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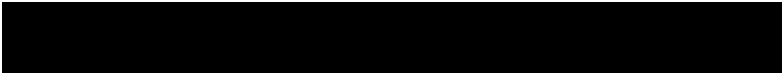
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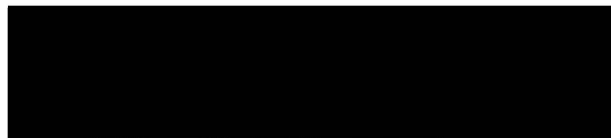


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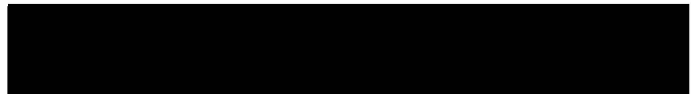
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**MICROTENSILE BOND STRENGTH OF A NOVEL ADHESIVE SYSTEM
CONTANING 0.2% CHLORHEXIDINE**

BY

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A THESIS

Submitted in partial fulfillment of the requirements
For the degree of Masters of Science in the
Department of Oral Biology
In the graduate School of
The Uniformed Services University of the Health Services

FORT BRAGG, NORTH CAROLINA
2015

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Abstract

Objective: This in-vitro study looks at the immediate and 16 week micro tensile bond strength (MTBS) of Peak Universal Bond (PUB), a novel adhesive system containing 0.2 % chlorhexidine (CHX), in a self etch (SE) application.

Materials and Methods: Thirty six non-carious extracted third molars were randomly assigned to one of three test group based on dentinal adhesive system. Group A: Clearfil SE, Group B: Clearfil SE +0.2 % CHX, Group C: PUB. All teeth were sectioned parallel to cemento-enamel junction (CEJ) mid-coronally. Dentin adhesive was applied to exposed dentinal surfaces per manufacturers instructions before adding composite resin (Filtek Z250, Shade A2). Samples were tested for immediate (24 hour) and for 16 week MTBS. Proc GLM of SAS protocol, Tukey tests, and studentized T-test were used for statistical analysis.

Results: Group A-0 displayed significantly less immediate MTBS than B-0 and C-0. At 16 weeks Group A-16 displayed a significant decrease in bond strength with a loss of 10.6 MPa. Groups B and C showed no significant decrease in bond strength at 16 weeks.

Conclusion: The addition of 0.2% CHX into Clearfil SE and PUB self etching primers acts to prolong dentin bond strength over a 16 week time period without significantly inhibiting immediate bond strength.

Table of Contents

Acknowledgements	iii
Abstract	iv
List of Tables.....	vi
List of Figures	vii
Introduction	1
Concise Review of Dentin Adhesives	3
Current Bonding Systems and Hybrid Layer.....	4
Matrix Metalloproteinases and Hybrid Layer Degradation.....	6
Chlorhexidine as an MMP Inhibitor	8
Novel Adhesive Systems Containing Chlorhexidine.....	10
Micro Tensile Bond Strength	11
Materials and Methods	13
Results	18
Discussion	24
Conclusions	26
Bibliography	27
Appendix ...	30

List of Tables

Table 1: Mean raw data for group A, B, C and 24 hr and 16 wk.....	18
Table 2: Comparison between groups of initial 24 hr MTBS.....	19
Table 3: Comparison between groups of 16 wk MTBS.....	21
Table 4: Degradation of dentinal bond strength over 16 wk.....	23
Table 5: Raw Data Group A-0	30
Table 6: Raw Data Group B-0	31
Table 7: Raw Data Group C-0	32
Table 8: Raw Data Group A-16	33
Table 9: Raw Data Group B-16	34
Table 10: Raw Data Group C-16	35

List of Figures

Figure 1: Comparison between groups of 24 hr MTBS.....23

Figure 2: Comparisons between groups at 16 wk.....25

Figure 3: Degradation of Bond strength over 16 wk period.....27

INTRODUCTION

Statement of Problem

Over the last 6 decades, the use of dental composites and other esthetic restorations has increased exponentially, due to an increase in esthetic demands and a pervasive fear of mercury in amalgam. The use of composite as a restorative material come the challenges of bonding to tooth structure. Unlike amalgam, which relies entirely on mechanical retention via undercuts in prep design, composite restorations must to bond to both enamel and dentin through micro-mechanical retention via dentinal bonding agents. (Milnar, 2011)

While bonding to enamel is very predictable, bonding to dentin has proven to be fraught with challenges. Manipulating the smear layer, managing moisture content, avoiding collapse of collagen fibrils are just a few of the difficulties in establishing a dentinal bond. Over time, the dentin bond is susceptible to enzymatic and hydrolytic breakdown of the collagen fibrils and a subsequent degradation of the overall bond. Multiple studies have shown significant loss of bond strength in as little as 6 months.

Significance: As the dentinal bond fails, several sequelae can develop. Initially, marginal leakage occurs and staining develops along the margins of the restoration. Ingress of cariogenic bacteria can lead to secondary caries, loss of retention and ultimate failure of the composite restoration. Extensive research

has been dedicated to preventing the breakdown of the dentinal bond. Matrix metalloproteinases (MMPs) are collagenases and implicated in the enzymatic breakdown of dentinal collagen fibrils resulting in the degradation of the hybrid layer and subsequent nano leakage of the restoration (Boushell, Kaku, Mochida, & Yamauchi, 2011) (Hannas & Pereira , 2007). This has the potential to precipitate failure of the restoration. Chlorhexidine digluconate (CHX) has been studied in great detail as a cross linking agent that inactivates dentinal MMPs allowing greater longevity of the hybrid layer and maintenance of bond strength. (Hannas & Pereira , 2007) (Varun, Nair, & Sivakumar) Numerous studies investigating the use of CHX in various etch-and-rinse and self-etch techniques have been conducted over the past several years (Zhou, Chen, & Tan, 2009) (Bracket & Tay, 2009) (Collares, Rodrigues, & Leitune, 2013). With few exceptions, CHX has been successful in inhibiting MMP enzymatic breakdown of the hybrid layer. With this in mind, new materials have sought to incorporate MMP inhibition into their products via the addition of CHX. However, current literature is sparse in regards to these new products.

Concise review of Dentin Adhesives

Dentin: Dentin is a hard tissue of the tooth characterized by multiple closely backed dentinal tubules that transverse its entire thickness. Mature dentin is composed of 70% inorganic material, 20% organic material, 10% water by weight. The inorganic component is primarily hydroxyapatite crystals, while the organic component is 90% type 1 collagen with small amount of type 2 and 3, collagen, along with non-collagenous matrix proteins and lipids that pack the space between collagen fibrils and accumulate along the periphery of the dentinal tubules. Type 1 Collagen acts as a scaffold that accommodates a large proportion of the hydroxyapatite mineral while the non-collagenous matrix proteins regulate mineral deposition during tooth maturation. (Nanci, 2008)

Dentinal Tubules are hollow communications that extend through the entire thickness of the dentin from the dentinoenamel junction (DEJ) to the pulp. They contain dentinal fluid and the cellular processes of the odontoblast's (Nanci, 2008). The tubules become narrow as they approach the DEJ. At the pulp there are approximately 60-70 thousand per square mm. At the DEJ there is approximately half that number (Nanci, 2008).

