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Title of Thesis: The Effect of Delayed Curing Time of a Self-Etch Primer on Shear Bond Strength of the Orthodontic Bracket

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Abstract

This study investigated if a delay in curing time of a self-etching primer (SEP) affects the shear bond strength (SBS) of orthodontic brackets and the Adhesive Remnant Index (ARI) score. In 2-Step group, the conventional two-step technique was used for enamel conditioning. In groups Delay0, Delay1, and Delay5, a SEP was used with various delays in curing time (0, 1, and 5 minutes). Stainless steel brackets were bonded to the specimens using composite resin adhesive and were tested for SBS. Then ARI scores were measured under naked eye, 2.5x, and 10x magnifications. The results showed no significant difference in mean SBS and the ARI scores between the SEP technique and the two-step technique. The findings also implied a delay up to 5 minutes in SEP curing time do not affect the SBS and ARI scores. Finally, there was no difference in the ARI scores measured under three levels of magnification.

The Effect of Delayed Curing Time of a Self-Etch Primer on Shear Bond Strength of the Orthodontic Bracket

by
Hyun San Lee

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Introduction

Debonded brackets, or broken brackets, are very commonly seen in orthodontic clinics. Replacing debonded brackets is a costly and time-consuming procedure for both the patient and the clinician and more importantly, broken brackets interrupt the continuity of orthodontic treatment and prolong treatment time (Bishara et al. 2002; Alavi and Ehteshami 2019). Therefore, adequate bond strength between orthodontic bracket and enamel surface is important to maintain the flow of orthodontic treatment without premature bond failures. To sustain such reliable bonding between tooth surfaces and orthodontic brackets during treatment, numerous studies have been done on dental materials especially on the adhesives for fixed orthodontic appliances. Self-etching primer is one of the materials that was developed for more efficient yet effective orthodontic bonding. Unlike the conventional two-step technique which involves two separate etching and priming procedures for the preparation of tooth surface, self-etching primers combine the two steps into one, simplifying the orthodontic bonding process and thus reducing the operator time and chance of operative error (Başaran et al. 2009; Türköz and Ulusoy 2012). In addition, previous studies have verified clinically acceptable bond strength of self-etching primers (Mandall 2002; Başaran et al. 2009; Türköz and Ulusoy, 2012; Zope et al. 2016). However, despite the advantages of self-etching primers, clinicians should be aware of the variables that may positively or negatively impact the bracket bond strength to consistently achieve successful bonding in the clinic.

One of the factors that may impact the mechanical properties of self-etching primers is the application time. For example, when teeth are conditioned using a self-etching primer, the amount of priming time can vary in each tooth depending on the pace or bracket placement technique of the operator, or the condition of the patient's dentition. Let’s say a clinician's bonding protocol starts with conditioning the maxillary teeth using a self-etch primer from the right second molars to the left second molars. The total time the self-etch primer is applied on the second molar will be different from that of the central incisors, or of the second molar on the contralateral side. In fact, depending on the clinician's technique or the bonding protocol of the orthodontic office, the total priming time for each tooth can differ by tooth or quadrant, at times exceeding the 2 minutes of application time suggested by the manufacturer (3M Unitek 2010). Therefore, it is valuable for orthodontists to understand the influence of delayed curing time of the self-etching primer since inconsistent curing time, which leads to variable application time of the self-etching primer is almost inevitable in a clinical setting. To date, however, the little is known about the relationship between the delayed curing time of a self-etching primer and bond strength. Therefore, the primary goal of this study is to evaluate the shear bond strength (SBS) between orthodontic brackets and enamel surfaces that are conditioned using a self-etching primer with varying delay time before final curing.

Literature Review

Orthodontic Bands to Brackets

Before the introduction of the bondable orthodontic brackets, circumferential bands were fitted around each tooth to engage archwires or place appliances and apply orthodontic forces for treatment. The multiple banding system was not only time-consuming to the clinicians, but also inconvenient to the patient since it required a separate pre-banding appointment to create interproximal space to accommodate the width of the band (Zachrisson and Buyukyilmaz 2012; Gange, 2015; Alavi and Ehteshami 2019). However, with significant improvements in dental materials, especially in adhesives and bracket designs, orthodontic brackets are often chosen as the current gold standard for treatment method by most orthodontists (Gorelick 1979; Fox et al. 1994). Nowadays, orthodontists routinely bond brackets on the tooth surfaces which significantly reduced the operator time for the bonding appointment. In addition to simplifying the bonding process to the clinicians, orthodontic brackets provide several advantages to the patients as well, such as improved hygiene, reduced gingival irritation and decalcification, better esthetics, and elimination of residual spaces from the bands (Boyd and Baumrind 1992; Chung et al. 2001; Zachrisson and Buyukyilmaz 2012; Gange 2015).

Bonding Technique

Orthodontic bracket bonding starts with preparation of the enamel surface, which often involves cleaning, etching, and priming immediately before bonding the brackets on the tooth (Zachrisson and Buyukyilmaz 2012). In the oral cavity, the enamel surface, which is the outermost layer of the tooth, is usually immersed in saliva, food particles, plaque, and a thin layer of amorphous glycoproteins and polysaccharides known as biofilm. Previous studies by Buonocore (1955) and Miura et al (1973) have argued that pumice prophylaxis of the tooth surface removes these substances on the outer layer, allowing better penetration of the acid etchant and therefore promoting mechanical bonding between the enamel and the adhesive. However, later studies have found that etched enamel surfaces without pumice prophylaxis were comparable to the pumiced surfaces (Lindauer et al. 1997). In addition, the studies did not find significant improvement in bond strength after pumice prophylaxis (Donnan and Ball 1988; Barry 1995; Lindauer et al. 1997). Furthermore, enamel loss of 6.9-14.38µm from pumice prophylaxis has also been reported (Thomas and Way 1981). While numerous studies have shown that pumice prophylaxis is unnecessary clinically, it continues to be frequently utilized in the laboratory and clinical settings, especially before the application of a self-etching primer (Lindauer 1997; Hosein et al. 2004; Wang 2004).

After prophylaxis of the tooth surface, orthodontists routinely utilize the acid-etch bonding technique, which employs an enamel conditioner, a primer solution, and adhesive resin to bond the brackets to the enamel surface. (Bishara et al. 1999; Başaran et al. 2009; Türköz and Ulusoy 2012). This direct bonding technique is often called the two-step technique because it involves two separate steps of etching and priming. The etching procedure of the two-step bonding technique usually involves 37% phosphoric acid which roughens the tooth surface by exposing the interprismatic enamel spaces (Retief 1973). After acid etching, the enamel surface becomes rough, showing irregular prism-like surface with microclefts, which serves as a network for the resin to penetrate and achieve micromechanical retention on the enamel (Diedrich 1981). Buonocore also stated in his study in 1955 that treating enamel surfaces with acid increases the surface area for adhesion by exposing a fresh enamel framework that is free of various substances from the saliva (Buonocore 1955). After the application of the etching agent on the tooth surface for 15 to 30 seconds, the teeth are rinsed copiously with water and dried with oil-free compressed air. Thorough irrigation of the tooth is crucial for adequate bond strength since calcium monophosphate and calcium sulfate deposits that may remain after etching can prevent the infiltration of resin between the microclefts on the enamel surface (Wang 2004). Once the tooth is dry, which is verified by the frosty white appearance of the enamel, an unfilled resin, often called a primer, is applied on the etched tooth surface. The primer works to create an intermediate layer that achieves a closer adaptation of the etched enamel and the resin adhesive, subsequently enhancing the bonding between the two layers (Joseph and Rossouw 1990). However, a laboratory study by O'Brien et al. did not find any significant effect of a primer on bond strength of the orthodontic brackets (O'Brien et al. 1991). Yet, despite the disagreement in the need for a primer during orthodontic bonding, priming agents are continuously used as a part of the two-step bonding procedure, one of the benefits being that it can act as a sealant to prevent enamel decalcification during treatment (Banks and Richmond 1994).

