Shear bond strength of a single use self-etching primer used multiple times over 24 hours

By

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

Objectives: To determine whether single use Transbond Plus™ self-etching primer (SEP) (3M Unitek, Monrovia, Calif) *in vitro* shear bond strength (SBS) will be affected if used multiple times over a prolonged period, for cost-effectiveness.

Materials and Methods: Transbond ™ Plus SEP was used to bond brackets to 120 extracted human teeth at three different time points after opening the SEP package: immediately- (as recommended by manufacturer), 4 hours-, and 24 hours after opening. Samples were debonded with a universal testing machine after 24 hours to assess short term SBS, and after 2 months to assess bond maturation.

Results: No statistically significant difference in SBS was found in the 24-hour debond group between the different time points of package opening, but there was a significant statistical difference for the 2-month debond group ($p<0.05$), which also showed an increased coefficient of variation range. SBS matured from the 24-hour debond group to the 2-month debond group but decreased or stayed the same when manufacturer guidelines were not followed.

Conclusions: Short term SBS is not affected when using an opened SEP package multiple times after 24 hours, but more long term SBS is affected, and shows more variable and inconsistent values. However, no group's minimum value decreased below the "clinically acceptable" standard of 6-8 MPa for *in vitro* SBS testing, suggesting a more cost-effective bonding protocol might be possible with Transbond ™ Plus SEP. It remains vital to pumice teeth before bonding with a SEP during the Covid-19 pandemic, even though pumicing is considered an aerosol generating procedure.

Acknowledgements

- My supervising committee:
    - Dr. William Wiltshire (Supervisor)
    - Dr. Robert Drummond (Internal Examiner)
    - Dr. Rodrigo França (External Examiner)
- GAC International (Don Grant) for the generous donation of products (Orthodontic buttons)
- All the staff, friends, and colleagues at the Graduate Orthodontics program at the University of Manitoba
- My family for all their support

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1. INTRODUCTION

Since Buoncore (1955) introduced the acid etching technique, several different concepts of bonding resins to enamel, have been developed. Today, conventional adhesive systems consist of 3 primary agents: an enamel conditioner, a primer and an adhesive resin (Bishara, Oonsombat, Ajlouni, 2004). This technique is also known as the total-etch technique or the conventional etching method (CEM) and is the most common bonding system used in orthodontics. (Ketona & Long, 2006). Over the years the primary etchant with the most retentive etching pattern has been phosphoric acid (30-40%) (Turk, Elekdag-Turk and Isci, 2007). Self-etching primers (SEPs), currently in the 6^{th} to 8^{th} generation, are adhesives which combine the acid etch and the primer simplifying orthodontic bonding procedures significantly, thus making it possible to eliminate the etching and rinsing step. This alteration has made it more attractive to clinicians in practise, for both simplicity and time saving. Another major advantage of SEP's is the shallower etch depth, this quality decreases the risk of enamel fractures during debonding (Hosein, Sheriff, Ireland, 2004). However, its effect on bond strength is a concern which potentially arises due to shallower etch depth.

In North America, Transbond™ Plus (3M Unitek, Monrovia, CA) is a widely used SEP for attaching brackets to both arches, repositioning brackets, and rebonding brackets. Transbond™ Plus combines the etchant and primer into one step, which eliminates the need for separate etching and drying afterwards. The active ingredient of Transbond™ Plus is a methacrylated phosphoric acid ester. The methacrylated group and the phosphoric group are combined into a molecule that can etch and prime at the same time (Elekdag-Turk, Cakmak, Turk, 2008).

According to manufacturer's, Transbond™ Plus is a SEP with a unique chemistry that allows multiple advantages above conventional etching methods. The SEP is less moisture sensitive due to the hydrophilic nature of the primer and it is an easy one-step application that allows for additional time to place brackets precisely. A shorter treatment window saves the clinician time, patient comfort is increased due to elimination of the etchant rinsing process, there is potentially no compromise in the bond strength, and less enamel is lost after etching due a shallower etch depth, which allows the etchant and primer to penetrate to the same depth (Hosein, Sherriff & Ireland, 2004).

Orthodontists want to utilize a material that is strong enough to withstand forces against accidental debonding for the course of the orthodontic treatment time but is also low enough to avoid enamel damage at debonding (Özcan, Finnema & Ybema, 2008). Wiltshire & Noble (2010) showed that for minimum reliable clinical bond strengths, *in vitro* testing should yield consistent values of at least 3 MPa to 4 MPa (Wiltshire & Noble, 2010).

According to Beckwith (1999), the second most important factor that affects treatment time is debonding of brackets, the first factor being missed appointments. It has also been observed in other studies that if two or more brackets debond, the treatment time will increase between 2.2 to 4.6 months (Skidmore, Brook, Thomson, 2006). Due to the frustration for the patient and the clinician, there are multiple contradictory *in vitro* studies published regarding the shear-bond strength of SEPs compared to CEMs.

Some studies have shown that SEPs have a lower bond strength than conventional etching methods, yet other studies show adequate bond strength with less enamel damage at debonding. Most studies have found the bond strength to be comparable with conventional etching methods (Reis, Santos, Loguercio and Bauer, 2008).

The instruction sheet of Transbond™ Plus clearly states that one SEP package is required for bonding of one arch only, and if both arches need to be bonded, two packages need to be used. Many clinicians prefer using Transbond™ Plus for full arch bondings of upper and lower teeth, due to the advantages of SEP's, and the simplicity of the product. Another reason is that it is not feasible or practical to keep multiple bonding agents in stock.

Transbond™ Plus is a single-use product as cross-contamination needs to be considered. Clinicians cannot use the SEPs on one patient and then insert a new applicator stick into a contaminated package from a previous patient (Cacciafesta, Sfondrini, Angelis et al., 2003). Clinicians can however theoretically use a new applicator stick for every tooth without contaminating the SEP package when used for repositioning and rebonding broken brackets. The average price for one Transbond™ Plus SEP package is $3.5 CAD, or $2.67 US. If a new package is used per patient for repositioning or rebonding of a few brackets, it would be significantly more expensive than using conventional etching methods and it would not be cost-effective.

2. LITERATURE REVIEW

2.1 Evolution of bonding

Buonocore is well known for opening the door to adhesive dentistry techniques (Bishara, Otsby, Ajlouni, Laffoon, & Warren, 2008). Buonocore (1955) showed that utilizing microscopic interlocks for bonding acrylic to enamel after acid etching with 85% phosphoric acid, has a 100-fold increase in retention and this is due to the formation of a mechanical bond (Bishara, Otsby, Ajlouni et al., 2008). According to Buonocore the increased adhesion is due to an increase in surface area, wettability, and an increase in polar bonding to the acrylic (Buonocore, 1955). This method of bonding was not widely accepted until the late 1970's (Dorminey, Dunn, & Taloumis, 2003).

The first generation of adhesives were studied by Buonocore and his colleagues, showing that using glycerophosphoric acid dimethacrylate-containing resin could bond to dentin if it was acid etched. The bifunctional resin molecule with calcium ions of hydroxyapatite was believed to be responsible for the bond (Kugal & Ferrari, 2000). The bond strength to enamel was high, but unfortunately dentinal adhesion was non-existent. Although some tubular penetration occurred, it was not sufficient as debonding would occur within several months after placement (Freedman, 2019). The bond strength ranged from 2 to 3 MPa, and therefore also required mechanical retention in the tooth preparation (Heyman, Swift et al., 2013). Unfortunately, this generation of adhesives were greatly affected by immersion in water and post-operative sensitivity was common (Freedman, 2019). In the late 1970's the second generation of adhesives were introduced as improvements were made in the adhesive coupling agents for composites. Bisphenol-A glycidyl methacrylate (bis-GMA) or hydroxyethylmethacrylate (HEMA) are halophosphorus esters of unfilled resins, they were incorporated to increase the adhesion to dentin (Kugal & Ferrari 2000). The bond strength ranged from 2-8 MPa and mechanical retention was also required in the tooth preparation. Ionic bonds between the positively charged calcium ions from the smear layer and the negatively charged phosphate ions were the mechanism of bonding for this generation of adhesives (Retief & Denys 1989, Kugal & Ferrari 2000). Extensive microleakage was reported in restorations with dentinal margins, and post-operative sensitivity was still reported in posterior occlusal restorations (Freedman, 2019).In the late 1980's the third generation adhesives were developed with the use of acid etch, and the revolutionary two-component primary/adhesive systems were introduced. The two components consisted of a hydrophilic primer and an unfilled resin adhesive which were dispensed separately. (Kugal & Ferrari 2000). The dentin bond strength ranged from 8-15 MPa and eliminated the need for mechanical cavity retention (Freedman, 2019). Less post-operative sensitivity was reported with posterior occlusal restorations, which initiated the launch of aesthetic and direct posterior occlusal restorations. Although weak, the third generation was also the first to bond dental metals and ceramics in addition to bonding to tooth structure (Freedman, 2019).

In the early 1990's the fourth generation, also known as the total-etch technique was developed by Fusayama and Nakabayashi in Japan, which completely transformed dentistry. The bond strength ranged from 17-25MPa and overcame polymerisation shrinkage (Freedman, 2019). The smear layer was completely removed by etching the preparation with 40 percent phosphoric acid for 15 to 20 seconds (Kugel & Ferrari 2000). The tooth surface was meant to be left moist ("wet bonding") so that the hydrophilic primer would infiltrate the exposed collagen and form the hybrid layer and resin tags. Hybridisation can be defined as the replacement of hydroxyapatite and water in the surface dentin with resin, which improved bond strength significantly. Within five years, in the mid 1990's, the fifth generation was developed with only two components: the etch, a pre-mixed primer combined with the adhesive in 1 bottle (Freedman, 2019). This generation reduced post-operative sensitivity, simplified the technique and generated more consistent bond strengths of 20-25 MPa. The sixth-generation adhesives are characterized by achieving sufficient bond strength bonding to enamel or dentin by using a one-step bonding system (Kugal & Ferrari 2000). In the year 2000 extensive efforts were made to eliminate the etching step so that no rinsing would be required.

Bonding protocols with no separate etching step is a sixth-generation adhesive characteristic. Although the bonding process is one step, there are typically two components that need to be mixed prior to use (Freedman, 2019). Self-etching primers (SEPs) such as Transbond™ Plus is an example of a sixth-generation adhesive.

In 2002 the seventh generation of adhesives were introduced. This generation simplified the two components into a one-component, one bottle system with no pre-mixing requirements. The bond strength ranges from 18-35MPa to dentin and similar micromechanical adhesion strength to unprepared and prepared enamel surfaces. (Freedman, 2019). For 17 years there has not been a major advancement in adhesion technology as the existing adhesion systems were predictable, effective and accepted universally. The only way adhesives could be improved was to create a zero-step adhesive, which led to the invention of the eight-generation adhesive in 2002.With this generation the adhesive is incorporated into the restorative material (Işman et al., 2012). The composite resin has the ability to etch, prime and bond to enamel and dentin, requiring just polymerization to finish the restoration. The development and advances made over the years were focused on restorative dentistry primarily, but orthodontics have benefitted greatly from the evolution of bonding. 2

Table 2.1 Evolution of bonding first-to eight-generation summary

Generation	Characteristics	Bond strength	Components	Brand names
1st	Very weak bond to dentin	2 MPa	1	Cervident (SS White Co), Cosmic Bond (Amalgamated Dental)
2nd	Weak adhesives, requires retentive preparations, prone to water degradation	2-8 MPa	2	Bondlite (Kerr), Scotchbond (3M)
3rd	Two-component primer and adhesive system, reduced sensitivity	8-15 MPa	2-3	Scotchbond II (3M), Prisma Universal Bond (Johnson and Johnson)
4th	Hybridization, total-etch	17-25 MPa	2-5	All Bond II (Bisco)
5th	Single component, moist bonding, no mixing, little sensitivity	20-24 MPa	1	Single Bond (3M), One-Step (Bisco)
6th	Multicomponent, self-etching, self-priming, very little sensitivity	18-23 MPa	2-3	Transbond Plus SEP (3M), Clearfil SE Bond (Kuraray)
7th	Single component, desensitizing, self-etching, self-priming, no mixing, bonds to metal, very little/no sensitivity	18-25 MPa	1	iBOND (Heraeus Kulzer), G-Bond (GC Corporation)
8th	Single component, self-etching, self-adhering flowable composite	Inconsonant results	1	Vertise Flow (Kerr), Maxcem Elite (Kerr)

Inspired from (Freedman 2019)

2.2 Enamel structure

Enamel is a highly mineralized crystalline structure creating a rigid structure that is both brittle and strong. Enamel is the hardest tissue of the human body. Hydroxyapatite is the main component of enamel, and constitutes for 90 to 92% of volume, the other constituents include 4 to 21% water and 1-2% of organic matrix proteins (Heymann, Swift, et al., 2013). Average calcium and phosphorus values can range from 37.5% and 17.5% respectively (Robinson, Weatherell et al., 1971). The principal structural element is the enamel rod, also referred to as the enamel prism. The enamel rod is enclosed by a rod sheath which is comprised of an organically rich interspace and a cementing inter-rod substance in certain areas. The average diameter of an enamel rod is about 8 μm at the outer enamel surface which decreases to about 4 μm at the dentino-enamel junction (Heymann, Swift, et al., 2013). Enamel is soluble in low pH environments and the solubility is variable and dependant on the amount of fluoride present in the enamel.

2.3 Enamel bonding

Orthodontists use the acid-etching technique to bond brackets to the enamel surface. The primary concern of the clinician is maintaining a sound and unblemished enamel surface after debonding (Bishara et al., 2004). When bonding to enamel it is desirable to have bond strengths high enough to resist forces of accidental debonding during the course of orthodontic treatment, but low enough to prevent enamel fracture at debonding (Özcan, Finnema, & Ybema, 2008). Bonding to enamel involves attention to preparation of the tooth surface, the attachment surfaces and the bonding material that joins the two (Proffit, 2013). Preparation of the tooth surface is typically initiated by removal of the enamel pellicle with a non-fluoridated pumice. Acid-etch is then used to convert the smooth enamel to a roughened surface area through dissolution of the interprismatic substance in enamel (Nanjannawar & Nanjannawar 2012). A porous enamel surface is created, and a resin primer-adhesive penetrates the porous surface to result in the formation of micro-mechanical interlocking or resin tags after polymerization of the adhesive. (Heymann, Swift et al., 2013).

2.4 Enamel etching

Thirty-seven percent phosphoric acid etching causes dissolution of interprismatic enamel which produces a porous and roughened enamel layer, ranging from 5 to 50 µm² (Nanjannawar and Nanjannawar 2012). The purpose of etching is to create surface irregularities that provide micro-mechanical retention with a primer-adhesive through dissolution of hydroxyapatite of enamel. Different etch patterns can be identified simultaneously on the enamel surface under scanning electron microscopy (SEM). Five types have been observed and classified (Silverstone, Saxton et al., 1975).

Table 2.2 Enamel etching patterns

Types	Enamel etching patterns observed
I	Honeycomb appearance, enamel prism cores preferentially removed
II	Cobblestone appearance Relatively unaffected prism cores, dissolution of interprismatic areas
III	Types I and II observed together
IV	Pitted enamel, unfinished maps appearance
V	Unaffected, flat smooth surface

Inspired from (Silverstone, Saxton et al. 1975)

Type II etching patterns cause the most enamel loss, and type I causes minimal enamel loss. Type II etching patterns correspond mostly to the conventional etching methods (CEMs) with phosphoric acid, and type IV corresponds with self-etching primers (SEPs), a conservative etching pattern leading to minimal loss of enamel (Nanjannawar & Nanjannawar 2012). SEPs produce shallower etching patterns due to inferior penetration of the acidic primer into the enamel porosities or due to the calcium precipitation on the enamel surface, masking the etching pattern. The acidic primer does not get rinsed off as with the CEM, therefore causing calcium and phosphorus ions released from hydroxyapatite to get suspended in the primer solution (Dorminey et al., 2003).

2.5 Conventional bonding to enamel

The conventional etching method (CEM) for bonding orthodontic brackets to the enamel requires three different agents: an enamel conditioner, a primer solution and an adhesive. The conventional bonding system is classified as a fifth-generation adhesive (e.g., Transbond™ Plus XT Light Cure Adhesive Primer) and is known as the gold standard for bonding orthodontic attachments to enamel. Phosphoric acid seems to be the most frequently used method for enamel conditioning (Turgut, Attar et al., 2011). One of the disadvantages of etching with phosphoric acid is demineralization of most of the superficial layer of enamel (Buyukyilmaz, Usumez and Karaman, 2007). Conventional bonding systems are often used as a control when evaluating the bond strengths of alternate adhesive methods like SEPs or self-adhering resin composites (Bishara, Oonsombat et al., 2004, Bishara, Ajlouni et al., 2006, Turk, Elekdag-Turk et al., 2007, Scougall Vilchis, Yamamoto et al., 2009, Scougall-Vilchis, Ohashi et al., 2009, Ho, Akyalcin et al., 2011).

Phosphoric acid gel or solution is the first component of the CEM, it is also the most commonly used enamel conditioner with the highest reported retentive etching pattern at a concentration of 30 to 40% (Craig, Powers and Sakaguchi, 2006). After etching for approximately 15 seconds, the enamel conditioner is rinsed thoroughly with water from the enamel, and then dried to produce a frosty white appearance that indicates dissolution of the hydroxyapatite. It has been recommended that each quadrant needs to be rinsed for 15 seconds, and that the rinsing time should be doubled when using an acid gel. The rinsing and drying stage can be difficult for both the clinician and patient due to the need for an uncontaminated enamel surface, free from saliva (Gwinnett, 1982). The second step has two components in one bottle: a primer and an adhesive. The primer consists of hydrophilic monomers, polymers, or oligomers. The solvents used in primers can be acetone, ethanol-water, or primarily water. Adhesives are hydrophobic dimethacrylate oligomers (e.g., Bis-GMA) that are diluted with a lower molecular weight monomer (e.g., Tryethylene glycol dimethacrylate- TEGDMA). This step involves placing a thin layer of primer-adhesive to freely flow into etched enamel porosities to form micro- and macro-tags once polymerized (Craig, Powers et al., 2012).

2.6 Bond failures and factors involved in a clinical setting

Bond failures can be due to multiple factors that can be challenging to identify. Bond failures could be due to protocol, operator technique, or the product itself (Solid, 2018). Protocol factors could range from contamination from saliva, blood, gingival crevicular fluid, bacteria on the tooth surface, tooth type and condition, poor oral hygiene and variations in process steps. Operator technique factors that could affect bond strength include application of the primer, adhesive and curing errors. Another factor to consider is the product used, factors like bracket base design, curing light performance, storage and expiration dates could also affect bond failures. Anatomical variations such as the amount of prismless enamel has also been reported more on posterior teeth, it has been proposed that this may also affect the quality of acid etch and the resultant bond (Hobson, Rugg-gunn, & Booth, 2002). By reducing the number of steps of a bonding procedure it becomes easier to identify the cause of bond failures, this can be very challenging with so many factors playing a role (Solid, 2018). Therefore, using SEPs has a reduced risk of debonding compared to CEMs, simply because it requires less steps and there is less room for error.

