EFFECTS OF FLUORIDE VARNISH APPLICATION AND PHOSPHATE BUFFERED SALINE SOLUTION WITH OR WITHOUT DEMINERALIZING CONDITIONS PRIOR TO BRACKET BONDING ON SUBSEQUENT ENAMEL-BRACKET SHEAR BOND STRENGTH

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MASTER OF SCIENCE

by
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EFFECTS OF FLUORIDE VARNISH APPLICATION AND PHOSPHATE BUFFERED SALINE SOLUTION WITH OR WITHOUT DEMINERALIZING CONDITIONS PRIOR TO BRACKET BONDING ON SUBSEQUENT ENAMEL-BRACKET SHEAR BOND STRENGTH

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ABSTRACT

This study examined the effect of sodium fluoride varnish (V) and phosphate buffered saline (PBS) solution with or without demineralization (DM) prior to bracket bonding on subsequent enamel-bracket shear bond strength (SBS). Sixty maxillary third molars were used for the testing procedures after the roots were embedded in acrylic resin. Specimen were randomly assigned to the following four groups: 1) fluoride varnish-PBS only; 2) fluoride varnish-PBS with demineralization; 3) no fluoride varnish-PBS only; 4) no fluoride varnish-PBS with demineralization. Immediately after varnish or no varnish application, all teeth were placed in PBS for four hours to simulate a time of no food or beverage consumption according to the manufacturer's protocols. All teeth were then brushed to simulate natural brushing removal. Teeth were then placed in their respective solutions of PBS only or PBS+DM for 7 days, and stored in an incubator at 37°C and subjected to a shaker set at 15 rpm to simulate oral circulation. A universal ten Cate demineralization solution was used. PBS was changed every day and DM three times daily. After 7 days, metal maxillary premolar brackets were bonded using a primer and resin adhesive to the mesial buccal surface of the molars.

Following a 24-hour dark cure, SBS was determined with a universal mechanical tester, and adhesive remnant index (ARI) values were then assigned. There was a significantly ($p=0.001$) greater SBS with varnish application as compared to no varnish with bracketed teeth stored in the PBS+DM. An effect size of 0.372 indicates that approximately 37% of the SBS difference in the PBS+DM group can be attributed to varnish application. There were no significant differences in ARI results based on varnish application or storage medium. Overall results suggest that metal brackets bonded to teeth treated with fluoride varnish and stored in a medium of PBS+DM exhibited a statistically higher SBS, as compared to teeth with no fluoride varnish application.

APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Dentistry have examined a thesis titled "Effects of Fluoride Varnish Application and Phosphate Buffered Saline Solution with or without Demineralizing Conditions Prior to Bracket Bonding on Subsequent Enamel-Bracket Shear Bond Strength," presented by Burt K. Kawamoto, candidate for the Master of Science degree in Oral and Craniofacial Sciences, and hereby certify that in their opinion it is worthy of acceptance.

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CHAPTER 1

INTRODUCTION

Orthodontic therapy is often utilized to address dental malocclusions and skeletal deformities to improve function, esthetics, and psychosocial well-being. The use of orthodontic treatment to correct dental discrepancies relies on a series of archwires, and/or springs and fixed brackets that are bonded to the enamel with an adhesive to concurrently move teeth to their desired location. Bonding procedures include isolation to keep the teeth dry, an optional thirty to ninety second acid etch (34% phosphoric acid, which is rinsed off), followed by the application of a self-etch adhesive primer (L-pop), and then application of a resin cement (Transbond) to the back of the orthodontic bracket which is light cured onto the enamel surface. After the initial bonding, patients have their fixed appliances adjusted every four to six weeks to move their dentition to their desired place according to the treatment plan. Premature debonding of a bracket in between appointments means that the tooth is not engaged to the archwire leading to no force application to that tooth which can lead to prolonged treatment time, excess time spent in the clinic chair replacing the loose bracket, and compromised results (Almosa and Zafar 2018). Therefore, it is important to use techniques that optimize bond strength to reduce misspent time and improve the predictability of treatment.

Factors Influencing Enamel-Bracket Bond Stability

Before and after the orthodontic bracket is bonded to the enamel, there are multiple factors that may permit detachment of the bracket during treatment. These factors include: enamel demineralization prior to bracket bonding caused by acid erosion, the buffering capacity of saliva and its impact on the enamel-bracket bond, and debonding failure rate forces acting on the enamel-bracket bond such as masticatory muscle forces and stress produced by the archwire system (Gorelick et al. 1982; Humphrey and Williamson 2001; Pickett et al. 2001; Bowen 2013).

Enamel Condition Prior to Bracket Bonding

A common complication with orthodontic treatment is related to white spot lesions (WSL), which are visible signs of early enamel demineralization that can occur around the bracket on the enamel surface. WSL are defined as areas of early enamel demineralization that have not yet cavitated, appear chalky white, and can potentially progress into dental caries (Farhadian et al. 2017). A previous investigation (Gorelick et al. 1982) states that 24% of patients that are referred to an orthodontic office already have pre-existing WSL, while another 50% have non-developmental WSL (Farhadian et al. 2017). In addition, it was reported that over 50% of 5 to 6 year olds, 25% of 11 to 14 year olds, and 77% between the ages of 20 and 25 exhibit significant enamel erosion. Furthermore, 79% of adults have at least moderate evidence of decay (Abou Neel et al. 2016). Not only can WSL potentially progress into caries, but they also create irregularities in the enamel surface weakening the bond strength between bracket and tooth (Sena et al. 2018).

An investigation (Bowen 2013) stated that enamel exposed to acidic levels at pH of 5.5 began to show demineralization. Following a diet composed of glucose or fructose, acidic conditions in plaque have been shown to reach levels of pH 3.9 or even lower as a byproduct of acid production from oral bacteria, suggesting that our oral cavity is constantly exposed to acidic environments that can demineralize enamel. When carbohydrates are ingested, soluble forms of polysaccharides, alpha 1-6 linked glucan and beta 2-6 linked fructose, are broken down by enzymes, dextranase and fructanase, into sugars. These sugars, as long as they are available, are then metabolized by the Emden Meyerhoff pathway into acid, lactic and acetic being the most common (Bowen 2013). In addition, as the oral environment drops below pH 5.5, the microbiome changes to the benefit of cariogenic bacteria such as *Streptococcus mutans* and *Lactobacillus rhamnosus* (Bradshaw and Marsh 1998; Marsh 2006).