While the conventional two-step bonding technique involves two separate etching and priming procedures, self-etching primers enable the tooth preparation process to be a one-step procedure by eliminating the rinsing stage of the etchant. Self-etching primer functions both as an etchant and a primer and thus eliminates the need for rinsing of the etchant (Türköz and Ulusoy 2012; Zope et al. 2016). Self-etching primer originally started to be used in restorative dentistry for bonding dentinal layer, but it was found to effectively bond to enamel surface as well, allowing its application in orthodontic bonding (Ireland et al. 2003). Self-etching primers help to simplify the steps for orthodontic bonding procedure by combining the etching and priming processes, subsequently reducing the chairside time and the risk of operator errors during the procedure (Dorminey et al. 2003).White in 2001 claimed that up to 65% reduction in chairside time during bonding can be achieved through the use of a self-etching primer (White 2001).

Self-Etching Primer

Self-etching primer, due to its easy manipulation and shorter operator time in the clinic during bonding procedures, has become an attractive bonding modality to orthodontists in the recent years (Başaran et al. 2009). Fleming et al. reported that 29.5% of orthodontists in the US routinely use the one-step bonding technique with a self-etching primer for the bonding procedure (Fleming et al. 2012). In the previous studies on self-etching primers, one of the commonly addressed advantages of self-etching primer is reduced amount of enamel loss during enamel conditioning. Studies have shown less amount of enamel loss from the one-step technique using self-etching primers compared to the two-step technique using an acid etchant, specifically the 37% phosphoric acid (Hosein et al. 2004; Sharma et al. 2014). Sharma et al. used the scanning electron microscope and inspected and compared the enamel surfaces of the teeth that were treated with self-etching primer and the 37% phosphoric acid after debonding. They have found smooth, even surfaces on the self-etching group and porous, rough surfaces on the acid etchant group (Sharma et al. 2014). Hosein et al. further supported this finding by measuring greater enamel loss from the conventional etch (1.11 – 4.57 μm) than that from the self-etching primer (0.03 – 0.74 μm) (Hosein et al. 2004).

There are various factors that can influence the bond strength of self-etching primers, including prophylaxis and pre-etching prior to priming, air-dispersion, and application time and mode of the self-etching primer. The study by Ireland et al. showed bond failure rates associated with a self-etching primer almost twice as those of conventional two-step technique when pumice prophylaxis was omitted (Ireland et al. 2003). This finding is further supported by a randomized clinical trial by Burgess et al. which found higher bond failure rate of 55.5% for no-pumice group and 33.2% for the pumice group when self-etching primer was used for the boding procedure (Burgess et al. 2006). Therefore, both studies showed a significant impact of pumice prophylaxis in reducing clinical bond failure rates of brackets when a self-etching primer is being used for bonding.

Pre-etching of the enamel surface prior to self-etching primer is also associated with higher bond strength. Due to shallower penetration of the self-etching primer than acid etchant in between the microclefts of the enamel, the idea of additional etching before the application of a self-etching primer arose. Van Landuyt et al. studied the effect of pre-etching with phosphoric acid prior to the use of self-etching primer and found more macro- and micro-resin tags on the enamel surface that was previously treated with phosphoric acid. Furthermore, improved bond strength at the enamel layer was also noted with additional etching (Van Landuyt et al. 2005). This agrees with the finding in the study by Erhardt et al. and Fitzerald et al., which found increased SBS to the enamel after pre-treatment with 37% phosphoric acid in the self-etching bonding systems that were tested (Erhardt et al. 2004; Fitzerald et al. 2011).

Another factor that is associated with the final bond strength of orthodontic brackets is the air-dispersion step of the self-etching primer. After the application of a self-etching primer, air-thinning step is usually recommended by the manufacturer (3M Unitek 2010). This step is investigated by an in-vitro study by Dorminey et al., which found significantly lower SBS when the air-dispersion was omitted (Dorminey et al. 2003). The authors suspected the increased thickness of the interface between the composite adhesive and the enamel could be contributing to the lower SBS values. However, the study found that adequate thinning of the self-etching primer resulted a comparable SBS values compared to the conventional 2-step bonding system.

Lastly, application time as well as application mode of self-etching primers can impact bond strength of self-etching primers. For Transbond Plus Self Etching Primer, the manufacturer’s instruction recommends the application of the product accompanied by rubbing motion for at least 3-5 seconds, which is followed by gentle air-dispersion and light curing (3M Unitek 2010). Studies have investigated if extended rubbing time or passive application time of the self-etching primer affected the final bond strength. Parrish et al. compared the difference in SBSs of orthodontic brackets after actively applying (rubbing) a self-etching primer for 0, 5, and 10 seconds. The result showed that the SBS of the brackets without the rubbing of the self-etching primer was significantly lower than that of which was bonded using the instructed application mode (Parrish et al. 2012). However, there was no significant difference in the SBSs between the 5 and 10-second active application. This finding is supported by the study by Iijima et al., which found no difference in the final bond strength after increasing the rubbing time up to 30 seconds (Iijima et al. 2009). However, the difference in the enamel surfaces among different treatment groups was observed through the scanning electron microscopy images, where more porosities and prominent enamel rods were seen in the groups that were exposed to longer application time and agitation of the self-etching primer on the enamel surface.

This study used Transbond Plus Self Etching Primer, often called the L-pop, which is comprised of two parts: methacrylated phosphoric acid esters and initiators; and the other which contains water, fluoride complex, and stabilizers (Sharma et al. 2014). The phosphate group of the methacrylated phosphate acid ester dissolves calcium from hydroxyapatite, which forms a complex with the phosphate group and becomes incorporated into the network when the primer polymerizes, instead of being rinsed away in the conventional two-step technique (Miller 2001; Cinader 2001). Immediately prior to usage, the two compartments of the self-etching primer are squeezed and mixed together to activate the product, which etches and primes the enamel surface simultaneously. After the activation, the self-etching primer is applied onto the tooth surface with rubbing motion for at least 5 seconds, lightly dried using the compressed air for 1-2 seconds, and light cured. Lastly, a bracket is placed on the tooth with the adhesive paste on the base. Then the bracket is manipulated to the ideal position on the tooth surface and is light cured, which completes the orthodontic bonding process (3M Unitek 2010).

Bond Strength of Orthodontic Brackets

As mentioned previously, dependable bond strength of orthodontic brackets is crucial for the continuity of the orthodontic treatment. Premature failure of bracket bonding results in extended chair time, additional cost of materials, increased number of appointments, and subsequently longer overall treatment time (Powers et al. 1997; Bishara et al. 2002). The cause of bond failure in orthodontics is multifactorial in nature. Some of the factors that contribute to orthodontic bond failure include the integrity of the tooth surface, enamel preparation procedure, type of adhesive, design and shape of the bracket base, patient care of the appliances, and moisture contamination (Fox et al. 1991; Chung et al. 2001; Burgess et al. 2006; Zachrisson and Buyukyilmaz 2012; Gange 2015).

However, while the bonding strength between the bracket and the enamel surface should be strong enough to withstand in the wet oral cavity during treatment, it should be weak enough to be broken with minimal iatrogenic damage to the tooth structure at the end of the treatment (Zhang et al. 2014; Zope et al. 2014). Therefore, at the completion of orthodontic treatment, one of the clinician’s concerns is to return the enamel surface to as near its original condition as possible. To sustain such reliable, yet safe bonding between tooth surfaces and orthodontic brackets during treatment, numerous studies have been done on dental materials especially on adhesives for fixed orthodontic appliances.

According Reynolds in 1975, the minimum clinically acceptable bond strength to resist masticatory forces for fixed orthodontic appliances ranges from 5.9-7.8 MPa. After Reynolds' study, there were numerous studies that investigated bond strength of orthodontic appliances using different types of forces, such as shearing, torsional, and tensile (Beech and Jalaly 1981; Powers 1997), and many authors disagreed on one type of force that most closely represented occlusal force. For example, Retief and Sadowski in 1975 stated that shearing force was more proper measure of occlusal force than other types forces, whereas Tavas and Watts in 1979 argued that combined shear and torsional force most closely simulated the debonding forces in the oral cavity. Among studies that investigated bond strengths of fixed orthodontic appliances, tensile bond strength and shear bond strength are often measured, with shear bond strength being the more prevalent method, since it replicates clinical debonding force more closely than tensile bond strength (Powers and Sakaguchi 2006; Parrish et al. 2011).

