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

BY

ARI CRAIG CYLUS

B.S. Muhlenberg College, Allentown, Pennsylvania, 2005
D.D.S. University of Maryland, Baltimore, Maryland, 2010

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Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

10 June 16
Date

[redacted]
LTC Stacy Larsen, DDS
Associate Professor
AEGD Program Director

10 June 16
Date

[redacted]
LTC Manuel Pelaez, DMD MS
Associate Professor
Research Director

10 JUNE 16
Date

[redacted]
CPT Jason Bullock, DMD
Associate Professor
AEGD Assistant Program Director

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Ari Craig Cylus
2-Year AEGD Program, Ft. Bragg
Uniformed Services University
Date: 05/20/2016

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_____	_____
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INTRODUCTION

Statement of Problem

Composite resins are a tooth colored restorative material commonly utilized today as an alternative to metallic restorations when full coverage is not required. The creation of a perfect tooth colored restorative material is not a new goal. Composite was developed to replace silicate cements (Paffenbarger, et al., 1938) due to their shortcoming: a four to five year lifespan due to acid decay (Bowen, et al., 1968). Unlike amalgam, which relies on mechanical retention within the preparation, composites require an adhesive bond for retention. This bond is complex and varies according to the histologic features of the adhering surface. Bonding to dentin has been a particular challenge. Even products labeled 'dentin-bonding agents' fail to produce a long lasting bond.

Observed bond weakening has been recorded in as little as 6 months after application. This is attributed to the hydrolytic breakdown of resin components and the enzymatic destruction of collagen at the bond interface. Nanoleakage at restorative margins is one of the major causes of failure in bonded restorations. Leakage can manifest as unaesthetic staining, dentin sensitivity, recurrent decay, and ultimately loss of the restoration due to lack of retention.

Significance

Hydrolytic breakdown of resin components and the enzymatic destruction of collagen at the bond interface are credited as the major sources of dentin-composite bond weakening over time. Current clinical research has focused on developing dentin bonding agents that are impervious to enzymatic bond degradation, thus prolonging the lifespan of bonded restorations. Matrix metalloproteinases (MMPs), a family of proteases involved in tissue remodeling and wound healing, have been the subject of much of this research due to their role in degrading dentin collagen fibrils at bonded dentin surfaces. Several studies have examined chlorhexidine digluconate (CHX) and its ability to inactivate dentinal MMPs (Stanislawczuk, et al., 2009). The results from these studies indicate CHX facilitates the formation of a stabilized hybrid layer essential in maintaining dentinal bond strength. Several products have incorporated CHX for this purpose but their efficacy has not been tested.

Review of Literature

In 1955, Buonocore, working at the Eastman Dental Center in Rochester, New York, discovered that he could alter the surface of enamel to "render it more receptive to adhesion by exposure to varying chemicals. After experimenting with different acrylic resins, he found that by conditioning enamel with 85% phosphoric acid, it was possible to produce a bond that could withstand stresses experienced in the mouth (Buonocore, 1955). Thus began the journey to create the perfect bonding agent. The major mechanism of bonding to tooth structure is based on a process in which resin monomers,

which replace the minerals removed from enamel and dentin (Van Meerbeek, et al., 2003), become micromechanically interlocked upon polymerization (Van Meerbeek, et al., 2006).