Currently, soft drinks are the most common source of exposing the oral environment to acidic conditions in the general population (Medeiros et al. 2018). Carbonated beverages have intrinsic sources of acid which includes citric acid, phosphoric acid, and maleic acid, contributing to a drop in pH immediately after consumption. The acids found in fruit juices are predominately citric acid and ascorbic acid, with the former having more erosive consequences on enamel, than the acid found in carbonated beverages (Pachori et al. 2018). On average, children between Kindergarten and the 12th grade take between 7 and 10 minutes to consume a meal (Conklin, 2002). Oral conditions return to physiologic pH following thirty minutes after the last meal consumption due to the buffering capacity of saliva (Bowen 2013; Pachori et al. 2018).

In general, a two-fold problem occurs when oral conditions drop below pH 5.5; demineralization of enamel occurs, and an oral environment that favors proliferation of acidogenic bacteria leads to a more virulent plaque causing detrimental modifications of the enamel surface weakening the bond strength of bracket to tooth (Marsh 2006).

Buffering Capacity of Saliva

Despite the enamel demineralization that can occur, saliva is important in oral health and improving enamel surface properties. Saliva is involved with enamel remineralization, initial carbohydrate breakdown following a meal, and maintaining oral pH through its buffering ability. Saliva is composed of electrolytes, immunoglobulins, proteins, enzymes, mucins, urea, and ammonia. Electrolytes found in saliva include sodium, potassium, calcium, magnesium, bicarbonate, and phosphate. The buffering capacity of saliva is due to the presence of bicarbonate, phosphate, and urea. The demineralization and remineralization cycle of enamel is modulated by the presence of calcium and phosphate in the saliva. Immunoglobulins, protein, and enzymes are involved in antibacterial activities (Humphrey and Williamson 2001).

Forces Acting on the Enamel-Bracket Bond

Orthodontic therapy relies on multiple treatment methods to correct dentofacial abnormalities. A common treatment plan includes the utilization of brackets, concurrently with archwires, composed of an amalgamation of different metals differing in shape and size, to align maligned teeth into a functional and esthetic occlusion. The enamel bracket bond strength needs to be strong enough to move teeth, overcome patient abuse, resist the stress produced by archwires, and resist the forces generated during mastication (Pickett et al. 2001). Orthodontic brackets are subject to tension, torsional, and shear forces produced by these various forces (Katona and Long 2006). Premature bracket detachment during treatment can lead to increased treatment time, damage to the enamel, and increased chair-time to replace the displaced bracket.

Enamel-Bracket Bond Strength

Brackets can potentially prematurely debond after initial placement with debonding occurring between the bracket and adhesive, within the adhesive, and between the adhesive and enamel. The incidence of brackets debonding range from 0.6% to 28.3% depending on the adhesive being used and technique of the provider (Almosa and Zafar 2018). Current literature suggests that optimal bracket bond strengths should be between 6 to 8 MPa, to provide enough strength to overcome the previously mentioned debonding forces (Verma et al. 2013). Bond strengths greater than 9.7 MPa have been shown to damage enamel surfaces when brackets are initially removed by debonding pliers during the debonding procedure after treatment is completed (Pickett et al. 2001). Therefore using systems that provide appropriate bond strengths is important for orthodontic treatment. One factor that might lead to premature debonding is the condition of the enamel prior to bracket bonding as previously discussed. For example, it has been previously reported that less than ideal enamel surface properties before bracket bonding can negatively impact shear bond strength (SBS) (Farhadian et al. 2017). Methods such as fluoride varnishes, fluoride-releasing adhesives, and laser irradiation to improve enamel properties become imperative to prevent further demineralization, but to also improve enamel properties to facilitate better bond strength (Farhadian et al. 2017).

Previous investigations studying the effects of fluoride on SBS used phosphate-buffered saline solution (PBS), as an artificial saliva solution to store teeth specimens. PBS contains sodium chloride, sodium phosphate, potassium chloride, and potassium phosphate. Although PBS does not contain the normal salivary proteins, their role in antibacterial activity and early enzymatic degradation of food would be negligible in studies that don't involve food degradation and bacterial metabolism. A previous investigation that placed teeth specimen in a pH-cycling model used a demineralizing solution, containing 2.20 mmol/L calcium, 2.20 mmol/L phosphate, and 0.05 mol/L acetic acid at 37 ^{0}C for 2 hours, following by a neutral solution at pH 7.0 (ten Cate and Duijsters 1982).

Effects of Fluoride on the Enamel Surface

Previous investigations have highlighted the positive remineralization effects that fluoride has on decalcified enamel (ten Cate and Featherstone 1991). Remineralization is the process in which calcium and phosphate, obtained from saliva, are deposited into demineralized enamel improving strength and function. Fluoride enhances the remineralization by adsorbing to the enamel surface, bringing in calcium ions. The hydroxyl ions (OH^-) are then replaced by fluoride ions (F^-) in the enamel hydroxyapatite [$Ca_{10}(PO_4)_6F_2$], creating a more acid resistant fluoroapatite [$Ca_{10}(PO_4)_6F_2$] (Mohd Said et al. 2017). Importantly, fluoride is able to bind to enamel creating fluorohydroxyapatite (FHA), $Ca_{10}(PO_4)_6F(OH)$, reducing enamel's solubility in acidic conditions (Langhorst et al. 2009; Ortiz-Ruiz et al. 2018) .

Fluoride varnish was introduced thirty years ago as a means to enhance contact time between fluoride and the enamel surface (Kimura et al. 2004). Topical fluoride varnish contains 5% sodium fluoride. A previous study that took into account simulated manual brushing and acidic oral challenges has illustrated that fluoride can be released from fluoride varnish for up to 28 weeks after application to tooth enamel surfaces (Kimura et al. 2004). Furthermore, fluoride is liberated from fluoride varnish when in an acidic environment, thus having a more proactive effect in demineralizing conditions. In addition, a previous study evaluating the surface of enamel has concluded that pretreatment with acidulated phosphate fluoride reduced the microporosity in permanent teeth (Leodido Gda et al. 2012). Along with remineralizing properties, fluoride is also able to inhibit enzymes involved in bacterial glycolysis, disrupting acid production following a meal (Marsh 2006).

Topical fluoride varnish has also been shown to increase microhardness when enamel was exposed to pH cycling conditions, pH 5.0 for 2 hours and pH 7.0 for 22 hours, compared to enamel without fluoride varnish. Additionally, it has been shown that fluoride varnish applied to enamel resulted in less mineral loss compared to enamel without fluoride varnish, when submersed in a pH cycling environment (Mohd Said et al. 2017).

Previous studies have investigated the effectiveness of fluoride varnish to prevent WSL and improve enamel surface properties when bonding brackets (Marquis et al. 2003; Leodido Gda et al. 2012; Medeiros et al. 2018). These investigations show that fluoride provides multiple benefits in preventing enamel erosion, such as incorporating fluoride into enamel creating a more acid resistant fluorohydroxyapatite, and also inhibiting bacterial glycolysis preventing acid formation (Ortiz-Ruiz et al. 2018). However, no studies to date have examined the effects of, fluoride varnish, on the SBS when teeth are exposed to natural occurring demineralizing and remineralizing conditions prior to bracket bonding.

