccal crown surface. This window of exposed enamel extended approximately 1 mm beyond the dimensions of the orthodontic bracket to allow adequate enamel etched surface prior to bracket bonding. Most importantly,

[7] Biocryl #040-016, Great Lakes, 200 Cooper Ave., Tonawanda, NY 14150
[8] Item #20-8180, Buehler Ltd., 41 Waukegan Rd., Lake Bluff, IL 60044
[9] Johnson Level & Tool Mfg. Co., Inc, 6333 W. Donges Bay Road, Mequon, WI 53092-4456
[10] Sally Hansen No Chip Top Coat, Coty US LLC, New York, NY 10118

this approach limited subsequent exposure to the demineralization storage solution to a small enamel area surrounding the bonded bracket to prevent demineralization of the entire crown, which may affect the pH of demineralization solution. In order to demarcate the area of exposed enamel, a standardized wire mesh template (5.5 mm by 5.0 mm) with a bonded composite handle was fabricated. The wire mesh template was held against the buccal crown surface and a thin line was drawn in pen to define the corners of the exposed enamel window. These marks served as a guide when coating the remaining crown and root surfaces with the acid-resistant varnish (fig. 2).

As per the bracket adhesive manufacturer's instructions, a 34% phosphoric acid etchant[11] was applied to the exposed enamel bonding surface for 20 seconds. Teeth were then rinsed with deionized water and dried until etched surfaces appeared chalky white. Following etchant conditioning, the mounted teeth were separated into two groups to receive a conventional primer[12] (CP) or a highly filled light-cured sealant[13] (HFLCS). Each tooth was assigned at random to an experimental group. The order of specimen bonding was also randomized. A thin, uniform coat of either CP or HFLCS was applied to the previously etched surfaces of teeth using a bristle brush, as per manufacturer instructions. The HFLCS layer was polymerized for 20 seconds with an LED curing light[14] prior to bracket bonding. The CP was not light cured prior to bracket bonding, per manufacturer instructions.

Bracket bonding was conducted in controlled conditions in an environmental chamber. Conditions were set at 33 °C (+/- 2 °C) and 85% (+/- 5%) humidity to simulate the

[11] Caulk® 34% Tooth Conditioner Gel, Dentsply, 38 West Clarke Avenue, Milford, DE 19963
[12] Transbond XT Primer, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016
[13] LED Pro Seal®, Reliance Orthodontic Products, 1540 West Thorndale Ave, Itasca, IL 60143
[14] OrtholuxTM Luminous Curing Light, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016

oral cavity (Plasmans et al. 1994). A light-cured resin adhesive[15] was applied evenly to the maxillary first premolar bracket base. The bracket was aligned using a bracket placing instrument on the mesio-buccal enamel surface coated with conventional primer or HFLCS. A hand instrument[16] was used to firmly press the bracket against the tooth surface, allowing excess adhesive to be expressed and carefully removed. A microbrush was then used to remove any excess material around the bracket perimeter. The remaining adhesive was light-cured for 10 seconds on the mesial and 10 seconds on the distal of the bracket. Each day, a radiometer[17] was used to ensure the curing light output is at least 1600 mW/cm^2.

Figure 1. Maxillary third molar mounted in self-curing acrylic resin.

[15] Transbond XT Light Cure Adhesive, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016
[16] Hollenbeck Carver, CVHL 1/2, Hu-Friedy, 3232 N. Rockwell, Chicago, IL 60618-5982
[17] 3H Dental Light Radiometer, Shenghua Industry Co,.107 He Dong Nan Road Jiang Pu St., Shijiazhuang , China