Factors Related to Orthodontic Bracket Bond Strength

Multiple factors determine sufficient or insufficient bond strength of orthodontic appliances. These aspects may originate from the clinician, patient, or materials being used for the bonding procedure. Numerous studies have supported this statement by reporting a wide range of factors that can impact bond strength of orthodontic appliances, for example, the initial condition of the enamel surface, type of adhesive, bracket base design, bracket position (maxilla vs. mandible, anterior vs. posterior), contamination of the enamel surface during bracket placement, operator error, and enamel preparation methods (etching and priming) (Zachrisson 1977; Fox et al. 1991; Millett and McCabe 1996; Mattick and Hobson 2000).

As previously mentioned, adequate bond strength of the orthodontic fixed appliances is critical during 20 to 28 months of active treatment in the oral cavity, which creates such a unique, rigorous environment (Fink and Smith 1992). Not only is it constantly wet due to the presence of saliva, the teeth on which the appliances are bonded to are under frequent masticatory forces, which are significantly higher in magnitude than orthodontic forces. According the previous studies, orthodontic forces range between 0.05-0.40kg while occlusal forces can reach as high as 50kg (Proffit et al. 2007). In addition, while the range of occlusal forces varies widely depending on the person's gender and age, it is known that the posterior teeth such as the molars are under heavier occlusal forces compared to the anterior teeth (Proffit et al. 2007). Knoll in 1986 also stated that the forces in the posterior part of the mandible estimated twice as large as the forces in the anterior region, which supports the higher failure rate of the molar tubes than the incisor and premolar brackets in a clinical setting (Jung 2014).

Studies have compared the resultant bond strengths of the conventional two-step technique and the one-step technique using a self-etching primer since its introduction to the market. The study by Bishara et al. in 1999 investigated SBS of orthodontic brackets that were bonded using the acid etchant/resin adhesive, acid etchant/glass ionomer adhesive, and self-etching primer/resin adhesive. The results from this study showed that the self-etching primer/resin adhesive presented the lowest SBS (2.8±1.9 MPa) compared to the other two groups which utilized two-step bonding technique (Bishara et al. 1999). Furthermore, due to the significantly lower bond strength of the self-etching primer, they concluded that more reliable bond strength is assured from using an acid etchant and a resin adhesive than a self-etching primer. However, with the advancement in dental adhesives, more recent studies have shown improved, more clinically adequate bond strength of self-etching primers. Multiple studies have found that bond strengths produced by self-etching primer are consistently lower in comparison to the two-step method but are clinically acceptable (Mandall 2002; Başaran et al. 2009; Türköz and Ulusoy, 2012; Zope et al. 2016). In-vitro study by Başaran et al., for example, showed lower bond strength when the enamel surfaces were prepared using self-etching primers compared to the conventional acid etchant, but they concluded that the values were still clinically adequate. Therefore, the suitability of self-etching primers has been widely accepted by more recent studies.

As a clinician, understanding the effect of variables on orthodontic bracket bond strength is crucial to achieve adequate bonding, especially the factors that are controlled by the operator, such as handling time of the materials during the procedure. Previous research has examined the effect of delayed polymerization time of the orthodontic adhesives and pre-cure bracket manipulation time on orthodontic bracket bond strength (Beebe 2011; Gilbert 2015). The effect of active (rubbing) application time of self-etching primer on bond strength is also another variable that can be regulated by the operator (Parrish et al. 2011). However, no previous research has investigated the effect of delay in final curing time, resulted by extended application time, of a self-etching primer on orthodontic bracket bond strength.

Orthodontic Bond Failure and Adhesive Remnant Index (ARI)

The location of the bond failure of orthodontic bracket can be at four different sites: 1) the adhesive-enamel interface; 2) within the adhesive; 3) adhesive-bracket base interface; and 4) bracket base-bracket. The site of the bond failure indicates the risk for potential enamel damage after bracket debond since it dictates the need for extensive or more conservative cleaning of the residual adhesives on the enamel surface. One of the primary concerns of the orthodontist is to return the enamel surface to its original, untreated condition at the completion of fixed appliance therapy (Hosein et al. 2004). Therefore, most clinically desirable location of bond failure is the adhesive-enamel interface due to decreased risk of enamel damage from polishing (Joseph and Rossouw 1990; Fox et al. 1994; Sharma et al. 2014). For example, the adhesive that tends to fail at the adhesive-enamel interface will reduce the need for the removal of remnant adhesive on the tooth surface because most of the adhesive will be debonded off the tooth surface at the time of bracket debond. Subsequently, less remnant adhesive on the tooth surface reduces the risk of damaging the enamel from removing the residual adhesive on the tooth surface (Montasser and Drummond 2009; Sharma et al. 2014).

In the past, most orthodontic bracket bond failures occurred commonly at the adhesive-bracket base interface due to inferior wettability of the mesh base of the brackets and high viscosity of the adhesive resin (Gwinnett and Gorlick 1977). However, with the advancement in bracket base design and adhesives, and consequent higher bond strengths between the adhesive and the brackets, the location of bond failure shifted from the adhesive-bracket base interface to the adhesive-enamel interface, the purpose being leaving less adhesive remnant on the tooth surface after bracket debonding (Bradburn 1992, Fox et al. 1994; Montasser and Drummond 2009). Therefore, accurate assessment of the remnant adhesive on the enamel is important factor in selecting an adequate orthodontic adhesive (Montasser and Drummond 2009).

In studies that have tested bond strength of orthodontic brackets, ARI

(Adhesive Remnant Index) scores are routinely used to inspect the enamel surface after debonding of the brackets. ARI is one of the most commonly used methods of assessing the quality of adhesion between the composite and tooth as well as between the composite and bracket base (Sharma et al. 2014). These scores, developed by Årtun and Bergland in 1984, quantifies the remnants of resin adhesives on the enamel and determines the location of bond failure and the following 4-point scale (0 to 3) was used for measurement in this study (Årtun and Bergland,1984). Lower ARI score, either 0 or 1, is more clinically desirable than 2 or 3, since it indicates lower bond strength between the enamel-adhesive interface, and thus less amount of residual adhesive on the tooth after debond. Subsequently, less remnant adhesive on the tooth surface reduces the risk of enamel damage during the removal of the residual adhesive on the tooth surface (Montasser and Drummond 2009; Sharma et al. 2014).

Purpose of the Study

The purpose of this study was to investigate the effect of various delayed curing times of the self-etching primer on the final bond strength of the orthodontic brackets and the location of bond failure.

Hypotheses

1. The SBS of the orthodontic brackets that were bonded using the two-step bonding technique will be higher than those that were bonded using the one-step technique with the self-etching primer.
2. The SBS of the orthodontic brackets will be higher as the curing time of the self-etching primer is delayed longer.
3. The ARI scores of the specimens in the two-step bonding group will be higher than the one-step group.
4. The ARI scores will be higher as the curing time of the self-etching primer gets delayed longer.
5. The ARI scores of the enamel surface will be higher with higher degree of magnification.

Method & Materials

Tooth Selection and Preparation

A total of 60 human extracted premolars were collected from various departments within the University of Maryland School of Dentistry. The tooth specimens were stored in a container of distilled water for up to 9 months prior to data collection. The selection criteria for the sample teeth consisted of intact buccal enamel surfaces which were free of previous restorations, visible cracks, enamel irregularities, or presence of decay. Each tooth was visually inspected and any specimens with surface damage and were discarded. After examination, each sample was measured for the length, and the root tip was sectioned as needed to fit the tooth in the 25 mm diameter acrylic wells. The specimens were then fixed horizontally in a customized 15-well using self-curing acrylic resin (Instant Tray Mix Powder and Liquid, Liang Dental mfg. Co., Inc., Wheeling, IL). The specimens were oriented so that the exposed buccal surfaces were parallel to the base of the well. The mounted teeth specimens were then randomly divided into four groups of fifteen teeth.

Conditioning and Bracket Bonding

The buccal surface of each tooth was mechanically polished with fluoride-free pumice and a rubber cup for 10 seconds, rinsed with water, and then dried with air for 5 seconds. After the preparation, the fifteen specimens of group 1 (2-Step) were conditioned following the two-step technique. 37% phosphoric acid was applied to the buccal surfaces of each tooth for 15 seconds and was thoroughly rinsed and dried with oil-free compressed air until frosty white appearance of the tooth enamel was noted. After etching, Assure Universal Bonding Resin (Reliance Orthodontic Products Inc) was applied to the buccal surface using a microbrush, rubbed on the tooth enamel for

5 seconds, and gently dried with air for 2 seconds. After confirming the shiny appearance of the tooth surface, the specimen was light cured.