Smear layer: Any form of tooth preparation involving the cutting of dentin or enamel by rotary instruments will generate cutting debris. Some of this debris will

unavoidably become compacted into the layer on the cut surface regardless of dental hard tissue (Roberson, 2006). This smear layer is typically 1-3 μm thick on the surface. When dentin is involved, the smear layer will extend into the dentinal tubules to a depth of approximately 2-5 μm known as smear plugs. If left alone, the smear layer provides some degree of sealing of the dentinal tubules. They are 25-30% porous allowing for long-term micro leakage. In regards to adhesive dentistry, the smear layer must be either removed, allowing access to dentinal tubules and etched dentin, or modified, incorporated into the adhesive bond. If the smear layer is left intact, bond strength is severely compromised. (Pashely, 1992)

Current Bonding systems and the Hybrid Layer

There are currently two approaches to bonding to dentin and interacting with the smear layer; etch-and-rinse (two-step and three-step), and self-etch (two-step and one-step). The first, etch-and-rinse (aka total etch), is characterized by first etching with 37% phosphoric acid followed by compulsory rinsing, resulting in complete removal of the smear layer (Cardoso, Neves, & Mine, 2011). This exposes a scaffold of collagen fibrils 5-8 μm deep. The rinse removes and replaces native water resulting in a makeup that is 20% collagen and 80% water. (Pashley D. H., 2011) The next step for the etch-and-rinse is to apply the primer which contains hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) dissolved in organic solvents such as acetone, ethanol or

water. The solvent is able to displace any remaining water thus preparing the collagen fibrils for resin infiltration. The third and final step is to apply a non-solvent, hydrophobic adhesive resin, leading to the penetration of the monomers not only into interfibrillar spaces of the collagen network but also into dentinal tubules. In situ polymerization leads to the formation of the hybrid layer, a hybridized bio composite of collagen fibrils entombed in resin (Pashley D. H., 2011) (Pashley, Horner, & Brewer, 1992).

In the two step system, the hydrophilic primer is combined with the adhesive resin. The two-step etch-and-rinse tends to have lower bond strengths due to inferior resin infiltration and increased hydrolytic degradation resulting from the hydrophilic nature of the adhesive (Cardoso, Neves, & Mine, 2011).

Self-etch adhesives interact with the smear layer from an entirely different approach. They do not require a separate etching step but instead use acidic monomers that simultaneously etch and prime the dental substrate. (Cardoso, Neves, & Mine, 2011) As self-etch adhesives interacts with the smear layer, rather than removing it, their pH may be buffered by the high pH of the basic mineral content of the remaining thick and dense smear layer. Depending on the initial pH, penetration depths may vary. In two-step self-etch, etch and primer are combined into one step, followed by application of the bonding agent. In the one step system, etch, primer, and bonding agent have been combined into one step. These adhesives have been made more acidic and more hydrophilic than their two step counter parts. One of the main disadvantages of one step self-etch

adhesives is related to their excessive hydrophilicity, increasing water sorption from the intrinsically wet dentinal substrate. (Cardoso, Neves, & Mine, 2011)

As previously discussed, in both the etch-and-rinse and self-etch adhesive systems, resin infiltration into the hybrid layer is the primary goal to ensure high bond strength. Ideally, intrinsic water would be entirely displaced from dentinal tubules and demineralized dentin allowing for complete adhesive infiltration. In reality, adhesive monomers are not able to fully encapsulate the exposed collagen matrix leaving partial or complete exposure of collagen fibrils at the bottom of the hybrid layer. (Tjaderhane, 2013) Due to the presence of residual solvents and fluid movement out of dentinal tubules, and increasing hydrophilicity of the adhesive layer depending on system used, resin infiltration is never ideal. There are often small local regions that are water rich and resin poor. (Pashley D. H., 2011) These fibrils lack the protection of the polymerized resin and are susceptible to hydrolytic degradation and enzymatic breakdown (Tjaderhane, 2013). One of the key enzymes implicated in the hybrid layer destruction are the matrix metalloproteinases.

Matrix Metalloproteinases and Hybrid Layer Degradation

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that can degrade extra-cellular matrix proteins, both in dental hard tissues and throughout the body. Their normal function involves the controlled and coordinated breakdown and remodeling of the extra-cellular matrix (Varun, Nair,

& Sivakumar). They are beneficial during tissue remodeling and dentin formation (Moon, Weaver, & Brooks, 2010). MMPs can also act during inflammation to increase adverse effects of cardiovascular disease, cancer metastasis, periodontal disease and caries. Normally, this enzymatic breakdown is regulated by Tissue Inhibitors of Metalloproteinases (TIMPs). If this balance is disturbed, or a much larger proportion are activated, it can lead to enzymatic degradation of the dentinal collagen fibrils and subsequent weakening of the hybrid layer. (Moon, Weaver, & Brooks, 2010)

There are 26 identified members of the MMP family; with three of particular relevance to hybrid layer failure, MMP 2, 8 and 9. MMP-8 is a collagenase that preferentially clips type I and II collagen into smaller pieces (Varun, Nair, & Sivakumar). Collagen is further degraded by MMP-2 and MMP-9, gelatinases. MMPs are initially secreted from connective tissue cells such as osteoblasts and odontoblasts as zymogens, inactive forms of the enzymes. The N-terminus is folded over blocking the active catalyst site so Zn and Ca cannot bind and activate the MMP (Kotra, 2001). The Inactive MMP are trapped in the dentin during dentin formation and can be released when pH reaches levels below 4.5. During acid etching, vast amount of MMPs are released, but in the zymogen (inactive) form. Activating proteins such as bone sialoprotein, osteopontin and dentin matrix protein 1 are responsible for converting MMPs from the zymogen to an active form in one of two ways: they either cleave the N-

Terminus or displace it. Zn and Ca are then free to bind to and activate the MMP (Kotra, 2001).

Numerous in vitro studies have demonstrated hybrid layer degradation over long periods of time via microtensile bond strength tests. In vitro and in vivo studies demonstrated enzymatic breakdown of collagen fibrils under adhesive resin. High levels of active MMPs present in the tested dentin were implicated in the hybrid layer degradation (Sorsa, Tjanderhane, & Salo, 2004). Koshiro demonstrated strength loss of 36% to 70% at 12 months and 14 months with both self-etch and etch-and-rinse adhesives and contributed it to high levels of MMPs (Koshiro, Inoue, & Tanaka, 2004). Carrilho demonstrated 38% reduction in Tensile Bond strength of 14 months (Carrilho, Ceraldeli, & Tay, 2007). Brackett demonstrated extensive destruction of the hybrid layer, including loss of collagen fibrils at 12 months (Bracket & Tay, 2009)

Chlorhexidine as an MMP inhibitor

Given the destructive nature of MMP 2,8 and 9 on hybrid layers, numerous studies have been conducted to determine clinically relevant MMP inhibitors. Chlorhexidine Digluconate (CHX) has repeatedly been shown to effectively inhibit MMP proteolytic activity. CHX acts through a cationic chelating mechanism that sequesters metal ions such as Ca and Zn, necessary MMP activators. (Hannas & Pereira , 2007) (Collares, Rodrigues, & Leitune, 2013) Numerous investigations have focused on 3-step and 2-step etch-and-rinse

adhesive in which CHX is applied as a conditioner after initial etching (Moon, Weaver, & Brooks, 2010) (Carrilho, Ceraldeli, & Tay, 2007) (Bracket & Tay, 2009), or as an addition to the phosphoric acid conditioner (Stanislawczuk & Amaral, 2009). Carrilho demonstrated that a 60 second application of 2% CHX after application of phosphoric acid and before application of primer resulted in maintenance of the hybrid layer over a 14 month period compared to a 38% reduction in control samples (Carrilho, Ceraldeli, & Tay, 2007). Brackett demonstrated 12 month hybrid layer maintenance following a 30 second CHX application to acid etched dentin. (Bracket & Tay, 2009) Adding CHX to etched dentin as a therapeutic primer is the most studied and currently the most widely accepted method of CHX use to maintain the hybrid layer (Moon, Weaver, & Brooks, 2010) (Sabatini, 2013).

Attempts have been made to reduce the number of steps involved in dentinal bonding by incorporating differing concentrations of CHX into various components of several bonding systems. CHX incorporation into acid conditioner was shown to prolong the integrity of the hybrid layer suggesting a potential for clinical relevance (Stanislawczuk & Amaral, 2009). Sabitini combined a 0.2% concentration with the adhesive component of a self-etch and an etch-and-rinse system and found no difference between the test and control (Sabatini, 2013). Munck added .05% CHX to the primer in a self-etch and etch and rinse adhesive system and found no difference between the test and control. (Munck & Van Den Steen, 2009) Zhou completed similar testing, demonstrating that the addition of

CHX to Clearfil SE Bond has no detrimental effects on the initial resin dentin bond strength when the CHX concentrations are 1.0% or less. (Zhou, Tan, Yang, & Cheng, 2010) Zhou then demonstrated significant difference in 12 month (MTBS) of Clearfil SE + CHX [0.10%-1.0%] compared to his control (Zhou, Chen, & Tan, The incorporation of chlorhexidine in a two step self-etching adhesive preserves dentin bond in vitro, 2009). This has become the gold standard for CHX incorporation into a self-etch adhesive system. With few exceptions, studies have supported the use of CHX for prolonged MMP inhibition.