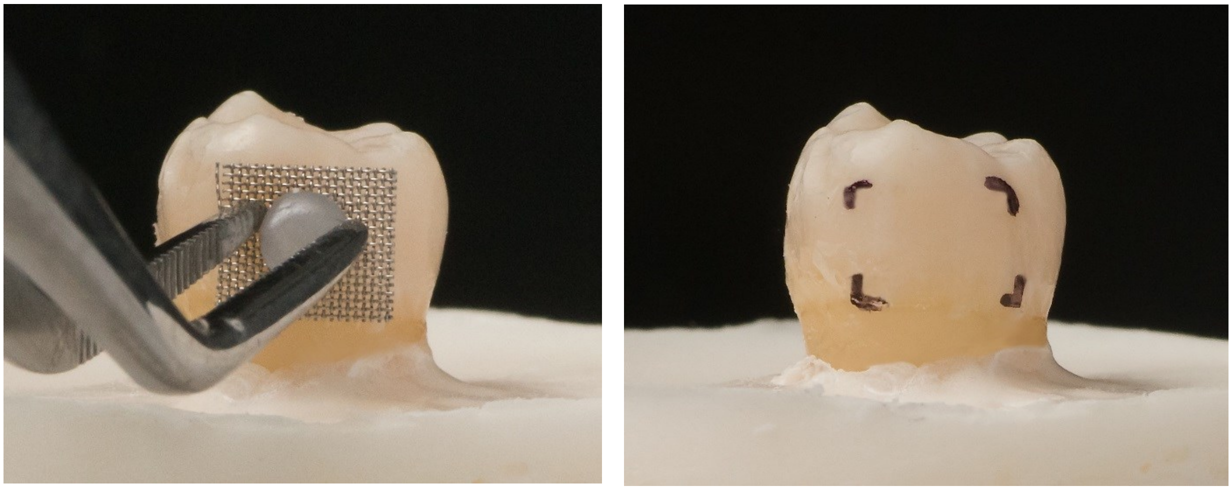

Figure 2. Preparation of exposed enamel area. A standardized wire mesh was held against the buccal surface of a maxillary third molar. A thin pen demarcated the corners of exposed enamel. Acid-resistant varnish was painted around the area of exposed enamel.

Storage or Demineralization Protocol

Following bracket bonding, the bracketed crowns of the teeth were suspended up to the level of the cemento-enamel junction in PBS (pH 7.4) at 37°C for 24 hours to ensure the bracket adhesive dark curing process is complete. The bracketed teeth were then removed from incubation, separated into four groups, and stored in their respective storage mediums for an additional 96 hours (N=10 per group) at 37°C. The 96-hr storage solutions were either PBS, as already described, or a demineralizing (DM) solution. The DM solution is a standard ten Cate solution composed of 2.20 mmol/L calcium, 2.20 mmol/L phosphate, and 0.05 mol/L acetic acid (ten Cate and Duijsters 1983). The DM solution was adjusted with KOH to the desired pH value of 4.2. It has been previously shown that exposure to constantly circulating ten Cate demineralization solution (pH 4.2) for 96 hours creates an average demineralization depth of 150 μm in unsealed enamel (Frazier et al. 1996). During the 96-hr storage period, containers with bracketed crowns in their respective solution were placed on a shaker set at 15 rpm for constant circulation. The storage solutions were changed every 24 hrs. Overall, the conventional primer or enamel sealer and 96-hr storage solution groups were as follows: 1) CP-PBS; 2) HFLCS-PBS; 3) CP-DM; 4) HFLCS-DM.

Bracket Shear Bond Strength Testing

After bonded teeth completed a 24-hr dark cure period in PBS, followed by 96 hours of storage in either PBS or DM solution, a universal mechanical tester[18] was used to debond brackets and record shear bond strength (SBS). Prior to testing, all teeth were removed from their storage solutions and rinsed with PBS. Teeth were tested for shear bond strength in the same order as bonding, which was randomized amongst all groups. All testing was performed by the primary investigator under ambient temperature and relative humidity conditions. The bonded teeth, mounted in acrylic, were stabilized on the universal testing machine platform with four locking screws. The teeth were positioned to allow the machine's stainless steel knife-edge rod attachment to contact the occlusal edge of the bonded bracket base. Load was applied with a crosshead speed of 1 mm per minute in the occlusal-gingival direction, parallel to the buccal surface of the tooth (fig. 3). The maximum load necessary to debond each bracket was recorded in Newtons (N) and converted into megapascals (MPa). Shear bond strength was calculated using the following equation:

$$\text{Shear bond strength (MPa)} = \frac{\text{Maximum compressive load (N)}}{(W*L)(\text{mm}^2)}$$

where W= width of bracket base (mm), L = height of bracket base (mm).

The dimensions of the universal maxillary premolar bracket are 3.05 mm by 3.53 mm, giving a bracket base surface area of 10.77 mm.^{2}