Effects of Fluoride Varnish on Enamel-Bracket Bond Strength

Various studies have investigated the enamel-bracket SBS with a focus on the interface between dental adhesive and enamel previously treated with fluoride, however certain limitations exist in these investigations. A recent investigation (Medeiros et al. 2018) looked at the application of fluoride varnish around orthodontic brackets after bonding to enamel. However, it did not take into account enamel surface imperfections subjacent to the bracket before bonding. Other studies that placed fluoride varnish on enamel prior to bracket bonding noted no differences in bracket SBS compared to teeth not treated with fluoride varnish (Kimura et al. 2004). However, these studies were done in a neutral artificial saliva solution (Kimura et al. 2004; Ortiz-Ruiz et al. 2018), and have not taken into account the changes in acidity of the oral environment when teeth are exposed to carbohydrates and sugars during daily meals. Furthermore, a neutral artificial saliva environment does simulate the fluoride liberating effects that an acidic solution has on sodium fluoride varnish.

Evaluation of Enamel-Bracket Bond

The enamel bracket bond can be measured by shear bond strength and adhesive remnant index (ARI). As discussed, the bond between enamel and bracket is exposed to forces produced by the bracket-archwire system and masticatory muscle activities. Shear forces surface occur in the y-axis, tension in the x-axis, and torsional in a moment around the z-axis. Two methods of recording enamel-bracket bond strength *in vitro* are tensile and shear forces during debonding. While both tests are reliable in measuring bond strength, shear bond strength is more commonly cited in the literature (Finnema et al. 2010). In addition, shear bond strength (SBS) between bracket and enamel has been studied to show greater strengths than other bonds such as tension (Scougall Vilchis et al. 2009).

Following orthodontic treatment, brackets are removed using a bracket removing pliers, and any remnant cement on the enamel is removed with a carbide bur in a slow- or high-speed handpiece. It is beneficial during this procedure, if most of the adhesive is removed attached to the bracket upon initial debracketing, leaving less on the enamel to be removed with a bur. Removal of cement on the enamel with a slow- or high-speed handpiece can expose the tooth to thermal damage, and lead to potential loss of enamel structure (Vukovich et al. 1991; Kley et al. 2016).

The Adhesive Remnant Index (ARI) was developed to qualitatively determine the amount of adhesive left on the tooth after debracketing (Montasser and Drummond 2009).

The ARI is recorded as follows, score 0 = no adhesive remaining on the tooth, score 1 = less than half of the adhesive remaining on the tooth, score 2 = more than half, but less than all of the adhesive remaining, and score 3 = all adhesive left on the tooth. A lower ARI score indicates that bond failure occurred between the adhesive and enamel, potentially indicating greater enamel decalcification (Naseh et al. 2017). To provide a more accurate score of remaining adhesive left after initial debracketing, studies have been developed to expand the ARI score into five or six scales (Montasser and Drummond 2009). In this study, ARI will be used to determine if there are differences in the amount of residual cement left on the bracket after debracketing between teeth treated with sodium fluoride varnish or without.

Problem Statement

Previous investigations that looked at the enamel-bracket SBS have studied the effects of fluoride varnish, when placed around the bracket after bonding, or on the enamel before bonding. However, those investigations did not place their teeth in a normal expected cycle of neutral, and demineralizing conditions as would be expected in a patient whose oral environment would become more acidic upon eating three meals a day. No study to date has evaluated the effect of fluoride varnish on SBS when the teeth are subjected to PBS with or without demineralization solution before bonding. The aim of this study is to evaluate the SBS of the bracket bonding to enamel, which has been treated by sodium fluoride varnish and stored in PBS with or without demineralization solution for seven days prior to bracket bonding. This investigation will consider the challenges that a demineralizing artificial salivary solution in combination with a neutral solution will have on enamel pre-treated with fluoride varnish and the subsequent SBS between enamel and bracket as would be relevant to a normal daily oral environment.

Hypotheses

1. Shear bond strength will vary for brackets bonded to enamel previously treated with or without fluoride varnish followed by tooth enamel exposure to PBS with or without demineralization solution.

2. Following bracket debonding, the adhesive fracture pattern between bracket and enamel measured by the adhesive remnant index will vary according to whether the teeth were treated with or without fluoride varnish followed by tooth exposure to PBS with or without demineralization solution.

CHAPTER 2

MATERIALS AND METHODS

Tooth Specimen Collection

Premolars have often been used in studies investigating tooth-bracket bond strength. Due to the difficulty of obtaining human premolar teeth because of a limited supply, this investigation used extracted maxillary third molars. A previous investigation has shown no statistically significant differences in SBS when maxillary premolar brackets are bonded to maxillary premolars or the mesial buccal segment of maxillary third molars (Ries 2010). Sixty de-identified maxillary third molars were collected according to the University of Missouri-Kansas City School of Dentistry IRB protocol. Teeth were visually inspected with magnifying loupes[1] for areas of decay, fluorosis, cracks, and/or restorations. Teeth meeting the inclusion criteria were cleaned of debris and stored in PBS containing 0.002% sodium azide at 4^{o}C for up to four months prior to testing procedures.

Orthodontic Brackets

This investigation used twin-wing universal maxillary premolar orthodontic bracket[2] with a 0.018-inch slot for the archwire. There is no adhesive or primer pre-pasted on the brackets to be used.

Fluoride Varnish

A 5% sodium fluoride varnish[3] was used and applied to enamel surfaces seven days prior to bracket bonding for appropriate groups, described later. This fluoride varnish

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[1] Nike Skylon Ace Loupes, Designs fir Vision, 4000 Veterans Memorial Hwy, Bohemia, NY 11716-1024
[2] Victory Series™ Low Profile MBT Metal Brackets, 3M Unitek, 2724 South Peck Rd., Monrovia, CA 91016
[3] Prevident Varnish 5% Sodium Fluoride, Colgate, 300 Park Avenue New York, NY 10022

contains 22,600 parts per million (ppm) of fluoride. Although the percent of other materials could not be found, other components in the varnish include hydrogenated rosin, ethyl alcohol, hexadecane phosphate, sodium fluoride, flavor, citric acid, polysorbate 80, sucralose, and xylitol. For purposes of application, typically approximately 1 ml of this solution is applied to the enamel surface, which would contain 50 mg. of sodium fluoride suspended in an alcoholic solution of synthetic resins. According to the manufacturer's directions, tooth surfaces should first be washed and dried, and then the sodium fluoride varnish applied to the tooth surface with a supplied brush. The varnish should be air thinned and allowed to dry (Colgate 2016). Clinically, abrasive actions such as eating or brushing should be delayed for 2 hours after fluoride varnish application.