Novel Adhesive Systems Containing Chlorhexidine

Several adhesive systems recently introduced to the market aim to incorporate CHX into their product. One such product, Peak Universal Bond by Ultradent (PUB), is a novel adhesive containing 0.2% CHX. Manufacturer's recommendation indicates use in either the etch-and-rinse system with 37.5% phosphoric acid or self-etch applications with Peak SE Ultadent. However, current literature on the ability of PUB to maintain hybrid layer integrity is sparse. Sabatini, one of the first to investigate this product, measured the shear bond strength (SBS) of PUB in both an etch- and-rinse and self-etch application over a 6 month period. He found no significant difference between PUB and his control in either application (Sabatini, 2013). One problem with this study is the use of SBS as the measured variable; SBS tests are known to be less discriminating in

their ability to discern small differences in adhesive bonds as compared to MTBS tests (Sabatini, 2013).

A recent study by Crites compares MTBS of PUB against Scotchbond multi-purpose bond + 2.0% CHX (Crites, 2014). This etch-and-rinse study looks at the initial and 6 month MTBS as an indication of MMP inhibition. He found that incorporation of CHX into his total etch adhesive system significantly improved the 6 month MTBS of Scotchbond multipurpose and PUB over his control.

The purpose of this study is to investigate the initial and 16 week MTBS of PUB in a self-etch application by measuring the MTBS of PUB + Peak SE (PUBSE) compared to Clearfil SE + CHX 0.2%.

Micro Tensile Bond Strength Test (MTBS)

Micro tensile tests are characterized by the use of small beam-shaped specimens with small bonding areas, generally less than 2mm, that generate perpendicular stress to the adhesive interface (Sano & al, 1994). Many believe this to be the most reliable method for evaluating the bond strength between the adhesive material and dental substrate as the stress is more uniformly distributed, as compared to micro-shear tests or macro shear/tensile tests. (DH, Sano , & Ciucchi, 1995) As a result of more uniform stress distribution, the occurrence of cohesive fractures within dentin or within the adhesive are greatly reduced. Such failures invalidate the sample by preventing the correct

assessment of the interfacial bond (Cardosa, Sadek, & et al, 2002). This test also allows the ability to obtain multiple specimens from a single tooth sample.

Specific Aim

1. Determine if addition of CHX to self-etching adhesives significantly alters immediate (24 hour) MTBS.
2. Determine if addition of CHX to self-etching adhesives significantly alters 16 week MTBS.
3. Determine if addition of CHX to self-etching adhesive significantly alters degradation of MTBS from initial (24 hour) to 16 week.

Null Hypothesis

1. No statistical difference exists between the initial MTBS of self-etch adhesive with and without CHX
2. No Statistical Difference exists between the 16 week of self-etch adhesive with and without CHX
3. No statistical difference exists in the rate of degradation of MTBS in groups with and without CHX.

Materials and Methods

Collection Period (4 weeks)

Non-carious human teeth indicated for extraction were collected immediately following extraction. All remaining soft tissue was removed via curette and specimens were stored in 0.5% solution of Chloramine T at 22 degrees Celsius for a minimum of 24 hours and no more than 4 weeks for disinfection (Munchow & Bossardi, 2013).

Tooth Preparation

In order to test the adhesion to dentin, the entire occlusal enamel layer was removed with a water-cooled diamond disc. In order to form a uniform smear layer, the dentinal layer was polished using progressively finer sandpaper; (220-, 320-, 400-grit) for 10 seconds each, followed by 600-grit for 60 seconds. The bonding surface was washed with water and gently air dried (Cardosa, Sadek, & et al, 2002).

Test Groups (Immediate and 16 week)

- Group A (Control Self Etch)
 - o Clearfil SE (Kuraray, Osaka, Japan)
- Group B (Control SE+0.2% CHX)
 - o Clearfil SE (Kuraray, Osaka, Japan)
 - o 20% Chlorhexidine Digluconate (Sigma-Aldrich, St. Louis, MO)

- Group C (Test Group, PUBSE)
 - o Peak Universal Bond (Ultradent Products Inc, South Jordan, UT)
 - o Peak SE (Ultradent Products Int, South Jordan, UT)

All teeth were categorized based on the following system to give each stick a unique identification number.

- a. Study Group(A,B,C)
 - i. 24 hour (A-0, B-0, C-0)
 - ii. 16 week (A-16, B-16, C-16)
- b. Individual tooth identifier (1, 2, 3,...)
- c. Individual beam identifier (a,b,c)

Addition of CHX to Clearfil SE

The addition of 20% Chlorhexidine digluconate followed the protocol indicated by Zhou to reach a final concentration of 0.2% CHX using serial dilution. Chlorhexidine was added directly to the acidic primer in Clearfil SE at the time of adhesive application (Zhou, Chen, & Tan, The incorporation of chlorhexidine in a two step self-etching adhesive preserves dentin bond in vitro, 2009).

- 5.0ul CHX digluconate (20%) + 995ul SE B primer

Application of Adhesive/ Composite

Adhesives were applied according the manufacturer's recommendations based on the assigned group; Group B Followed the same adhesive protocol as Group A. Following application of the adhesive, a 5x5x5 mm block was bonded to the adhesive layer via the following protocol as indicated by Cardoso (Cardosa, Sadek, & et al, 2002)

1. Composite: Filtek Z250 Universal Microhybrid Composite Shade A2 (3M/ESPE, St. Paul, MN, USA) was applied to the bonding surface through sequential application of 1-2mm thick layers of the material.
2. Each layer was cured for 20 seconds at 600nm based on manufacturer's recommendations.
3. This process was repeated to a final thickness of 5mm.
4. Samples were stored in artificial saliva for either 24 hours (Immediate) or 16 weeks.

Sample Sectioning and MTSB test

Each sample was sectioned and tested based on the following procedure:

- 1 After 24 hours/ 16 week storage in saline 37° C, each tooth was mounted into an acrylic jig to facilitate sectioning
2. Using Isomet 1000 at 300rpm with water irrigation, each tooth was multiply cross- and longitudinally sectioned in 1mm increments perpendicular to

the adhesive interface with a diamond blade, obtaining 1mm² beams approximately 10 mm in length.

3. Prior to MTBS testing, each sample was observed to verify that the adhesive interface was perpendicular to the long axis (necessary for application of pure tensile force) and to remove any samples displaying micro fractures. All Samples with slanted adhesive interface or fractures were discarded.
4. All cut samples were stored in artificial saline manufactured by Dr. Pashley.
5. All samples were adhered to Bisco MTBS testing jig with cyanoacrylate adhesive and tested for MTBS on Instron 5943.
6. Each sample was observed for method of fracture: Adhesive, Cohesive Dentin, Cohesive Composite

Sample Size Calculation

- Sample Size was based on the Power Analysis as described by James Aden and ISR Statistical and Epidemiology Services. The following factors were included.

- $$n = (2 * STD^2) / E^2 * 7.8$$

1. Based on a desired power of 80% (Beta =.2) and a 95% Confidence interval (alpha = 5%),

2. Expected Standard Deviation of 12 MPa (assumed from previous studies performed by Zhou)
3. Expected size Difference of 10 MPa between two independent study groups to be compared. (assumed from previous studies performed by Zhou)
4. Sample Size required per group= 23 samples.