2.7 Self-etching primers and bonding to enamel

Self-etching systems contain ester monomers with carboxylic or phosphate acid groups that are dissolved in water. The principal component of SEPs is methacrylated phosphoric acid ester, which comprises both the acidic component for etching and the monomer component for priming (Bishara, VonWald, Laffoon, & Warren, 2001). These systems can be classified according to their aggressiveness and can be divided into strong (pH of 1 or less), moderate (pH between 1 and 2), or mild (pH between 2 and greater). Self-etching systems can be categorized as a two-step-system or an all-in-one system. The two-step system has a hydrophobic bonding resin from a separate bottle, these systems are known as self-etching primers (Craig and Powers et al., 2012). SEPs include the sixth-, seventh- and eight generation adhesives. These products do not require a separate etching step. The only difference between the two generations of SEPs are the number of components and the mixing requirements prior to use. The sixth generation necessitates mixing of a primer and adhesive prior to application on the tooth and the seventh generation has simplified the two components into a one-component, one bottle system with no pre-mixing requirements at all. The seventh generation is also known as the no-mix or the premixed SEPs (Bishara, Oonsombat et al., 2004, Bishara, Otsby et al. 2008), they are described as “all-in-one” products (Farah & Powers, 2005). Most bonding agents are light-cure systems and contain camphorquinone as an activator, along with an organic activator amine (Craig and Powers et al., 2012). The conventional etching method with 37% phosphoric acid is useful in orthodontics, but there is still a need for improvement in order to maintain clinical bond strength, while also minimizing enamel loss and reducing the number of steps (Cal-Neto, Quintão, de Oliveira Almeida, & Miguel, 2009). In 2002 it was reported that 20% of orthodontists use SEPs in North America (Keim, Gottlieb, Nelson et al., 2002).

2.7.1 Self-etching primer advantages

Self-etching primers are popular in orthodontics bonding as they eliminate the washing and drying steps required with the conventional etching method. SEPs will therefore have the advantage of saving clinical time, reducing procedural errors, minimizing technique sensitivity, reducing the risk of salivary contamination, producing a more conservative etching pattern and minimizing enamel loss at debond (Cal-Neto, Quintão, de Oliveira Almeida, & Miguel, 2009).

The main advantages of SEPs are less steps, less operator technique-sensitivity, while still maintaining adequate bond strengths (Cal-Neto, Miguel et al. 2006, Vilchis, Hotta et al. 2007, Paschos, Westphal et al. 2008, Turgut, Attar et al. 2011, Nanjannawar and Nanjannawar 2012). SEPs were reported to save 10.2 seconds per tooth, a total of 204 seconds (3.4 minutes) when bonding both arches. The benefits of fewer bonding steps, less chair time, and a more conservative etch pattern should be weighed against the increased cost of SEPs (Pasquale, Weinstein, Borislow et al., 2007). Bonding is a technique sensitive procedure, and moisture contamination has been reported to be the most common cause of bond failures (Cacciafesta et al., 2003, Rajagopal et al., 2004) Traditional composite resin bonding materials require dry surfaces to obtain a clinically acceptable bond strength. However, there are difficult clinical situations that do not permit ideal isolation, especially when bonding brackets or attachments on second molars, close to the gingiva, or when attaching buttons to impacted or partially erupted teeth (Öztoprak, Isik, Arun et al, 2007). When teeth get contaminated the porosities caused by the etching gets plugged, resulting in insufficient in number of resin tags and lengths, this results in decreased micromechanical retention (Öztoprak et al., 2007). Conventional etching methods (CEMs) do not offer adequate bond strengths under moisture contamination conditions as they are hydrophobic. Transbond™ Plus Self-Etching Primers are considered hydrophilic and will therefore potentially perform more reliably in moisture contaminated environments (Rajagopal et al, 2004).

It has been demonstrated that the hydrophilic solvents, such as alcohol and acetone, are capable of displacing water from the enamel surface and facilitate the penetration of the adhesive into the enamel microporosities created by the etching (Cacciafesta et al., 2003, dos Santos, Quioca et al., 2006). *In vitro* studies have demonstrated that the relative humidity of the mouth is considerably high and it is known that this also has a profound effect on the bond strengths of CEMs, however SEPs are not as sensitive to this variable (dos Santos, Quioca et al. 2006). It has been reported that SEPs offer adequate bond strengths under dry and contaminated environments, however, the bond strength did decrease with contamination, but it was reported to still be adequate for successful bonding (Rajagopal et al., 2004, Oztoprak, Isik et al., 2007, Prasad, Mohamed et al., 2014).

2.7.2 Transbond™ Plus Self Etching Primer

In the late 2000's 3M Unitek (Monrovia, California) introduced Transbond™ Plus Self-Etching Primer, a sixth-generation adhesive, which combines the conditioning, rinsing and priming steps. The primer comes in a single-use foil package, which contains two bubbles that need to be pressed and folded or mixed with the 3M™ Easy Roller in order to combine the components prior to use (Grubisa, Heo, Raboud et al., 2004). The primary indication for use is orthodontic bonding to enamel (Holzmeier, Schaubmayr, Dasch et al., 2008). Once the components have been mixed and activated, the primer is rubbed on the enamel for three seconds with an applicator stick that is provided, followed by a gentle 1 to 2-second air burst. The bracket is ready for placement after this step as no rinsing is required (Grubisa et al., 2004). The two compartments that require mixing contain polyalkenoic acid, 2-Hydroxyethyl methacrylate (HEMA), water and stabilizers in one compartment and methacrylate phosphoric acid esters, bisphenol A-glycidyl methacrylate (Bis-GMA), photo-initiators and stabilizers in the other. (Holzmeier et al., 2008).

2.7.3 Transbond™ Plus Self Etching primer mechanism of action

Methacrylated phosphoric acid is the active ingredient in Transbond™ Plus Self-Etching Primer (Grubisa et al., 2004). The methycrylate group and the phosphoric acid are combined into a molecule that etches and primes at the same time (Buyukyilmaz, et al., 2003). The etching component is classified as a strong acid etch with a pH of 1.0, which also contains no volatile organic compound content. The etching and monomer penetration into the open enamel rods are simultaneous, and the depth of the etch is identical to that of the primer penetration (Buyukyilmaz, et al., 2003). Calcium gets dissolved and removed from the hydroxyapatite by the phosphate group of the methacrylated phosphoric acid ester, rather than being rinsed away as with conventional etching (Buyukyilmaz, et al. 2003, Grubisa, Heo et al. 2004, Pasquale, Weinstein et al. 2007). There are three processes that need to occur in order to arrest the action of the acid etch. The first process is the same as phosphoric acid, the phosphate group forms a complex with the calcium from the hydroxyapatite, but the calcium gets incorporated into the primer upon polymerization and not washed away as with the CEM. The second process is the gentle air burst that drives the solvent away from the primer, this increases the viscosity and therefore slows down the acid etching mechanism (Grubisa, Heo et al. 2004, Pasquale, Weinstein et al. 2007). Thirdly, when the primer is light cured, the monomers are polymerized and the transport of the acid groups to the enamel is finalized.

The importance of rubbing the SEP and the airburst is explained by the mechanism of action, the rubbing provides a fresh etch, and the airburst removes the solvent and slows down the etching. These two steps are essential in order to provide reliable and reproducible bond strengths (Grubisa, Heo et al. 2004, Pasquale, Weinstein et al. 2007). The primer contains solvent such as water, ethanol and acetone that needs to be removed, if air drying is omitted it could inhibit resin polymerization accounting for the decreased lower bond strengths (Miyazaki, Hirohata, Takagaki et al., 1999).

A uniform frosty appearance does not appear after etching so it can be difficult to determine if enamel is adequately etched. It has been recommended that the suggested etching time provided by the manufacturer might not offer sufficient shear bond strengths (Dorminey et al., 2003).

Table 2.3: Principal ingredients Transbond Plus ™ Self-Etching Primer as provided by manufacturer:

Ingredients	% by Wt Trade Secret*
2-Propenoic acid, 2-methyl-,phosphinicobis (oxy-2,1- ethandiyl) ester	30-45
Methacrylated pyrophosphates	15-35
Mono HEMA Phosphate	15-30
TRIS[2-(methacryloyloxy)ethyl]phospate	1-10
4-Methoxyphenol	<2
dl-Camphorquinone	1-2
Ethylene dimethacrylate	<2
N,N-Dimethylbenzocaine	<2
Phosphoric Acid	<2
2-hydroxyethyl methacrylate	<1

(3M Unitek Safety Data Sheet 02/26/20)

2.7.4 Transbond™ Plus Self Etching Primer *in vitro* testing

Transbond™ Plus Self Etching Primer (TB+SEP) has been reported to perform well with *in vitro* experiments. Transbond™ XT Primer, the conventional etching primer (CEP) is normally used as a control to compare results in a lot of studies. Significantly higher SBS for Transbond™ Plus Self Etching Primer of 16.0 ± 4.5 MPa was found, compared to 11.5 ± 3.3 MPa attained with a CEM. (Buyukyilmaz, et al., 2007). Two studies showed decreased bond strength of Transbond™ Plus Self Etching Primer, of which one was tested as an immediate debond strength (Grubisa, Heo et al., 2004, Ho, Akyalcin et al., 2011). Multiple other studies have reported that *in vitro* tests exhibited no statistical difference between Transbond™ Plus Self Etching Primer and CEM methods (Arnold, Combe et al., 2002, Cal-Neto, Miguel et al., 2006, Vicente, Bravo et al., 2006, Turk, Elekdag-Turk et al., 2007, Scougall Vilchis, Yamamoto et al., 2009, Scougall-Vilchis, Ohashi et al., 2009, Ho, Akyalcin et al., 2011). It been found that better shear bond strengths are obtained as time elapsed (Turk, Elekdag-Turk et al., 2007, Ho, Alyalcin et al., 2011).

The most reasonable explanation for increased bond strength with time is that most of the free radicals initially produced are at the periphery of the resin and bracket where there is complete light exposure, and further diffusion of these free radicals require time to continue polymerizing under the bracket base (Turk, Isci et al., 2007). Studies have also shown that CEM and TB+SEP are both negatively affected by the presence of saliva, but the experiments showed that TB+SEP performed superiorly in that environment while still exhibiting acceptable clinical bond strengths compared to CEP (Rajagopal, Padmanabhan et al., 2004, Oztoprak, Isik et al., 2007, Prasad, Mohamed et al., 2014).

Transbond™ Plus Self Etching Primer is also commonly used as a reference to compare new SEPs on the market (Buyukyilmaz, Usumez et al., 2003, Bishara, Otsby et al., 2008, Scougall-Vilchis, Ohashi et al., 2009, Ho, Akyalcin et al., 2011). Studies have shown that TB+SEP performs equally or better than other SEPs (Buyukyilmaz, Usumez et al., 2003, Bishara, Oonsombat et al., 2004, Bishara, Otsby et al. 2008, Scougall Vilchis, Yamamoto et al., 2009, Scougall-Vilchis, Ohashi et al., 2009). It has also been reported that G-Bond produced significantly higher bond strengths than TB+SEP immediately after bonding (Ho, Akyalcin et al., 2011) and that Clearfil SE Bond produced better bond strengths after 48 hours (Arhun, Arman, Sesen et al., 2006).

2.7.5 Transbond™ Plus Self Etching Primer-clinical trails

Although *in vitro* studies can provide a more standardized method for evaluating the performance of dental materials, these tests do not simulate the oral environment closely, and therefore cannot be used in isolation to predict how the material will perform clinically. Factors that are not taken into consideration during *in vitro* testing are the possibility of salivary contamination, masticatory forces on brackets, temperature fluctuations due to food or beverages, degradation of the adhesive on exposure to saliva, plaque, as well as clinician clinical skills (dos Santos, Quioca et al., 2006). The performance of a bonding agent during *in vivo* studies can be determined by the bond failure rate. Bond failure rate is calculated by dividing the number of debonded brackets by the number of bonded brackets over a period of time, and then multiplied by one hundred percent (Powers, Kim et al., 1997). Transbond™ Plus Self Etching Primer has been compared to CEMs in multiple randomized controlled trails over six to 12 months (Aljubouri, Millett et al., 2004, Manning, Chadwick et al., 2006, Banks and Thiruvenkatachari 2007, Elekdag-Turk, Isci et al., 2008, Reis, dos Santos et al., 2008, Cal-Neto, Quintão et al., 2009, Dominguez, Tortamano et al., 2013). Most of these studies used the split mouth technique where teeth in the maxillary left and mandibular right quadrants received one pre-treatment, and teeth in the maxillary right and mandibular left received the alternative pre-treatment (Ireland, Knight & Sheriff, 2003).

Transbond™ Plus Self Etching Primer has been shown to have higher clinical bond failure rates by 3 studies (Ireland, Knight et al., 2003, Murfitt, Quick et al., 2006, Elekdag-Turk, Cakmak et al., 2008) and only one study showed that the clinical bond failure rate was lower with Transbond™ Plus Self Etching Primer compared to CEM (dos Santos, Quioca et al., 2006). In this study the manufacturer guidelines of brushing the enamel for 3 seconds was not followed as in the other studies, this study brushed the enamel for 10 to 15 seconds, three to five times the recommended time. This study indicates that the duration of brushing the enamel is of essence when using Transbond™ Plus Self Etching Primer. Previous reports with SEPs have shown that the agitation of the SEP on the enamel surface for double the recommended time can increase the resin-enamel bond strength, improving sealing and the etching pattern of the enamel significantly (dos Santos, Quioca et al., 2006). The majority of *in vivo* studies concluded that Transbond™ Plus Self Etching Primer has no significant difference in clinical failure rates when compared to CEMs

(Aljubouri, Millett et al., 2004, Manning, Chadwick et al., 2006, Banks and Thiruvenkatachari 2007, Elekdag-Turk, Isci et al., 2008, Reis, dos Santos et al., 2008, Cal-Neto, Quintão et al., 2009, Dominguez, Tortamano et al., 2013). Most of the studies identified that the SEP one step procedure was significantly less time consuming than CEMs with more steps.

2.8 Bond strength testing

In vitro studies have the ability to standardize procedures and accurately determine the strength of a material, but it cannot be used to predict clinical performance of a material, neither can it substitute *in vivo* studies use (Finnema, Özcan, Post et al., 2010). *In vivo* testing in randomized controlled trails is the most reliable way to test the effectiveness of a bonding system and the effects on enamel, however, it is almost impossible to independently determine the potential of a bonding material due to all the variables that can affect the quality and longevity of bonding to enamel. There are several methods that can cause bracket debonds when studying bond strength, these methods include shear bond strength, tensile strength and torsional strength tests. Both shear and tensile testing are valid ways to assess orthodontic bond strengths, but torsional testing is more challenging to perform and not used often (Katona & Chen 1994, Powers et al., 1997). Advantages of *in vitro* testing are the simplicity of the methodology, the ability to measure a specific variable while maintaining other parameters, speed of data collection, low cost and the ability to compare the strength of new materials to previously tested materials (Van Meerbeek, Peumans, Poitevin et al., 2010).

In order to use and compare results from *in vitro* studies accurately, experimental conditions need to be standardized with clear and uniform guidelines. When *in vitro* results are commendable for a specific material, it is also important to always be evaluated by *in vivo* randomized clinical trials for clinical use (Finnema et al., 2010).

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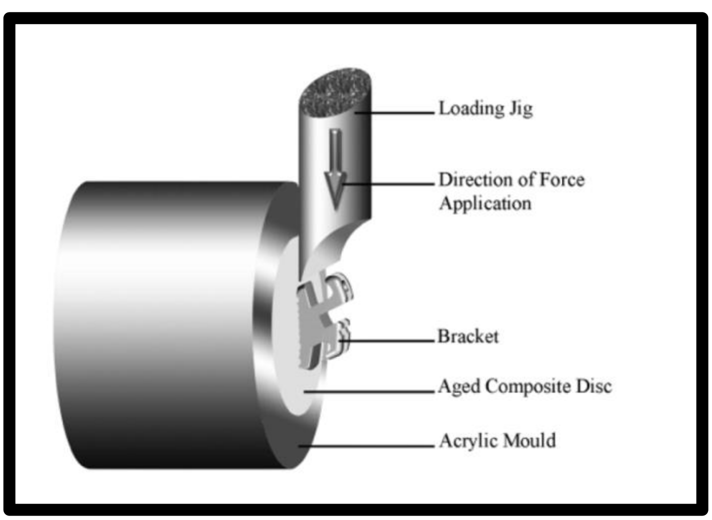
2.8.1. Shear bond strength

Pure shear loading is difficult to achieve as most of the time shear testing has a component of peeling, torsion and tension that is an unavoidable during the test phase (Katona 1994). Shear-bond strength testing is still the most widely used experiment used for debonding brackets despite the unavoidable element of bending during testing (Finnema et al., 2010). Most studies reporting shear bond strengths are testing shear-peel bond strength in reality, due to the difficulties of obtaining a pure shear bond strength at the bracket-enamel interface (Katona, 1994, Powers et al., 1997). With shear bond testing, a load is applied to the bracket-enamel interface with a blade under stress of a load cell (kN) at a given speed measured in millimeters per minute (mm/min). The crosshead speed when debonding the bracket can vary from 0.1 to 5 mm per minute, but most studies use a speed of 0.5 mm per minute. In order to produce a shear force, the location of the force should be at the bracket-enamel interface, with the shearing blade sliding parallel to the enamel surface of the tooth at 0 degrees (Katona, 1994; Powers et al., 1997).

Figure 2.1 Schematic illustration of shear bond strength testing



(Bayram, Yesilyurt et al, 2011)

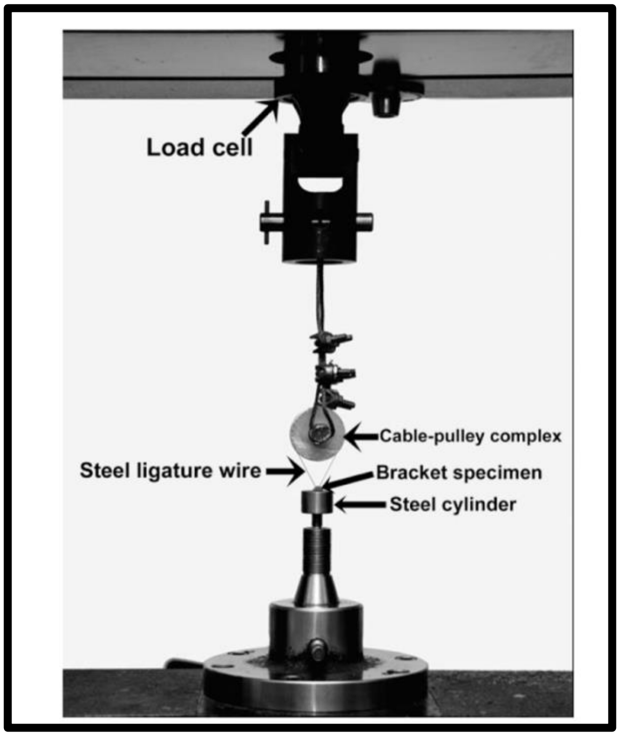
2.8.2 Shear-peel bond strength

Shear-peel bond strength, very similar to shear bond strength, is also measured in N/mm^2= MPa. To obtain the peel component the force has to be applied parallel to the enamel surface but a distance away from the bracket-bonding interface. The amount of peel incorporated is directly proportional to the distance the force is applied away from the bracket-bonding interface. The further the force is applied from the enamel surface; the greater the generated moment will be (Katona 1994). It is difficult to determine the exact magnitude of shear and peel forces, but in reality, all articles that are reporting shear bond strength, are reporting shear-peel bond strength (Katona 1994, Katona 1997, Eliades and Brantley 2000).