The one-step bonding groups (Delay0, Delay1, Delay5) were conditioned using the Transbond Plus Self Etching Primer (3M Unitek), which was activated following the manufacturer's instructions immediately before application. The activated primer was applied to the buccal surface of each tooth using a microbrush and was rubbed for 5 seconds and gently dried with air for 3 seconds to evaporate the solvent and obtain a thin coating on the tooth surface. After conditioning, the specimens were treated according to the assigned group.

For Delay0 group, the buccal surfaces were light cured immediately after the application of the self-etching primer by applying the curing unit for 5 seconds directly to the tooth surface. For Delay1 group, the final curing time was delayed for 1 minute after application and air-drying of the self-etching primer. For Delay5 group, the final curing time was delayed 5 minutes after application and air-drying of the self-etching primer. The 1-minute and 5-minute time delays were chosen based on the manufacturer's instructions which recommended curing within 2 minutes after the application of self-etching primer.

Once all of the specimens were conditioned according to one of the four assigned protocols, Transbond XT light-cured adhesive paste (3M Unitek) was dispensed onto the base of the stainless steel standard edgewise premolar brackets (Discovery, Dentaurum Inc., Langhorne, PA) and positioned on the FA point of each tooth using a college plier. Force was applied on the bracket using a dental scaler and the excess adhesive around the periphery was removed with the explorer, and the samples were cured by applying the light curing unit for 10 seconds each at the mesial and distal sides of the brackets (20 seconds per tooth). After bonding of the brackets, the specimens were stored in distilled water for 24 hours at a temperature of 37 ºC until they were tested for SBS (Zhang et al. 2016; Ma et al. 2017).

Figure 1. 37% phosphoric acid etchant, Assure Universal Bonding Resin, Transbond Plus Self Etching Primer, Transbond XT adhesive.

Shear Bond Strength Testing (MPa)

After 24-hour storage in distilled water at 37 ºC, the brackets were debonded using a universal testing machine (MTS, Eden Prairie, MN). For SBS testing, a chisel was connected to the computer-controlled universal testing machine and the knife-edge tip was positioned on the occlusal edge of the bracket base for the shear force to be applied to the bracket-tooth interface. A representative image of the setting for a SBS testing is presented in Figure 2. As the chisel vertically moves down, an occlusogingival load parallel to the base of the bracket was applied to the bracket at a displacement rate of 0.5 mm/min. The moment each bracket was debonded off the tooth surface, the maximum shear force was measured. Then SBS was recorded for each specimen in Newtons and were converted to Megapascals. SBS values were calculated using the equation: Shear bond strength (MPa) = shear force (N) / surface area of bracket base (mm^2). The surface area of the bracket base ($9.0mm^2$) was calculated using the width from the manufacturer's manual (2.8mm) and the length measured using a Boley Gauge (3.2mm) (Zhang et al. 2016; Ma et al. 2017).

After debonding of the brackets, the tooth surfaces were inspected for the presence of enamel damage. Specimens with damaged enamel surface after SBS testing were selected and omitted from the data collection. A total of three brackets, sample #11 in 2-Step group, sample #5 in Delay0 group, and sample #15 in Delay1 group were excluded from data analysis due to possible errors in the SBS measurements (See Appendix Table A). In order to maintain the total of 15 specimens per group, additional tooth was added to each group.

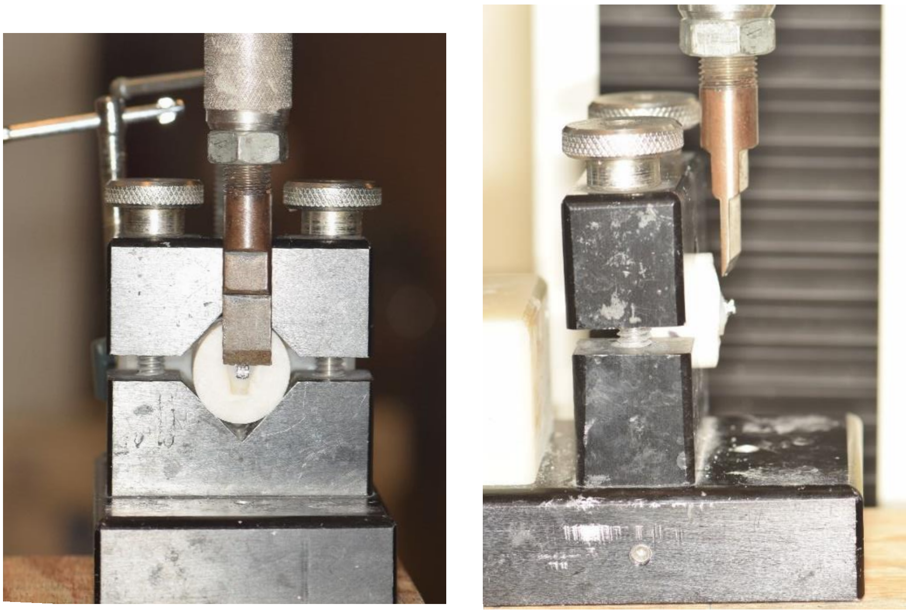

Figure 2. Mounted and bonded specimen ready for SBS testing

Adhesive Remnant Index (ARI) Score

After debonding the brackets, each tooth enamel surface was inspected for ARI scores, which is one of the most commonly used methods of assessing the quality of adhesion between the composite and tooth as well as between the composite and bracket base (Sharma et al. 2014). These scores, developed by Årtun and Bergland in 1984, quantified the remnants of resin adhesives on the enamel and determined the location of bond failure and the following 4-point scale (0-3) was used for measurement: 0 = no adhesive remained on enamel, suggesting bond failure at the adhesive-enamel interface, 1 = <50% of the bonding area on enamel was covered with adhesive, suggesting bond failure within the adhesive but mainly at the adhesive-enamel interface, 2 = >50% of the bonding area on enamel was covered with adhesive, suggesting bond failure within the adhesive but mainly at the adhesive-bracket interface, and 3 = the adhesive entirely covered the bonding area on the enamel, suggesting bond failure at the adhesive-bracket interface (Montasser and Drummond 2009). Representative images of each ARI score are presented in Appendix Figure B.

The ARI scores of all the specimens were collected by the same evaluator under three different levels of magnification; under the naked eye, 2.5x magnification using dental loupes, and 10x magnification using a microscope. The specimens were randomly evaluated under each magnification in a separate session.

<u>Statistical Analysis</u>

All the SBS measurements were tested for the presence of possible outliers using Microsoft Excel prior to statistical analyses. One outlier was found in 2-Step group (40.0 MPa), which was omitted resulting 14 specimens. Two dependent variables were used to measure bonding of the orthodontic brackets: SBS in MPa and the ARI score. Two independent variables were also measured. The first was bonding technique, which included two-step and 3 one-step bonding techniques. The one-step bonding procedures included a delay of 0, 1, and 5 minutes in the curing time of the self-etching primer. The second independent variable was magnification level, which was 0, 2.5x, and 10x. Kruskal-Wallis test and one-way analysis of variance test was performed using MYSTAT, and the Chi-square test was performed using Microsoft Excel.

(1) A Kruskal-Wallis test was used to determine if there were significant differences in the SBS of the orthodontic brackets to enamel when using conventional two-step bonding technique (2-Step) and one-step technique with a self-etching primer without delaying the curing time (Delay0).

(2) The SBS as a function of delay in curing time of the of the self-etching primer were compared. Intervals were 0 second (Delay0), 1 min (Delay1), and 5 min (Delay5), and one-way ANOVA was performed for the analyses.

(3) For the analysis of the ARI scores, a Chi-square test was used. The test was used to determine if there were significant differences in the ARI scores between the two types of bonding technique and different delays in curing time of the self-etching primer. The ARI scores measured under 10x magnification were used in this analysis as it was considered the most accurate.