[18] Model 5967, Instron Corporation, 825 University Ave., Norwood MA 02062-2643

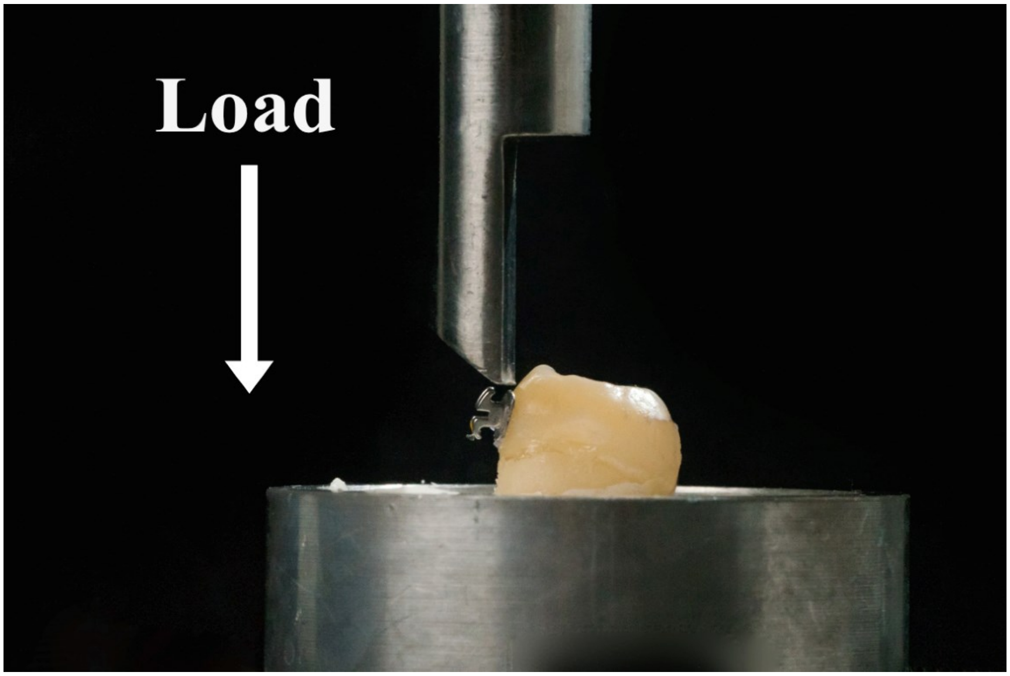

Figure 3. Shear bond strength testing setup. Shear load was applied to the bracket base by a stainless steel rod in the universal testing machine.

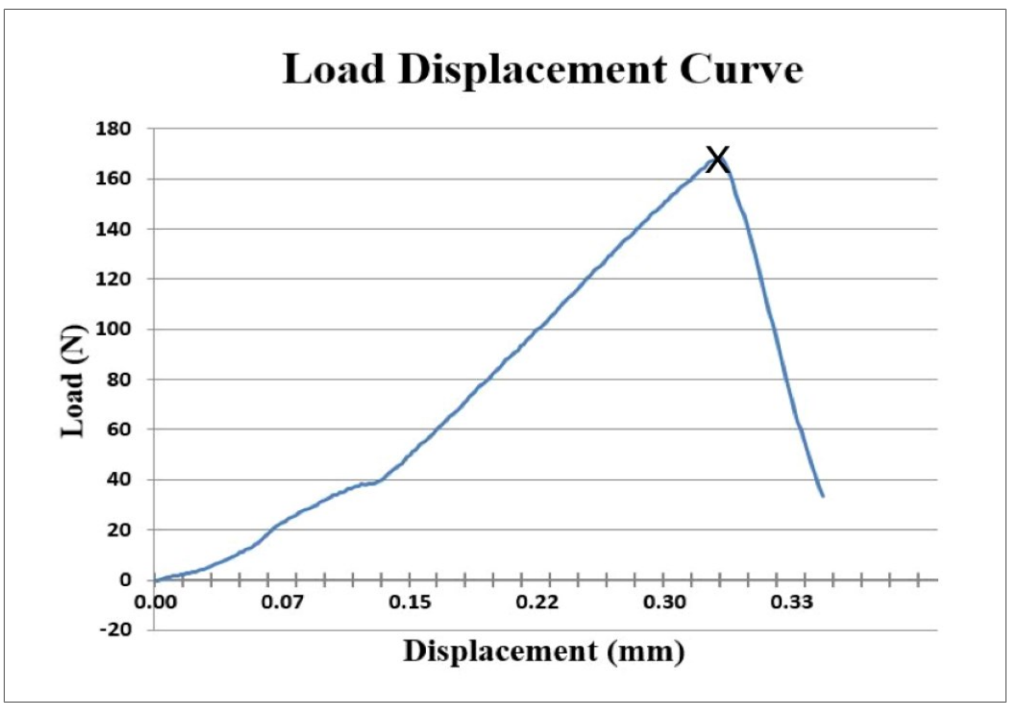

Figure 4. Representative load-displacement curve for shear bond strength testing. Maximum load (X) was used to calculate shear bond strength.