2.8.3 Tensile bond strength

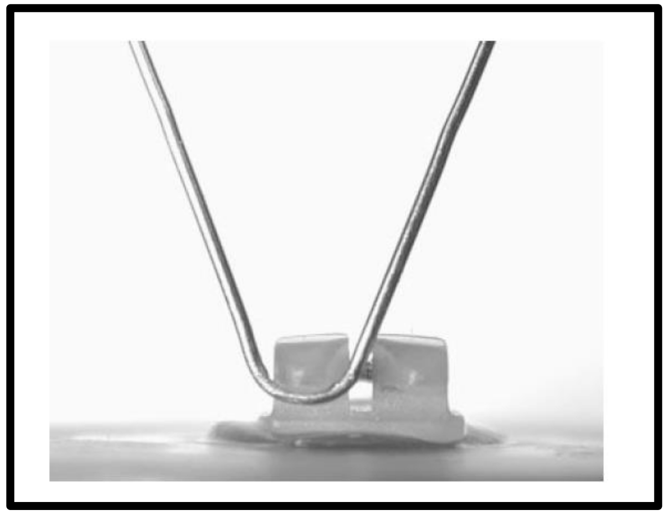
Tensile strength can be defined as the ratio of maximum load a material can withstand when being stretched or elongated without fracturing (Johnson, Walker & Kula, 2004). In clinical tensile testing the bracket is pulled perpendicularly from the enamel surface (Phan, Akyalcin, Wiltshire & Rody, 2011). The orthodontic attachments are removed by pulling a wire loop that is harnessed around the bracket (Katona and Chen 1994, Reicheneder, Gedrange et al. 2009). All measurements are taken in the central part of the specimen, away from the clamping location to produce a uniform stress field. The local tensile stress is then calculated by dividing the load by the cross-sectional area of the bracket. The loop harness adaptation and frictional resistance may make interpretation of results more complicated, therefore it was proposed by Katona and Chen (1994) to use long and thin wires in such experimental models (Eliades and Brantley 2000).

Figure 2.2 Tensile bond strength testing



(Johnson et al., 2004)

Figure 2.3 A 0.014 steel ligure wire looped under disto-incisal wing for tensile testing



(Johnson et al., 2004)

2.8.4 Testing machine

In vitro experiments often utilize a mechanical testing machine that is either servo-hydraulic or screw-driven. The classification is determined by the movement of the crosshead and the application of load on the samples. Servo-hydraulic machines move the crosshead by pumping oil pressure into a hydraulic piston, whereas screw-driven machines have screws on either end of the crosshead (Phan, Akyalcin, Wiltshire, & Rody, 2011). In either type, the crosshead is driven with a predetermined load towards the specimen that is being tested, and the load cell will sense the force applied and relay the raw data on a computer.

Generally, the weight of the load cell is approximately 1 kN for bracket adhesion and heavier loads are used for bigger objects, however, researchers over the years have used load cells of up to 20 kN (Wiltshire, 2021-Personal communication).

The software will plot the force applied over the time or distance of the crosshead movement, and if desired, stress versus strain plots can be generated using software that has been calibrated with the surface area of the orthodontic attachment (Brantley and Elaindes, 2000). These machines are often referred to as universal testing machines as they can be used for compression, shear, bending, torsion and tension experiments. Both machines are also referred to as constant strain-rate machines, as the rate of movement of the crosshead can be set to a designated displacement speed. For orthodontic testing the crosshead speed typically ranges from 0.1 to 0.5 mm per minute (Cheba, 2012). Two of the most common testing machines used in dentistry and orthodontics include the Instron (Norwood, MA) and the Zwick Universal Testing Machine (Ulm, Germany) (Brantley & Eliades, 2000)

2.8.5 Standardization and experimental conditions of in vitro bond strength testing

In vitro experiments allow for more standardization for testing a specific bonding system, however, there are various test conditions that can hamper the comparison of different *in vitro* results. A meta-analysis by Finnema et al. from 2010, showed that there are 27 experimental conditions that can affect the results of *in vitro* bond strength testing. The parameters identified by Finnema et al. were the enamel origin (i.e., bovine vs human), type of teeth, pre-treatment of enamel surface (e.g., grinding and means of cleaning), substate storage before bonding (e.g., physiologic saline solution or water), storage time before bonding, storage temperature, bracket material, type of bracket, type of etchant, time of etching, adhesive type, force at bracket placement, total polymerization time, curing light device type, light direction, sample storage time, sample storage solution, sample storage temperature, thermocycling, testing machine, test mode (e.g., tensile or shear testing), crosshead speed, force location on bracket, blade design, Adhesive Remnant Index (ARI), magnification used to determine ARI and bond strength reported in MPa. (Finnema, Ozcan et al. 2010).

These different testing conditions can explain why there are contradictive outcomes between *in vitro* bond strength studies. Unfortunately, many studies omit to mention these experimental conditions, and therefore uniform guidelines and standardization of experimental conditions of *in vitro* bond strength research is essential to accurately compare results and conclusions from different studies (Finnema, Ozcan et al. 2010).

In most experiments, storage time can range from no storage, in immediate testing experiments, to 5 years (Williams & Svare, 1985). Rueggeberg suggested that 6 months should be the normal storage time (Rueggeberg 1991), however, some experiments start testing shear bond strength from 2.5, 5, 10, 15 and 30 minutes, arguing that testing after 24 hours does not simulate clinical practice as a load is applied immediately after tying in an arch wire (Turk, Eledag-Turk et al., 2007). Storage solutions usually include thymol, formalin, saline, aqueous chloramine, tap or distilled water at varying temperatures and concentrations (Eliandes and Brantley, 2000). Enamel of extracted premolars or third molars are the most commonly used substrate in these experiments. The teeth used should preferably be sound, uncut, non-fluoridated enamel with no restorations or anatomical abnormalities (Eliades and Brantley 2000). The crosshead speed that has been suggested for uniformity is 0.5 mm/min (Elaindes, Viazis et al. 1991, Kao, Eliandes et al. 1995). Photopolymerization time, water storage and crosshead speed were shown to have the most significant effect on shear bond strength results out of all the experimental conditions according to Finnema et al, but according to Cheba et al. there is no significant difference in shear bond strength results, even with varying photopolymerization times and crosshead speeds (Cheba, 2012).

Table 2.4: Experimental conditions that can influence results of shear bond strength results

27 Experimental conditions
1. Substrate origin (bovine or human teeth)
2. Type of teeth (premolars or wisdom teeth)
3. Storage time before bonding
4. Storage temperature before bonding
5. Storage solution before bonding (saline, thymol, distilled water etc.)
6. Cleaning of specimens (grinding enamel)
7. Bracket material
8. Type of bracket
9. Type of etchant
10. Time of etching
11. Adhesive type
12. Amount of force at bracket placement
13. Curing light device type
14. Total polymerization time
15. Light directions
16. Sample storage time
17. Sample storage solution
18. Sample storage temperature
19. Thermocycling
20. Testing machine
21. Shear testing as test method
22. Crosshead speed
23. Force location on bracket (at bracket-enamel interface or bracket wings)
24. Blade design (shearing blade or a wire loop)
25. ARI
26. Magnification used to determine ARI
27. Bond strength in MPa

Inspired by Finneman *et al*, 2010

2.8.6 Bond strength and debonding force

Bond strength can be defined as a measure of interfacial adhesion between the bonded material and the substrate, facilitated by an adhesive material (Eliandes, 2012). Units of bond strength are often expressed in MegaPascals (MPa), kilograms per square inch (kg/cm^2), as well as pounds per square inch (lb/in^2 or psi). MegaPascal (MPa) is currently accepted as the preferred unit when reporting bond strength (Wiltshire and Noble, 2010). Bond force is often recorded in Newtons (N), kilograms (kg) and pounds (lb), but bond strength is calculated by the bond force divided by the surface area of the bracket base in mm^2. A typical bracket has a nominal bonding surface of 16 mm^2 (Powers, Kim et al., 1997). The bond force is normally measured in shear or tension with a universal testing machine, but torsion forces have also been observed. The goal of bond testing is to achieve a coefficient of variation that ranges from 20-30% (Powers, Kim et al., 1997).

2.9 Optimum bond strength in orthodontics

It is difficult defining the "ideal bond strength", as every patient is unique with respect to enamel etching ability, intraoral factors and their individualized masticatory functions (Wiltshire and Noble, 2010). The ideal orthodontic adhesive should have adequate bond strength to retain orthodontic brackets for the desirable treatment duration, while also maintaining unblemished enamel (dos Santos, Quioca et al. 2006). The average force transferred to a bracket during mastication has been reported to range from 40-120 Newtons (N); thus, the adhesive bracket system should be able to withstand an applied force of 7.5 MPa with a typical bracket surface area of 16 mm^2 (Powers, Kim et al. 1997). More specifically, Wiltshire and Noble reported the average force of mastication on anterior brackets to be close to 5 MPa, and approximately 20 MPa on posterior teeth. (Wiltshire and Nobel, 2010). Bond strength ranging from 6 to 8 MPa has been reported in multiple *in vitro* studies as the "clinically acceptable" standard for shear bond strength (Reynolds 1975, Joseph and Rossouw 1990, Whitlock, Eick et al., 1994, Finnema, Ozcan et al.

2010, Wiltshire and Noble 2010). For minimal reliable clinical bond strengths to occur, i*n vitro* shear bond strength testing needs to yield at least 3 or 4 MPa for the lowest values of the range, based on clinical data using glass ionomer adhesives (Wiltshire and Noble, 2010, Fricker, 1994). To determine whether a bonding material could perform consistently in a clinical setting it is important for the researcher to consider the range of shear bond strength values *in vitro*, and not just the means (Wiltshire and Noble 2010). According to Retief, bond strength should be less than 14 MPa in order to prevent enamel fractures, however enamel fractures were recorded at values as low as 9.7 MPa in this study (Retief, 1974). Higher bond strengths are not necessarily more optimal clinically as bond strengths that are too high may do nothing but result in iatrogenic damage (Wiltshire and Noble, 2010).

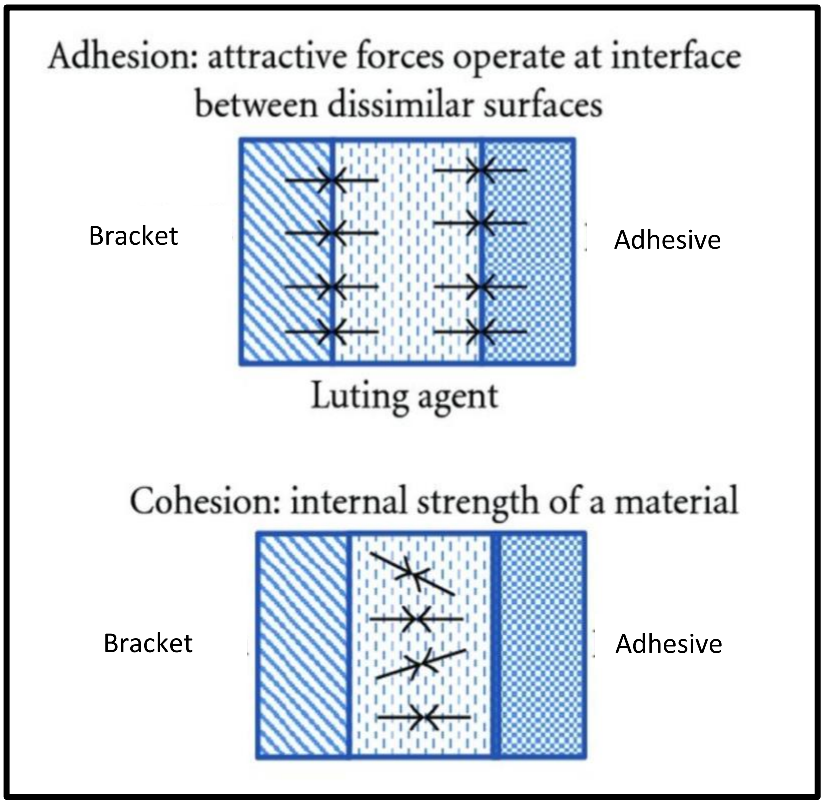
2.10 Adhesive Remnant Index and bonding failure site

Bracket bond failures are one of the most frustrating aspects of any orthodontic practice, resulting in increased cost, staff, treatment time and additional visits by the patient. If the bond failure site can be identified it is easier for the orthodontist to modify his or her bonding technique (Powers, Kim et al., 1997). The location of the bond failure makes it easier to identify the cause, therefore it is important to understand the significance of bond strength in a clinical setting. All orthodontic bonding systems have at least two interfaces, the enamel/adhesive interface and the bracket/adhesive interface (Powers, Kim et al. 1997). The Adhesive Remnant Index (ARI) is a grading system that indicates the cohesive or adhesive nature of the orthodontic bond. failure

Adhesive forces are attractive forces between substances of different molecular species that have been brought into direct contact, e.g., a tooth surface and adhesive material in direct contact. Cohesive forces are intermolecular forces such as Van der Waals forces that cause like-molecules to resist separation. Cohesion may be defined as the internal strength of a material. (Fraunhofer, 2014). Failure at the enamel might indicate reduced depth of demineralization and, therefore less adhesive remains on the tooth surface, decreasing the amount of time required to clean the adhesive at debond (dos Santos, Quioca et al. 2006).

With CEMs the failure is normally cohesive, and according to Bishara and Velo et al., self-etching primers have more adhesive bond failures rather than cohesive detachment at debond. (dos Santos, Quioca et al. 2006).

Figure 2.4 Adhesion and Cohesion between tooth, adhesive and bracket



Inspired by (Fraunhofer 2014)

In 1984 Årtun and Bergland developed the Adhesive Remnant Index with a gradation of 0 to 3, this was used to qualify the type of bond failure (Årtun and Bergland 1984). In 1999 Bishara modified the index, with a score ranging from 1 to 5 (Bishara, VonWald et al. 1999). A microscope is used to observe the tooth surface and the remaining adhesive at a magnification of X10.

Table 2.5 Adhesive Remnant Index (Årtun and Bergland)

Score	Criteria
0	No adhesive remaining on tooth surface
1	Less than half of adhesive remaining on tooth surface
2	More than half of adhesive remaining on tooth surface
3	All adhesive remaining on tooth, distinct impression of bracket mesh

(Årtun and Bergland 1984)

Table 2.6 Modified Adhesive Remnant Index (Bishara et al.)

Score	Criteria
1	All composite remaining on tooth with impression of bracket base
2	> 90% composite left on tooth
3	10-90% composite left on tooth
4	< 10% composite left on tooth
5	No composite left on enamel

(Bishara, VonWald et al. 1999)

Bond failure between the bracket and the adhesive resin or within adhesive resin are the preferred fracture modes as they minimize the potential for enamel fractures, which can occur if the bond fails at the enamel adhesive resin interface (Holzmeier et al., 2007). Failure mode of the brackets depends on various factors like cohesive strength of adhesive, brackets base morphology, the bond strengths achieved (Diedrich, 1981). Bonding materials with low bond strengths generally show debonding at the adhesive-enamel interface, and materials with higher bond strengths show adhesive-bracket failures or cohesive failures (dos Santos, Quioca et al. 2006). A scanning electron microscopy (SEM) examination of the enamel surfaces revealed that Transbond™ Plus Self Etching Primer showed a less aggressive etching pattern, and a reduced amount of adhesive remained on the teeth (Montasser, Drummond et al. 2008). More enamel damage was found with the CEP technique (Zhang, Yao et al. 2014). It must also be borne in mind that the removal of adhesive remnants from enamel with hand or rotary instrumentation, may also cause damage to the enamel (Wiltshire 2021- Personal communication).

2.11 Enamel loss and self-etching primers

Significant differences have been reported regarding enamel loss after acid etching due to the variation of the etchant type, concentration, and contact time in different experiments (Hosein and Sheriff et al., 2004). It has been reported that the total amount of enamel loss after etching, debonding and polishing is 55 µm. The amount off enamel loss after 15 to 30 seconds of etching with 37% phosphoric acid ranges from 8.8 µm to 16.4 µm (Legler, Retief et al, 1990), however, according to a more recent study the range of enamel loss ranges from 1.11 µm and 4.57 µm (Hosein, Sheriff, Ireland et al., 2004). In the same study it was reported that SEPs have reduced the depth of enamel etching significantly, with enamel loss ranging from 0.03 µm to 0.74 µm (Hosein, Sheriff et al., 2004).

The shallower etching depths from self-etching materials are due to their low acidity; furthermore, hydroxyapatite extraction is hindered by a higher calcium and phosphate content within the primer's suspended aqueous solution. The higher concentration of calcium and phosphate limits the etching depth of the enamel surface (Holzmeier et al. 2007). Due to the shallower etching depth, SEPs produce less adverse effects to enamel, and also simplifies the debonding appointment for the orthodontist (Øgaard and Fjeld 2010). Hosein and Sheriff et al. concluded that CEMs lead to more enamel loss than Transbond™ Plus self-etching primer, and at debond more adhesive remains on the enamel surfaces with CEMs than with SEPs (Hosein, Sheriff et al., 2004). That said, there is no agreement on the inherent enamel repair process between shallower and deeper etching processes and the caries development process between the two variables. Suffice to say that it is presumed that the shallower etch depths are the preferred biologically prudent option (Wiltshire 2021- Personal communication).

3. PURPOSE

The purpose of this study was to evaluate whether the shear bond strength (SBS) of single use self-etching primer (SEP) (Transbond ™ Plus) will be affected after using it over a prolonged period after being opened, in opposition to the manufacturer's instructions, for cost-effectiveness.

In practise clinicians are known to use Transbond ™ Plus self-etching for orthodontic bonding procedures over prolonged periods, in contravention to the manufacturer's instructions, which are strictly for single use. The purpose of this study, accordingly, was to evaluate whether clinicians can utilize this specific SEP over prolonged periods of time without negatively affecting shear bond strength.

4. NULL HYPOTHESES

1) There is no statistically significant difference in the shear bond strength between using Transbond™ Plus self-etching primer as a single use product (manufacturer guidelines), or multiple times over 24-hours

2) There is no statistically significant difference in shear bond strength (SBS) from the 24-hour debond group to the 2-month debond group.

3) There is no statistically significant difference in the Adhesive Remnant Index (ARI) scores with varying opening time of the package or associated debonding times.

4)The mode of bond failure of Transbond™ Plus self-etching primer is of a mixed adhesive cohesive nature.

5. MATERIALS AND METHODS

Orthodontic brackets were bonded with Transbond™ Plus self-etching primer to non-carious extracted human third molars. Three different groups were tested, and two *in vitro* shear bond strength tests were performed on each tooth.

5.1 Ethical Considerations

Ethics approval was acquired through Health Research Ethics Board of the University of Manitoba to get approval prior to commencing this study. Ethics was approved on December 11^{th}, 2019 (Appendix 11.1).