Statistical Analysis

The outcome variables for this study are continuous and measured as MPa. All groups are independent of one another. Each group has a normal distribution and the variances of the groups are equal. Two variables are considered: group allocation (Group A, B, and C) and individual tooth within each group. Ergo, Proc GLM of SAS, a two factor analysis of variance was done to evaluate if there are differences between the three groups and if there are differences between individual teeth. Since each group and tooth has significantly effect size, Tukey's method was used to assess which pairs had statistically significant differences.

Results

The means and standard deviations for samples collected at 24 hour and 16 weeks are presented in Table 1.

Group/ Timepoint	N	Mean MPa	Std Dev MPa	Std Err
A-0	43	29.71	13.23	2.01
B-0	50	41.07	16.64	2.35
C-0	41	40.48	18.92	2.95
A-16	47	19.14	14.81	2.16
B-16	48	39.82	15.62	2.25
C-16	44	34.81	18.07	2.72

Table 1. Means of Raw data for groups A, B, and C at initial 24 hour time point and 16 week time point.

Specific Aim #1: Determine if addition of CHX to self-etching adhesives significantly alters immediate (24 hour) MTBS.

In order to determine if the addition of CHX to self etching adhesives (Clearfil SE and Peak universal bond) significantly decreased the initial (24 hour) bond strength, A-0, B-0 and C-0 were investigated under Proc GLM of SAS protocol, a two factor analysis of variance. Tukey's method was used to assess which pairs had a significant difference. The results, tabulated in table 2, indicated that group A-0 had a significantly lower initial (24 hour) MTBS when compared to B-0 and C-0. This is also seen graphically in figure 1.

Group Comparison	Difference of means (MPa)	P Value
A-0 to B-0	-11.36	<0.05
A-0 to C-0	-10.77	<0.05
B-0 to C-0	0.59	>0.05

Table 2. Comparison between groups of Initial (24 hour) MTBS.

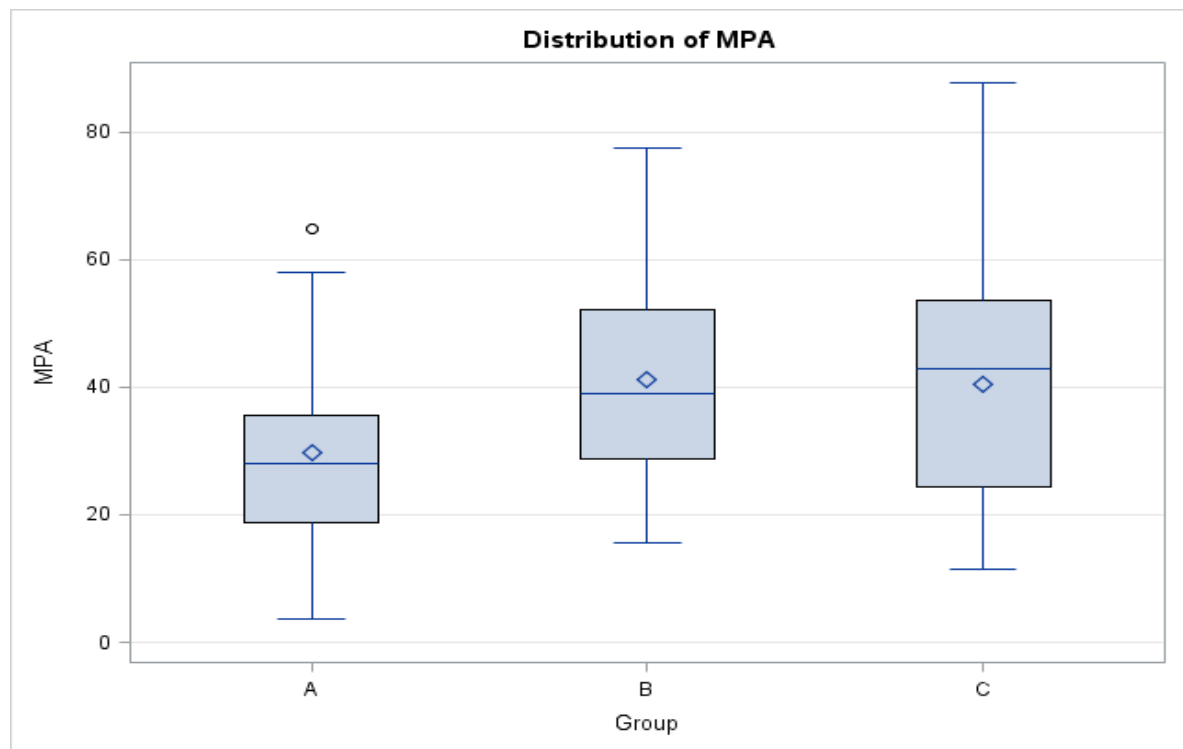


Figure 1. Comparison between groups of initial (24 hour) MTBS

Specific aim #2: Determine if addition of CHX to self-etching adhesives significantly alters 16 week MTBS between A-16, B-16, and C-16.

In order to determine if the addition of CHX to self etching adhesives (Clearfil SE and Peak universal bond) significantly decreased the 16 week MTBS, A-16, B-16 and C-16 were investigated under Proc GLM of SAS protocol, a two factor analysis of variance. Tukey's method was used to assess which pairs had a significant difference. Statistical analysis indicates that A-16 had significantly lower MTBS as compared to B-16 and C-16 as indicated in Table 3. This relationship is represented graphically in figure 2.

Group Comparison	Difference of means (MPa)	P Value
A-16 to B-16	-20.67	<0.05
A-16 to C-16	-15.66	<0.05
B-16 to C-16	5.01	>0.05

Table 3: Comparison between groups of 16 week MTBS. Group A shows significant difference from Group B and C No significant difference exists between Group B and C

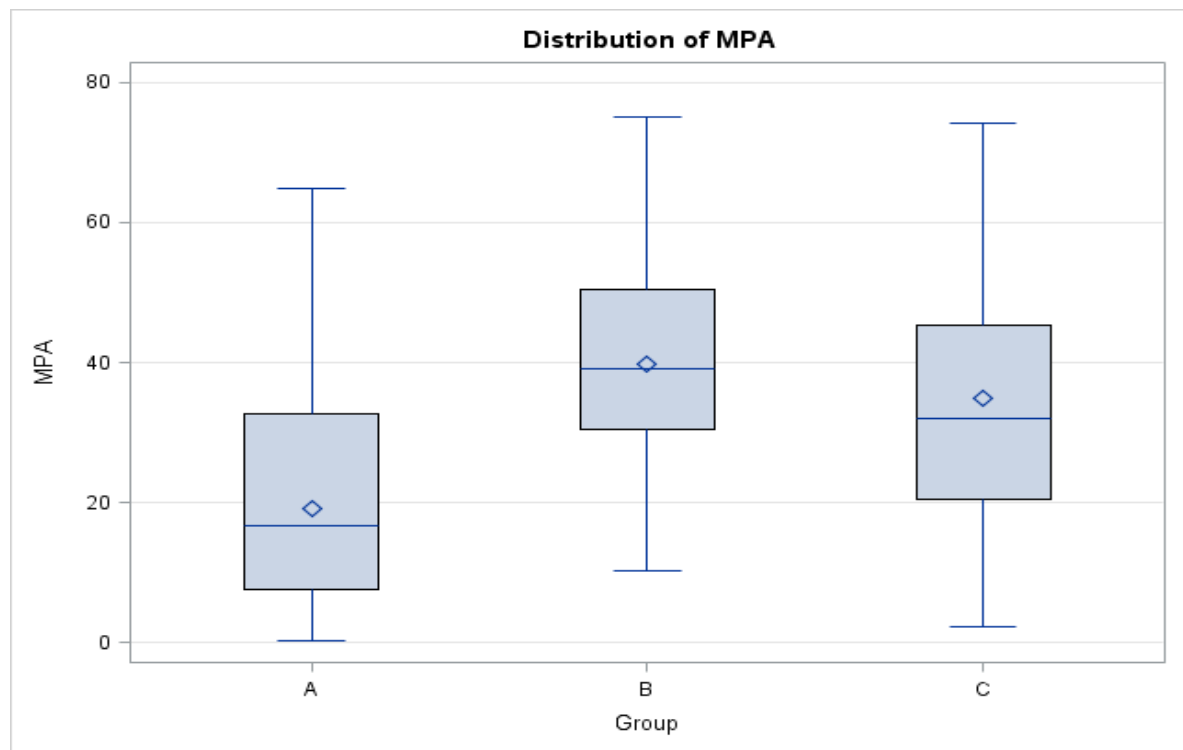


Figure 2. Comparison between groups of 16 week MTBS

Specific aim #3: Determine if addition of CHX to self-etching adhesive significantly alters degradation of MTBS from initial (24 hour) to 16 week between groups A, B, and C.