Light-Cured Resin Primer and Adhesive

This investigation used a self-etching primer[4] and universal resin adhesive[5] to bond the bracket to the enamel surface. The self-etching primer is composed of, by weight, 10-25% methacrylated pyrophosphates, 0-2% ethylene dimethacrylate, 0-2% phosphoric acid, <1% 2-hydroxyethyl methacrylate (HEMA), 25-40% 2-propenoic acid, 2-methyl-,phosphinicobis (OXY-2,1-ethanydiyl) ester, 15-25% water, 10-25% mono HEMA phosphate, 1-10% tris [2-(methacryloyloxy)ethyl] phosphate, and <3% dl-camphorquinone (Unitek 2018).

The universal adhesive resin contains by weight, 70-80% silane treated quartz, 10-20% bisphenol a diglycidyl ether dimethacrylate (BISGMA), 5-10% bisphenol a bis(2-

[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

hydroxyethyle ether) dimethacrylate, <2% silane treated silica, and <0.2% diphenyliodonium hexafluorophosphate (Unitek 2017).

Remineralizing and Demineralization Solutions

The neutral solution is composed of neutral PBS solution to simulate normal salivary conditions. The demineralizing solution is composed of a 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 1982). Demineralizing solution will be adjusted with KOH to pH 4.1.

Mounting Teeth and Fluoride Varnish Application Protocol

To accommodate the various steps of this study, teeth were individually mounted in acrylic to be stabilized during bracket debonding to test SBS. Utilizing a plastic mounting ring, the teeth were submerged into the acrylic such that the cemento-enamel junction was approximately 2 mm above the acrylic resin block.

Mounted teeth were then randomly assigned to four groups with 15 teeth per group. Two groups had 5% sodium fluoride varnish (V) applied to the mesial buccal surface of the third molar crown according to manufacturer directions. The other two groups did not have fluoride varnish applied. Fluoride varnish application was completed in a controlled environmental chamber to simulate the oral cavity temperature and humidity during bracket bonding when the patient’s mouth is open (Plasmans et al. 1994). Chamber conditions were set to 33^{o}C (+/-2^{o}C) and 85% humidity (+/-5%).

Remineralizing and Demineralization Solution and Tooth Storage Protocol Prior to Bracket Bonding

Immediately after varnish or no varnish application, all teeth were placed in neutral PBS for four hours to simulate a period of no food or beverage consumption according to manufacturer protocols. Following the four-hour neutral PBS storage, the two varnish tooth groups had their 5% V brushed off, in the previously described controlled environment chamber, with a toothbrush[6] and toothpaste[7] to simulate natural brushing removal. Teeth without fluoride varnish applied were also brushed with a toothbrush and toothpaste prior to being placed in their respective solutions. One group of teeth with varnish (V) and another without varnish (NV) were subjected to a cycle of PBS with demineralizing solution, for 7 days at 37°C to simulate natural intra-oral environment conditions and temperature when the mouth is closed. The remaining varnish and non-varnish (NV) tooth groups will be stored in neutral PBS only for 7 days at 37°C.

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[6] Oral-B Manual Toothbrush, Oral-B, 600 Clipper Drive, Suite 200 Belmon, CA 94002
[7] Crest Cavity Protection Toothpaste, Crest, 600 Clipper Drive, Suite 200 Belmon, CA 94002

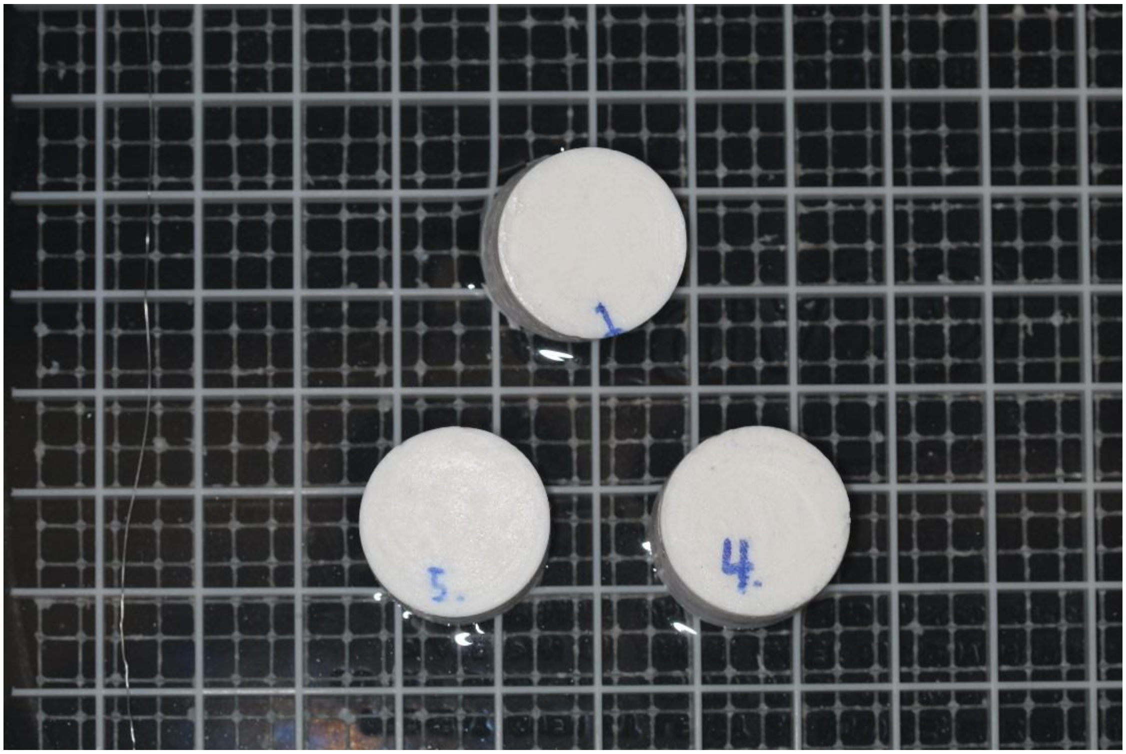

Figure 1. Mounted teeth in acrylic placed upside down in their respective solution. Crown of teeth are placed upside down in their acrylic block in their respective solution (PBS with or without demineralization solution). Teeth are placed in an incubator at 37°C and subjected to a shaker set at 15 rpm to simulate intra oral conditions.