5.2 Teeth collection and storage

The sample included 120 sound extracted human third molars. The teeth were collected from Oral and Maxillofacial Surgeons in Winnipeg, Canada. The teeth were stored in 0.5% Chloramine T until the teeth were used in the study. The teeth were carefully selected in order to exclude teeth with cavities, restorations and/or abnormalities. Only healthy teeth with anatomically similar flat buccal and/or lingual surfaces were selected.

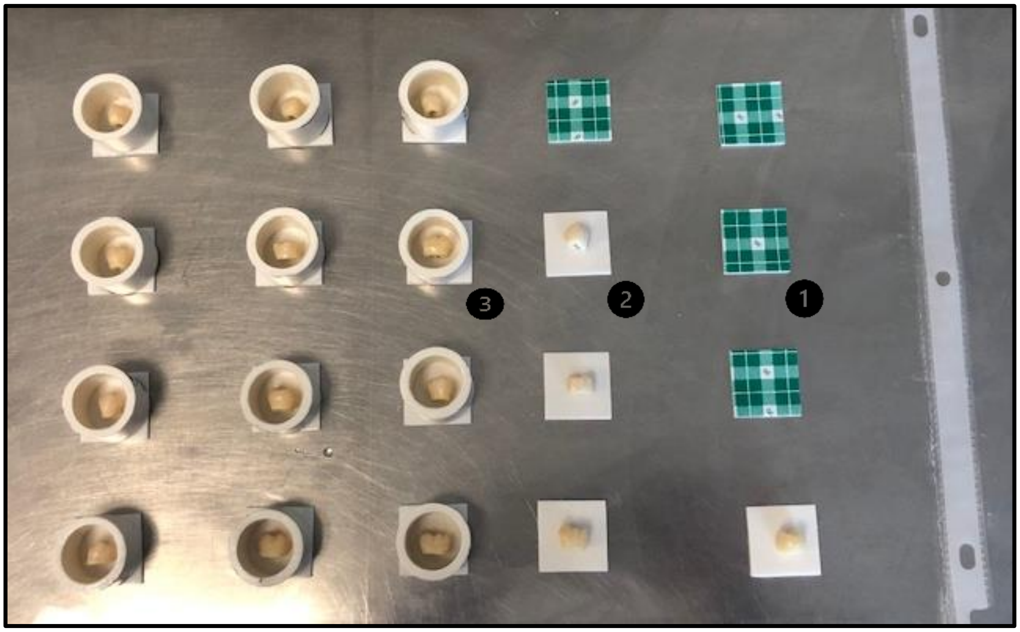
Table 5.1 Materials used in the experiment

Material	Manufacturer	Reference number
Tooth preparation		
NSK 500 Ultimate Handpiece	NSK America Corp.	
Circular diamond separating disc		
Polyvinyl chloride (PVC) cylindrical moulds		
Bosworth Fastray	Bosworth, IL	0921375
Monomer liquid		0921375
Polymer powder		0921375
Prophy Paste	Ortho Technology, Tampa Bay, Florida	15486
Scotch® Permanent Mounting Squares	3M Unitek, Monrovia, California	
Bonding Agents		
Transbond™ Plus Self Etching Primer	3M Unitek, Monrovia, California	5011222
Transbond ™XT Adhesive Paste	3M Unitek, Monrovia, California	933317
Bonding materials		
MicroArch® brackets	GAC International, Central Islip, NY	72-612-60
Loading apparatus gauge	Federal: Miracle Movement 0.001” C81S, Providence,	
Applicator sticks (fine tips) for each tooth	Microbrush® International, Gafton, USA	MFP400
3M™ Easy Roller to mix SEP	3M Unitek, Monrovia, California	
Ortholux™ Luminous Curing Light	3M Unitek, Monrovia, California	
Debonding materials		
Universal testing machine	MTS Landmark® Servohydraulic Test System, Eden	
Bencor Multi-T testing apparatus	Danville Engineering, San Ramon, CA	
Leica EZ4 Stereo microscope	Ontario, Canada	2942700
Storage chemicals		
Distilled water		
Chloramine-T trihydrate 98%	Acros Organics, NJ	
Other		
Incubator 37°C	Thelco/Canlab Model 2, Precision Scientific, Chicago,	

5.3 Tooth preparation

The extracted teeth were rinsed with distilled water and wiped with gauze to remove remnant soft tissue, and then sectioned with a circular diamond disc on a straight hand piece. The teeth were sectioned at the cemento-enamel junction. After the roots were discarded, the crowns were embedded into Bosworth Fastray (Bosworth, IL), a self-curing acrylic, within polyvinyl chloride (PVC) cylindrical moulds. To assure the buccal or lingual surfaces are parallel to the horizontal plane, the teeth were placed on adhesive tape (Scotch® Permanent Mounting Squares), which were positioned on a flat surface (e.g., a table or a flat working bench) to secure the teeth in a parallel position (Figure 5.1). When the position of the surface was parallel to the horizontal plane, the self-cure acrylic was flowed over the teeth until the PVC mould was three quarters full (Figure 5.2). After approximately 8 minutes of cure time, the set acrylic with the embedded teeth was removed from the PVC moulds. When the PVC moulds are flipped to the other side, the flat, parallel and uncovered surface of the 3rd molar was be visible (Figure 5.3). After the acrylic moulds have been removed, they were stored in distilled water and placed into an incubator 37°C at 100% Relative Humidity for 24 hours. This kind of storage was chosen to ensure complete polymerization of the acrylic at oral conditions, as well as to ensure adequate water sorption equilibration of the bonding material.

Figure 5.1 Teeth positioned horizontally on flat surface; polyvinyl chloride (PVC) cylindrical moulds placed on Scotch® Permanent Mounting Squares



1) Adhesive tape (Scotch® Permanent Mounting Squares)
2) Flat surface of teeth placed on adhesive tape
3) Polyvinyl chloride (PVC) cylindrical moulds

Figure 5.2 Self-cure acrylic flowed over the teeth until the PVC mould is three quarters full

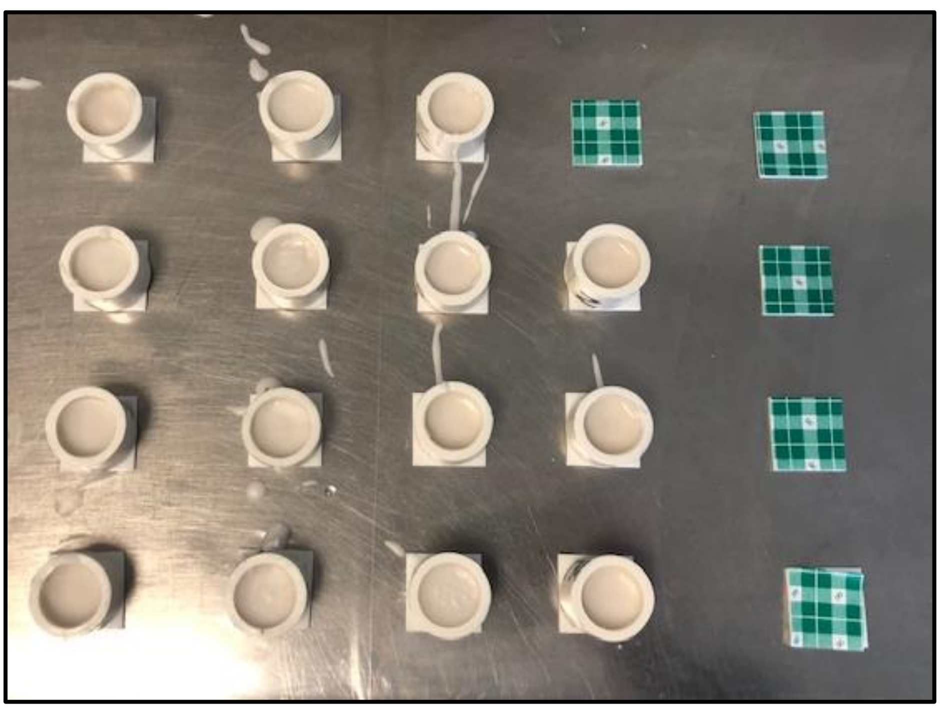


Figure 5.3 Teeth imbedded in acrylic removed from the PVC moulds, with the flat surface visible

5.4 Adhesive materials

Transbond™ Plus Self Etching Primer

Transbond™ Plus Self Etching Primer that was used, is a hydrophilic sixth generation light-cured bonding agent. This eliminates the need for conventional etching prior to application of the primer. The product consists of two liquids in two separate reservoirs that requires mixing before application on the teeth. In this experiment the reservoirs were mixed with the 3M™ Easy Roller. The product comprises of Ingredients in table 5.2 (MATERIAL SAFETY DATA SHEET 3M Unitek Transbond Plus Self Etching Primer (712-090, 712-091) 02/26/20.

Figure 5.4 Transbond™ Plus Self Etching Primer being mixed with 3M™ Easy Roller

Table 5.2 Composition/information on ingredients of Transbond™ Plus Self Etching Primer

Ingredients	% by Wt Trade Secret*
2-Propenoic acid, 2-methyl-, phosphinicobis (oxy-2,1- ethandiyl) ester	30-45
Methacrylated pyrophosphates	15-35
Mono HEMA Phosphate	15-30
TRIS[2-(methacryloyloxy)ethyl]phospate	1-10
4-Methoxyphenol	<2
dl-Camphorquinone	1-2
Ethylene dimethacrylate	<2
N, N-Dimethylbenzocaine	<2
Phosphoric Acid	<2
2-hydroxyethyl methacrylate	<1

(3M Unitek Safety Data Sheet 02/26/20)

Transbond™ XT Light Cure Paste Adhesive

The adhesive paste consists of a light-cured resin composite used for direct and indirect bonding of orthodontic attachments such as metallic and ceramic brackets. Its delivery is available in syringes and capsules. The components consist of the ingredients in table 5.3. (MATERIAL SAFETY DATA SHEET 3M Unitek Transbond XT Light Cure Pate Adhesive (712-036) 07/30/20).

Figure 5.5 Transbond™ XT Light Cure Paste Adhesive

Table 5.3 Composition/information on ingredients of Transbond™ XT Light Cure Paste Adhesive

Ingredients	% by Wt Trade Secret*
Silane treated quartz	70-80
Bisphenol A Diglycidylether Methacrylate (Bis-GMA)	45-55
Triethylene Glycol Dimethacrylate (TEGDMA)	45-55
Silane treated silica	<2
4-(Dimethylamino)-Benzeneethanol	<0.5

(3M Unitek Safety Data Sheet 07/30/20)

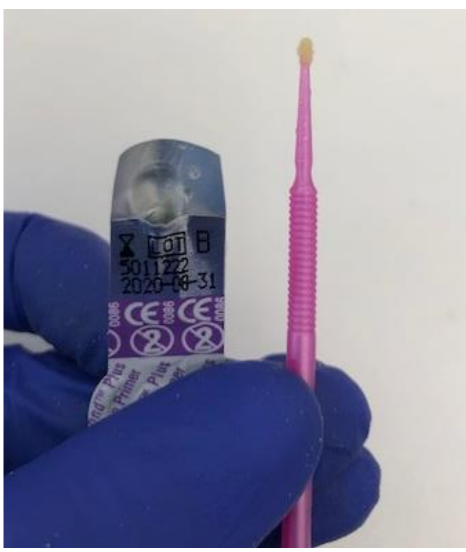
5.5 Bonding protocol

Before starting bonding, it was decided to remove some acrylic superior to the bonding surface of the tooth, this was done to facilitate a resistance free movement for the blade, so the blade touches the bracket alone (Figure 5.8). Lower incisor MicroArch® brackets provided by Dentsply GAC International (Islandia, NY, USA) were used as the orthodontic attachments in this study. The surface area of the bracket is 8.85 mm^2, as disclosed by the manufacturer. One hundred and twenty teeth were randomly divided into 3 test groups. Prior to bonding, the teeth were cleaned with a non-flavored, non-fluoridated prophy paste (Ortho Technology, Tampa Bay, FL) for 10 seconds and then washed and dried. The bonding and rebonding protocols for each group were performed as follows:

Bonding procedure with Transbond ™ Plus self-etching primer

After cleaning the teeth, one hundred and twenty teeth were bonded with the sixth-generation adhesive system, Transbond ™ Plus self-etching primer (3M Unitek, Monrovia, California) at different time points after opening the package (Table 5.4). After the package was opened and used, the end of the package was folded between bonding individual teeth (Figure 5.6). The Transbond ™ Plus self-etching primer was mixed as per the manufacturer instructions with the 3M™ Easy Roller. After mixing the SEP was applied on the tooth surface with a microbrush (Microbrush® International, Gafton, USA) for 5 seconds, making sure the tip of the brush was saturated. A gentle airburst was delivered onto the SEP or tooth surface for 2 seconds. A new mircobrush was used for every tooth to simulate the clinical procedure where cross contamination is avoided. A uniform amount of Transbond XT Adhesive Paste was placed on the mesh pads and the brackets were placed on the surfaces of the teeth mounted in acrylic. A 500g vertical loading apparatus (Miracle Movement) was used to provide a homogeneous seating of the brackets to the teeth. The excess resin was removed using an explorer probe, and the teeth were light cured at an intensity of 1000 mW/cm^2 for 5 seconds mesial and 5 seconds distal to the brackets. An Ortholux LED curing light lamp (3M Unitek, Monrovia, California) was used as per recommendation by the manufacturer.

<u>Figure 5.6 Saturated tip of microbrush</u>



<u>Figure 5.7 Folded package between individual bondings</u>

<u>Figure 5.8 Acrylic removed superior to bracket/ bonded surface to facilitate a resistance free movement for the sheering blade</u>

Test groups and Shear bond strength test groups

The total 120 human extracted teeth were divided into 3 different test groups according to the time elapsed from when the SEP has been opened to when the brackets were bonded.

<u>Table 5.4 Summary of bonding protocols for the 3 test groups and their debond times:</u>

Group	Time elapsed from when SEP package was opened:	Number of teeth	Storage Time/ Debonding time	Number of teeth
A	0 hours (control)/ bonded brackets immediately	40	24 hours	20
			2 months	20
B	4 hours / brackets bonded after package was open for 4 hours	40	24 hours	20
			2 months	20
C	24 hours/ brackets bonded after package was open for 24 hours	40	24 hours	20
			2 months	20

Rationale for exposure times chosen:

Group A represents 0 hours of exposure, or immediate bonding of the brackets after the SEP package was opened. This group was chosen as a control. Group A is used as a reference point to assess the shear bond strength of the Transbond ™ Plus self-etching primer if used as a single use product, as recommended by the manufacturer, as well as to compare to group B and C.

Group B, at 4 hours of exposure or 4 hours of delaying bracket bonding after the SEP package was opened. This group represents the morning from 8am-12pm of a theoretical working day in an orthodontic practice.

Group C, at 24 hours of exposure represents or delaying bracket bonding for 24 hours after the SEP package was opened. This group represents the next morning/ the next working day at 8am, and was chosen as an extreme to evaluate if there will be major changes in the shear bond strength compared to group A and B.

Table 5.5 Summary of the experimental conditions

Experimental conditions	
Substrate origin	Human Enamel
Type of teeth	Extracted third molars (120)
Storage time before bonding	Variable
Storage temperature before bonding	4°C (refrigerator)
Storage solution before bonding	Distilled water with 0.5% Chloramine T
Cleaning of specimens	Prophy Paste (Ortho Technology) for 10 seconds
Bracket material	Stainless steel
Type of orthodontic attachment	MicroArch® brackets GAC International, Islandia, NY
Type of etchant	Transbond™ Plus SEP
Time of etching	5 seconds (Transbond™ Plus SEP)
Adhesive type	Composite resin, Transbond™ XT Adhesive Paste
Amount of force at bracket placement	500g vertical loading apparatus (Miracle Movement)
Light device type	Ortholux LED curing light lamp (3M Unitek, Monrovia, California)
Total polymerization type	1000 mW/cm^2
Light directions	5 seconds mesial, 5 seconds distal
Sample storage time	24 hours and 2 months
Sample storage solution	Distilled water
Sample storage temperature	37°C, 100% humidity
Thermocycling	No
Testing machine	MTS Landmark® Servohydraulic Test System, Eden Prairie, MN
Shear testing as test method	Yes
Crosshead speed	0.5 mm/min
Force location	At the tooth-bracket interface
Blade design and load cell	Knife edged shearing blade, 1 kN
ARI	Yes, score 1 to 5
Magnification used to determine ARI	10X magnification
Bond strength in MPa	Yes

5.6 Storage conditions

After bonding, all teeth were stored in glass containers that were filled with distilled water and placed in an incubator at 37°C at 100% Relative Humidity. The teeth were stored for the same period of time as the proposed shear bond strength test times (described below). i.e., T1 for 24 hours, and T2 for 2 months after the initial bonding. The rationale for these selected debonding times was to test short-and long term SBS.

5.7 Debonding procedure and data collection

Two shear bond strength tests were completed as follows:

T1: First shear bond strength test, 24 hours after initial bonding (60 teeth).

T2: Second shear bond strength test, 2 months after initial bonding (60 teeth).

All the teeth were mounted into the **Bencor Multi-T testing castle** (Danville Engineering, San Ramon, CA) and placed in the MTS Landmark® Servohydraulic Test System (Eden Prairie, MN) device, which was used to record the SBS with a crosshead speed of 0.5 mm/min using a 1 kN load cell. The measurements were recorded on a computer (Dell, Round Rock, TX) and linked to the testing machine. The data was collected in MegaPascals (MPa). As mentioned before, the surface area of the brackets used was provided by the manufacturer. The total bracket surface area is 8.85mm^2.