Adhesive Remnant Index

Unmagnified photos of debonded bracket bases were taken to analyze the adhesive remnant index (ARI). ARI categorization was used to quantify the amount of cement left on the tooth following debonding of the bracket, according to the following criteria:
0 = all adhesive remained on the bracket base; 1 = >50% of the adhesive remained on the bracket base; 2 = <50% of the adhesive remained on the bracket base; and 3 = no adhesive present on the bracket base (Artun and Bergland 1984). A grid was superimposed onto images to accurately determine the amount of residual adhesive on the bracket base.

The examiner (BC) was calibrated prior to ARI evaluation. The examiner assigned an ARI score to ten photographs of debonded bracket bases during two scoring sessions, one week apart. The examiner was blinded to any identifying information for each image. Intra-rater reliability was calculated and there was 100% agreement between both scoring sessions. Following calibration and reliability testing, actual ARI evaluation was completed.

Enamel Demineralization Evaluation

Following debonding of brackets, unmagnified photos of teeth specimens were taken to conduct a qualitative analysis of enamel demineralization. The photos were visually evaluated for presence of enamel demineralization in the form of white spot lesions within the window of exposed enamel. Teeth were scored based using a white spot lesion (WSL) scoring system developed by Gorelick (Gorelick et al. 1982). The WSL Index includes four categories: 1, no white spot formation; 2, slight white spot formation (thin rim); 3, excessive white spot formation (thicker bands); 4, white spot formation with cavitation.

Experimental Design and Sample Size

This study utilized a two-factor design with independent variables of bonding protocol (conventional primer or HFLCS) and 96-hr storage medium (PBS or demineralization solution). Due to costs of brackets, bracket adhesive, and the HFLC sealer, a convenience sample of 10 teeth per group was used with a total of N =40. An overview of the experimental design is depicted in table 1.

TABLE 1

EXPERIMENTAL DESIGN

Groups (N=10 teeth/group)	**Bonding Protocol**	**96-Hr Storage Medium**	**Shear Bond Strength (MPa)**	**Adhesive Remnant Index (0-3)**	**White Spot Lesion Score (1-4)**
1. CP-PBS	Conventional primer	PBS			
2. HFLCS-PBS	HFLCS	PBS			
3. CP-DM	Conventional primer	DM solution			
4. HFLCS-DM	HFLCS	DM solution			

Data Analysis

A two-factor ANOVA was used to determine if shear bond strength differs as a function of CP vs HFLCS use and storage in DM solution vs PBS. If a significant difference was found, a Tukey's post hoc test followed to determine where differences existed. A Kruskal-Wallis two-way analysis of variance was used to analyze if ARI scores and White spot lesion scores vary as a function of HFLCS use or storage in DM solution. A Mann-Whitney paired comparison would be used as a post-hoc evaluation approach. All statistical analysis were performed using a statistical analysis software program[19], with a significance level of $\alpha = 0.05$ for all tests.

[19] SPSS version 23, 233 S. Wacker Dr., Chicago IL 60606

CHAPTER 3

RESULTS

Bracket Shear Bond Strength Measurements

Mean shear bond strengths and standard deviations for each bonding protocol and storage medium are presented in figure 5. Specimen shear bond strength values ranged from 4.2 to 20.1 MPa. Based on the two-factor ANOVA, there was no significant difference in shear bond strength between bonding protocols and storage mediums ($p>0.05$). This does not support the hypothesis that shear bond strength would vary as a function of bonding with or without HFLCS following bonded bracket exposure to demineralization conditions or PBS.