While a bond to both enamel and dentin are achievable (Cardoso, et al., 2011), it is the enamel bond, which is most predictable enamel bonding, (Brackett, et al., 2011). This occurs because, relative to other human hard tissues, enamel is the most mineralized and most homogenous (Perdigao, et al., 2002). Roughly 95%-98% of enamel is calcium and phosphate ions making up the hydroxyapatite crystals. 1%-2% of enamel is composed of organic materials including enamelins, which are proteins with a high affinity for hydroxyapatite crystals. Water makes up the difference in enamel's weight. These elements are all highly organized into long thin rods that are 4μm to 8μm in diameter (Robinson C, et al., 1998). Between 5 million (in mandibular incisors) and 12 million (in maxillary molars) of these rods are estimated to be present (Robinson C, et al., 1998). Demineralization of the enamel rods creates micro-porosities to which resin monomers are attracted. When polymerized, these monomers are responsible for the micro-mechanical adhesion that makes enamel bonding so strong (Gwinnett & Matsui, 1967). The predictable bonding to this highly organized enamel is responsible for sealing the restoration margins and protecting the more vulnerable dentin bond (De Munck, et al., 2003). While little has changed in the way we bond to enamel, the majority of research in 'adhesive dentistry' has centered on how to obtain a strong and lasting bond to dentin (Brackett, et al., 2011).

While enamel is the most homogenous hard tissue, dentin is one of the most complex. It has a greater concentration of water and organic material, 10% and 20% respectively, permitting this great variation). The majority (90%) of the organic materials in dentin is type 1 collagen. In addition to these differences in composition, the microanatomy and histology of dentin is greatly variable (Perdigao, et al., 2010). Dentin is composed of hollow communications, called dentin tubules, that course through the entire thickness of dentin, from the dentino-enamel junction (DEJ) to the pulp. Dentin tubules contain a fluid and the cellular processes of odontoblasts, the cells responsible for the formation of dentin, and are lined by a hyper-mineralized layer known as 'peritubular dentin' (Nanci, 2008) While this type of dentin is particularly difficult to condition and results in weak bonding, 'intertubular dentin', which is located between dentinal tubules, results in stronger bonds (Perdigao, et al., 2002).

The diameter of dentin tubules varies between 2.5μm near the pulp to 0.9μm at the DEJ. The density of dentin tubules is estimated to range from 59000 to 76000 tubules/mm^2 near the pulp (Schilke, et al., 2000). Thus, characteristics vary throughout the dentin with tubules being narrower in diameter and numbering fewer in density closer to the DEJ than the pulp (Mjor, et al., 2002). This variation has a significant impact on surface area. While dentinal tubules near the DEJ make up just 1% to2% of the surface area, they make up nearly 22% of the surface area at the Pulp (Srinivasulu, et al., 2012). Another unique property is the varying hydrostatic pressures inside each dentinal tubule. The variation in pressure establishes the outward flow of water and sustains a state of constant state of dehydration within the dentinal tubules (Cardoso, et al., 2011). The

large tubule diameter, increased density, and outward water flow makes bonding increasingly difficult closer to the pulp resulting in weaker bonds (Srinivasulu, et al., 2012).

Another inhibitor of achieving a great bond is the smear layer. This microcrystalline and organic particle debris, only 2-5 μm thick, was noted by Brannstrom and Johnson on dentinal walls following cavity preparation (Brannstrom, 1974). The smear layer can be described as two phases and two layers. The organomineral phase is made of collagen residues and glycosaminoglycans from the extracellular matrix of pulp cells, and acts as a matrix for the inorganic phase. The first layer is 1-2 μm thick, loosely covers the canal walls, and is easily removed. The second layer strongly adheres to the canal walls (Pashely, 1984) penetrating the dentinal tubules on average 5μm (Mader, et al., 1984). Their porous structure does not completely occlude the canals resulting in long term microleakage . Adhesive dentistry approaches the smear layer by either removal or modification. Failure to remove or modify the smear layer results in a significantly weakened bond. (Pashely, 1984).

Complete removal of the smear layer is accomplished with the etch-and-rinse approach. This is done by treating the tooth with phosphoric acid and rinsing prior to the application of adhesive. The phosphoric acid promotes dentin demineralization resulting in exposed collagen fibrils a depth of 3-5μm (Van Meerbeek, et al., 1992), that is nearly completely depleted of hydroxyapatite (Perdiga˜o, et al., 1996). This step is followed by the application of a primer that contains specific monomers with hydrophilic properties such as 2-hydroxy ethyl methacrylate (HEMA) dissolved in organic solvents. While the

HEMA acts as a wetting agent and promotes re-expansion of the collagen network (Nakabayashi & Takarada, 1992), the solvents displace water from the dentin surface (Carvalho, et al., 2003). In the final step, bonding, a solvent-free adhesive resin is applied to the prepared surface. This results in the penetration of hydrophobic monomers in both the interfibrillar spaces of the collagen network and into the dentin tubules themselves, providing a micromechanical retention once polymerized. This layer is the 'hybrid layer' because it includes components of both dentin and resin (Nakabayashi, et al., 1982).