The data was automatically be converted by the computer in MegaPascals using the equation:

$$1\ \frac{N}{mm^2} = 1\ MPa$$

Figure 5.9 Tooth mounted onto the Bencor Multi-T Apparatus

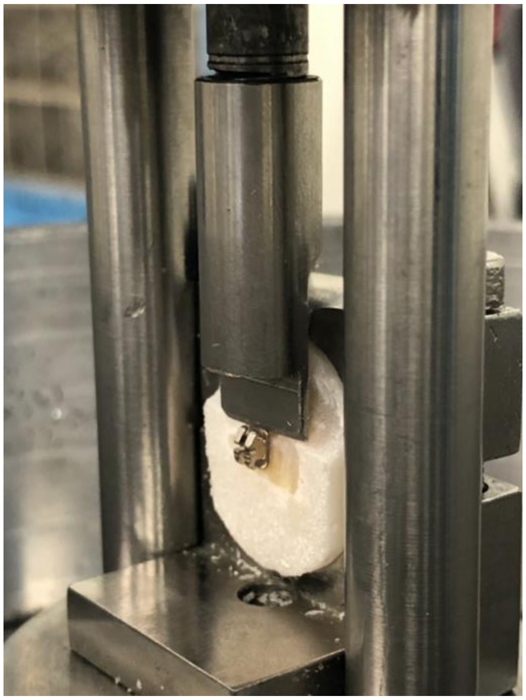


Figure 5.10 MTS Landmark® Servohydraulic unit

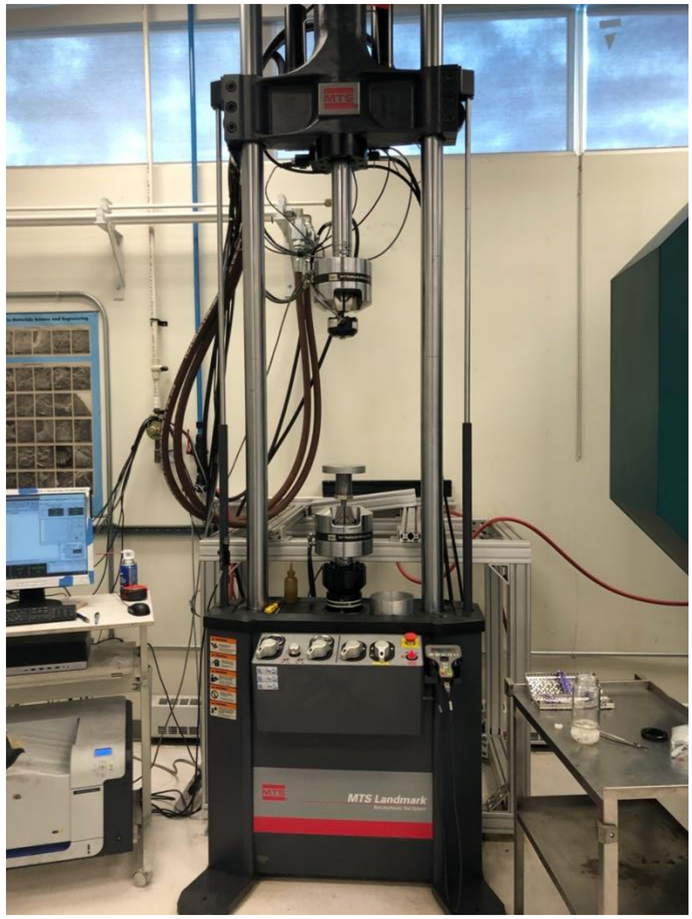


Figure 5.11 Testing machine linked to computer

5.8 Evaluation of the residual adhesive

Each tooth was observed under a Leica EZ4 Stereo microscope (Wetzlar, Germany) at 10X magnification after each debonding to assess the amount of resin composite left on the tooth. The Adhesive Remnant Score Index (ARI) described by Bishara *et al* (Bishara, VonWald et al., 1999), modified from the original Adhesive Remnant Index by Årtun & Bergland, was used.

The ARI evaluation was completed twice on all 120 teeth after each debonding. Of the total of 240 readings, 33 percent of the sample (i.e., 40/120) was randomly selected and then subjected to inter-rater and intra-rater reliability test for ARI evaluation, this was done to ensure results are reliable. The inter-reliability test included a qualified third party (a Graduate Orthodontic resident), who evaluated ten percent of the sample after adequate training, for consensus. The intra-reliability testing was done by the primary investigator, re-evaluating the ten percent 4 weeks after the initial scoring. The results were statistically compared to the original to ensure valid results were obtained. Variability equal or less than five percent was considered acceptable in this study.

<u>**Figure 5.12 Adhesive Remnant Index (ARI) Score 1 to 5**</u>

Figure 5.13 Leica EZ4 Stereo microscope (Wetzlar, Germany) at 10X magnification

5.9 Statistical Analysis

A descriptive statistical analysis including the mean, standard deviation, coefficient of variation, median, minimum, and maximum values and the range was calculated for each group at the three time points. A two-way ANOVA test was utilized to compare the mean shear bond strength values among the groups described in Table 5.4. Pairwise comparisons were done to determine the statistical significance between each group. *Kruskal Wallace test* was used to determine if there was a significant difference among the *Adhesive Remnant Index* scores of each group. Pairwise comparisons were done to determine the statistical significance between each group. The inter- and intra-rater agreement used the *Cohen's weighted kappa statistic* to calculate rater agreement. Significance level for all statistical tests $p \leq 0.05$ was be used.

6. RESULTS

6.1 Shear bond strength statistical analysis

Descriptive data including the mean, standard deviation, minimum, maximum, range, and coefficient of variation of the shear bond strength was determined for each group as in Table 6.1. The two-way ANOVA test was used to determine if there was a significant effect detected between the test groups. The interactions were examined in more detail in pairwise comparison tables. Significance was predetermined at a probability value of ≤ 0.05.

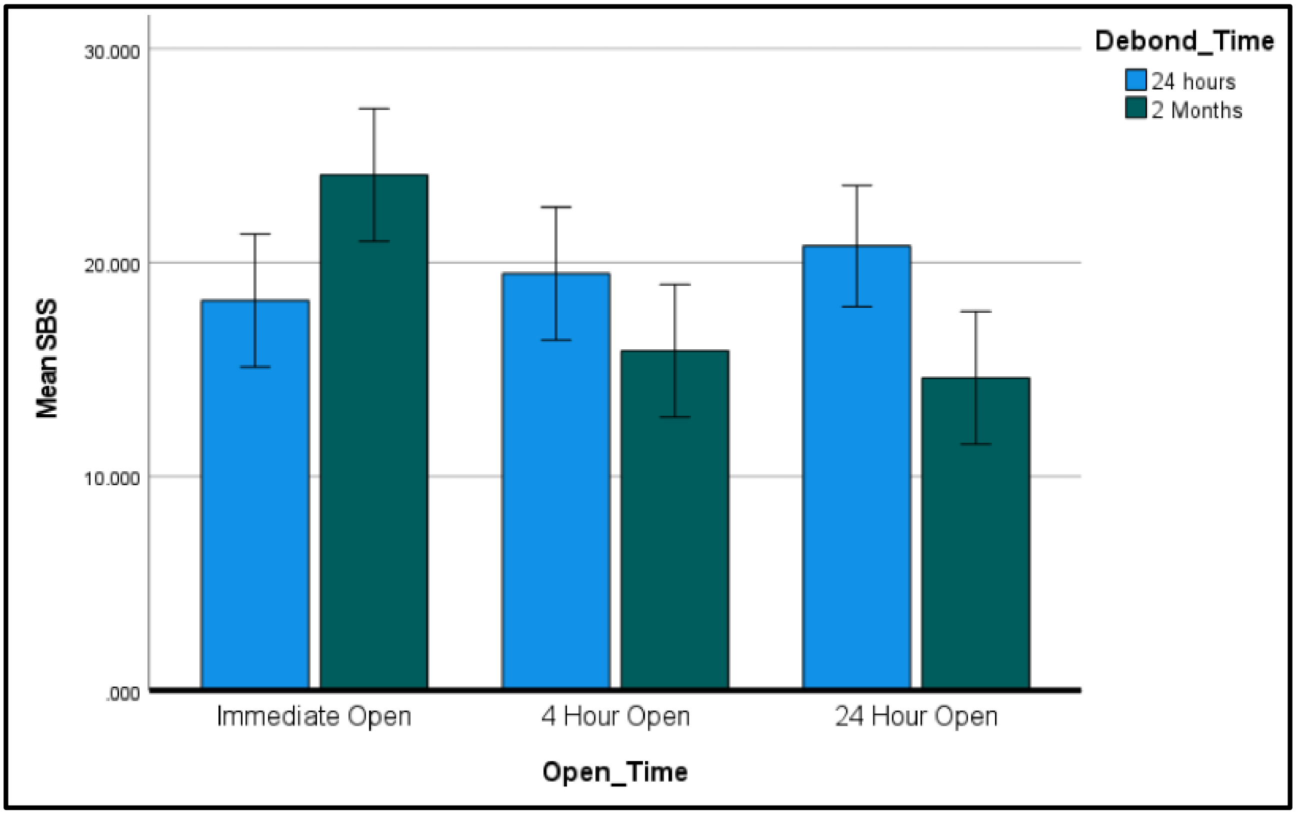
Table 6.1 Descriptive data of shear bond strength

Debond Time/Open Time of Transbond™ Plus SEP package	N	Mean SBS (MPa)	SD	Minimum (MPa)	Maximun (MPa)	Range (MPa)	CV %
24 hours/Immediate	20	18.28	6.38	11.25	31.62	20.37	34.90
24 hours/4 hours	20	19.49	5.60	11.17	31.55	20.38	28.73
24 hours/24 hours	20	20.77	7.04	11.04	38.55	27.51	33.89
2 months/Immediate	20	24.09	7.69	11.09	35.32	24.23	31.92
2 months/4 hours	20	15.88	8.38	8.03	33.97	25.94	*52.77
2 months/24 hours	20	14.61	6.62	7.51	27.21	19.70	*45.31

SD: standard deviation, CV: coefficient of variation, N: number.

*. Significantly higher CV %, indicating a significantly greater level of dispersion of values around the mean (highlighted in yellow)

Figure 6.1 Means of Shear Bond Strength (SBS): Package open time of the Transbond ™ Plus SEP and the debond time at 24 hours and 2 months



The group with the highest Shear Bond Strength (SBS) (MPa) from the 24-hour sample was the 24-hour open group, with a mean SBS of 20.77 MPa, however, there is no statistical difference ($p>0.05$) in mean SBS between the different package opening times within this sample (see Table 6.3).

The group with the highest Shear Bond Strength (SBS) (MPa) from the 2-month sample was the immediate package opening group, with a mean SBS of 24.09 MPa, in this group there was a significant statistical difference between the groups ($p<0.05$) (Table 6.4). The coefficient of variation from the 24-hour debond group ranges from 28.73% to 34.9%, this is considered an acceptable dispersion of values around the mean, but it is on the higher end.

The coefficient of variation from the 2-month debond group ranges from 31.92% to a high 52.77% value. CV values higher than 32% indicate that there is greater dispersion around the mean for that group. For the 2-month group, all the minimum values of the SBS values were above the clinically acceptable standard of 6-8 MPa.

Table 6.2 Two-way ANOVA comparing Shear Bond Strength (MPa) by Debond and Open Times

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1190.704[a]	5	238.141	4.845	.000
Intercept	43830.328	1	43830.328	891.735	.000
Debond Time	52.349	1	52.349	1.065	.304
Open Time of SEP package	326.562	2	163.281	3.322	.039*
Debond_Time * Open_Time	824.376	2	412.188	8.386	.000*
Error	5799.904	118	49.152		
Total	51315.244	124			
Corrected Total	6990.607	123			

a. R Squared = .170 (Adjusted R Squared = .135)
df. degrees of freedom
*. The mean difference is significant at the 0.05 level (highlighted in yellow).

Based upon a 3x2 ANOVA (Table 6.2) there was a significant effect detected for the debond times and the open times of the Transbond™ Plus Self Etching Primer (F=8.386, p= 0.000, p<0.05).

There was also a significant main effect detected for the different opening times of the Transbond™ Plus Self Etching Primer package (F= 3.322, p= 0.039, p<0.05). The interaction effect was examined in detail in the pairwise comparison Tables 6.3 and 6.4.

Table 6.3 Pairwise comparison of the different open times of Transbond™ Plus Self Etching Primer package at debond time 24 hours and 2 months

Debond Time	Open Time of SEP package	Open Time	Mean Difference	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
24 hours	Immediate Open	4 Hour Open	-1.258	2.217	1.000	-6.642	4.126
		24 Hour Open	-2.547	2.123	.698	-7.702	2.608
	4 Hour Open	Immediate Open	1.258	2.217	1.000	-4.126	6.642
		24 Hour Open	-1.289	2.123	1.000	-6.444	3.866
	24 Hour Open	Immediate Open	2.547	2.123	.698	-2.608	7.702
		4 Hour Open	1.289	2.123	1.000	-3.866	6.444
2 Months	Immediate Open	4 Hour Open	8.218*	2.217	.001	2.834	13.602
		24 Hour Open	9.484*	2.217	.000	4.100	14.868
	4 Hour Open	Immediate Open	-8.218*	2.217	.001	-13.602	-2.834
		24 Hour Open	1.266	2.217	1.000	-4.118	6.650
	24 Hour Open	Immediate Open	-9.484*	2.217	.000	-14.868	-4.100
		4 Hour Open	-1.266	2.217	1.000	-6.650	4.118

*. The mean difference is significant at the 0.05 level (highlighted in yellow).
b. Adjusted for multiple comparisons: Bonferroni

The difference in the mean shear bond strengths (SBS) was only statistically significant in the 2 months debond group. From the 24-hour debond group, none of the pairwise comparisons were found statistically significant ($p>0.05$) for all pairwise comparisons in the 24-hour group).

From the 2-month debond group in Table 6.3, two mean SBS differences were found highly statistically significant. The first SBS mean difference of 8.218 MPa was comparing the immediate opening group to the 4-hour opening group, considered very highly statistically significant at $p<0.001$.

The second SBS mean difference of 9.484 MPa was comparing the immediate opening group to the 24-hour opening group, considered highly statistically significant at ($p<0.05$).

The difference in the mean shear bond of the 4-hour opening group and the 24-hour opening group was not found statistically significant ($p>0.05$).

Table 6.4 Pairwise comparison of Transbond™ Plus Self Etching Primer debond times at 24 hours and 2 months at every open time of package

Open Time of SEP package	Debond_Time	Debond_Time	Mean Difference	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	95% Confidence Interval for Difference[b] Upper Bound
Immediate Open	24 hours	2 Months	-5.866*	2.217	.009	-10.257	-1.476
	2 Months	24 hours	5.866*	2.217	.009	1.476	10.257
4 Hour Open	24 hours	2 Months	3.610	2.217	.106	-.781	8.000
	2 Months	24 hours	-3.610	2.217	.106	-8.000	.781
24 Hour Open	24 hours	2 Months	6.164*	2.123	.004	1.961	10.368
	2 Months	24 hours	-6.164*	2.123	.004	-10.368	-1.961

*. The mean difference is significant at the 0.05 level (highlighted in yellow).
b. Adjusted for multiple comparisons: Bonferroni

In this table the 24-hour debond sample and its respective package open times was compared to the 2-month sample and the same SEP packet opening times. For the immediate open group, a SBS mean difference of 5.866 MPa was found between the 24-hour debond and 2-month debond group, this was considered statistically significant ($p<0.05$).

For the 4-hour group (Table 6.4), SBS mean difference of 3.610 MPa was found between the 24- hour debond and 2-month debond group, this was not considered statistically significant ($p> 0.05$).

For the 24-hour open group (Table 6.4), a SBS mean difference of 6.164 MPa was found between the 24-hour debond and 2-month debond group, this was considered statistically significant ($p<0.05$).

6.2 Adhesive Remnant Index

Statistical comparison of the ARI scores distribution among the different groups was accomplished using the *Kruskal Wallace Test*. Pairwise comparisons were then carried out to determine where the significant statistical differences existed. Table 6.5 reiterates the criteria for the Adhesive Remnant Index scores (Bishara, VonWald et al. 1999).

Table 6.5 Modified Adhesive Remnant Index score (1-5)

Score	Criteria
1	All composite remaining on the tooth with the impression of bracket base
2	> 90% composite left on the tooth
3	10-90% composite left on the tooth
4	< 10% composite left on the tooth
5	No composite left on the tooth

(Bishara, VonWald et al. 1999)

Table 6.6 Frequency and percentage of ARI Scores for this experiment

Group	ARI Score					Total
Debond time/ Open Time	**1**	**2**	**3**	**4**	**5**	
24 Hour/Immediate	4 (20%)	4 (20%)	2 (10%)	3 (15%)	7 (35%)	20
24 Hour/4 Hours	1 (5%)	0 (0%)	1 (5%)	13 (65%)	5 (25%)	20
24 Hours/24Hours	0 (0%)	0 (0%)	6 (30%)	13 (65%)	1 (5%)	20
2 Months/Immediate	2 (10%)	2 (10%)	10 (50%)	5 (25%)	1 (5%)	20
2 Months/4 Hours	1 (5%)	3 (15%)	6 (30%)	10 (50%)	0 (0%)	20
2 Months/24 Hours	8 (40%)	10 (50%)	1 (5%)	1 (5%)	0 (0%)	20

From the ARI results in Table 6.6 it is evident that in all the groups, mixed adhesive-cohesive fractures occurred. From Table 6.6 it evident that the 24 hour/Immediate the ARI scores are evenly distributed with ARI scores 1 and 2 (40% of the sample) and ARI scores 3, 4 and 5 (60% of the sample), but the most prevalent score was 5 (35%), where no composite was left on the tooth surface. For group two, 24 hour/4 hours the most common ARI scores were 4 and 5 (90% of the sample). In Group three, 24 hours/ 24 hours the most prevalent ARI scores were 3 and 4 (95% of the sample). For the group of 2 months/ Immediate ARI scores 3 and 4 were the most prevalent (75% of the sample). For the 2 months/ 4 hours group the ARI scores 2, 3 and 4 were the most prevalent (95% of the sample). For the 2 months/ 24 hours group, the most prevalent ARI score was 2 (50% of the sample), followed by ARI score 1 (40% of the sample).

The Kruskal Wallace Test was used to compare the ARI across the SEP package opening times and debond times. The test statistic shows there are significant differences in ARI scores across the groups (p=0.000). Table 6.7 shows the ranks of ARI across the SEP package opening and debond times.

Table 6.7: Kruskal Wallace ranks and means of ARI ratings

SEP package opening Time	Removal Time	Mean Rank	Mean ARI rating
4 Hours	24 Hours	102.88	4.08
24 Hours	24 Hours	84.67	3.63
Immediate	24 Hours	73.29	3.21
4 hours	2 Months	69.22	3.13
Immediate	2 Months	62.87	3.00
24 Hours	2 Months	31.00	1.87

Table 6.7 illustrates that the highest mean ARI ranking is in group 4 hours/24hours with a mean of 4.08 and the lowest mean is 1.87 in the 24-hours/2-month group. The ARI scores range from 3 to 3.63 for the remaining groups.

Pairwise comparisons were done to determine where the statistically significant differences existed between the groups. Table 6.8 shows that statistically significant ARI rating differences were found in 5 comparisons (highlighted in yellow) ($p<0.05$).

<u>Table 6.8 Pairwise comparisons of debond and opening times of SEP package-ARI ratings</u>

Comparison of sample 1 to sample 2	**Sig.**	**Adj. Sig.[a]**
2 Months debond /24 Hours open - 2 Months debond /Immediate open	.006	.092
2 Months debond /24 Hours open - 2 Months debond /4 Hours open	.001	.015*
2 Months debond /24 Hours open - 24 hours debond /Immediate open	.000	.004*
2 Months debond /24 Hours open - 24 Hours debond /24 Hours open	.000	.000*
2 Months debond /24 Hours open - 24 Hours debond/4 Hours open	.000	.000*
2 Months debond /Immediate open - 2 Months debond/4 Hours open	.585	1.000
2 Months debond /Immediate open - 24 Hour debond/Immediate open	.365	1.000
2 Months debond /Immediate open - 24 Hour debond/24 Hours open	.058	.874
2 Months debond /Immediate open - 24 Hours debond/4 Hours open	.001	.008*
2 Months debond /4 Hours open - 24 Hours debond /Immediate open	.723	1.000
2 Months debond /4 Hours open - 24 Hours debond /24 Hours open	.180	1.000
2 Months debond /4 Hours open - 24 Hours debond /4 Hours open	.003	.052
24 Hours debond /Immediate open - 24 Hours debond/24 Hours open	.318	1.000
24 Hours debond /Immediate open - 24 Hours debond/4 Hours open	.009	.141
24 Hours debond /24 Hours open - 24 Hours debond/4 Hours open	.110	1.000

*. Significant at the 0.05 level (highlighted in yellow).
Adj. Sig.[a]: Adjusted Significance: Bonferroni

<u>The following statistically significant differences found in 5 comparisons from Table 6.8 are highlighted:</u>

1) 2 Month debond /24 Hour open – 2 Month debond /4 hour open (p=.015)

2) 2 Month debond/24 Hour open – 24 Hour debond/Immediate open (p=.004)

3) 2 Month debond/24 Hour open – 24 Hour debond/24 Hour open (p=.000)

4) 2 Month debond/24 Hour open – 24 Hour debond/4 Hour open (p=.000)

5) 2 Month debond/Immediate open – 24 Hour debond/4 Hour open (p=.008)

6.2.1 Intra-rater and Inter-rater agreement for the Adhesive Remnant Index (ARI)

Of the total ARI score readings (120) done in this study, 5 ARI scores (20%) per group were re-evaluated four weeks after the initial readings by the principal investigator to determine intra-rater agreement. In total 40/120 (33%) were re-evaluated. The initial scores of the ARI were compared to the ARI scores done 4 weeks later by utilizing *a weighted Kappa test*, this test takes into account the distance between categories and penalizes those with large disagreements. The re-assessment found that the sample evaluated 4 weeks later was 100% the same as the initial sample with a *weighted kappa statistic* of 1.00, which indicates perfect agreement (Table 6.9).