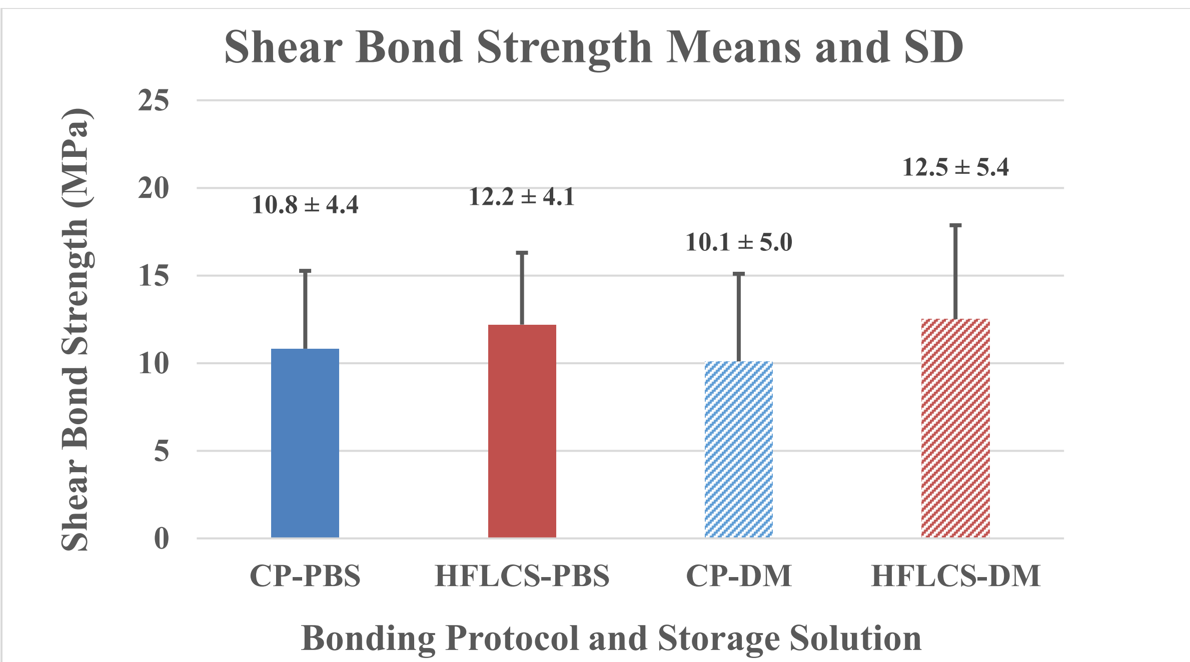

Figure 5. Means and standard deviations of shear bond strength. There were no statistically significant differences between bonding protocol of using a conventional primer (CP) or highly filled light cured sealant (HFLCS) and storage medium of PBS or demineralizing solution (DM).

Adhesive Remnant Index Measurements

Representative images of brackets with each Adhesive Remnant Index (ARI) score are shown in figure 6. The ARI frequency distribution is depicted in table 2. As noted in the table, majority of the specimens received an ARI score of 1 across all study groups. Based on the Kruskal-Wallis two-way analysis of variance, there was no significant difference in ARI scores as a function of bonding protocol or storage medium ($p>0.05$). This does not support the hypothesis that the adhesive fracture pattern will vary as a function of HFLCS use and storage medium in demineralization solution or saline.

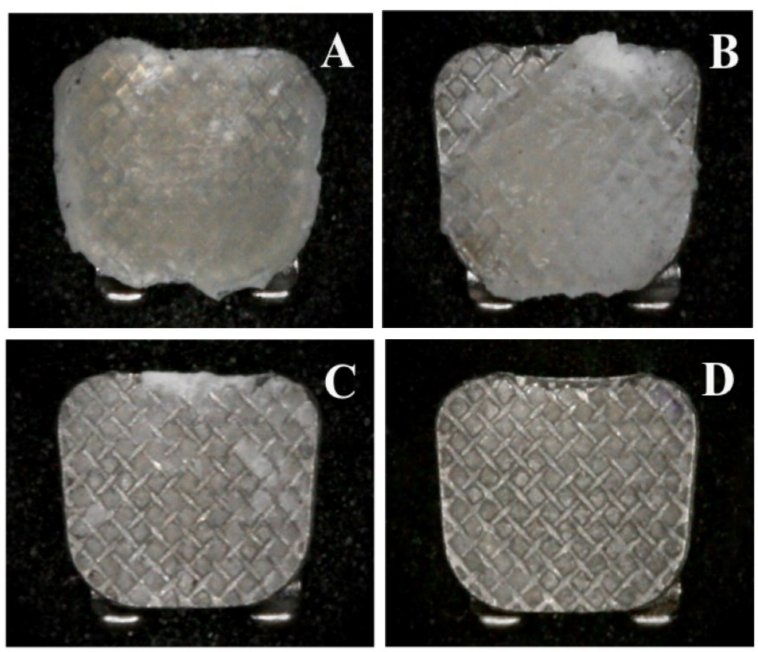

Figure 6. Representative images of debonded bracket base ARI scoring. ARI Score 0 (A), ARI Score 1 (B), ARI Score 2 (C), and ARI Score 3 (D).

TABLE 2.