(4) The ARI scores measured under three levels of magnification were analyzed using a Chi-square test to investigate if the ARI scores vary as a function of different level of magnification. All statistical analyses were performed using significance set at $p = 0.05$.

Results

Shear Bond Strength (SBS)

Descriptive statistics such as the means, standard deviations, maximum and minimum SBS values for each of the four groups are presented in Table 1. Prior to data analysis, outlier calculation was performed which indicated the presence of an outlier in the SBS measurements in 2-Step group (Sample #8), and thus this data was not included in the statistical analysis (See Appendix Table 1.)

The mean SBS values of the two-step group (2-Step) and the one-step group (Delay0) were 15.6 ± 6.5 and 16.9 ± 8.5 MPa, respectively. Based on the Kruskal-Wallis test, there was no statistically significant difference ($p > 0.05$) in the SBS between 2-Step and Delay0 groups, which did not support the hypothesis that higher SBS will be achieved by using the conventional two-step technique than the one-step technique with the self-etching primer.

Among the three self-etching groups, the highest mean SBS was measured in Delay1 group (17.0 ±8.4 MPa), although it was only minutely different from that of Delay0 group (16.9 ± 8.5 MPa). Delay5 group, which was subjected to the longest delay (5 minutes) in curing time of the self-etching primer, showed the lowest mean (12.0 ± 6.4 MPa) as well as the lowest range of SBS values compared to the other two self-etching groups (See Table 1. and Figure 3.). Yet, the ANOVA showed that the SBS within the self-etching primer groups (Delay0, Delay1, Delay5) were not significantly different, rejecting the hypothesis that the SBS of the orthodontic brackets will increase with longer delay in the curing time of the self-etching primer.

Shear Bond (MPa)		2-Step	Delay0	Delay1	Delay5
	Mean	15.6	16.9	17.0	12.0
	SD	6.5	8.5	8.4	6.4
	Minimum	5.9	6.6	4.5	2.1
	Maximum	27.2	30.0	32.4	24.6
	Sum	14	15	15	15

Table 1. The mean, standard deviations, the lowest and the highest SBS measurements of the four groups.

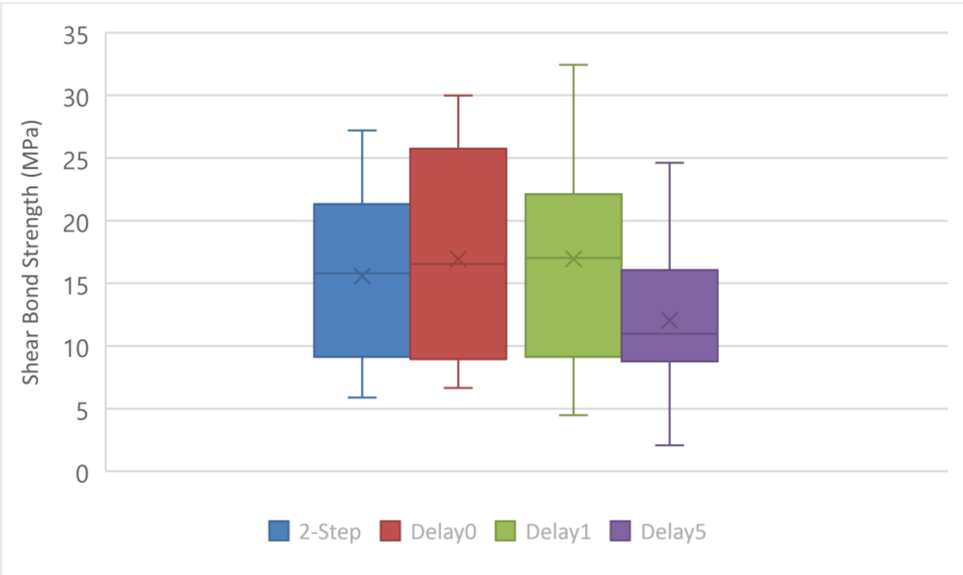

Figure 3. Box and whisker plot showing means, median, interquartile range, and maximum and minimum SBS measurements of the four groups.

Adhesive Remnant Index (ARI)

The number of specimens and the percentage for each ARI score per group are presented in Table 2, 3, and 4, grouped by the level of magnification; naked eye (0x), dental loupes (2.5x), and microscope (10x). For the statistical analyses, the ARI measurements under 10x magnification were chosen as it was considered the most accurate compared to the naked eye and 2.5x magnification.

The distribution of the ARI scores of all four groups showed a similar pattern with higher numbers of score 1s and 2s than 0s and 3s. This corresponding pattern of ARI scores of the four groups under 10x magnification is shown in Figure 4. Based on the Chi-square test, there was no statistically significant difference ($p>0.05$) in the ARI scores between the two-step bonding group (2-Step) and the one-step bonding group (Delay0). In addition, no significant difference ($p>0.05$) was found in the ARI scores among the one-step system groups with different delays in curing time (Delay0, Delay1, and Delay5). These results rejected the hypotheses that the ARI scores will be significantly different between the two types of bonding technique and different delays in curing time of the self-etching primer.

ARI Score	Number of specimens with each ARI Score per group (0x)				
	2-Step	Delay0	Delay1	Delay5	Sum
0	1 (7%)	3 (20%)	1 (7%)	3 (20%)	8
1	11 (73%)	6 (40%)	11 (73%)	8 (54%)	36
2	3 (20%)	5 (33%)	3 (20%)	2 (13%)	13
3	0 (0%)	1 (7%)	0 (0%)	2 (13%)	3

Table 2. Adhesive remnant index measurements under the naked eye (NE).

ARI Score	Number of specimens with each ARI Score per group (2.5x)				
	2-Step	Delay0	Delay1	Delay5	Sum
0	1 (7%)	3 (20%)	1 (7%)	2 (13%)	7
1	9 (60%)	6 (40%)	10 (66%)	8 (54%)	33
2	5 (33%)	5 (33%)	4 (27%)	3 (20%)	17
3	0 (0%)	1 (7%)	0 (0%)	2 (13%)	3

Table 3. Adhesive remnant index measurements under 2.5x magnification using dental loupes (DL)

ARI Score	Number of specimens with each ARI Score per group (10x)				
	2-Step	Delay0	Delay1	Delay5	Sum
0	0 (0%)	2 (13%)	1 (7%)	1 (7%)	4
1	9 (60%)	7 (47%)	9 (60%)	8 (53%)	33
2	6 (40%)	4 (27%)	5 (33%)	5 (33%)	20
3	0 (0%)	2 (13%)	0 (0%)	1 (7%)	3

Table 4. Adhesive remnant index measurements under 10x magnification using microscope (MS).

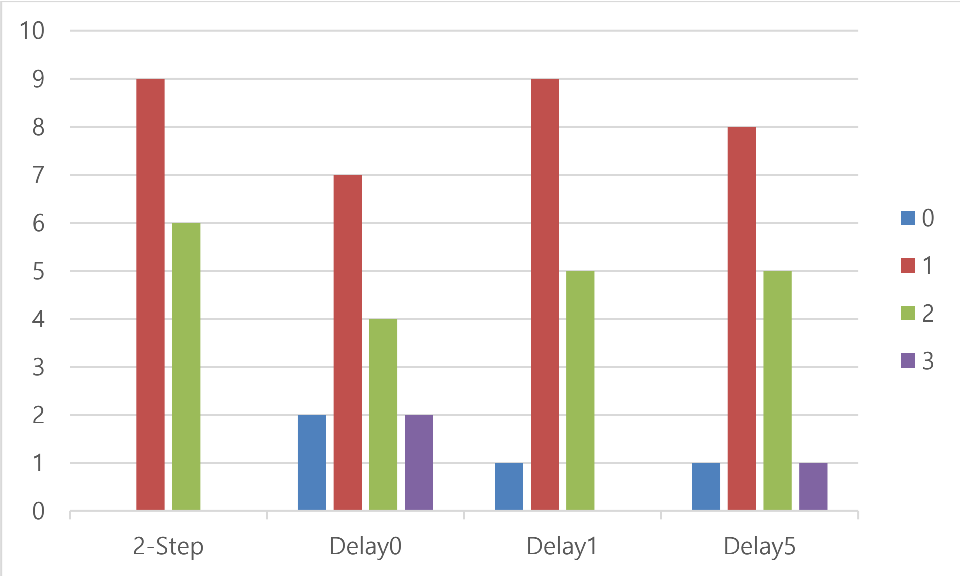

Figure 4. Frequencies of the ARI scores of the four groups under 10x magnification.