For subject group one, sodium fluoride varnish was applied and removed, and teeth were placed in a neutral PBS solution only for seven days. After 5% V application and removal, teeth in the second experimental group were placed in the demineralizing solution three times a day to simulate breakfast, lunch, and dinner meals, for a period of 15 minutes each time. Between demineralizing conditions, sample teeth were washed and placed in the neutral PBS solution. For subject group three, no sodium fluoride varnish was applied, and teeth were placed in a neutral PBS solution only for seven days. For subject group four, no sodium fluoride varnish was applied, and teeth were placed in PBS with demineralization (DM) for seven days. PBS storage solutions were changed every 24 hours and demineralizing solutions were changed after every cycle. During the seven-day storage, teeth in their respective storage container solutions were placed in an incubator at 37°C and subjected to a shaker set at 15 rpm to simulate constant oral circulation.

To clarify, the 4 groups will be the 5% sodium fluoride varnish or no sodium fluoride varnish groups to be cycled in PBS with or without demineralization for 7 days were organized as follows: 1) fluoride varnish-PBS only; 2) fluoride varnish-PBS with demineralization; 3) no fluoride varnish-PBS only; 4) no fluoride varnish-PBS with demineralization.

Bracket Bonding Protocol

After all groups were stored in their respective solution for seven days, the orthodontic brackets were bonded to the enamel surfaces. Bracket bonding was completed in the previously described controlled environmental chamber to simulate the oral cavity temperature and humidity during bracket bonding. According to Unitek's protocol, the universal self-etch primer was mixed for 5 seconds and rubbed onto the prepared enamel surface with a provided brush for 3-5 seconds, followed by a gentle air burst for 1-2 seconds. The universal adhesive resin cement was applied to the back of the bracket mesh pad, and then pressed firmly onto the mesial buccal enamel surface of the maxillary third molars. Location of bracket placement on the mesial buccal cusp was determined by a vertical line from the mesial buccal cusp tip of the maxillary third molar to the cemento enamel junction. Horizontal placement was determined by a line halfway between the mesial buccal cusp tip and cemento enamel junction, perpendicular to the vertical line. Excess cement is removed with an explorer tip, and then light cured (430-480 nm) with an LED light[8] for 3 seconds

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

from both the mesial and distal of the metal bracket. Following bracket placement, teeth in all four groups were placed back in neutral PBS for 24 hours at 37°C to simulate the dark curing process of the resin cement prior to SBS testing.

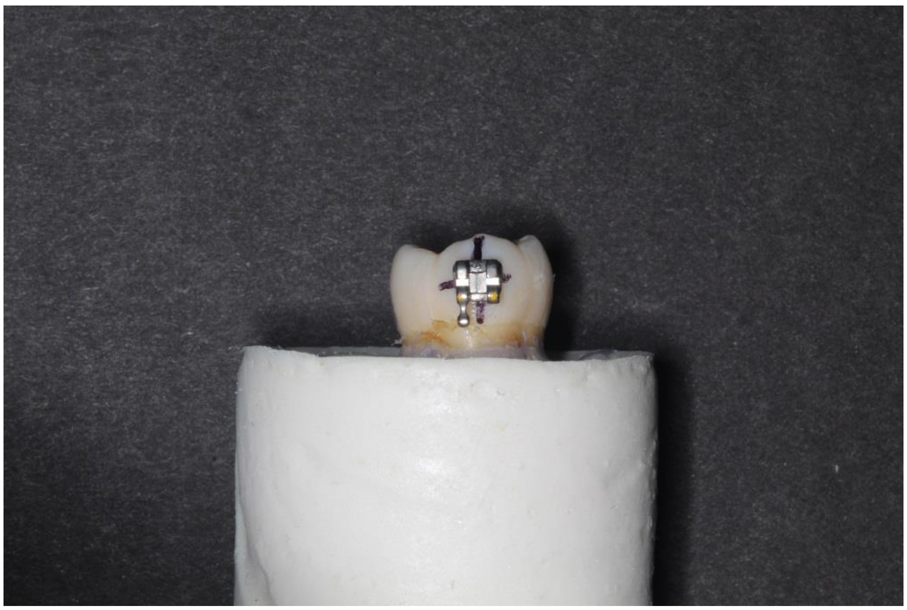

Figure 2. Bracket bonded to mesial buccal of a maxillary third molar.

Enamel-Bracket Shear Bond Strength Testing

After the 24-hour dark cure, teeth from their respective groups were washed with neutral PBS and randomly assigned to be debonded by the primary investigator using the universal mechanical tester[7]. All teeth, in their acrylic block, were stabilized with four locking screws during the debonding process. Debonding with the mechanical tester[7] was done in a 37°C water bath. The teeth were positioned such that the mechanical tester's stainless-steel knife-edge shear debonding rod would contact the occlusal edge of the base of the bonded bracket. Debonding load was applied at rate of 1 mm per minute in an occlusal-gingival direction, parallel to the bracket base. Maximum load to debond the bracket from the enamel surface was recorded in Newtons (N). Shear bond strength, in megapascals (MPa) was then calculated as follows:

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

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

The area of the universal maxillary premolar bracket is 10.77 mm^2 given by its width of 3.05 mm and 3.53 mm.

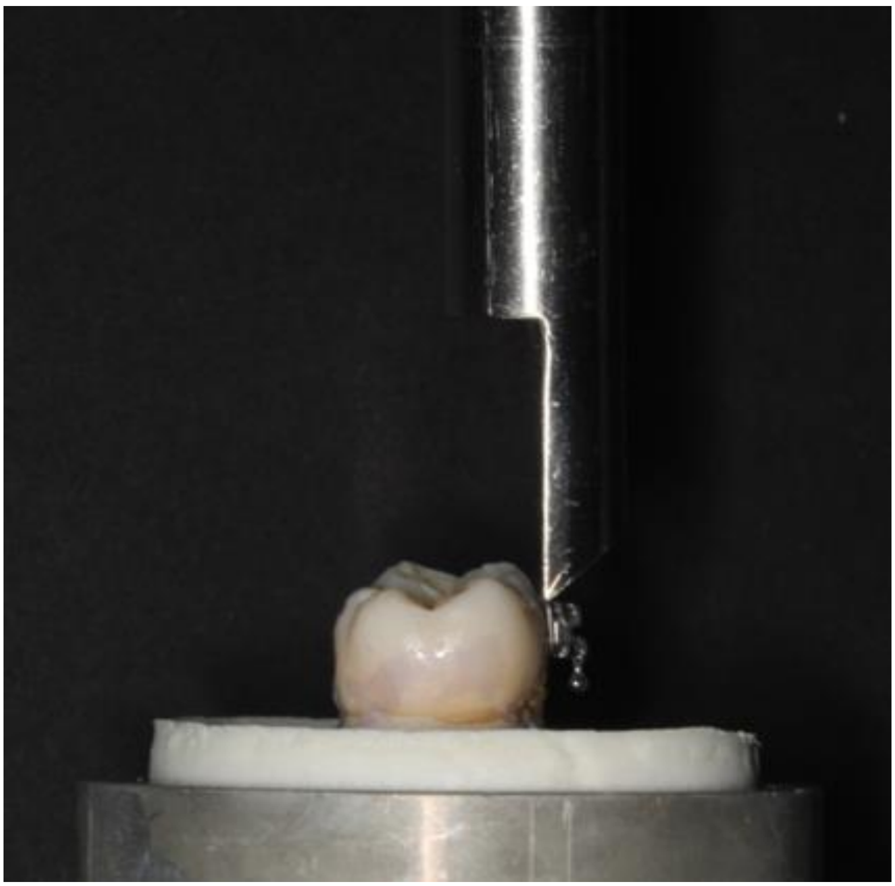

Figure 3. Shear bond strength testing setup. Shear load was applied parallel to the bracket base by the mechanical testing machine.