The two-step adhesives are a simplified version of the traditional three-step etch-and-rinse. While these adhesives combine the primer and adhesive resin into one solution resulting in fewer steps, they typically have inferior bond strength in comparison to their traditional three-step predecessors. This is because the primer-adhesive combination has a reduced penetration of the demineralized dentin substrate producing an inferior hybridization (Finger & Balkenhol, 1999). Additionally, their hydrophilic nature leaves them more susceptible to hydrolytic degradation and impairs the evaporation of the solvent frequently entrapping it within the adhesive layer (Van Meerbeek, et al., 2005).

Self-etch adhesives do not require a separate etching step because they contain an acidic monomer that simultaneously etches and primes the dental surfaces. This acidic characteristic allows self-etch adhesives to dissolve the smear layer and demineralize the underlying dentin and enamel (Tay & Pashley, 2000). Mild self-etch adhesives partially demineralize dentin leaving a large amount of hydroxyapatite crystals around the collagen fibrils to which the adhesive can chemically interact (De Munck, et al., 2005). It

was originally thought that the combination of both micromechanical and chemical adhesion would be advantageous with respect to bonding durability and effectiveness (Yoshida , et al., 2004). However, the hybrid layer formed was less than 1μm and resin tags were not significant (De Munck, et al., 2005). Recently, the assumption that self-etch adhesives demineralize and infiltrate the dentin surface to the same extent preventing incomplete penetration of the adhesive into the exposed collagen network, has been challenged (Tanumiharja & Burrow, 2000). Perhaps the greatest concern about one-step self-etch adhesives is their hydrophilicity, resulting in the attraction of moisture from intrinsic tooth structure (Tay & Pashley, 2003). The outcome is, even after polymerization, the adhesives have been reported to act as a semi-permeable membrane allowing water to move throughout the adhesive layer. (Tay , et al., 2002) This results not only in a direct decrease of bond strength but contributes to the hydrolysis of resin polymers and tooth-resin bond degradation (Hashimoto, et al., 2003).

The goal of both etch-and-rinse adhesives and self-etch adhesives is to infiltrate resin into the hybrid layer resulting in the greatest bond strength between the tooth and composite restorative material. Unfortunately water is never entirely displaced from the dentinal tubules, and demineralization of the dentin and resin infiltration is inconsistently to the same depth. As a result, adhesive monomers fail to fully encapsulate the exposed collagen matrix leaving partial or complete exposure of collagen fibrils at the deepest level of the hybrid layer (Tjaderhane, et al., 2013). The presence of residual solvents, the action of fluid movement out of dentinal tubules, and the increased hydrophilicity of the adhesive layer consistently result in bonds that are less than ideal but, at least initially,

clinically acceptable (Talic, 2003). Some regions are often completely devoid of polymerized resin and therefore lack protection from the hydrolytic degradation and enzymatic breakdown that occurs over time (Tjaderhane, et al., 2013). Matrix metalloproteinases are one of the key enzymes identified in destruction of the hybrid layer.