ARI Assessment under Different Magnifications

The ARI scores for different magnifications are distributed for each group in Figure 5, 6, 7, and 8 and the measurements are shown in Appendix Table B, C, D, and E. The Chi-square test showed that the ARI score evaluation was not significantly different ($p>0.05$) under different levels of magnification, rejecting the hypothesis that the ARI scores of the enamel surface will be higher with higher degree of magnification.

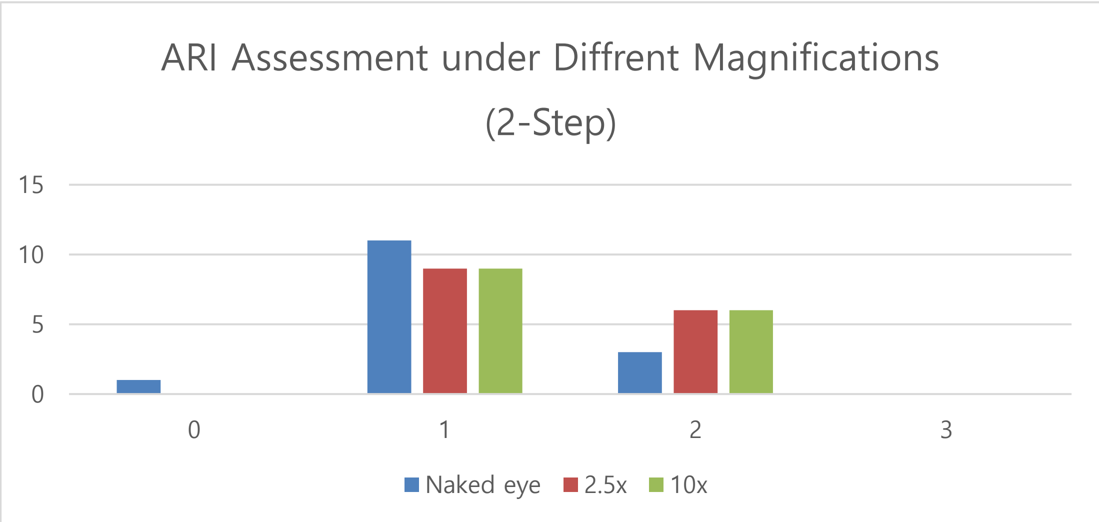

Figure 5. ARI score distribution under 3 levels of magnifications (naked eye, 2.5x, and 10x) for 2-Step group.

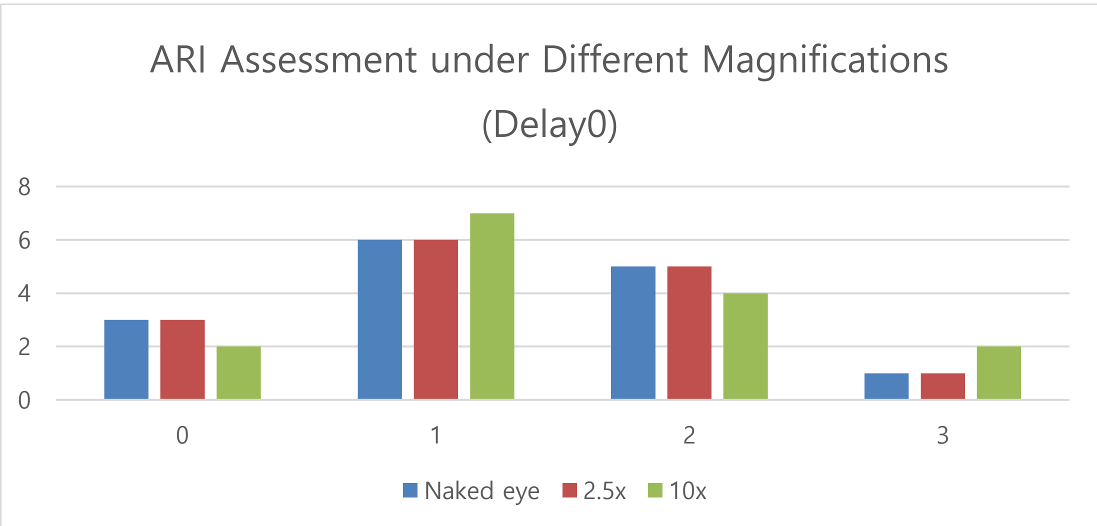

Figure 6. ARI score distribution under 3 levels of magnifications (naked eye, 2.5x, and 10x) for Delay0 group.

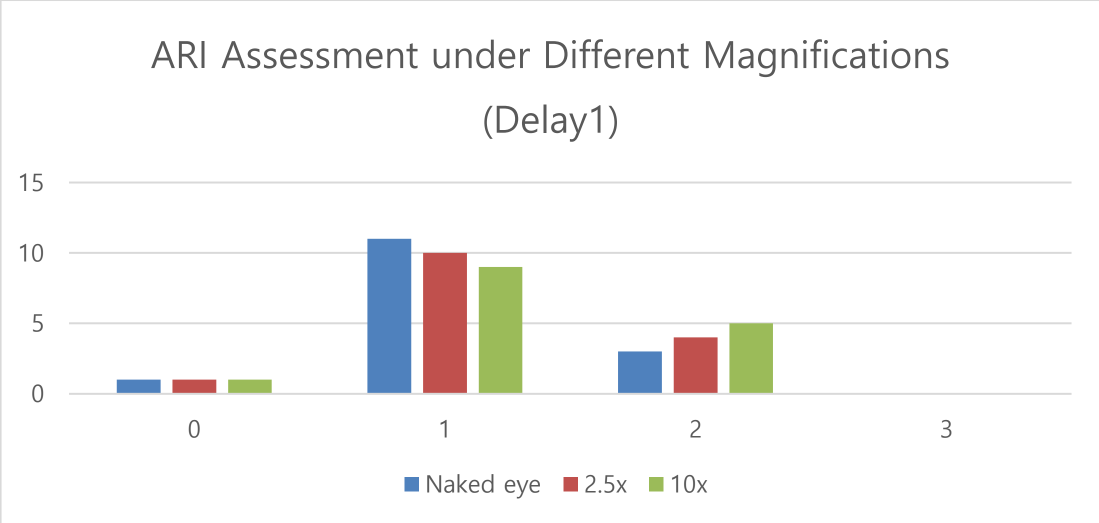

Figure 7. ARI score distribution under 3 levels of magnifications (naked eye, 2.5x, and 10x) for Delay1 group.

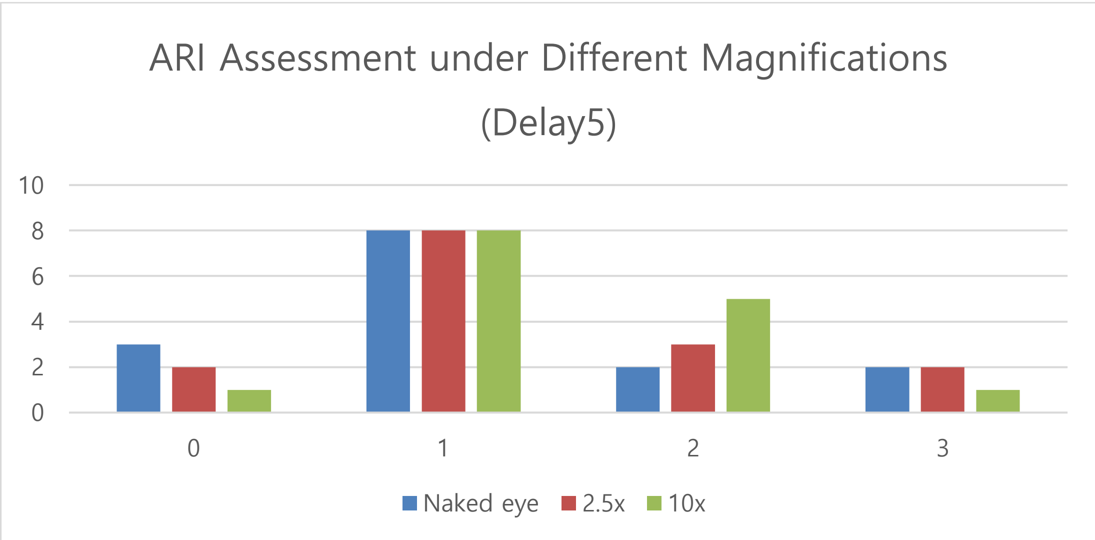

Figure 8. ARI score distribution under 3 levels of magnifications (naked eye, 2.5x, and 10x) for Delay5 group.