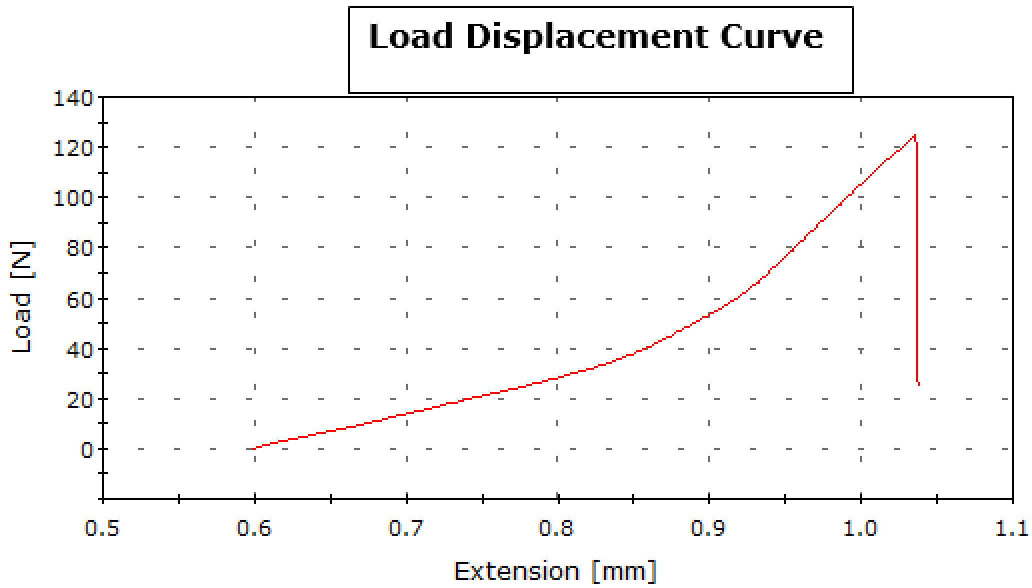

Figure 4. Representative load-displacement curve for shear bond strength testing. Maximum load (Newtons) was used to calculate shear bond strength (MPa).

Adhesive Remnant Index

Images were taken of the back of the bracket mesh pad after debonding to qualitatively determine the ARI. Using Image J, an area of the remaining adhesive on the bracket mesh pad was outlined. From this, the percent of adhesive left on the mesh pad (using the area of the bracket mesh pad) were calculated. As discussed, ARI was used to qualitatively determine the amount of adhesive left on the bracket base after debonding. As previously mentioned, a score of 0 = all of the adhesive left on the bracket base, 1 = more than half of the adhesive left on the base, 2 = less than half of the adhesive left on the base, and 3 = no adhesive remaining on the bracket base.

Experimental Design

This investigation used a two-factor design with independent variables of fluoride varnish application or no application before bracket bonding, and a 7-day storage medium of PBS with or without demineralization. Dependent variables included shear bond strength and adhesive remnant index. Due to financial considerations of brackets, adhesive, and fluoride varnish, a convenience sample of 15 teeth per group were used with a total sample of N=60. The experimental design is shown in table 1.

TABLE 1

EXPERIMENTAL DESIGN

Varnish Application Prior to Bracket Bonding	7-day Storage Medium at 37°C	Groups (N=15 teeth/group)	Shear Bond Strength (MPa)	Adhesive Remnant Index (0-3)
Sodium Fluoride Varnish (V)	PBS only	V-PBS		
Sodium Fluoride Varnish (V)	PBS with demineralization solution exposure (PBS+DM)	V-PBS+DM		
No Fluoride Varnish (NV)	PBS only	NV-PBS		
No Fluoride Varnish (NV)	PBS with demineralization solution exposure (PBS+DM	NV-PBS+DM		

Data Analysis

A two-factor ANOVA was used to determine if shear bond strength differed as a function of fluoride varnish application prior to bracket bonding following storage in PBS storage with or without demineralization. If a significance was detected, 1-factor ANOVAs were used to identify where the differences existed. An effect size analysis was used to determine if our data is clinically relevant. Frequency distributions and Mann-Whitney tests were used to determine if ARI scores vary as a function of fluoride varnish application prior to storage in PBS with or without demineralization. All statistical analyses were performed with a statistical analysis software program[9], using a significance level of $\alpha = 0.05$.

[9] SPSS version 25, 233 S. Wacker Dr., Chicago IL 60606

CHAPTER 3

RESULTS

Bracket Shear Bond Strength Measurements

Mean shear bond strengths and standard deviations for bonding protocols and storage mediums are presented in figure 5. Based on a 2-factor ANOVA, there was a significant interaction between varnish application and storage medium ($p=0.018$) and a significant main effect of varnish application ($p=0.001$) on shear bond strength (SBS), but no significant effect of storage medium ($p=0.18$) on SBS. To better understand where significant differences existed, 1-factor ANOVAs based on varnish application were done within each storage medium group, phosphate buffered saline solution (PBS) with demineralization solution (DM) and PBS only.

The 1-factor ANOVAs indicated that SBS was significantly higher with varnish (V) application as compared to no varnish (NV) in the PBS+DM group ($p=0.001$). Based on the effect size (partial eta squared) value of 0.372, approximately 37% of the SBS difference between the PBS+DM groups could be linked to varnish application. Effect sizes, as previously described (Cohen 1988), range from small (0.1–0.3), medium (>0.3–0.5), large (>0.5). Thus, a 37% effect size would be considered medium and clinically relevant. In contrast, there was no significant difference in SBS between varnish or no varnish application in the PBS only group ($p=0.296$).

This supports the hypothesis that shear bond strength will vary for brackets bonded to enamel previously treated with or without fluoride varnish followed by tooth enamel exposure to PBS and a demineralization solution.