The same sample (40/120 debonded teeth) was evaluated by a third party to determine the inter-rater agreement. A *weighted Kappa statistic* of 0.828 was found when the third-party ARI scores were compared to the initial group and the group assessed 4 weeks later by the principal investigator (Table 6.9). This score indicates strong agreement between the third party and the principal investigator initially and 4 weeks later. The intra- and inter-rater agreement were considered highly valid.

Table 6.9 Interpretation of weighted Cohen's Kappa statistic

Value of Kappa	Level of Agreement	% of Reliable data
0-0.20	None	0-4%
0.21- 0.39	Minimal	4-15%
0.40- 0.59	Weak	15-35%
0.60-0.79	Moderate	35-63%
0.80-0.90	Strong	64-81%
Above 0.90	Almost perfect	82-100%

McHugh M. L. (2012). Interrater reliability: the kappa statistic. *Biochemia medica*, *22*(3), 276–

7. DISCUSSION

Orthodontic treatment relies heavily, among other factors, on reliable and successful longer-term (24-36 months) bonding of orthodontic brackets to the enamel. Conventional etching methods (CEM) have proven to have adequate bond strength when bonding to enamel, but unfortunately, the CEM is also very sensitive to moisture contamination and is a time-consuming protocol (Dos Santos et al., 2006). Orthodontists have continued to look for a one-step, reliable, stable, affordable, and "user-friendly" adhesion promoter that can effectively bond to enamel surfaces, in moist conditions, with adequate immediate bond strength that can be sustained through the entire course of treatment (Wiltshire & Noble, 2010).

The present study evaluated whether the shear bond strength (SBS) of single-use Transbond ™ Plus self-etching primer (SEP) will be affected after using it for a more prolonged period after being opened. In practice, clinicians are known to use Transbond ™ Plus self-etching for orthodontic bonding procedures over prolonged periods, in contravention to the manufacturer's instructions, which are strictly for single use, for the economy of scale purposes and reducing practice overhead costs.

The purpose of this study, accordingly, was to evaluate whether clinicians can utilize a popular SEP, Transbond ™Plus, over prolonged periods without negatively affecting shear bond strength. The main reason for prolonged, multiple-use in private practice for this product is because it is effective and advantageous for many reasons, but unfortunately, it is not cost-effective if used for repositioning of brackets or repair of broken brackets as a single-use product for every patient and procedure.

The pandemic outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) in 2019/2020 has forced clinicians to rethink bonding and debonding protocols to efficiently manage and minimize aerosol production. The nature of the virus' infectious route has revealed potential hazards underlying standard orthodontic procedures due to the implication of airborne droplets. The most common categories of orthodontic-related applications that produce aerosols are bonding and debonding strategies (Eliandes & Koletski, 2020). Minimization of water-pray syringe utilization during bonding needs to be prioritized to reduce aerosol production. According to Elaindes and Koletsi (2020), CEMs gels with increased viscosities require higher water and/or spray pressure to be rinsed off, increasing the spatter and droplet formation, and has increased working times. CEMs with low viscosities or liquid gels should be prioritized for CEMs. Self-etching primers are a good alternative, which eliminates the rinsing phase, thus reducing aerosol production (Eliandes & Koletski, 2020). However, according to manufacturers, it is required to first pumice the teeth to remove the pellicle layer and plaque before bonding for any bonding agent. Pumicing is also considered an aerosol-generating procedure as it needs to be rinsed off (Camba et al., 2020), controversially, pumicing is unnecessary by Ireland *et al.* (2002) as it has no significant effect on *in vivo* bond failure rate before using CEM for direct bonding (Ireland and Sheriff 2002, Barry, 1995). However, in a clinical study where pumicing was omitted, bond failure rates of Transbond™ Plus self-etching primer were almost double that of CEMs (Hosein, Sheriff, and Ireland, 2002). 3M manufacturers recommend choosing a bonding protocol with fewer steps to decrease bond failures, but this concept is also true to minimize aerosol-generating procedures (Solid, 2018). It is clear orthodontists now need to consider purchasing products not only for cost-effectiveness, efficiency, or personal preference, but also to minimize aerosol-generating procedures to prevent the spread of SARS-Cov-2.

7.1 Shear bond strength

Multiple studies have shown that 6 to 8 MPa is the “clinically acceptable” standard for *in vitro* shear bond strength testing (Reynolds 1975, Joseph and Rossouw 1990, Whitlock, Eick, et al. 1994, Finnema, Ozcan et al. 2010, Wiltshire and Noble 2010). However, Wiltshire & Noble (2010) found that glass ionomers often perform inferiorly when compared to composite resins *in vitro*. Shear bond strengths as low as 2.2 MPa were recorded, nevertheless, the bonding agent showed acceptable performance during *in vivo* testing (Fricker, 1994, Wiltshire &Noble, 2010).

The minimum shear bond strength value is very important to assess, it is even more important to assess the minimum than the mean of the SBS values to evaluate the performance. (Wiltshire &Noble, 2010). Although higher SBSs can reduce the number of bond failures during orthodontic treatment, higher SBS are not always better (Kusy, 1994). Higher SBS values have been associated with increased enamel fractures and more patient discomfort during the debonding appointment (Zhang, Yao, et al. 2014). Retief (1974) reported enamel fractures at SBS values as low as 9.7 MPa. It has been recommended that SBS values of less than 14 MPa are more likely to prevent enamel fractures (Retief, 1974). Powers *et al.* (1997) recommended that SBS studies should aim to have a coefficient of variation that ranges from 20-30%, this percentage range indicates that there is less dispersion of values around the mean, demonstrating the results are more reliable and don’t have a lot of variation (Powers, Kim et al. 1997).

Overall, the results from our research have indicated that the mean shear bond strengths of all the groups were above the clinically acceptable range of 6-8 MPa as proposed by Reynolds (1975). All the results were also above the minimal reliable clinical SBS values of 3-4 MPa range (Wiltshire & Noble, 2010, Fricker, 1994).

7.1.1 Coefficient of variation

The coefficient of variation of 20-30% suggested by Powers (1997) was only found in 1 group of the 6 groups, the 24 hours/4 hours group (28.73%) (Table 6.1). For 24 hours/ Immediate and 24 hours/24 hours the coefficient of variation was 34.90% and 33.92% respectively, close to the maximum range advised by Powers *et al*. (1997) This indicates the amount of variability relative to the mean was still within an acceptable range, but on the higher end of the coefficient of variation recommend. For the 2-month debond group the only group that had an acceptable coefficient of variation was the 2-month immediate group (as recommended by the manufacturer) (31.92%), the coefficient of variation for the other two groups (2 months/ 4 hours & 2 months/ 24 hours) was 52.77% and 45.31% respectively. Both groups far exceeded the preferred range proposed by Powers (1997), indicating that the amount of variability relative to the mean was high. The high values are concerning as it makes it challenging to assess the highest and lowest ends of the bond strength spectrum accurately. This result could also indicate that consistent SBS values cannot be guaranteed when choosing to use the product multiple times, over a prolonged period of time for long-term reliable SBS bonds.

7.1.2 Mean, minimum, and maximum shear bond strength of the 24-hour debond group

There is no statistical difference in the mean SBS between the different package open times within the 24-hour debond sample, indicating that SEP package can be used up to 24 hours without affecting short-term SBS (see Table 6.3).

The minimum values of all three 24-hour debond groups (24 hours/ Immediate, 24 hours/ 4 hours, 24 hours/ 24 hours) were very similar, ranging from 11.04 to 11.17 MPa, well above the accepted clinical bond strength of 6-9 MPa recommended in the literature (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010).

The maximum values of all three 24-hour debond groups were also very similar, ranging from 31.55-38.55 MPa. These values are significantly higher than the proposed 14 MPa proposed by Retief (1975) to prevent enamel fractures., however, this has never been clinically verified as a major disaster of enamel fracture during debonding. The higher SBS can be associated with increased enamel fractures and more patient discomfort during the debonding procedure (Zhang, Yao et al. 2014). According to Buyukyilmaz *et al*. (2003) the high bond strengths can be attributed to the simultaneous etching and priming, where the primer penetrates the entire depth of the etch to result in excellent mechanical interlocking (Buyukyilmaz et al., 2003). The *in vitro* situation may not necessarily reflect the clinical situation (Wilshire 2021-Personal communication).

The mean SBS, minimum as well as maximum values (Table 6.1) were very similar when comparing the 24-hour groups to each other, therefore we can conclude that short term SBS is not affected after keeping the package open for up to 24 hours in this experiment, and that the practitioner can still use the same SEP package after a full day without affecting short term SBS. A similar mean and minimum SBS value for Transbond™ Plus SEP after 24 hours debonding time was demonstrated by Turk *et al.* (2007), with the mean at 19.11 ± 3.4 MPa and the minimum at 14.68MPa (Turk, Elekdag-Turk and Isci, 2007). Buyukyilmaz *et al*. (2003) also demonstrated a similar mean SBS of 16.0 ± 3.4 MPa after 24 hour debond (Buyukyilmaz,Usumez and Karaman, 2003.

7.1.3 Mean, minimum, and maximum shear bond strength of the 2-month debond group

Within the 2-month debond sample, there was a significant decrease in the mean SBS and the minimum SBS values from the immediately opened package (24.09 MPa) to the package that was open for 24 hours (14.61 MPa) (Table 6.1), however, the SBS decrease is not clinically significant as all the values from this group, including the minimum values were still above the clinically acceptable standard for SBS recommended (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010). Accordingly, our study indicates that practitioners can still use the same SEP package after 24 hours without affecting the long-term SBS clinically. A possible explanation for the decrease in SBS when used after prolonged periods could be evaporation of the solvent, according to the manufacturer water is the only solvent used in Transbond Plus SEP. However, a study by Abate *et al*, (2000) reported that products with acetone and ethanol-based solvents have low boiling temperatures compared to water, and therefore products that have only water as their solvent are more stable under the same conditions, whereas products with acetone and ethanol require careful conservation and should be used immediately after dispensing.

Any loss of mass-produced by solvent evaporation could affect the adhesive behavior (Abate, Rodriguez & Macchi, 2000). This might explain why Transbond™ Plus is used for a prolonged period with clinical success in practise without affecting SBS clinically, if the package is folded properly and is not stored in conditions that cause increased evaporation (e.g., excessive heat).

However, there still would have been some degree of evaporation of the water solvent when the package was left open. Evaporation leads leads to an increased viscosity of adhesive materials, increasing the contact angle, which ultimately results in decreased wettability/hydrophobicity (Marchall,Bayne, Baier et al., 2009).

This might explain the decrease in SBS from the 0-hour open group to the 24 hour-open goup in the 2-month debond sample. Another possible reason for the decrease in SBS in the 2-month sample could be that the SEP becomes less acidic with time, leading to an even further reduction in the etching depth, resulting in decreased SBS values.

It is important to keep in mind that the coefficient of variation exceeds the suggested 20-30% by Powers in this group (1997), it is hard to determine how accurate and reliable the data is to make the same assumption *in vivo*, since it cannot be demonstrated *in vivo* without clinical testing. This also applies to the 2-month/4 hour and 2-month/ 24-hour groups with a coefficient of variation percentages of 52.77% and 45.31% respectively.

7.1.4 Pairwise comparison of debond groups at 24 hours and 2 months of Transbond™ Plus Self Etching Primer and their respective opening times

In Table 6.4 the 24-hour debond sample and its respective packet opening times were compared to the 2-month sample and the same open times. The SBS increased from the 24-hour debond /immediate open group (18.28 MPa) to the 2-month debond/immediate open group (24.09 MPa) when the manufacturer's instructions were followed (Table 6.1). According to Wiltshire and Noble (2010) the clinical association of bond maturation can be explained by water sorption equilibration (Wiltshire & Noble, 2010). However, it has also been stated that the composite has reached its maximum strength after 24 hours by a few studies (Rock & Abdullah, 1997, Klocke, Shi, Vaziri et al., 2004, Turk, Elekdag-Turk and Isci, 2007). According to Turk *et al.* (2007) the most reasonable explanation for the increase in SBS up to 24 hours is that most of the free radicals are initially produced at the border of the resin, and then later diffusion of these free radicals will require time to continue polymerization under the bracket base (Turk, Elekdag-Turk and Isci, 2007).

For the 4-hour open group, SBS mean difference of 3.610 MPa was found between the 24-hour debond and 2-month debond group, this was not considered statistically significant as the mean SBS changed from 19.49 MPa to 15.88 MPa, respectively (Table 6.1). The difference in SBS between the 24-hour and 2-month group is not clinically significant either, but it is worth noting that there was no bond maturation in this group, suggesting that when the manufacturer guidelines are not followed, the expected bond maturation might not occur.

For the 24-hour open group, the mean SBS decreased from the 24-hour debond group (20.77 MPa) to the 2-month debond group (14.61MPa) (Table 6.1). Our study suggests that bond maturation will only occur when the manufacturer guidelines are followed, when they are not followed the SBS might stay the same or decrease over time, however, the mean long term SBS only decreased by 6.164 MPa and still has a clinically acceptable SBS well above 6-8 MPa *in vitro* (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010), and is certainly above the minimum recommended clinical SBS values of 3-4 MPa (Wiltshire & Noble, 2010). It would be valuable for this study to be done *in vivo* for the duration of treatment to assess the long-term results, for at least 24-month, in a more realistic environment with saliva, fluctuating temperatures, bacteria, and masticatory forces to assume this experiment's *in vitro* results are reliable.

7.2 Adhesive Remnant Index

SEP's gained popularity in orthodontics because they reduce chair time due to the etch that is less aggressive and results in less enamel loss while also decreasing the amount of enamel that needs to be removed at the debonding appointment (Wiltshire & Noble, 2010). ARI determination indicates the cohesive and adhesive nature of the orthodontic bond (dos Santos, Quioca, Loguurcio et al., 2006). The failure mode of the bracket depends on various factors such as cohesive strength of the adhesive, bracket base morphology, as well as the resin-enamel bond strength from the bonding system (dos Santos et al., 2006).

7.2.1 Frequency and percentage of ARI Scores

All the bond failures from Table 6.6 were mixed adhesive-cohesive fractures. Bonetti *et al.* (2011) suggested that, generally, most bracket failures are combinations of adhesive and cohesive failures, and that the latter results in retention of resin on the tooth surface and bracket surfaces (mixed failure) (Bonetti, Parenti, Lattuca et al., 2011). In our study, the majority of each group's ARI scores ranged from 3-5, but there were also infrequent lower scores of 1-2. Although the entire sample had mixed failures, a bigger percentage of failures per group were adhesive, most of the scores from Table 6.6 ranged from 3-5.

It has been reported that SEP's have more adhesive bond failures (adhesive-enamel fractures) rather than cohesive bond failures (Bishara, Gordan, VonWald et al., 1999, Velo & Carano, 2002), which suggests that the ARI are higher (between ARI score 4-5), to leave less than 10% resin on the tooth surface.

Another study by Hosein *et al.* (2004), presented similar mixed-mode failures for Transbond™ Plus SEP, and they mentioned that the reason for mixed-mode failures is still not explained as reports have shown no difference in measured force for CEMs and Transbond ™Plus self-etching primers (Hosein, Ireland and Sheriff, 2004).

A combination of the two modes of failures was seen in our study, but no enamel fractures occurred during debonding of any of the groups, this might be due to the reduced depth of demineralization of SEP's. Bonding systems with higher bond strengths, have more adhesive bracket debonding (cohesive failure), leaving more resin on the tooth surface, decreasing the risk of fracture. Lower bond strengths have more adhesive enamel debonding (adhesive failure), leaving more resin on the bracket than on the tooth surface, increasing the risk of fracture (dos Santos et al., 2006), however, in our study, SBS values did not necessarily correspond with specific failure mode.

7.2.2 Kruskal Wallace ranks and means of ARI ratings

Table 6.7 illustrated that the highest mean ARI ranking is in group 4 hours/24hours with a mean of 4.08, an adhesive bond failure with less than 10% of the resin remaining on the tooth surface. A study by dos Santos *et al*. 2006 suggested that adhesive failures that are at the enamel might be the result of the reduced depth of demineralization of SEP’s, and therefore less resin remains on the tooth surface, decreasing the time required to clean the enamel at the debonding procedure (dos Santos et al., 2006, Øgaard & Fjeld, 2010). Due to the decreased depth of demineralization, the risk of enamel fractures is decreased when compared to CEMs with higher ARI scores, as CEMs have deeper penetration of resin tags which would require more force to remove the resin at the debonding procedure. Instead of solely focussing on the magnitude of bond strengths, clinicians should be mindful of iatrogenic damage to the tooth surface, such as enamel tearing, crazing, microfractures, and gross enamel fractures (Wiltshire and Noble, 2010). Another consideration is compromised aesthetics, due to staining from the remaining resin tags after debonding. Since the resin tags of SEPs penetrate more superficially, it would be easier to prevent and remove any residual resin stains from the enamel surface.

The lowest mean of the sample was 1.87 in the 24 hours/2-month group. This was the only group that showed a more cohesive bond failure, with more than 90% of the composite remaining on the tooth surface. For this group, it will take longer to clean the adhesive from the enamel, but there would be less of a risk of fracture compared to the other groups, and the shallower penetration of resin tags with SEP’s would also decrease the risk of enamel fractures compared to CEMs, therefore this group had the least risk of fracturing the enamel (dos Santos et al., 2006, Øgaard & Fjeld, 2010). This group had the lowest mean SBS out of all the groups in the experiment (Table 6.1), therefore the reason for the cohesive failure mode could not be related to increased SBS as stated by dos Santos *et al*. 2006 in this case. The ARI scores range from 3 to 3.63 for the remaining groups, indicating that 10-90% of the adhesive remained on the tooth surface and, that these groups were more adhesive in nature as reported in multiple studies (Bishara, Gordan, VonWald, et al., 1999, Velo & Carano, 2002).