Discussion

One-step bonding technique using a self-etching primer has become an attractive bonding armamentarium to the current orthodontists (Fleming et al. 2012). By combining the etching and priming steps, self-etching primers simplified the bonding procedure, saving the chair time and reducing the chance of operator error. In addition to the procedural advantages of the self-etching primer, numerous studies have verified clinically sufficient bond strength of the orthodontic brackets using the self-etching primer (Türköz and Ulusoy, 2012; Zope et al. 2016). While acknowledging the clinical suitability of the self-etching primer, it is equally important to understand the factors that may impact its bond strength, especially those that may be modified in different clinical practices. In this study, various delays in curing time of the self-etching primer were investigated for its impact on the SBS and ARI scores of the orthodontic brackets.

Shear Bond Strength (SBS)

The results of this study showed that there was no significant difference in the SBS between the orthodontic brackets that were bonded using the two-step technique and the one-step technique using the self-etching primer. The SBS measurements of the 2-Step group and the one-step group (Delay0) from this study were 15.6±6.5 and 16.9±8.5MPa, respectively, with the value of the self-etching primer group being slightly higher than that of the 2-Step group. These values contradict the results from the previous studies that showed consistently lower SBS of the self-etching primer compared to the conventional phosphoric acid etchant and a primer (Başaran et al. 2009; Türköz and Ulusoy, 2012; Zope et al. 2016). In addition, the SBS values of the self-etching primer groups of this current study were greater than the measurements presented in a recent study by Zope et al., whom reported mean SBS of 10.93±4.02MPa for the one-step technique. The lowest mean SBS obtained from this present study in Delay5 group (12.0±6.4 MPa) was still higher than the measurement of the one-step technique presented by Zope et al. Despite the difference in the SBS values compared to the previous studies, more recent studies with similar experimental designs have reported similar bond strength values of the one-step system to the conventional system as well as bond strengths that are greater than the previously reported values (Fleming et al., 2012; Zhang et al., 2014; Schauseil et al., 2016; Alavi and Ehteshami, 2019). Therefore, comparable SBS measurements of the two bonding techniques as well as higher overall bond strengths measured in this present study compared to the previous studies may be contributed to improved adhesives due to recent advancement in dental materials. For example, improved infiltration of the self-etching primer in between the enamel rods during enamel conditioning, or enhanced bond strength between the primer and the resin adhesive could be the advancement that was implemented to the more recent self-etching primer.

As mentioned previously, the mean SBS of the 5-min delay group (Delay5) was the lowest (12.0±6.4 MPa) compared to those of Delay0 and Delay1, 16.9±8.5 and 17.0±8.4 MPa, respectively. However, statistical analysis of the results of this study showed that there was no significant difference in the SBS when the curing time of the self-etching primer was delayed for 0 second, 1, or 5 minutes. Based on this finding, the hypothesis that the SBS of the orthodontic brackets will be higher with longer delay in the curing time of the self-etching primer was rejected. It was speculated that longer delay in curing time, therefore longer application time of the self-etching primer, might increase the etching time of the tooth surface, allowing deeper penetration of the primer in between the enamel rods and subsequently improving the bond strength of the orthodontic brackets. However, the results of this study did not support this proposition. This result agrees with the findings of the study by Iijima et al., which found no difference in the final SBS with longer application time of the self-etching primer up to 30 seconds (Iijima et al. 2009).

Additionally, the mean SBS of all four groups of this study showed values that were above the clinically adequate range of 5.9-7.8 MPa reported by Reynolds. These values, however, were also all above 9-11 MPa which is found to be the lowest range of bond strength at which enamel fracture can be observed upon debonding of the orthodontic brackets (Diedrich 1981; Chun et al. 2014). Thus, the enamel fracture that occurred during SBS testing in three of the specimens in this present study can be explained by the SBS values that were greater than 9-11 MPa.

The three specimens, one in each 2-Step, Delay0, and Delay1 groups, were excluded from the data analysis due to enamel fracture that occurred during the SBS testing. The size of the fractures ranged from a small chip of the buccal surface to the size of a bracket, extending to the cemento-enamel junction (See Appendix Figure A). The damaged samples were excluded from the data collection and additional specimens were added to each group accordingly and tested again for SBS. This occurrence might have happened due to SBS values greater than 9-11 MPa, which is known to be the range of SBS for potential enamel fracture. Although the SBS values for these specimens were not included for the statistical analyses, they were 31.6 (2-Step), 26.7 (Delay0), and 22.6 (Delay1) MPa, which were all above the mean SBS values of each group.

Another possibility might be operator error during selection process by including specimens with small craze lines. These could have led to enamel fracture during the application of shearing force during SBS testing. The other factor could have been the age of the selected teeth, which was not controlled for the course of the sample collection. The older the tooth, the more likelihood for it to be damaged in response to shearing forces due to the presence of small cracks from years of mastication.

Adhesive Remnant Index (ARI)

In this study, the ARI scores were not significantly different between the two-step technique group and the one-step technique group. This finding disagrees with the results by Zeppieri et al and Montasser et al., which showed higher ARI scores with conventional two-step system compared to the one-step system (Zeppieri et al, 2003; Montasser et al. 2009). However, the result of this current study is supported by the study by Zhang et al., which also found no significant difference in the ARI scores between the conventional phosphoric acid etchant and primer group and the self-etching primer group (Zhang et al. 2014). In addition, the site of bond failure for the four groups occurred most commonly within the adhesive, either at the adhesive-enamel interface or at the adhesive-bracket interface, suggested by higher numbers of ARI score 1s and 2s. The ARI score 1 was seen most frequently in all four groups, which indicates weaker bond at the enamel-adhesive interface than at the adhesive-bracket interface (Figure 4). This further suggests that bond failure occurred most commonly at the enamel-adhesive interface and that less adhesive remained on the enamel surface after debonding of the bracket.

The ARI scores were not significantly different among the self-etching primer groups with different curing time. This finding of the study rejected the hypothesis that the ARI scores of the tooth surfaces will be higher with longer delay in the curing time of the self-etching primer. It was hypothesized that longer delay in curing time of the self-etching primer will result a longer application time of the primer on the tooth, therefore enhancing the mechanical union between the enamel rods and the composite resin. However, the results of this study did not support this theory. Despite the lowest mean SBS value of Delay5 group, its ARI score distribution was comparable to that of the other two self-etching groups. This suggests that the discrepancy between the SBS values of Delay5 and the other three groups might not be significant enough to impact the pattern of bond failure. Therefore, one of the discoveries of this study suggests that the type of enamel conditioning technique or the presence of delay in curing time of the self-etching primer does not impact the site of bond failure of the orthodontic brackets.

ARI Assessment under Different Magnifications

The ARI scores showed an upward trend as the level of magnification increased. When the ARI scores under 0 and 10x magnifications were compared, increased frequency of higher ARI scores were seen (Figures 5, 6, 7, and 8). One interesting finding was that the frequency of score 0 decreased when the ARI assessment was performed under 10x magnification compared to the naked eye in all groups except for Delay1, which showed equal number of score 0 under all three levels of magnification. This finding suggests that inspection for residual adhesive under lower magnification, especially naked eye, most likely underestimates the actual amount of remaining resin on the tooth surface. Therefore, this result advises the clinicians to be more thorough when cleaning up the adhesives after bracket debonding since there is a likelihood of inadequate removal of the remnant adhesives under low magnification.