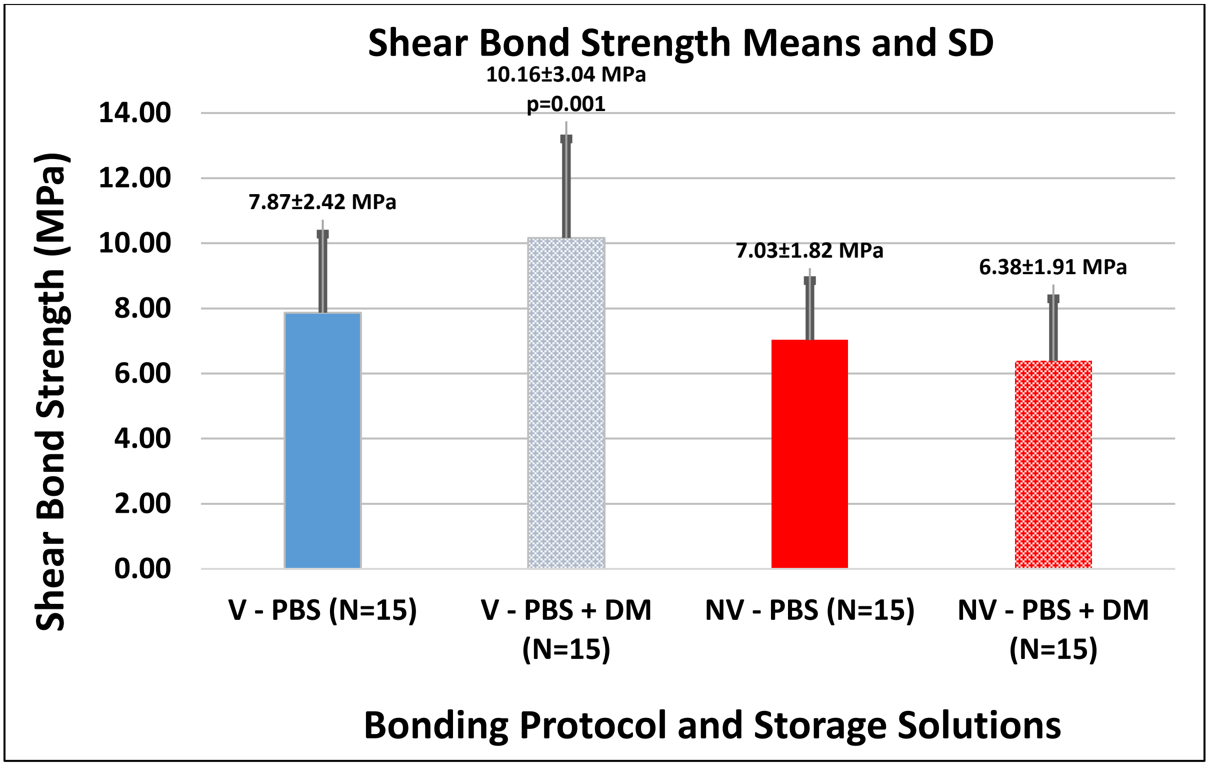

Figure 5. Means and standard deviations value of shear bond strength. There was a statistically significant difference between varnish (V) application versus no varnish (NV) application in specimen stored in phosphate buffered saline solution (PBS) with a demineralization solution (DM).

Adhesive Remnant Index Measurements

Based on a Mann-Whitney test there was no significant difference in ARI results (table 2) based on varnish application within the PBS+DM or PBS groups. This does not support the hypothesis that following bracket debonding, the adhesive fracture pattern between bracket and enamel measured by the adhesive remnant index will vary according to whether the teeth were treated with or without fluoride varnish followed by tooth exposure to PBS with or without demineralization solution.

TABLE 2

ARI FREQUENCY DISTRIBUTIONS

Bonding Protocol	7-Day Storage Medium	Number of Specimens (%) with each ARI Score			
		0	1	2	3
Varnish	PBS only	6/15 (40%)	7/15 (47%)	0/15 (0%)	2/15(13%)
Varnish	PBS+DM	6/15 (40%)	5/15 (33%)	2/15 (13%)	2/15 (13%)
No Varnish	PBS only	6/15 (40%)	6/15 (40%)	2/15 (13%)	1/15 (7%)
No Varnish	PBS+DM	6/15 (40%)	6/15 (40%)	1/15 (7%)	2/15(13%)

* Adhesive remnant index (ARI) score of teeth with or without varnish application stored in phosphate buffered saline (PBS) with or without demineralization (DM). Specimens showed a trend where more adhesive was left on the back of the bracket base mesh pad after debonding.

CHAPTER 4

DISCUSSION

Enamel demineralization has been shown to negatively impact the bond strength between orthodontic brackets and tooth surfaces (Sena et al. 2018). Previous studies have reported that over 50% of 5 to 6 year olds, 25% of 11 to 14 year olds, and 77% of 20 to 25 year olds display significant enamel erosion (Abou Neel et al. 2016). Sodium fluoride varnish (V) is one method to promote enamel remineralization and improve enamel surfaces prior to bonding. Although V has been shown to induce enamel remineralization, no known studies have investigated the effects of V, prior to bonding, on the enamel bracket shear bond strength (SBS) under simulated oral conditions. This investigation studied the effects that V has on the enamel bracket SBS and adhesive remnant index (ARI) following an exposure to a storage medium of phosphate buffered saline (PBS) solution with or without demineralization (DM).

Enamel Bracket Shear Bond Strength

The results of this study showed a statistically significantly higher SBS between bracket and enamel surface with varnish application, as compared to no varnish (NV) application in the PBS+DM groups; 10.16 ± 3.04 MPa for V-PBS+DM and 6.38 ± 1.91 MPa for NV-PBS+DM ($p=0.001$). Based on these results, V significantly increased the SBS between bracket and enamel surface in a simulated oral environment. The higher SBS between V-PBS+DM compared to V-PBS group can be explained due to the fluoride liberating effects that a more acidic solution presents (Leodido Gda et al. 2012). Ultimately, fluoride should reduce the microporosity on the enamel surface and induce remineralization

(Leodido Gda et al. 2012; Medeiros et al. 2018), improving the surface properties prior to bracket bonding. This indicates that V may be used to increase bond strength when bonding brackets to enamel in a natural oral environment.

Previous studies have reported no differences in SBS when fluoride was applied prior to bracket bonding compared to no fluoride application. However, these previous investigations used a neutral salivary solution, which did not take into account the fluoride liberating effects of an acidic solution that would be expected in the normal oral flora (Kimura et al. 2004; Ortiz-Ruiz et al. 2018). This was also evident in this current study in which there was no statistically significant differences in SBS between the V application and NV application groups stored in PBS only. Instead, there was a significantly higher SBS observed between the V-PBS+DM and V-PBS groups. This difference in SBS can be attributed due to the fluoride liberating effects that an acidic solution presents (Leodido Gda et al. 2012) The group without V application and stored in the PBS+DM had on average the lowest SBS. This in part can be explained by a previous study, which demonstrated that demineralization can lead to enamel irregularities, weakening the bond strength between bracket and tooth (Sena et al. 2018). In addition, the lack of V application in this group may have led to no improvement in the enamel surface properties, resulting in a lower SBS.