7.2.3 Pairwise comparisons of debond and opening times of SEP packet-ARI ratings

The first 4 statistically significant differences between the groups all compared the 2 months/ 24-hour open group, which had a mean ARI rating of 1.87 (cohesive), to the rest of the groups that had ARI ratings ranging from 3-4.08 (adhesive). However, the mean SBS of the 2-month/24-hour open group was the lowest of all the groups (14.61 MPa), which would indicate a more adhesive failure according to the literature, but a score of 1.87 suggests a cohesive failure.

This inconsistent finding can be explained by the coefficient of variation that was 45.31% (Table 6.1) for group 2 Months/ 24 hours. This high value indicates that there is a large dispersion of values around the mean, therefore the results for that group are more unreliable and might not be a true reflection of the clinical situation. The high coefficient of variation range could be the reason this group has a significantly different ARI. It can be concluded that keeping the SEP packet open does not decrease the SBS to a point where it is below the clinically acceptable standard of 6-8 MPa (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010), but it does cause inconsistent SBS values which can lead to ARI scores that range significantly.

The 5th statistically significant comparison was the 2-month debond/Immediate open with 24 hours/ 4 hours open group, the mean ARI was 3.00 and 4.08 respectively (Table 6.7). The reason for significance might be that the mean SBS of the 2 months/ Immediate group (24.09 MPa) was 4.6 MPa more than the 24 Hours/ 4 hours open group (19.49 MPa) (Table 6.1). Bonding systems with higher resin-enamel bond strengths have more adhesive-bracket debonding (cohesive failure), leaving more resin on the tooth surface (low ARI score (dos Santos et al., 2006).

7.3 Evaluation of the null hypotheses

1. The null hypothesis states that there is no statistically significant difference in the shear bond strength between using Transbond™ Plus self-etching primer as a single-use product following the manufacturer's instructions, or multiple times, over a 24-hour period, is rejected as there was a statistically significant difference ($p<0.05$) in the 2-month debond group.

2. The null hypothesis that states there will be no statistically significant difference in shear bond strength (SBS) from the 24-hour debond group to the 2-month debond group is rejected, as there was a statistically significant difference between the 2 groups ($p<0.05$).

3. The null hypothesis that states that there is no statistically significant difference in the Adhesive Remnant Index (ARI) scores with varying opening time of the package or associated debonding times is rejected, as there were 2 groups with statistically significant differences ($p<0.05$).

4.The null hypothesis that states that the mode of bond failure of Transbond™ Plus self-etching primer is of a mixed adhesive cohesive nature is accepted, as all groups showed mixed-mode bond failures.

7.4 Potential clinical applications

Some of the potential clinical applications after analyzing the outcomes of the present study would be chair time and financial considerations. Transbond™ Plus self-etching primer is an expensive product when compared to the CEMs, but the simplicity and clinical advantages such as saving clinical time, reducing procedural errors, minimizing technique sensitivity, reducing the risk of salivary contamination, producing a more conservative etching pattern, and minimizing enamel loss at debonding while maintaining adequate bond strengths, outweighs the cost of the product for some practitioners (Cal-Neto, Quintão, de Oliveira Almeida, & Miguel, 2009). Previous clinical reports have shown that using SEPs can reduce chair time during bonding up to 65% (Ireland, Knight, and Sheriff, 2003).

The outcome of the present study explains why Transbond™ Plus self-etching primer is being used successfully in clinical practice. All the samples that were used for a prolonged period of time in this *in vitro* study, had to shear bond strengths above the "clinically acceptable" standard of 3-6 MPa or 6-8 MPa, even at their minimum (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010).

The most important clinical application would be cost. If a practitioner could use the same Transbond™ Plus self-etching primer package for rebonding broken brackets, and repositioning brackets over a prolonged period, up to 24 hours, it would be a lot more cost effective than opening a new package of Transbond™ Plus self-etching primer for each patient and each procedure. Inventory can also be limited to Transbond™ Plus self-etching primer supplies, which can also be more cost-effective than stocking multiple bonding products that can expire if not used regularly.

Based on discussions from social media platforms for orthodontists, it has been confirmed that some practitioners have been using this protocol successfully for an entire working day, without noticing increased bond failures (Women in Orthodontics, 2021).

7.5 Limitations of the present research

Due to the absence of experimental parameter standardization *in vitro* experiments, it can be very challenging to compare results and conclusions from other orthodontic bonding studies. A total of 27 experimental parameters for *in vitro* shear bond strength experiments have been identified in a systematic review by Finnema *et al.* (2010) as necessary parameters to mention, in order to assess the validity of the results (Finnema, Ozan et al. 2010). Other sources of variability in the bonding protocol that can affect SBS can range from premolar/molar crown anatomy/contour variations, the method of adhesive removal, the interfacial characteristics of the bracket adhesive complex, and the quantitative features of the adhesive and force used during bonding (Bishara, Gordon et al. 1998). However, our study protocol simulated similar norms at the University of Manitoba for the past 24 years and are aimed at attempting to follow clinical procedures as far as possible.

In our study, the debonding procedures were completed at 24 hours and 2 months. The 24-hour debond sample does not necessarily reflect the actual clinical situation, where an archwire is tied to the brackets within minutes of bonding. The 2-month debond sample also does not reflect the actual duration of orthodontic treatment, which ranges from 1.5 to 3 years.

The debond group of 2 months could have been longer if time was not a limitation, orthodontic brackets must be able to withstand masticatory forces throughout the duration of the treatment, therefore testing up to 24 months could be considered. Artificial saliva as the medium of storage could have been considered, to mimic the oral cavity environment as closely as possible within the boundaries of *in vitro* testing. However, 2 months of storage/debonding was considered adequate for the purpose of our study to glean short term information. In addition, storage in distilled water is a well-known protocol, and therefore it was chosen as our storage medium.

Another consideration would have been to expose the teeth to temperature fluctuation, to simulate the temperature fluctuation from cold and hot food and/or beverages consumed by the patient, but the present study did not include it in its protocol as it may be considered too excessive compared to the clinical situation.

Precautions were taken to ensure the testing was done with the shearing blade as parallel and as close as possible to the tooth surface in this study, but some variation may still exist in the angulation of the blade and/or distance, which could have included varying in the shear bond strength values. Finally, scanning electron microscopy of the enamel surfaces could have helped to quantify the extent of enamel damage at a microscopic level, but this was not part of the main purpose of this research project.

8. CONCLUSIONS

Based on our *in vitro* study, which evaluated the shear bond strength of a single use self-etching primer (Transbond™ Plus Self Etching Pirmer) used multiple times, over a prolonged period of time, the following conclusions can be drawn:

1. Short term SBS, up to 24 hours, is not affected after keeping the package open for up to 24 hours in this experiment, therefore the practitioner may still be able to use the same opened SEP package after a full day (24 hours) without affecting short term SBS.

2. After 2 months, the SBS decreases after keeping the SEP package open for 24 hours, but the SBS remains above the clinically acceptable standard of 6-8 MPa *in vitro* (Reynolds, 1975, Fricker 1994, Wiltshire & Noble, 2010). Therefore, practitioners may still be able use the same SEP package after 24 hours without affecting the long term SBS clinically.

3. SBS values can become more inconsistent and variable in the long term when the manufacturer guidelines are not followed, when the SEP is used multiple times over a prolonged period of 24 hours.

4. Pumicing is an essential clinical step, as per the manufacturer's instructions, when using a SEP, supported by two clinical studies (Hosein et al. 2002, Lill et al., 2008) to ensure adequate clinical success. Considering the above, even though attempting to reduce the additional pumicing step for decreased chair time, decreased technique sensitively, and patient comfort, the pumicing step remains vital for successful clinical bonding.

5. Reducing aerosol generating procedures, such as pumicing, during the Covid-19 pandemic has become an important consideration for orthodontists, however, it is crucial that the pumicing step is not omitted when using SEPs, despite the fact that this step generates aerosols, as the clinical success of bonding will be affected negatively according to two in vivo studies (Hosein et al. 2002, Lill et al., 2008).

6. Bond maturation will occur ideally when the manufacturer guidelines are followed, but when they are not followed the SBS could either stay the same or decrease over time.

7. The debonding of Transbond™ Plus Self-Etching Primer is adhesive and cohesive in nature, and has mostly mixed mode bond failures, regardless of how long the SEP package is open.

9. RECOMMENDATIONS

1. Future efforts by researchers should be directed towards stating all testing parameters utilized and the standardization of *in vitro* bond strength testing to facilitate improved data interpretation and comparison among different orthodontic bonding studies.

2. *In vivo* testing could be done for the duration of orthodontic treatment (2-3 years), to assess the results in the oral environment.

3. Only use opened SEP packages for rebonding broken brackets and emergency rebonding, and not for universal use until *in vivo* testing has shown that the SBS is not affected clinically.

4. When reusing SEP again or over a prolonged period, ensure that the package is folded properly and not stored in warm temperatures (above 18°C) that can increase evaporation of the solvent, and therefore negatively affect SBS.

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11. APPENDIX

11.1 Ethics Approval

University of Manitoba | Research Ethics and Compliance

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HEALTH RESEARCH ETHICS BOARD (HREB)
CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. Nadine Williams	**INSTITUTION/DEPARTMENT:** U of M/Dentistry/Orthodontics	**ETHICS #:** HS23516 (H2019:517)
HREB MEETING DATE (If applicable):	**APPROVAL DATE:** November 30, 2020	**EXPIRY DATE:** **December 11, 2021**
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): Dr. William Wiltshire		

PROTOCOL NUMBER: NA	**PROJECT OR PROTOCOL TITLE:** Shear bond strength of a single use self etching primer used multiple times
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: NA	

Submission Date of Investigator Documents: November 16, 2020	**HREB Receipt Date of Documents:** November 16, 2020

REVIEW CATEGORY OF ANNUAL REVIEW: **Full Board Review** ☐ **Delegated Review** ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(if applicable)	Version(if applicable)	Date

Annual approval

*Annual approval implies that the most recent **HREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.*

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM HREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

A unit of the office of the Vice-President (Research and International) umanitoba.ca/research

11.2 Journal Article and Submission Confirmation

THE ANGLE ORTHODONTIST

ONLINE MANUSCRIPT SUBMISSION AND PEER REVIEW

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Detailed Status Information

Manuscript #	051921-401
Current Revision #	0
Submission Date	2021-05-19 19:30:47
Current Stage	Initial QC Started
Title	Shear bond strength of a single-use self-etching primer used multiple times over 24-hours
Running Title	Single-use self-etching primer used multiple times
Manuscript Type	Original Article
Special Section	N/A
Corresponding Author	Nadine Williams (University of Manitoba)
Contributing Authors	William Wiltshire , Robert Drummond , Rodrigo Franca
Financial Disclosure	I have no relevant financial interests in this manuscript.
Abstract	Objectives: To determine whether single use Transbond Plus™ self-etching primer (SEP) (3M Unitek, Monrovia, Calif) in vitro shear bond strength (SBS) is affected if used over a prolonged period, for cost-effectiveness in orthodontic practices. Many orthodontists already use the SEP multiple times or for prolonged periods in clinical practise. Materials and Methods: Transbond ™ Plus SEP was used to bond brackets to 120 extracted human teeth at three different time points after opening the SEP package: immediately- (as recommended by manufacturer), 4 hours-, and 24 hours after opening. Samples were debonded with a universal testing machine after 24 hours and 2 months. Results: No statistically significant difference in SBS was found in the 24-hour debond group between the different time points of package opening, but there was a significant statistical difference for the 2-month debond group ($p<0.05$). SBS increased from the 24-hour debond group to the 2 month debond group, but decreased or stayed the same when manufacturer guidelines were not followed. Conclusions: Short term SBS is not affected when using an opened SEP package multiple times after 24 hours, but more long term SBS is affected, and shows more variable and inconsistent SBS values. However, no group's minimum SBS value decreased below the "clinically acceptable" standard of 6-8 MPa for in vitro SBS testing, which could explain why some orthodontists have clinical success in practice when using Transbond ™ Plus SEP for prolonged periods. Recommendations: it remains vital to pumice teeth before bonding with a SEP during the Covid-19 pandemic.
Associate Editor	Not Assigned
Key Words	Shear Bond Strength, Transbond ™ Plus self-etching primer, Single-use, Multiple use, Prolonged period

Shear bond strength of a single-use self-etching primer used multiple times over 24-hours

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ABSTRACT

Objectives: To determine whether single use Transbond Plus™ self-etching primer (SEP) (3M Unitek, Monrovia, Calif) *in vitro* shear bond strength (SBS) will be affected if used multiple times over a prolonged period, for cost-effectiveness.

Materials and Methods: Transbond ™ Plus SEP was used to bond brackets to 120 extracted human teeth at three different time points after opening the SEP package: immediately- (as recommended by manufacturer), 4 hours-, and 24 hours after opening. Samples were debonded with a universal testing machine after 24 hours to assess short term SBS, and after 2 months to assess bond maturation.

Results: No statistically significant difference in SBS was found in the 24-hour debond group between the different time points of package opening, but there was a significant statistical difference for the 2-month debond group ($p<0.05$), which also showed an increased coefficient of variation range. SBS matured from the 24-hour debond group to the 2-month debond group but decreased or stayed the same when manufacturer guidelines were not followed.

Conclusions: Short term SBS is not affected when using an opened SEP package multiple times after 24 hours, but more long term SBS is affected, and shows more variable and inconsistent values. However, no group's minimum value decreased below the "clinically acceptable" standard of 6-8 MPa for *in vitro* SBS testing, suggesting a more cost-effective bonding protocol might be possible with Transbond ™ Plus SEP. It remains vital to pumice teeth before bonding with a SEP during the Covid-19 pandemic, even though pumicing is considered an aerosol generating procedure.

Keywords: Shear Bond Strength, Transbond ™ Plus self-etching primer, single-use, multiple use, prolonged period

INTRODUCTION

Self-etching primers (SEPs) are adhesives which combine the acid etch and the primer simplifying orthodontic bonding procedures significantly, thus making it possible to eliminate the etching and rinsing step. SEPs are popular in orthodontic bonding as they eliminate the washing and drying steps required with the conventional etching method (CEM). SEPs will therefore have the advantage of saving clinical time, reducing procedural errors, increasing patient comfort, minimizing technique sensitivity, reducing the risk of salivary contamination due to the hydrophilic primer, producing a more conservative etching pattern, therefore minimizing enamel loss at debond, while still maintaining adequate bond strengths. [1-6]

Bonding is a technique sensitive procedure, and moisture contamination has been reported to be the most common cause of bond failures.[7,8] CEMs do not offer adequate bond strength under moisture contamination conditions as they are hydrophobic. Transbond™ Plus SEPs are considered hydrophilic and will therefore potentially perform more reliably in moisture contaminated environments.[8] Due to the frustration of bracket debonds for the patient and the clinician, there are multiple contradictory *in vitro* studies published regarding the shear-bond strength (SBS) of SEPs compared to CEMs. Some studies have shown that SEPs have a lower bond strength than CEMs, yet other studies show adequate bond strength with less enamel damage at debonding. The majority of *in vivo* studies concluded that Transbond™ Plus SEP has no significant difference in clinical failure rates when compared to CEMs. [9-15]

Orthodontists want to utilize a material that is strong enough to withstand forces against accidental debonding for the course of the orthodontic treatment time but is also low enough to avoid enamel damage at debonding.[16] Wiltshire et al[17] showed that for minimum reliable clinical bond strengths *in vitro* testing should yield consistent values of at least 3 MPa to 4 MPa, based on clinical studies on glass ionomer bonding in orthodontics. Multiple *in vitro* studies have also reported 6 to 8 MPa as the “clinically acceptable” standard for SBS. [16,22,30,31]

Transbond™ Plus SEP is a single-use product according to the manufacturer, as cross-contamination needs to be considered. However, clinicians can theoretically use a new applicator stick for every tooth without contaminating the package, when used for repositioning and rebonding broken brackets between different patients. According to resources from orthodontic online platforms, orthodontists in North America already use Transbond™ Plus SEP for prolonged periods in practise with clinical success [34,35] but, there is no current evidence to support whether clinicians can use that bonding protocol without compromising the SBS.

Accordingly, the aim of this study is to assess whether clinicians can use the same Transbond™ Plus SEP package over a longer period of time without affecting the SBS negatively, for economy of scale purposes and reducing practise overhead costs. It may also present advantages during the Covid-19 pandemic.

METHODS AND MATERIALS

Teeth

One hundred and twenty extracted sound human third molars with similar lateral surfaces were collected and stored in 0.5% Chloramine T solution until use. The teeth were rinsed with distilled water and wiped with gauze to remove remnant soft tissue, and then sectioned at the cemento-enamel junction with a circular diamond disc on a straight hand piece. The crowns were embedded into Bosworth Fastray (Bosworth, IL) a self-curing acrylic, within polyvinyl chloride (PVC) cylindrical moulds. In order to assure the tooth surfaces are parallel to the horizontal plane, the teeth were placed on adhesive tape (Scotch® Permanent Mounting Squares), which were positioned on a flat surface. (Figure 1). Self-cure acrylic was flowed over the teeth until the PVC mould was three quarters full. After 8 minutes of cure time, the set acrylic with the embedded teeth was removed from the PVC moulds.

When the PVC moulds are flipped over, to the side facing the flat surface/table, the uncovered flat surface of the 3rd molar was visible (Figure 2). After the acrylic moulds had been removed, they were stored in distilled water and placed into an incubator 37°C at 100% Relative Humidity for 24 hours to ensure complete polymerization of the acrylic at oral conditions.

Brackets used

Lower incisor MicroArch® brackets provided by Dentsply GAC International (Islandia, NY, USA) were used. The surface area of the bracket was 8.85 mm^2.

Shear bond strength test groups

The total 120 human extracted teeth were divided into 3 different test groups according to the time elapsed from when the SEP package was opened to when the brackets were bonded (Table 1)

Bonding preparation

Prior to bonding, the teeth were cleaned with non-fluoridated prophy paste (Ortho Technology, Tampa Bay, FL) for 10 seconds and then washed and dried. Acrylic superior to the bonding surface of the tooth was removed with an acrylic bur to facilitate a resistance free movement of the shear testing blade (Figure 3).

Bonding procedure

All teeth were bonded with Transbond ™ Plus SEP (3M Unitek, Monrovia, California) at the different time points described in (Table I). The package was folded between bonding individual teeth to prevent any evaporation of the solvent (Figure 4). The Transbond ™ Plus SEP was mixed with the 3M™ Easy Roller. The SEP was applied on the tooth surface with a microbrush (Microbrush® International, Gafton, USA) for 5 seconds. A gentle airburst was delivered onto the SEP for 2 seconds.

A new mircobrush was used for every tooth to simulate the clinical procedure where cross contamination is avoided. A thin uniform coat of Transbond XT Adhesive Paste (3M Unitek, Monrovia, California) was placed on the mesh pads of the brackets.

A 500g weight was used to provide a homogeneous seating of the brackets to the teeth. Excess resin was removed with an explorer probe, and the teeth were light cured at an intensity of 1000 mW/cm^2 for 5 seconds mesial and distal to the brackets with an Ortholux LED curing light lamp (3M Unitek, Monrovia, California).

Debonding procedure

Prior to each debonding, the teeth were kept in distilled water in an incubator at 37°C, 100% humidity for the same period of time as the proposed shear bond strength test times.

Two shear bond strength tests were completed as follows:

T1: 24 hours after initial bonding (60 teeth), simulating short term SBS.