Currently there are 26 identified members of the MMP family with three implicated in the hybrid layer failure: MMPs 2, 8 and 9. Initial breakdown of types I and II collagen has been observed by MMP-8 and further breakdown is by MMP-2 and MMP-9 (Varun, et al.). MMPs are secreted by connective tissue cells such as osteoblasts and odontoblasts as zymogens, an inactive form of the enzymes. The N-terminus is initially folded which blocks the active catalyst site so Zn^{2+} and Ca^{2+} cannot bind and activate the MMP (Kotra, 2001). The inactive MMPs which are incorporated into the dentin during dentin formation and can be released when pH levels drop below 4.5. During adhesive application, acid etching results in MMP release. The released MMPs are still in the zymogen form until they are activated by proteins such as bone sialoprotein, osteopontin, and dentin matrix protein 1. This occurs over time when the N-Terminus is either cleaved or displaced (Kotra, 2001).

The degradation of microtensile bond strength has been examined in several notable studies both in vitro and in vivo. Koshiro discovered a loss of 36% to 70% bond strength at 12 months and 14 months with self-etch and etch-and-rinse adhesives attributing this loss to high levels of MMPs (Koshiro, et al., 2004). Carrilho has since replicated these findings (Carrilho, 2007).

Since discovering the destructive effects of MMPs 2, 8, and 9 on the hybrid layers, several studies have been conducted to search for ways to prevent this degradation. One chemical that has been shown to be effective is Chlorhexidine Digluconate (CHX). CHX effectively inhibits the MMPs by sequestering the metal ions (Zn^{2+} and Ca^{2+}) (Hannas & Pereira , 2007) required for their activation (Collares, et al., 2013). The application of CHX as a conditioner following the etching steps of both 4^{th} and 5^{th} generation bonding agents (Moon, et al., 2010), or as an addition to the phosphoric acid conditioner (Stanislawczuk, et al., 2009) has yielded positive outcomes. Brackett was able to maintain the hybrid layer for 12 months following a 30 second CHX application to acid etched dentin (Bracket, et al., 2009). Virtually a complete maintenance of the hybrid layer over a 14 month period was achieved after a 60 second application of 2% CHX following phosphoric acid and prior to application of primer compared to a 38% reduction in controls (Carrilho, 2007). Currently, the most accepted method of CHX application is by adding the CHX to previously etched dentin as a "therapeutic primer" (Moon, et al., 2010). Following the trend of bonding agents, researchers are now examining the idea of incorporating CHX into the bonding agents to minimize the number of steps.

More recently, studies have focused on the effects of differing concentrations of CHX have incorporated bonding systems. Zhou demonstrated a statistically significant microtensile bond strength (MTBS) differences after 12 months between Clearfil SE + CHX [0.10%-1.0%] and his control (Zhou, et al., 2009). Other researchers have examined varying CHX quantities and with mixed results with some (Munck & Van Den Steen,

2009) finding no beneficial effect. Despite the mixed outcomes, Zhou's study has become the gold standard for incorporation of CHX into a self-etch adhesive.

Recently, several adhesive systems have been introduced claiming to harness CHX's ability to inhibit the deleterious effects of MMPs. Peak Universal Bond (PUB) is an adhesive containing 0.2% CHX that can be applied either as an etch-and-rinse system with 37.5% phosphoric acid, or a self-etch application. However, limited evidence exists to support PUB's ability to maintain hybrid layer integrity over time. One of the first investigators of this product was Sabatini, who measured the shear bond strength (SBS) of PUB used as both etch- and-rinse and self-etch application over a 6 month period. The findings indicated that there was no significant difference between his control and PUB after 6 months (Sabatini, 2013). This may be the result of evaluating SBS instead of MTBS, which is noted for its ability to detect smaller differences in adhesive bonds compared to SBS (Sabatini, 2013). When the same study was done investigating the MTBS of PUB and against Scotchbond multi-purpose bond + 2.0% CHX initially and after 6 months, it was found that incorporation of CHX into his total etch adhesive system significantly improved the 6 month MTBS of Scotchbond multipurpose and PUB (Crites, 2014). Most recently, a 16 week study examining the addition of 0.2% CHX into Clearfil SE and PUB self-etching primers concluded it prolongs dentin bond strength without significantly inhibiting immediate bond strength (Craig, 2015).