Another previous investigation demonstrated no statistically significant changes in SBS when V was applied (Medeiros et al. 2018). However, that investigation utilized bovine incisors, and applied the fluoride around the bracket after it was bonded to the tooth, thus disregarding the effects of V on the enamel surface prior to bracket bonding (Medeiros et al. 2018). This current study investigated the effects of V on enamel subjacent to the bracket prior to bonding and demonstrated a higher SBS in both V groups compared to NV groups in their respective storage medium. However, it is important to note that the increased SBS was not statistically significant between the V and NV in PBS only groups. A likely explanation for an increase in SBS in the V-PBS+DM group could be due to the remineralization and reduction in enamel microporosity effects of V (Leodido Gda et al. 2012).

The average SBS of three of the following groups (V-PBS, NV-PBS and NV-PBS+DM) fell within the clinically relevant range of 6-8 MPa (Verma et al. 2013) However, the average SBS of the V-PBS+DM group, 10.16±3.04 MPa, is minimally higher than the recommended maximum SBS value of 9.7 MPa (Pickett et al. 2001). However, while this difference is likely not clinically significant, it is important to take into consideration since higher bond strengths can potentially lead to enamel damage when debonding the bracket with a debonding plier. The clinician should thus utilize care during the initial bracket debonding stage.

Because this current investigation is the first to study the effects of V, prior to bracket bonding, and PBS with or without DM, there is no literature to which the results can be compared. However, the effect size value of 0.372, suggests that 37% of the SBS difference in PBS+DM groups can be attributed to V application and can be considered clinically relevant. Although this study suggests clinical relevance, it still remains difficult to compare an *in vitro* simulation to natural *in vivo* conditions.

Adhesive Remnant Index

The adhesive remnant index (ARI) evaluation did not demonstrate statistical significant differences with V use and storage medium. All groups demonstrated a trend where the ARI scores were mostly of 0 or 1, indicating that most of the resin adhesive was left on the back of the bracket after debonding. This indicates that bond strengths were greater between the bracket and resin adhesive interface than the resin adhesive and enamel interface. This can be beneficial to the clinician because it means that there is less adhesive to remove after initial debonding, which requires less chair time, less use of a high speed handpiece to remove remaining adhesive, and subsequent less chance of enamel damage.

Clinical Implications

The results of this study demonstrated that V can be applied prior to bracket bonding to increase the SBS. Although the effect size of 37% indicates clinical significance, care must be considered upon initial debonding to not damage the enamel surface due to an increase in bond strength. The consistent ARI score that consisted mostly of 0 or 1 also indicates that most of the adhesive was left on the bracket mesh pad after debonding. This results in less adhesive to remove on the enamel after initial debonding by the clinician. Many patients present to the orthodontist with pre-existing enamel demineralization which can lead to a reduction in bond strength and may potentially progress to dental caries (Farhadian et al. 2017; Sena et al. 2018). The use of fluoride has been shown to inhibit bacterial glycolysis and also promote enamel remineralization (Marsh 2006). Along with the aforementioned effects of fluoride, this current study suggests that clinicians may find that V can also improve the bond strength between bracket and enamel leading to fewer premature brackets debonding during treatment.

Study Limitations

This study was designed to approximate oral conditions during testing, however all *in vitro* investigations have their limitations compared to an *in vivo* environment. For example, sample teeth were not subject to any forces after brackets were bonded for 24 hours, compared to immediate force loads placed by the archwire and masticatory muscle system that would be experienced *in vivo*. In addition, a universal ten Cate solution (pH 4.1) was interchanged with neutral PBS three times a day, whereas it can be expected that pH values would have a gradual increase and decrease throughout the day dependent on the person's diet regimen. Furthermore, teeth were stored in a solution of 0.002% sodium azide at 4°C for up to 4 months prior to testing to inhibit microbial growth. This solution has different constituents than normal saliva, which may affect the enamel surface and subsequent SBS and ARI testing.

Brackets are also subject to forces in the y-axis (shear), x-axis (tension), and z-axis (torsion). In this study, brackets were only debonded with a mechanical tester at a crosshead speed of 1mm/min in a vertical vector (shear). This was to allow for comparison to other studies, which used SBS to discuss bond strength. However, it does not test for other potential force systems placed on the bracket enamel bond as would be expected *in vivo*.

Lastly, this study bonded maxillary premolar brackets onto maxillary third molars. However, this may be less of a concern, since a previous study has shown no statistically significant differences in SBS when maxillary premolars brackets are bonded to the mesial buccal portion of maxillary third molars (Ries 2010).

Future Investigations

While this study concluded that the application of V prior to bracket bonding can increase SBS, future studies could investigate if there is an optimal ppm of fluoride concentration to obtain optimal SBS (between 6-8 MPa) without drastically improving the bond strength, which may lead to enamel damage during initial debonding with a debonding plier. For example, various products such as fluoridated toothpaste, silver diamine fluoride, ACT mouthwash, and Prevident all have different concentrations of fluoride and may influence the SBS differently. Another factor to consider is how long before bracket bonding should V be applied to increase SBS to the desired level.

In addition, the bond strength with ceramic brackets compared to the metal brackets used in this study should be considered. Many patients currently seek a more esthetic approach to treatment, especially with their maxillary anterior teeth, which are visible when smiling. It is of clinical relevance to consider the effects of V application prior to ceramic bracket bonding on SBS for those patients who desire a more esthetic treatment approach.

Finally, another aspect to consider is the morphological change in the enamel surface before bonding and after debonding. This study determined that the average SBS in the V-PBS+DM group was 10.16 MPa. The recommended SBS is between 6-8 MPA, with a maximum SBS of 9.7 MPa in order to prevent enamel damage during debonding. Use of a scanning electron microscopy to evaluate the enamel surface can determine if there is any enamel damage after debonding when V is applied. In addition, an elemental analysis could be used to better understand if fluoride is incorporated into the varnished enamel surface.

CHAPTER 5

CONCLUSION

1. Shear bond strength was significantly increased for metal brackets bonded to enamel previously treated with fluoride varnish followed by storage in PBS with demineralization solution (V-PBS+DM) as compared to the other groups, V-PBS, NV-PBS, or NV-PBS+DM
2. Following bracket debonding, the adhesive fracture pattern between bracket and enamel measured by the adhesive remnant index did not vary significantly whether the teeth were treated with or without fluoride varnish followed by exposure to PBS with or without demineralization solution.

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2016	Class of 1974 Scholarship
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