Given the normal distribution, the Studentized T-test was used to determine if significant difference exists between timepoint 24-hour and 16 week within each of the three groups. As seen in Table 4, Group A, Clearfil SE, had an initial MTBS of 29.7 MPa and a 16 week MTBS of 19.1 MPa. That is a loss of 10.6 MPa 35.7 %. This constitutes a statistically significant loss of bond strength over the 16 week period with $p=0.0006$. Group B had an initial MTBS of 41.1 MPa and a final MTBS of 39.8 MPa. This is a loss of only 1.3 MPa or 3.2%. There is no significant loss over 16 weeks. Group C had an initial MTBS of 40.5 MPa and a final MTBS of 34.8 MPa, a loss of 5.7 MPa or 14.1%. Again, no statistically significant difference exists between 24 hour and 16 weeks for group C. This relationship is shown graphically in figure 3.

	24 Hour	16 week	% loss	P value
Group A	29.7	19.1	35.69	0.0006
Group B	41.1	39.8	3.16	0.7
Group C	40.5	34.8	14.07	0.16

Table 4: Degredation of dentinal bond strength over 16 week period. Group A displayed significant loss of MTBS over 16 week period. Group B and C showed no significant loss of MTBS over 16 week period.

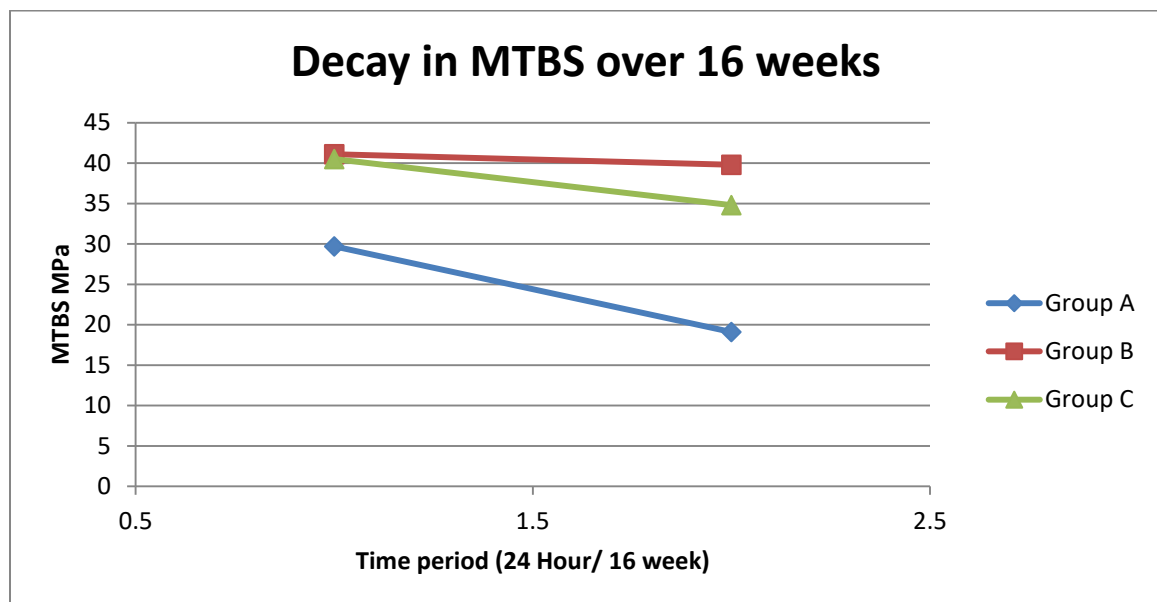


Figure 3. Degradation of bond strength over 16 week perior. Graph indicates groups B and C maintained their initial MTBS compared to Group A, which had a significant reduction in MTBS.

Discussion

This study was undertaken to determine if the addition of chlorhexidine into a self etch adhesive protocol significantly altered the immediate MTBS (24 hour), the 16 week MTBS, and the rate at which the dentinal bond degrades. The study produced some surprising results. Firstly, the immediate bond strength of A-0 of (29.7 MPa) was significantly less than B-0 and C-0 (41.1 MPa and 40.5 MPa respectively). It is surprising to see the significant difference between A-0 and B-0. Both groups contained Clearfil SE. Group B-0 included 0.2% CHX and the bonding protocols were identical. Prior to undertaking this study, it was assumed that the addition of CHX would, at best, have no influence on the immediate MTBS of A-0 and B-0. As shown in previous studies Sabitini found no significant difference in immediate bond strength following the addition of 0.2% CHX (Sabatini, 2013). Previous studies of CHX suggest it only acts on the MMPs which should have no impact on immediate bond properties, only exerting beneficial effects over a longer time frame. Regardless of the perceived improvement in immediate bond strength, the important point is that the addition of CHX to Clearfil SE did not inhibit the immediate MTBS. Further investigation into this phenomenon should be conducted.

When looking at the MTBS of A-16, B-16, and C-16, there was a significant difference between group A-16 and B-16 with a difference of mean MTBS of -20.67 MPa ($p < 0.05$) and also between A-16 and C-16 with a difference

of mean MTBS of -15.66 ($p < 0.05$). These results are not surprising as they follow the same pattern as the 24 hour timepoint.

When looking at each group over time, an interesting result emerges. A-0 vs. A-16 displayed a significant decrease in MTBS with a total loss 10.6 MPa (35.69%). No significant difference was seen between B-0 vs B-16 with a total loss of 1.3 MPa (3.16%) and C-0 vs. C-16 with a total loss for 5.7 MPa (14.1%). These results closely align with those reported by Koshiro et al who found a 36% reduction in MTBS over a 12 month period in the absence of CHX (Koshiro, Inoue, & Tanaka, 2004). A previous Crites found a loss of 12 MPa in his control group over a 6 month period while his test groups, which contained CHX, had no significant decrease in their MTBS (Crites, 2014). These results support the assumption that the addition of CHX into a self etch adhesive inhibits the enzymatic degradation of the dentinal bond.

One major limitation of this study is the time interval. Although dentinal bond strength for PUB was maintained over a 16 week period, very little data indicates bond strength for PUB is maintained beyond 6 months. Further research is needed to observe MTBS at a 12 month time period or longer.

Conclusion:

Within the confines of this study, it can be concluded that the addition of CHX into self-etch adhesive systems (Clearfil SE + 0.2%CHX and PUB) may have a significant effect in increasing initial bond strength. It can also be concluded that CHX may significantly improve the longevity of the dentinal bond.