T2: 2 months after initial bonding (60 teeth), simulating more long term SBS.

A shearing blade attached to the Bencor Multi-T testing castle (Danville Engineering, San Ramon, CA) was loaded into the MTS Landmark® Servohydraulic Test System (Eden Prairie, MN) device in a shear force mode at the enamel-bracket interface and using a crosshead speed of 0.5mm/min with a 1kN load cell and tested until bracket bond failure occurred. The SBS was recorded on a computer (Dell, Round Rock, TX) and linked to the testing machine. The data was collected in MegaPascals (MPa).

Adhesive Remnant Index

The enamel surface of each tooth was observed under a Leica EZ4 Stereo microscope (Wetzlar, Germany) at 10X magnification after each debonding to assess the amount of adhesive left on the tooth. A modified adhesive remnant index[32] was used to quantify the amount of remaining resin composite using the following scale: 1 = All the composite left on tooth (100%) with the impression of the bracket base; 2 = > 90% composite left on tooth; 3 = between 10-90% composite left on tooth; 4 = < 10% composite left on tooth; 5= No composite left on tooth.

Statistical Analysis

A two-way ANOVA test was utilized to compare the mean shear bond strength values among the groups. Pairwise comparisons were done to determine the statistical significance between each group. *Kruskal Wallace test* was used to determine if there was a significant difference among the *Adhesive Remnant Index* scores of each group. The inter- and intra-rater agreement used the *Cohen's weighted kappa statistic* to calculate rater agreement. Significance level for all statistical tests $p \leq 0.05$ was be used.

RESULTS

Descriptive data including the mean, standard deviation, minimum, maximum, range, and coefficient of variation of the SBS was determined for each group as in Table II.

Shear bond strength

The group with the highest SBS (MPa) from the 24-hour sample was the 24-hour open group (20.77 MPa), however, there is no statistical difference ($p>0.05$) in mean SBS between the different package opening times within the 24-hour debond group. The group with the highest SBS(MPa) from the 2-month sample was the immediate package opening group (24.09 MPa), in this group there was a significant statistical difference between the groups ($p<0.05$) (Table 6.4). The minimum mean SBS values were above the clinically acceptable standard of 6-8 MPa for all groups.

Coefficient of variation

The coefficient of variation (CV) (Table II) from the 24-hour debond group is considered an acceptable dispersion of values around the mean, but it is on the higher end. The CV from the 2-month debond group ranges from 31.92% to a high 52.77% value. COV values higher than 32% indicate that there is greater dispersion around the mean for that group.

The pairwise comparison of the package open times in Table III. show that the difference in the mean shear bond strengths (SBS) were only statistically significant in the 2 months debond group. None of the pairwise comparisons were found statistically significant in the 24-hour group ($p>0.05$). In the 2-month debond group from Table III, two mean SBS differences were found highly statistically significant.
The first SBS mean difference of 8.218 MPa was comparing the immediate opening group to the 4-hour opening group, considered very highly statistically significant at $p<0.001$. The second SBS mean difference of 9.484 MPa was comparing the immediate opening group to the 24-hour opening group, considered highly statistically significant at ($p<0.05$). The difference in the mean SBS of the 4-hour opening group and the 24-hour opening group is not found statistically significant ($p>0.05$).

In Table IV. the 24-hour debond sample was compared to the 2-month sample. For the immediate open group, a SBS mean difference of 5.866 MPa was found between the 24-hour debond and 2 month debond group, and is statistically significant ($p<0.05$).

For the 4-hour group (Table IV.), SBS mean difference of 3.610 MPa was found between the 24-hour debond and 2-month debond group, this is not considered statistically significant ($p> 0.05$). For the 24-hour open group (Table 6.4), a SBS mean difference of 6.164 MPa was found between the 24-hour debond and 2-month debond group, this is statistically significant ($p<0.05$).

Adhesive Remnant Index

From the frequency distribution ARI results in Table V., it is evident that in all the groups, mixed adhesive-cohesive fractures occurred. The Kruskal Wallace Test was used to compare the ARI across the SEP package opening times and debond times and shows there are significant differences in ARI scores across the groups ($p=0.000$). Table VI. shows the ranks of ARI across the SEP package opening and debond times. Pairwise comparisons were done to determine where the statistically significant differences existed between the groups. Table VII shows that statistically significant ARI rating differences are found in 5 comparisons (highlighted in yellow) ($p<0.05$).

DISCUSSION

Due to the frustration of bracket debonding, orthodontists continue to look for a one-step, reliable, stable, affordable, and “user-friendly” adhesion promoter that can effectively bond to enamel surfaces, in moist conditions, with adequate immediate bond strength that can be sustained through the entire course of treatment. [17] The present study evaluated whether the SBS of single use Transbond ™ Plus SEP will be affected after using it for a prolonged period after being opened

The main reason for prolonged, multiple use in private practice for this product is because it is effective and advantageous for many reasons, but unfortunately, it's not cost-effective if used for repositioning of brackets or repair of broken brackets as a single use product for every patient and procedure. The pandemic outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) in 2019/2020 has also forced clinicians to rethink bonding protocols to efficiently manage and minimize aerosol production. SEPs are a good alternative to CEM, which eliminates the rinsing phase, thus reducing aerosol production. [18] However, manufacturers recommend to first pumice the teeth to remove the pellicle layer and plaque before bonding. Pumicing is considered an aerosol generating procedure as it needs to be rinsed off [18], controversially, pumicing has been shown to be unnecessary for CEMs .[19]However, if the pumicing step is omitted when using SEPs, SBS may be affected negatively by increasing bond failure rates up to 5 times .[20,21]

The mean SBS, minimum as well as the maximum values (Table I.) were very similar when comparing the 24-hour groups to each other, therefore we can conclude that short term SBS is not affected after keeping the package open for up to 24 hours, this might may explain why practitioners have clinical success in orthodontic practice with this bonding protocol.

Within the 2-month debond sample, there was a significant decrease in the mean SBS and the minimum SBS values from the immediately opened package (24.09 MPa) to the package that was open for 24 hours (14.61 MPa) (Table I.), however, the SBS decrease is not clinically significant as all the values from this group, were still above the clinically acceptable standard for SBS. [17,22,23] Accordingly, our study indicates that practitioners can still use the same SEP package after 24 hours without affecting the long term SBS clinically. It is important to keep in mind that the CV exceeds the suggested 20-30% by Powers et al [33] in this group, it is hard to determine how accurate and reliable the data is in order to make the same assumption *in vivo*, since it cannot be demonstrated *in vivo* without clinical testing. This result could also indicate that consistent SBS values cannot be guaranteed when choosing to use the product over a prolonged period.

A possible explanation for the decrease in SBS when used after prolonged periods could be evaporation of the solvent because according to the manufacturer water is the only solvent used in Transbond Plus SEP. A study by Abate et al [24] reported that products with acetone and ethanol-based solvents have low boiling temperatures compared to water, and therefore products that have only water as their solvent are more stable under the same conditions, whereas products with acetone and ethanol require careful conservation and should be used immediately after dispensing. This might explain why Transbond™ Plus SEP can be used for a prolonged period without affecting SBS clinically if the package is folded properly and is not exposed to conditions that cause increased evaporation. However, there still would have been some degree of evaporation of the water solvent when the package was left open. Evaporation leads to an increased viscosity of adhesive materials, increasing the contact angle, which ultimately results in decreased wettability. [36] This might explain the decrease in SBS from the 0-hour open group to the 24 hour-open group in the 2-month debond sample. Another possible reason for the decrease in SBS in the 2-month sample could be that water evaporation alters the chemical equilibrium of the solution and the SEP becomes less acidic with time, leading to an even further reduction in the etching depth, resulting in decreased SBS values. [37]

When the debond groups were compared (Table IV), the only group that showed a SBS increase was the immediate open group, where manufacturer's instructions were followed. According to Wiltshire et al [17] the clinical association of bond maturation can be explained by water sorption equilibration. Our study suggests that bond maturation will only occur when the manufacturer guidelines are followed. When they are not followed the SBS may stay the same or even decrease over time. It would be valuable for this study to be done *in vivo* for the duration of treatment to assess the long-term results, for at least 24 months, in the oral environment, to assume our study's *in vitro* results are reliable.

Potential clinical applications after analyzing the outcomes of the present study would be chair time, but the most important application would be cost. If an orthodontist could use the same Transbond™ Plus SEP over a prolonged period, up to 24 hours, it would be much more cost-effective than opening a new package of Transbond™ Plus self-etching primer for each patient and each procedure. Inventory can also be limited to Transbond™ Plus SEP supplies, which can also be more cost effective than stocking multiple bonding products that can expire if not used on a regular basis.

All the bond failures were mixed adhesive-cohesive fractures in our study. Bonetti et al [25] suggested that, generally, most bracket failures are combinations of adhesive and cohesive failures, and that the latter results in retention of resin on the tooth surface. It has been reported that SEP's have more adhesive bond failures due to reduced depth of demineralization. [32]

A combination of the two modes of failures were seen in our study, but no enamel fractures occurred during debonding of any of the groups, possibly due to the reduced depth of demineralization of SEP's.

CONCLUSIONS

1. Short term SBS, up to 24 hours, is not affected after keeping the package open for up to 24 hours in this experiment, this might explain why Transbond ™ Plus SEP is used successfully in clinical orthodontic practice after multiple-use or prolonged periods after opening the SEP package.
2. After 2 months, the SBS decreases after keeping the SEP package open for 24 hours, but the SBS remains above the clinically acceptable standard of 6-8 MPa *in vitro.* [17, 22, 23] This might explain why Transbond ™ Plus SEP is used successfully in clinical orthodontic practice after multiple-use or prolonged periods after opening the SEP package.
3. SBS values can become more inconsistent and variable in the longer term (> 2 months) when the manufacturer guidelines are not followed, when the SEP is used multiple times over a prolonged period.
4. Pumicing is an essential clinical step when using a SEP, supported by two clinical studies to ensure adequate clinical success. [20,21] In light of the above, even though attempting to reduce the additional pumicing step for decreased chair time, decreased technique sensitively, and patient comfort, the pumicing step remains vital for successful clinical bonding.
5. Reducing aerosol generating procedures, such as pumicing, during the Covid-19 pandemic has become an important consideration for orthodontists, however, it is crucial that the pumicing step is not omitted when using SEPs, despite the fact that this step generates aerosols, as the clinical success of bonding will be affected negatively according to two *in vivo* studies. [20,21]

6. Bond maturation will occur ideally when the manufacturer guidelines are followed, but when they are not followed the SBS could either stay the same, or decrease over time, but are still above what is considered minimum values.
7. The debonding of Transbond™ Plus SEP is adhesive and cohesive in nature, and has mostly mixed mode bond failures, regardless of how long the SEP package is open, and is not anticipated to incur enamel fractures during debonding.

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Table I. Summary of bonding protocols for the 3 test groups and their debond times:

Group	Time elapsed from when SEP package was opened:	Number of teeth	Storage Time/ Debonding time	Number of teeth
A	0 hours (control)/ bonded brackets immediately	40	24 hours	20
			2 months	20
B	4 hours / brackets bonded after package was open for 4 hours	40	24 hours	20
			2 months	20
C	24 hours/ brackets bonded after package was open for 24 hours	40	24 hours	20
			2 months	20

Group A (control): represents immediate bonding of the brackets after the SEP package was opened. Used as a reference point to assess the SBS as recommended by the manufacturer.
Group B: represents 4 hours after the SEP package was opened, simulating the morning from 8am-12pm of a theoretical working day in practice.
Group C: represents 24 hours after the SEP package was opened, simulating the next working day at 8am, to test the extremes.

Table II. Descriptive data of shear bond strength

Debond Time/Open Time of Transbond™ Plus SEP package	N	Mean SBS (MPa)	SD	Minimum (MPa)	Maximun (MPa)	Range (MPa)	CV %
24 hours/Immediate	20	18.28	6.38	11.25	31.62	20.37	34.90
24 hours/4 hours	20	19.49	5.60	11.17	31.55	20.38	28.73
24 hours/24 hours	20	20.77	7.04	11.04	38.55	27.51	33.89
2 months/Immediate	20	24.09	7.69	11.09	35.32	24.23	31.92
2 months/4 hours	20	15.88	8.38	8.03	33.97	25.94	*52.77
2 months/24 hours	20	14.61	6.62	7.51	27.21	19.70	*45.31

SD: standard deviation, CV: coefficient of variation, N: number.
*. Significantly higher CV %, indicating a significantly greater level of dispersion of values around the mean (highlighted in yellow)

Table III. Pairwise comparison the different open times of Transbond™ Plus Self Etching Primer package at debond time 24 hours and 2 months

Debond Time	Open Time of SEP package	Open Time	Mean Difference	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
24 hours	Immediate Open	4 Hour Open	-1.258	2.217	1.000	-6.642	4.126
		24 Hour Open	-2.547	2.123	.698	-7.702	2.608
	4 Hour Open	Immediate Open	1.258	2.217	1.000	-4.126	6.642
		24 Hour Open	-1.289	2.123	1.000	-6.444	3.866
	24 Hour Open	Immediate Open	2.547	2.123	.698	-2.608	7.702
		4 Hour Open	1.289	2.123	1.000	-3.866	6.444
2 Months	Immediate Open	4 Hour Open	8.218*	2.217	.001	2.834	13.602
		24 Hour Open	9.484*	2.217	.000	4.100	14.868
	4 Hour Open	Immediate Open	-8.218*	2.217	.001	-13.602	-2.834
		24 Hour Open	1.266	2.217	1.000	-4.118	6.650
	24 Hour Open	Immediate Open	-9.484*	2.217	.000	-14.868	-4.100
		4 Hour Open	-1.266	2.217	1.000	-6.650	4.118

*. The mean difference is significant at the 0.05 level (highlighted in yellow).
b. Adjusted for multiple comparisons: Bonferroni

Table IV. Pairwise comparison of Transbond™ Plus Self Etching Primer debond times at 24 hours and 2 months at every open time of package

Open Time of SEP package	Debond Time	Debond Time	Mean Difference	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Immediate Open	24 hours	2 Months	-5.866*	2.217	.009	-10.257	-1.476
	2 Months	24 hours	5.866*	2.217	.009	1.476	10.257
4 Hour Open	24 hours	2 Months	3.610	2.217	.106	-.781	8.000
	2 Months	24 hours	-3.610	2.217	.106	-8.000	.781
24 Hour Open	24 hours	2 Months	6.164*	2.123	.004	1.961	10.368
	2 Months	24 hours	-6.164*	2.123	.004	-10.368	-1.961

*. The mean difference is significant at the 0.05 level (highlighted in yellow).
b. Adjusted for multiple comparisons: Bonferroni

Table V. Frequency and percentage of ARI Scores for this experiment

Group	ARI Score					Total
Debond time/ Open Time	**1**	**2**	**3**	**4**	**5**	
24 Hour/Immediate	4 (20%)	4 (20%)	2 (10%)	3 (15%)	7 (35%)	20
24 Hour/4 Hours	1 (5%)	0 (0%)	1 (5%)	13 (65%)	5 (25%)	20
24 Hours/24Hours	0 (0%)	0 (0%)	6 (30%)	13 (65%)	1 (5%)	20
2 Months/Immediate	2 (10%)	2 (10%)	10 (50%)	5 (25%)	1 (5%)	20
2 Months/4 Hours	1 (5%)	3 (15%)	6 (30%)	10 (50%)	0 (0%)	20
2 Months/24 Hours	8 (40%)	10 (50%)	1 (5%)	1 (5%)	0 (0%)	20

Table VII. Kruskal Wallace ranks and means of ARI ratings

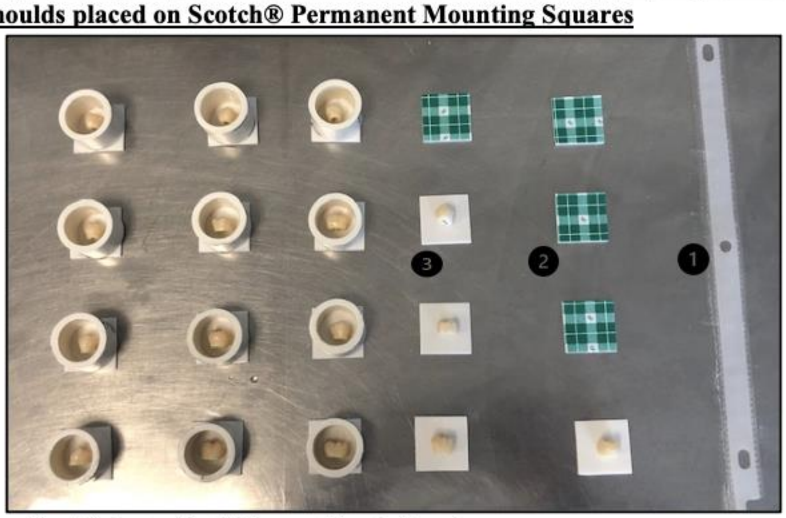
SEP package opening Time	Removal Time	Mean Rank	Mean ARI rating
4 Hours	24 Hours	102.88	4.08
24 Hours	24 Hours	84.67	3.63
Immediate	24 Hours	73.29	3.21
4 hours	2 Months	69.22	3.13
Immediate	2 Months	62.87	3.00
24 Hours	2 Months	31.00	1.87

Table VII. Pairwise comparisons of debond and opening times of SEP package-ARI ratings

Comparison of sample 1 to sample 2	Sig.	Adj. Sig.[a]
2 Months debond /24 Hours open - 2 Months debond /Immediate open	.006	.092
2 Months debond /24 Hours open - 2 Months debond /4 Hours open	.001	.015*
2 Months debond /24 Hours open - 24 hours debond /Immediate open	.000	.004*
2 Months debond /24 Hours open - 24 Hours debond /24 Hours open	.000	.000*
2 Months debond /24 Hours open - 24 Hours debond/4 Hours open	.000	.000*
2 Months debond /Immediate open - 2 Months debond/4 Hours open	.585	1.000
2 Months debond /Immediate open - 24 Hour debond/Immediate open	.365	1.000
2 Months debond /Immediate open - 24 Hour debond/24 Hours open	.058	.874
2 Months debond /Immediate open - 24 Hours debond/4 Hours open	.001	.008*
2 Months debond /4 Hours open - 24 Hours debond /Immediate open	.723	1.000
2 Months debond /4 Hours open - 24 Hours debond /24 Hours open	.180	1.000
2 Months debond /4 Hours open - 24 Hours debond /4 Hours open	.003	.052
24 Hours debond /Immediate open - 24 Hours debond/24 Hours open	.318	1.000
24 Hours debond /Immediate open - 24 Hours debond/4 Hours open	.009	.141
24 Hours debond /24 Hours open - 24 Hours debond/4 Hours open	.110	1.000

*. Significant at the 0.05 level (highlighted in yellow).
Adj. Sig.[a]: Adjusted Significance: Bonferroni

Figure 1. Teeth positioned horizontally on flat surface; polyvinyl chloride (PVC) cylindrical moulds placed on Scotch® Permanent Mounting Squares



1) Adhesive tape (Scotch® Permanent Mounting Squares)
2) Flat surface of teeth placed on adhesive tape
3) Polyvinyl chloride (PVC) cylindrical moulds

Figure 2. Teeth imbedded in acrylic removed from the PVC moulds, with the flat surface visible