ARI FREQUENCY DISTRIBUTIONS

Bonding Protocol	**96-Hr Storage Medium**	**Number of Specimens (%) with each ARI Score**			
		0	**1***	**2**	**3**
Conventional Primer	PBS	1/10 (10%)	7/10 (70%)	1/10 (10%)	1/10 (10%)
HFLCS	PBS	1/10 (10%)	8/10 (80%)	1/10 (10%)	0/10 (0%)
Conventional Primer	DM solution	4/10 (40%)	5/10 (50%)	1/10 (10%)	0/10 (0%)
HFLCS	DM solution	2/10 (20%)	8/10 (80%)	0/10 (0%)	0/10 (0%)

*Most specimen fell within the ARI 1 group for each bonding protocol and storage medium.

Enamel Surface Demineralization

Representative images of tooth specimen enamel surfaces and the associated WSL score following bracket debonding and are shown in figure 7. Teeth in groups CP-PBS and HFLCS-PBS exhibited sound enamel (WSL score =1), as they were not subjected to demineralization conditions. After storage in demineralization solution, the CP-DM group enamel appeared chalky and white around the perimeter of the debonded bracket base (WSL score =2 or 3), indicating the formation of white spot lesions. However, with HFLCS-DM group, a chalky surface was not noted within the boundary of the sealer (WSL score =1).

The severity and frequency distribution of WSL scores is depicted in table 3. With exposure to demineralization conditions, HFLCS-DM group demonstrated no WSL formation, which was significantly different ($p<0.05$) than the CP-DM group. All specimen in the CP-DM group exhibited enamel demineralization. WSL scores for the CP-DM group indicate that 60% of specimen had severe WSL formation and 40% had slight WSL formation.

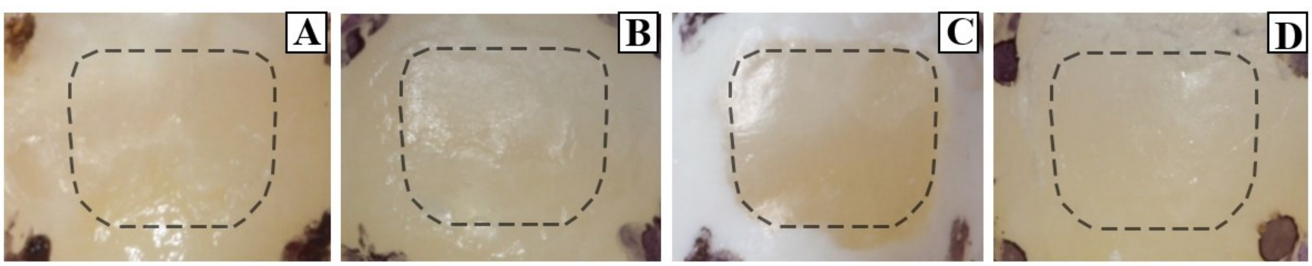

Figure 7. Representative images of enamel surface following debracketing. Tooth specimen and associated WSL scores from group **A)** CP-PBS: WSL score 1; **B)** HFLCS-PBS: WSL score 1; **C)** CP-DM: WSL score 3 and **D)** HFLCS-DM: WSL score 1. Perimeter of bracket base is outlined by dotted lines. Note the majority CP-DM enamel exhibits a chalky white appearance peripheral to bracket base (score 3), indicating enamel demineralization.

TABLE 3.

WHITE SPOT LESION SCORE FREQUENCY DISTRIBUTIONS

Bonding Protocol	**96-Hr Storage Medium**	**Number of Specimens (%) with each WSL score**			
		1 None	**2 Slight**	**3 Severe**	**4 Cavitation**
Conventional Primer	PBS	10/10 (100%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
HFLCS	PBS	10/10 (100%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
Conventional Primer	DM solution	0/10 (0%)	4/10 (40%)	6/10 (60%)	0/10 (0%)
*HFLCS	DM solution	10/10 (100%)	0/10 (0%)	0/10 (0%)	0/10 (0%)

*There was a significant difference between the conventional primer and HFLCS in terms of WSL when exposed to the DM solution. With HFLCS, there were no WSL as compared to conventional primer with varying levels of WSL and no teeth without WSL. With PBS exposure, there were no WSL with either conventional primer or HFLCS.