Bibliography

- Boushell, L. W., & Swift, E. J. (2011). Dentin Bonding: Matrix Metalloproteinases. *23*(5), 347-352.
- Boushell, L. W., Kaku, M., Mochida, Y., & Yamauchi, M. (2011). Distribution and relative activity of matrix metalloproteinase-2 in human coronal dentin. *Int J Oral Sci*, *3*, 192-199.
- Bracket, M., & Tay, F. (2009). In Vivo Chlorhexidine Stabilization of Hybrid Layers of an Acetone-based dentin Adhesive. *Operative Dentistry*(34), 379-83.
- C, S. (2013 Nov-Dec). Effect of a Chlorhexidine-containing Adhesive on Dentin Bond Strength Stability. *Oper Dent*, 607-17.
- Cardosa, P., Sadek, F., & et al. (2002). Adhesion testing with the microtensile method: Effects of Dental Substrate and adhesive systems on bond strength measurments. *Journal of Adhesive Dentistry*, *4*(4), 291-297.
- Cardoso, M., Neves, A., & Mine, A. (2011). Current aspects on bonding effectiveness and stability in adhesive dentistry. *Australian Dental Journal*(56), 31-44.
- Carrilho, M., Ceraldeli, S., & Tay, F. (2007). In Vivo Preservation of the Hybrid Layer by Chlorhexidine. *Journal of Dental Research*(86), 529-33.
- Collares, F., Rodrigues, S., & Leitune, V. (2013). Chlorhexidine Application in Adjesive Procedures: A Meta-Regression Analysis. *J Adhes Dent*(15), 11-18.
- Crites, P. (2014). Microtensile Bond Strength of an Adhesive System Containing 0.2% Chlorhexidine.
- DH, P., Sano , H., & Ciucchi, B. (1995). Adhesion testing of dentin bonding agents: A review. *Dent Mater*, *11*, 117-25.
- Ferracane, J. L. (2010). Resin Composite-State of the Art. *Dental materials* .
- H, S., & et al. (1994). Relationship between surface area for adhesion and tensile bond strength-evaluation of micro tensile bond test. *Dent mater*, *10*, 236-40.
- Hannas, A., & Pereira , J. (2007). The Role of matrix metalloproteinases in the oral enciornment. *Acta Odontol Scand*(65), 1-13.
- Koshiro, K., Inoue, S., & Tanaka, T. (2004). In Vivo degredation of resin-dentin bonds produced by self-etch vs. a total-etch adhesive system. *Eur J Oral Sci*, *112*(4), 368-75.
- Kotra, L. (2001). Insight into the complex and dynamic process of actication of matrix metalloproteinases. *J Am Chem Soc*, *123*(13), 3108-13.

- Milnar, F. (2011, Feb). The Evolution of Direct Composites. *Compendium of Continuing Education in Dentistry*, 32(1).
- Moon, P. C., Weaver, J., & Brooks, C. (2010). Review of Matrix Metalloproteinases Effect on teh hybrid dentin bond layer stability and chlorhexidine clinical use to prevent bond failure. *The Open Dentistry Journal*, 147-152.
- Munchow, E., & Bossardi, M. (2013). Microtensile Bersus microshera bond strangth between dental adhesives adn the dentin substrate. *International Journal of Adhesion and Adhesives*, 46, 95-99.
- Munck, J. D., & Van Den Steen, P. (2009). Inhibition of enzymatic Degredation of Adhesive-Dentin Interfaces. *Journal of Dental Research*(88), 1101-06.
- Nakabayash, N. i., & Pashley, D. H. (1998). Hybridization of Dental Hard Tissues. 65-67.
- Nanci, A. (2008). Ten Cate's Oral Histology. In *Dental Pulp Complex* (7th ed.). St. Louis, Missouri: Mosby.
- Pashely, D. (1992). Smear Layer: Overview of structure and function. *Proc Finn Dent Soc*, 88, 215-24.
- Pashley, D. H. (2011). State of the Art etch and rinse adhesives. *Dental materials*.
- Pashley, D., Horner, J. A., & Brewer, P. O. (1992). Interactions of conditioners on the dentin surfaces. *Operative Dentistry*, 17(5), 127-50.
- Roberson, T. M. (2006). Art and Science of Operative Dentistry. In *Biomaterials* (pp. 176-177). St. Lous, Missouri: Mosby.
- Sabatini, C. (2013). Effect of a Chlorhexidine-Containing adhesive on Dentin Bond Strength Stability. *Operative Dentistry*, 38(5).
- Sano, & al, e. (1994). Relationship between surface area for adhesion and tensile bond strength-evaluation of micro tensile bond test. *Dent mater*, 10, 236-40.
- Sorsa, T., Tjanderhane, L., & Salo, T. (2004). Matrix metalloproteinases in oral diseases. *Oral Dis*(10), 311-18.
- Stanislawczuk, R., & Amaral, R. (2009). Chlorhexidine-Containing acid conditioner perserves teh longevity of resin-dentin bonds. *Operative Dentistry*, 34(4), 481-490.
- Tjaderhane, L. (2013). Strategies to prevent hydrolytic degradation of the hybrid layer-A review. *Dental materials*.

- Varun, B., Nair, B., & Sivakumar, T. (n.d.). Matrix Metalloproteinases and their role in oral disease: A Review.
- Zhor, J. (2009). The incorporation of Chlorhexidine in a two-step self-etching adhesive preserves dentin bond in vitro. *Journal of Dentistry*, 37(2), 807-812.
- Zhou, J., Chen, L., & Tan, J. (2009). The incorporation of chlorhexidine in a two step self-etching adhesive preserves dentin bond in vitro. *Journal of Dentistry*, 37(10), 807-12.
- Zhou, J., Tan, J., Yang, X., & Cheng, C. (2010). Effect of chlorhexidine application in a self etching adhesive on the immediate resin dentin bond strength. *Journal of Adhesive Dentistry*, 12(1), 27-31.
- Zhou, J., Yang, X., Chen, L., & et al. (2013). Pre-treatment of radicular dentin by self etch primer containing chlorhexidine can improve fiber post bond durability. *Dental materials Journal*, 32(2), 248-255.

Appendix

	MTBS	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	38.68	0.98	0.92	1	Cohesive Dentin
2	64.80	0.89	0.94	1	Adhesive
3	25.01	0.89	0.92	1	Adhesive
4	17.68	0.91	0.99	2	Adhesive
5	18.81	0.88	1.00	2	Adhesive
6	20.51	1.00	1.00	2	Cohesive Composite
7	3.70	1.00	1.00	2	Adhesive
8	17.93	0.91	0.92	3	Adhesive
9	18.38	0.88	0.97	3	Adhesive
10	25.72	0.96	0.96	3	Adhesive
11	26.11	1.00	0.87	3	Adhesive
12	26.42	1.00	0.93	4	Adhesive
13	14.46	1.00	0.93	4	Adhesive
14	27.59	1.00	0.87	4	Adhesive
15	28.15	0.88	0.97	5	Adhesive
16	8.54	1.00	1.00	5	Cohesive Dentin
17	13.71	0.95	1.00	5	Adhesive
18	21.31	1.01	0.93	5	Adhesive
19	31.88	0.89	0.93	6	Adhesive
20	25.87	1.00	0.92	6	Adhesive
21	15.58	0.95	0.88	6	Adhesive
22	35.68	0.87	0.96	6	Adhesive
23	16.18	0.98	0.99	6	Adhesive
24	20.06	0.97	1.05	7	Adhesive
25	34.56	1.04	0.90	7	Cohesive Composite
26	39.77	0.90	0.92	7	Adhesive
27	46.71	1.00	0.98	7	Cohesive Dentin
28	35.40	0.89	0.91	8	Adhesive
29	31.34	0.90	0.93	8	Adhesive
30	32.21	0.93	0.88	8	Adhesive
31	52.09	0.88	0.95	9	Adhesive
32	25.67	0.91	0.94	9	Adhesive
33	31.78	0.95	1.10	9	Adhesive
34	28.05	0.99	0.92	9	Adhesive
35	33.92	1.00	1.00	9	Cohesive Composite
36	41.84	0.93	1.10	10	Cohesive Composite
37	41.66	0.97	0.91	10	Adhesive
38	32.12	0.88	0.93	10	Adhesive
39	30.82	0.90	0.93	10	Adhesive
40	42.00	1.00	0.93	11	Adhesive
41	39.58	0.87	0.90	11	Adhesive
42	31.82	0.98	0.92	11	Adhesive
43	53.03	0.95	0.95	11	Adhesive
44	41.56	0.90	0.86	11	Adhesive
45	51.63	0.91	0.94	11	Adhesive
46	35.13	0.91	0.94	12	Adhesive
47	9.67	0.91	0.94	12	Adhesive
48	12.88	0.94	0.99	12	Adhesive
49	58.01	0.87	0.91	12	Adhesive
50	26.38	0.92	0.97	12	Adhesive
Maximum	58.01	1.01	1.10		
Mean	29.28	0.93	0.94		
Median	27.82	0.92	0.93		
Minimum	9.67	0.87	0.86		
Standard deviation	11.61	0.05	0.05		
Mean + 1 SD	40.89	0.98	0.99		
Mean - 1 SD	17.67	0.89	0.89		