Despite the differences in the number of ARI scores in each group, the results of this study showed that there was no significant difference between scores when the ARI evaluation was performed at different levels of magnifications. Therefore, the hypothesis that larger number of the higher ARI scores will be seen under higher magnification was rejected. This was an interesting finding as it was initially speculated that closer evaluation of the tooth surface under higher magnification will allow assessment of even minute amount of adhesive residue, resulting in increased number of higher ARI scores than under lower magnification. This finding agrees with the study by Montasser and Drummond (Montasser and Drummond 2009), which found no significant difference in the ARI scores when evaluated with the naked eye and under 10x magnification.

Clinical Suggestions

The results from this study infer that a delay of up to 5 minutes in curing time of the self-etching primer after its application does not significantly affect the final bond strength of the orthodontic brackets. However, since there appears to be a decreasing trend to the SBS with longer than 1-minute delay of the curing time, clinicians should be mindful of their working time during orthodontic bracket bonding to restrain from excessively delaying the curing time of the self-etching primer.

The analyses on the ARI scores also indicate that delay in curing time of the self-etch primer does not significantly impact the mode of bracket bond failure. However, despite the findings in the study, postponed curing time of self-etch primer in vivo may imply a different outcome. Extended intra-oral working time during orthodontic bonding procedure will increase the duration that the tooth surface is exposed to the humidity in the oral cavity as well as the chance of contamination of the bonding site, consequently increasing the risk of premature bracket bond failure. Therefore, efficient preparation of the enamel surface followed by bracket bonding are most advised to achieve more reliable bracket bond strength and hence uninterrupted orthodontic treatment.

Study Limitations

One factor that could have affected the results of this study is the storage time of the extracted premolars. The specimens were collected over a 9-month period, which could have impacted the condition of the enamel surface and therefore the bonding site for the brackets. Specimens that were collected earlier during the collection process were exposed to the distilled water for a longer period of time compared to the samples that were collected later, which could have affected the test results.

This present study attempted to simulate the intra-oral environment by immersing the tooth specimens in distilled water at 37ºC for 24 hours prior to SBS testing. Additionally, the masticatory forces were reproduced by applying vertical shearing forces to the teeth using the Universal Testing Machine. Though these attempts were valuable, the innate limitation of in-vitro study is that it is impossible to perfectly reproduce the environment of a living organism. The tooth specimens in this study were prepared and bonded in an environment with reduced humidity and without saliva, which provided an advantageous environment for bonding compared to the oral cavity. Therefore, such advantages during sample preparation could have contributed to higher SBS values of this study than the values measured in-vivo. Moreover, the shearing forces applied by the Universal Testing Machine is limited to testing one type of force. The intra-oral forces that the orthodontic brackets are exposed to are rather dynamic and are applied in various directions.

Future Studies

The present study demonstrated that a delay up to 5 minutes in final curing of the self-etching primer does not significantly impact the SBS of the orthodontic brackets or the ARI scores after debond. Despite the results from this current study, including more samples to further investigate the decreasing trend of the SBS values with 1 to 5-minute delay in curing time may be an interesting topic for a future study. In addition, future studies could examine the changes that may occur on the enamel surface with delays up to 5 minutes in curing time of the self-etching primer to see if any changes in the enamel surface are in correlation with the SBS results from this study. Lastly, another possibility for a future study is to investigate the SBS of the attachments for clear aligners using different adhesive systems. The attachment bonding procedure for clear aligner therapy is similar to indirect bonding procedure but is quite different from the conventional direct bonding. As of now, little is known about the attachment bonding in general, not to mention the newly developed bonding materials that are specifically designed for attachments.

Conclusion

This study concluded that the two-step bonding technique and the one-step bonding technique resulted in similar SBS. Though the mean SBS of the one-step technique was slightly higher than that of the two-step technique, the difference was not significant. This finding may be due to improved self-etching primer with advancements in dental materials. Furthermore, the result of this study supports the self-etching primer to be a suitable alternative to the conventional acid etchant and primer.

Secondly, the results of this study showed that delays up to 5 minutes in curing time of the self-etching primer did not significantly affect the SBS of the brackets or the amount of residual adhesive that remain on the tooth surface after debond. In addition, the mean SBS values of the one-step technique with and without delay in curing time of up to 5 minutes were above the minimum clinically adequate bond strength of 5.9-7.8 MPa.

Lastly, the degree of magnification did not significantly impact the ARI scores. There was no significant difference in the ARI scores when the specimens were assessed under three different magnifications (naked eye, 2.5x, and 10x).

Appendix

Shear Bond (MPa)	Sample #	Two-step	Delay0	Delay1	Delay5
	1	5.9	25.7	17.0	11.1
	2	23.1	6.9	4.5	16.0
	3	21.6	29.5	21.6	8.8
	4	14.3	14.5	13.1	9.0
	5	21.2	26.7**	20.5	24.6
	6	9.4	8.9	8.6	16.1
	7	14.4	11.1	26.4	9.7
	8	40.0*	16.5	20.8	16.7
	9	17.2	30.0	9.1	11.0
	10	18.3	16.9	28.0	3.1
	11	31.6**	19.6	13.2	6.8
	12	27.4	29.2	6.9	9.5
	13	8.4	8.4	22.1	23.4
	14	10.1	10.1	32.4	12.6
	15	8.2	6.6	12.6**	2.1
	16	18.9	19.7	9.8	

Table A. SBS measurements of the four group.

* indicates an outlier which was excluded from the data analysis

** indicates specimen on which enamel damage had occurred during SBS testing and was excluded from the data analysis

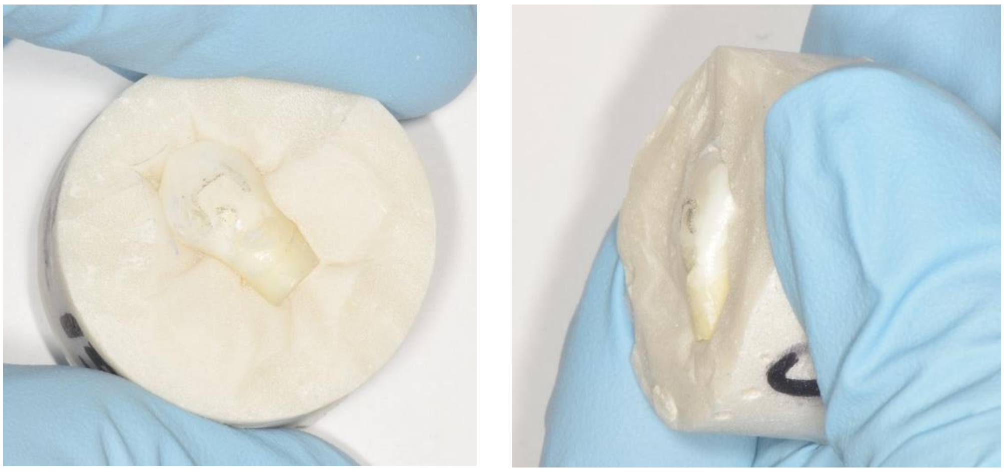

Figure A. Enamel fracture that occurred during shear bond strength testing.

0 1 2 3

Figure B. Examples of each ARI score; 0 = no composite remained on the enamel; 1 = <50% of composite remained on the tooth surface; 2 = >50% but < 100% of the composite remained on the tooth; 3 = 100% of the composite remained

Magnification	ARI Score			
	0	1	2	3
Naked Eye	1	11	3	0
2.5x Mag	0	9	6	0
10x Mag	0	9	6	0

Table B. ARI measurements of 2-Step group under 3 levels of magnification (naked eye, 2.5x, and 10x).

Magnification	ARI Score			
	0	1	2	3
Naked Eye	3	6	5	1
2.5x Mag	3	6	5	1
10x Mag	2	7	4	2

Table C. ARI measurements of Delay0 group under 3 levels of magnifications (naked eye, 2.5x, and 10x).

Magnification	ARI Score			
	0	1	2	3
Naked Eye	1	11	3	0
2.5x Mag	1	10	4	0
10x Mag	1	9	5	0

Table D. ARI measurements of Delay1 under 3 levels of magnifications (naked eye, 2.5x, and 10x).

Magnification	ARI Score			
	0	1	2	3
Naked Eye	3	8	2	2
2.5x Mag	2	8	3	2
10x Mag	1	8	5	1

Table E. ARI measurements of Delay5 group under 3 levels of magnifications (naked eye, 2.5x, and 10x).

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