CHAPTER 4

DISCUSSION

The use of highly filled light-cured resin sealants (HFLCS) is one method of protecting teeth from white spot lesions during orthodontic treatment. Previous studies have demonstrated that coating teeth with HFLCS prior to bracket bonding can prevent enamel demineralization (Hu and Featherstone 2005; Buren et al. 2008; Baysal et al. 2015). It has also been shown that bonding to compromised tooth surfaces, such as demineralized enamel, can adversely affect bond strength (Attin et al. 2012; Ekizer et al. 2012; Shahabi et al. 2012). Although HFLCS can prevent enamel demineralization, a known factor for decreased bond strength, no known studies have investigated if HFLCS may have a protective effect on bond strength under demineralization conditions. Therefore, this study examined the effect of HFLCS on bracket shear bond strength and subsequent adhesive fracture pattern following exposure to demineralization medium.

Bracket Shear Bond Strength

The data showed no significant effect of HFLCS or storage in demineralization medium on SBS. Based on these results, using HFLCS had no protective or stabilizing effect on the bracket shear bond strength following exposure to demineralization medium. Instead, when HFLCS was used with either PBS or demineralization solution storage, the SBS values were very similar, 12.2±4.1 MPa for HFLCS-PBS and 12.5±5.4 MPa for HFLCS-DM. When comparing CP bonding combined with PBS or demineralization medium storage, the shear bond strengths were also similar, 10.8±4.4 MPa for CP-PBS and 10.1±5.0 MPa for CP-DM. With high standard deviation values, there was no significant difference between any of the groups.

These results conflict with some previous investigations that have suggested that use of HFLCS decreases SBS when compared to conventional resin bonding agents (Lowder 2008, Tarvade 2014). Rather, the results of the current study are coincident with research from Mahajan and Bishara et al., which show that HFLCS use had no significant effect on bond strength. In fact, in the current study, there was a slight trend for higher bond strength when HFLCS was used with either storage medium compared to the CP. While the relatively higher SBS of HFLCS in this study was not statistically significant, this does challenge the idea that the use of HFLCS compromises bond strength compared to conventional primers.

Previous studies have demonstrated that bonding to demineralized enamel decreases bond strength (Attin et al. 2012; Ekizer et al. 2012; Shahabi et al. 2012). However, the results of the current study showed that storing bracketed teeth in demineralization solution had no significant effect on SBS. There are a couple of possible explanations for this data. First, it is possible that the storage time in the demineralization medium was insufficient to cause adequate enamel demineralization. However, this is an unlikely explanation, as it has been shown that storage in ten Cate demineralization solution for 96 hours creates an average demineralization depth of 150 μm in unsealed enamel (Frazier et al. 1996). This is the same demineralization protocol used in this study. In addition, the window of exposed enamel for CP-DM specimens all exhibited a chalky white appearance after storage in demineralization medium, consistent with formation of WSLs from enamel demineralization (fig.7). An alternative explanation is that the bonded bracket acted as a physical barrier, protecting the area under the bracket base from demineralization. If the bonded enamel surface under the bracket base was not demineralized, bond strength should not be affected by demineralization conditions. This explanation is supported by the visual observation of debonded specimens in group CP-DM (fig.7). The chalky white demineralized enamel appearance only appeared around the bracket base, but not under it, similar to the rectangular white spot lesions observed clinically after orthodontic treatment.

The reported shear bond strengths of this study ranged from 4.2-22.7 MPa (mean, 11.4; SD, 4.7). This falls within the clinically relevant range of 3.5-27.8 MPa (mean, 13.4; SD, 5.7) which was previously reported in a systematic review of orthodontic bond strength testing (Finnema et al. 2010). However, while the present study results suggest clinical relevance, it is always challenging to directly relate *in vitro* laboratory studies to the *in vivo* environment.

Adhesive Remnant Index

Adhesive fracture pattern in the current study did not vary significantly with HFCLS use and storage medium. Regardless of experimental group, majority of specimens were given an ARI score of 1, indicating that most of the adhesive remained on the bracket base following debond. This data suggest that bond strengths were higher between the bracket-adhesive interface compared to the tooth-adhesive interface across all experimental groups. The low ARI scores may be beneficial to clinicians because the orthodontist has less adhesive to remove from tooth after debonding. This not only results in decreased chair time for the clinician but also reduces the chance of enamel damage when removing adhesive with a bur.

Enamel Surface Demineralization

Previous investigations have demonstrated the effectiveness of HFLCS in preventing enamel demineralization (Hu and Featherstone 2005; Buren et al. 2008; Knosel et al. 2012).

The frequency distribution of WSL formation in this study supports this idea (Table 3). Following storage in demineralization medium and bracket debonding, all teeth in the HFLCS-DM group exhibited sound enamel with no WSL formation (WSL score 1). In contrast, every specimen in the CP-DM group developed a WSL (WSL score 2 or 3). Majority (60%) of these defects were classified as a severe or excessive WSL (WSL score 3). Thus, the HFLCS protected teeth from developing visible white spot lesions, while the CP was not effective in preventing demineralization.