Table 5. Raw Data Group A-0

	MTBS	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	37.19	0.87	0.92	1	Adhesive
2	62.21	1.00	1.00	1	Cohesive Dentin
3	59.68	0.88	0.94	1	Adhesive
4	55.42	0.97	0.88	1	Adhesive
5	48.26	0.82	0.98	1	Adhesive
6	31.91	0.92	0.95	2	Adhesive
7	52.06	0.85	0.97	2	Adhesive
8	35.13	0.96	0.92	2	Adhesive
9	49.11	0.96	0.93	2	Cohesive Dentin
10	27.56	0.91	0.97	2	Adhesive
11	38.70	0.92	0.97	2	Adhesive
12	30.22	0.99	0.99	3	Adhesive
13	34.52	0.94	1.00	3	Adhesive
14	26.10	0.95	0.93	3	Adhesive
15	17.68	0.97	0.95	3	Cohesive Dentin
16	10.09	1.00	1.00	3	Cohesive Dentin
17	46.82	0.88	0.92	3	Adhesive
18	20.73	0.96	0.97	4	Adhesive
19	15.54	0.88	1.00	4	Adhesive
20	17.90	0.98	1.00	4	Adhesive
21	28.69	0.90	0.92	4	Adhesive
22	63.52	0.89	0.94	5	Adhesive
23	31.58	0.96	1.00	5	Adhesive
24	70.59	0.92	0.96	5	Cohesive Dentin
25	19.77	0.95	0.95	6	Adhesive
26	46.85	0.92	0.94	6	Adhesive
27	19.60	0.82	0.88	6	Adhesive
28	30.74	0.86	0.92	6	Adhesive
29	31.48	1.00	1.00	6	Adhesive
30	74.17	0.93	0.99	6	Adhesive
31	61.82	0.99	0.98	7	Adhesive
32	65.56	0.98	0.93	7	Cohesive Dentin
33	77.41	0.89	0.80	7	Adhesive
34	66.21	0.94	0.83	7	Adhesive
35	48.26	0.91	0.96	8	Adhesive
36	21.22	0.90	0.93	8	Adhesive
37	18.94	0.99	0.97	8	Adhesive
38	30.57	0.88	0.94	8	Adhesive
39	33.18	0.87	0.92	9	Adhesive
40	44.90	0.94	0.93	9	Adhesive
41	18.18	0.83	0.91	9	Adhesive
42	16.91	1.09	0.92	9	Adhesive
43	67.06	0.99	0.92	9	Cohesive Dentin
44	63.25	0.93	1.00	10	Adhesive
45	41.43	1.00	0.93	10	Adhesive
46	58.57	0.93	1.02	10	Cohesive Composite
47	60.20	1.00	1.00	10	Cohesive Dentin
48	46.01	1.00	1.00	11	Adhesive
49	49.13	1.02	0.94	11	Adhesive
50	37.85	0.96	1.02	11	Adhesive
Maximum	47.60	0.96	0.93		
Mean	47.77	0.96	0.93		
Median	47.94	0.96	0.93		
Minimum	48.12	0.96	0.93		
Standard deviation	48.29	0.96	0.93		
Mean + 1 SD	48.46	0.96	0.93		
Mean - 1 SD	48.63	0.96	0.93		

Table 6: Raw Data Group B-0

	MTBS [MPa]	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	35.61	0.94	0.98	1	Cohesive Composite
2	71.30	0.93	0.94	1	Cohesive Composite
3	45.85	1.00	0.96	1	Adhesive
4	32.18	1.00	1.00	1	Cohesive Composite
5	51.83	1.00	1.00	1	Adhesive
6	38.67	1.00	0.96	1	Cohesive Composite
7	87.70	0.81	0.88	1	Adhesive
8	16.06	0.92	0.97	2	Adhesive
9	67.72	0.88	0.86	2	Adhesive
10	58.69	0.95	0.93	2	Adhesive
11	40.38	0.93	1.00	2	Adhesive
12	59.20	0.85	0.89	3	Adhesive
13	55.37	0.85	0.89	3	Adhesive
14	49.36	0.93	1.00	3	Adhesive
15	57.88	0.90	0.89	3	Adhesive
16	12.77	0.90	0.95	4	Adhesive
17	11.83	0.95	0.95	4	Adhesive
18	21.67	0.87	0.90	4	Adhesive
19	42.77	0.91	0.92	4	Adhesive
20	34.20	1.04	0.93	5	Adhesive
21	38.49	1.02	0.93	5	Adhesive
22	24.28	0.91	0.93	5	Adhesive
23	51.80	0.83	0.93	5	Adhesive
24	26.74	0.95	0.83	5	Adhesive
25	12.58	1.00	1.02	5	Adhesive
26	26.55	0.90	0.90	6	Adhesive
27	27.43	0.89	0.94	6	Adhesive
28	25.20	0.81	0.83	6	Adhesive
29	11.43	0.95	0.98	7	Adhesive
30	17.13	0.86	0.87	8	Adhesive
31	19.50	0.89	0.90	8	Adhesive
32	14.02	0.86	1.00	8	Adhesive
33	20.62	0.93	0.85	8	Adhesive
34	42.23	0.87	0.91	9	Adhesive
35	42.48	0.86	0.86	9	Adhesive
36	58.05	0.87	0.92	10	Adhesive
37	49.93	0.92	0.85	10	Adhesive
38	43.36	0.87	0.83	10	Adhesive
39	61.67	0.88	1.00	11	Adhesive
40	64.87	0.89	0.88	11	Adhesive
41	62.88	0.93	0.87	11	Adhesive
42	53.47	0.86	0.85	11	Adhesive
43	48.34	0.90	0.93	12	Adhesive
44	50.11	0.90	0.87	12	Adhesive
45	53.49	1.00	1.00	12	Adhesive
Maximum	87.70	1.04	1.02		
Mean	40.84	0.91	0.92		
Median	42.48	0.90	0.93		
Minimum	11.43	0.81	0.83		
STDEV	18.69	0.06	0.05		
Mean + 1 SD	59.53	0.97	0.98		
Mean - 1 SD	22.15	0.86	0.87		

Table 7: Raw Data Group C-0

	MTBS [MPa]	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	34.73	0.98	1.10	1	Adhesive
2	33.90	0.97	0.94	1	Adhesive
3	39.54	1.10	0.94	1	Adhesive
4	0.22	1.00	1.00	2	Adhesive
5	0.50	1.02	0.99	2	Adhesive
6	2.99	1.00	1.00	2	Adhesive
7	0.74	1.00	1.00	2	Adhesive
8	14.70	0.96	0.97	3	Adhesive
9	1.97	1.00	0.97	3	Adhesive
10	2.80	1.00	1.00	3	Adhesive
11	7.21	1.00	1.00	3	Adhesive
12	16.65	0.80	0.98	4	Adhesive
13	6.31	0.98	0.97	4	Adhesive
14	7.49	1.00	1.01	4	Adhesive
15	20.30	1.00	1.00	4	Adhesive
16	8.12	1.00	1.00	5	Adhesive
17	8.82	1.10	0.94	5	Adhesive
18	5.32	0.92	0.97	5	Adhesive
19	10.16	1.00	1.00	5	Adhesive
20	9.99	0.99	0.96	6	Adhesive
21	6.61	1.10	0.98	6	Adhesive
22	12.17	0.97	1.10	6	Adhesive
23	9.87	1.10	0.97	6	Adhesive
24	37.48	0.99	0.98	7	Adhesive
25	39.36	0.93	0.96	7	Adhesive
26	24.09	0.96	0.97	7	Adhesive
27	19.48	1.10	0.95	7	Adhesive
28	25.98	1.01	0.97	8	Adhesive
29	13.01	0.92	0.97	8	Adhesive
30	18.08	0.97	0.95	8	Adhesive
31	20.04	0.95	0.94	8	Adhesive
32	39.39	1.10	0.94	9	Adhesive
33	49.27	0.97	0.99	9	Adhesive
34	41.50	0.93	0.99	9	Adhesive
35	30.63	1.00	1.00	9	Adhesive
36	35.26	0.89	0.96	9	Adhesive
37	33.51	1.13	1.20	10	Adhesive
38	32.76	0.96	0.91	10	Adhesive
39	19.46	0.87	0.88	10	Adhesive
40	51.08	1.00	1.10	10	Cohesive Composite
41	10.29	0.91	0.94	11	Adhesive
42	18.37	0.94	0.95	11	Adhesive
43	13.53	0.90	0.95	11	Adhesive
44	64.99	0.93	0.96	11	Adhesive
45	17.92	0.93	1.04	12	Adhesive
46	1.23	0.94	0.97	12	Adhesive
47	18.39	0.90	1.00	12	Adhesive
48	14.81	0.90	0.96	12	Adhesive
Maximum	64.99	1.13	1.20		
Mean	19.81	0.98	0.98		
Median	17.28	0.98	0.97		
Minimum	0.22	0.80	0.88		
STDEV	15.37	0.07	0.05		
Mean + 1 SD	35.18	1.05	1.04		
Mean - 1 SD	4.45	0.91	0.93		