Clinical Implications

The current study demonstrated that bracket SBS values were not significantly altered by the use of HFLCS when compared to a conventional primer. There was a slight trend for higher bond strength with HFLCS compared to the CP irrespective of storage medium, but with an effect size of 4%, there is likely little clinical significance to this trend. While the HFLCS did not enhance bond strength under demineralization conditions, it also did not adversely affect bracket SBS as some previous research has suggested. The current study also demonstrated that HFLCS is effective in preventing the formation of WSLs under demineralization conditions, unlike the conventional primer. This is relevant to clinicians who may be searching for a method to minimize WSL formation during orthodontic treatment. The use of HFLCS appears to effectively protect against WSL formation without decreasing bond strength. The HFLCS is used in place of a conventional primer, so incorporating it into a clinician's bonding protocol requires minimal extra effort or time. Unlike other adjunctive fluoride therapies like fluoride varnish, which requires multiple applications, HFLCS needs just one application

The current study showed that majority of specimen were categorized as ARI 1 across all groups, indicating that less than 50% of adhesive remained on the tooth surface following bracket debonding. Clinicians may find this preferable because minimal adhesive remnant on enamel translates to less time spent manually removing adhesive and a reduced risk of damaging enamel during removal. The present study suggests that clinicians need not be concerned about HFLCS affecting the amount of adhesive remnant on teeth following bracket removal.

Study Limitations

This study was designed to approximate oral conditions as closely as possible, but as with all *in vitro* studies, there are limitations. For example, brackets were debonded with a crosshead speed of 1mm/min using vertical force only, which does not replicate the intra-oral forces exerted on brackets. This protocol was chosen for the purpose of comparison to previous studies. Furthermore, brackets *in vivo* experience forces immediately, without a 96 hour storage delay.

During collection of teeth specimen, teeth were stored for up to four months in PBS with 0.02% sodium azide to prevent microbial growth. This solution has different properties than saliva which may have an effect on shear bond strength and subsequent adhesive fracture pattern. Differences in storage time over the four-month collection period may also affect bond strength.

A standard ten Cate solution (pH 4.2) was used to simulate demineralization conditions. Again, this solution has different properties from saliva which may affect bond strength. Quantitative analysis of enamel demineralization was not conducted to ensure adequate demineralization occurred. It is possible that more demineralization could significantly affect bond strength. However, previous studies have shown that storage in ten Cate solution for 96 hours creates an average of 150 µm of demineralized enamel and the current study showed formation of severe white spot lesions upon visual examination.

Lastly, this study selected maxillary third molars for use with maxillary premolar brackets. However, previous research has suggested that maxillary third molars can be substituted for premolars in benchtop bonding studies, since the mesial portion of the third molar has a similar morphology to the premolar and allows for adequate fit of the bracket (Ries 2010).

Future Investigations

While research has shown that bonding to demineralized enamel decreases bond strength, the current study suggests that exposure to demineralization conditions does not affect SBS of brackets bonded to sound enamel. However, this may be because demineralization only occurred around, not under, the bracket base. Future research could investigate conditions similar to the current study, but with varying exposure times to demineralization solutions. This would potentially allow the evaluation of whether increasing demineralization severity negatively impacts the SBS of bonded brackets. Future investigations could also measure quantitative changes in the microhardness of the demineralized areas around the bracket to determine if there increased likelihood of an impact on the bracket SBS as hardness decreases. For future studies, it may be beneficial to expand the area of exposed enamel to greater than 1mm beyond the bracket perimeter. This may make visual evaluation of WSL severity easier by allowing for thicker bands of demineralized enamel.

The current study showed that the use of HFLCS does not affect adhesive remnant index, as majority of specimen across all groups fell into ARI 1 category. However, with ARI scoring alone, it is unclear whether the location of bond failure occurred at the sealant/tooth interface or the sealant/adhesive interface. Since HFLCS fluoresces under UV light, future investigations could more precisely determine the location of bond failure by using fluorescence microscopy to examine debonded brackets for the presence of HFLCS.

CHAPTER 5

CONCLUSIONS

1. Although the HFLCS prevented enamel demineralization around the bonded brackets, there was no significant difference in shear bond strength for brackets bonded with or without HFLCS following bonded bracket exposure to demineralization conditions or saline.
2. Similarly, there was no significant different difference in adhesive remnant index as a function of HFLCS use and storage medium.

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