Table 8: Raw Data Group A-16

	MTBS [MPa]	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	33.89	0.95	0.96	1	Adhesive
2	42.59	0.96	1.00	1	Adhesive
3	38.21	0.99	0.84	1	Adhesive
4	36.47	0.78	1.00	1	Adhesive
5	37.78	1.01	0.90	2	Adhesive
6	45.80	1.02	0.91	2	Adhesive
7	32.84	1.01	0.98	2	Adhesive
8	10.32	0.96	0.97	2	Cohesive Dentin
9	33.20	0.96	0.93	3	Adhesive
10	16.28	0.93	0.91	3	Adhesive
11	10.46	0.96	0.94	3	Cohesive Dentin
12	28.23	0.95	1.00	3	Adhesive
13	11.29	0.84	0.86	4	Adhesive
14	23.00	0.97	0.91	4	Adhesive
15	10.16	1.00	1.00	6	Adhesive
16	59.60	1.00	1.00	5	Adhesive
17	64.82	0.91	0.97	5	Adhesive
18	39.96	0.94	1.00	5	Adhesive
19	36.36	1.00	1.00	6	Adhesive
20	48.02	0.94	0.97	6	Adhesive
21	29.56	0.82	0.84	6	Adhesive
22	31.16	0.86	0.95	6	Adhesive
23	34.62	1.00	1.00	7	Adhesive
24	74.79	1.00	0.97	7	Adhesive
25	75.05	0.93	0.99	7	Adhesive
26	53.57	0.96	0.92	7	Adhesive
27	54.22	0.98	0.95	8	Adhesive
28	36.58	0.91	0.92	8	Adhesive
29	30.13	1.00	1.00	8	Cohesive Dentin
30	54.19	0.93	0.97	8	Adhesive
31	31.72	0.93	0.97	8	Cohesive Composite
32	16.51	0.93	0.97	9	Adhesive
33	57.38	1.02	0.96	9	Adhesive
34	44.20	0.94	0.97	9	Adhesive
35	18.00	0.97	0.94	9	Adhesive
36	18.48	1.00	1.00	9	Adhesive
37	64.29	0.95	0.95	10	Adhesive
38	41.80	0.98	0.97	10	Adhesive
39	43.86	0.97	0.94	10	Adhesive
40	37.05	0.98	0.92	10	Adhesive
41	32.87	0.98	0.96	11	Adhesive
42	21.84	0.96	0.92	11	Adhesive
43	47.40	0.98	0.97	11	Adhesive
44	24.43	0.95	0.99	11	Adhesive
45	42.55	0.98	0.97	12	Adhesive
46	56.18	0.96	1.04	12	Adhesive
47	48.48	0.96	0.96	12	Adhesive
48	42.35	0.90	0.94	12	Adhesive
49	20.79	0.96	0.94	13	Adhesive
50	55.86	1.00	1.60	13	Adhesive
51	42.49	1.03	1.00	13	Adhesive
52	52.42	1.00	1.00	13	Adhesive
Maximum	75.05	1.03	1.60		
Mean	38.35	0.96	0.97		
Median	37.42	0.96	0.97		
Minimum	10.16	0.78	0.84		
STDEV	16.12	0.05	0.10		
Mean + 1 SD	54.47	1.01	1.07		
Mean - 1 SD	22.23	0.91	0.87		

Table 9: Raw Data Group B-16

	MTBS [MPa]	Width [mm]	Thickness [mm]	Tooth #	Breakage Type
1	51.78	0.96	0.97	1.00	Adhesive
2	58.58	0.87	0.80	1.00	Adhesive
3	41.09	0.96	0.97	1.00	Adhesive
4	30.87	1.04	0.97	1.00	Cohesive Composite
5	31.83	1.00	0.98	2.00	Adhesive
6	32.33	0.95	0.97	2.00	Adhesive
7	23.30	0.97	0.96	2.00	Cohesive Dentin
8	66.33	0.97	0.96	2.00	Adhesive
9	67.05	0.94	0.91	3.00	Adhesive
10	56.52	0.96	0.91	3.00	Adhesive
11	44.94	0.96	1.00	3.00	Adhesive
12	74.21	0.92	0.96	3.00	Adhesive
13	13.24	0.96	1.10	4.00	Adhesive
14	7.48	0.97	0.84	4.00	Adhesive
15	2.34	1.00	1.00	4.00	Adhesive
16	24.04	1.00	1.00	4.00	Adhesive
17	18.33	0.93	0.90	5.00	Adhesive
18	23.43	0.95	0.96	5.00	Adhesive
19	44.62	1.01	0.96	5.00	Adhesive
20	20.49	1.00	1.00	5.00	Adhesive
21	45.78	0.96	0.90	6.00	Adhesive
22	35.30	1.00	0.88	6.00	Adhesive
23	42.15	0.97	0.94	6.00	Adhesive
24	30.64	0.86	0.86	6.00	Adhesive
25	23.07	0.94	0.95	7.00	Adhesive
26	20.52	0.94	0.94	7.00	Adhesive
27	13.02	1.00	1.00	8.00	Adhesive
28	9.96	0.96	0.90	8.00	Adhesive
29	19.67	0.92	0.96	8.00	Adhesive
30	17.21	0.92	0.96	8.00	Adhesive
31	44.17	0.92	1.00	9.00	Adhesive
32	40.85	0.82	0.86	9.00	Adhesive
33	44.92	1.00	1.00	9.00	Adhesive
34	46.19	0.93	0.98	9.00	Adhesive
35	9.40	0.96	0.94	10.00	Adhesive
36	24.80	1.00	1.03	10.00	Adhesive
37	30.28	0.96	0.99	10.00	Adhesive
38	38.90	0.96	0.88	10.00	Adhesive
39	49.46	1.00	1.00	11.00	Adhesive
40	28.01	0.93	0.98	11.00	Adhesive
41	62.95	0.95	0.97	11.00	Adhesive
42	67.60	0.99	1.00	11.00	Adhesive
43	28.77	0.98	0.97	12.00	Adhesive
44	14.69	0.97	1.10	12.00	Adhesive
45	29.74	0.92	0.96	12.00	Adhesive
46	35.22	1.00	1.00	12.00	Adhesive
Maximum	74.21	1.04	1.10		
Mean	34.48	0.96	0.96		
Median	31.35	0.96	0.97		
Minimum	2.34	0.82	0.80		
STDEV	17.76	0.04	0.06		
Mean + 1 SD	52.24	1.00	1.02		
Mean - 1 SD	16.72	0.92	0.90		

Table 10: Raw Data Group C-16