The purpose of this study is to evaluate the MTBS of Peak® Universal Bond + SE (PUBSE) and Clearfil SE + CHX 0.1% compared to a control over a 32 week period.

Specific Aims

1. Determine if the addition of CHX to self-etching adhesives significantly alters immediate (24 hour) MTBS.
2. Determine if the addition of CHX to self-etching adhesives significantly alters 16 or 32 week MTBS.
3. Determine if the addition of CHX to self-etching adhesive significantly alters degradation of MTBS from initial (24 hour) to 32 week.

Hypotheses

1. The initial microtensile bond strength of a self-etch adhesive with chlorhexidine will be equal to a self-etch adhesive without chlorhexidine.

2. The microtensile bond strength of a self-etch adhesive with chlorhexidine will be greater than a self-etch adhesive without chlorhexidine after 16 and 32 weeks.

3. The rate of microtensile bond strength degradation will be greater for bonding agents without chlorhexidine compared to those with chlorhexidine.

Materials and Methods

Collection of Teeth

Non-carious human molars indicated for extraction were collected immediately following extraction. Soft tissue was removed via curette and specimens were disinfected and stored in a solution of 0.5% chloramine-T at 22^{o}C for a minimum of 24 hours, and no longer than 4 weeks.

Tooth Preparation

Teeth were sectioned using a Isomet Low Speed precision saw at 300RPM. A uniform smear layer was produced by polishing the dentinal layer with 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. Teeth there then randomaly assigned to one of three groups: Group A, Group B, and Group C.

- Group A (Control Self Etch)
    - Clearfil SE (Kuraray, Osaka, Japan)
- Group B (Control SE+0.2% CHX)
    - Clearfil SE (Kuraray, Osaka, Japan)
    - 20% Chlorhexidine Digluconate (Sigma-Aldrich, St. Louis, MO)
- Group C (Test Group, PUBSE)
    - Peak Universal Bond (Ultradent Products Inc, South Jordan, UT)
    - 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)
   iii. 32 week (A-32, B-32, C-32)
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 outlined by Zhou using serial dilutions to reach a final concentration of 0.2% CHX. Chlorhexidine was added directly to the acidic primer in Clearfil SE at the time of adhesive application (Zhou, Et al., 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 and C followed the same adhesive protocol as Group A.

Following application of the adhesive, a 5x5x5 mm block was bonded to the adhesive layer following the 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 per manufacturer's recommendations.
3. This process was repeated until a final thickness of 5mm was obtained.
4. Samples were stored in artificial saliva for either 24 hours (immediate), 16 weeks, or 32 weeks.

Sample Sectioning and MTSB test

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

1. Using Isomet 1000 at 300rpm with water irrigation, each tooth was cross- and longitudinally sectioned in 1mm increments perpendicular to the adhesive interface with a diamond blade, obtaining 1mm^2 beams approximately 10 mm in length.

2. All cut samples were divided into three equal groups for testing at different time points and were stored in artificial saliva manufactured by Dr. Pashley.

Chemical	**Name**	**mmol/L**	**g/L**
KCl	potassium chloride	12.92	0.9639

KSCN	potassium thiocyanate	1.95	0.1892
$Na_2SO_4 \cdot 10\ H_2O$	sodium sulfate, decahydrate	2.37	0.763
NH_4Cl	ammonium chloride	3.33	0.178
$CaCl_2 \cdot 2H_2O$	calcium chloride, dehydrate	1.55	0.2278g
$NaHCO_3$	sodium bicarbonate	7.51	0.6308g
$ZnCl_2$	zinc chloride	0.02	10 ml of 2mM $ZnCl_2$ solution
HEPES		5	1.186
NaN_3	sodium azide	0.3	19.503

Table 1. Pashley's simulated body fluid (artificial saliva) composition.

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 samples were adhered to Bisco MTBS testing jig with cyanoacrylate adhesive and tested for MTBS on Instron 5943.

5. Each sample was observed for method of fracture: Adhesive, Cohesive Dentin, Cohesive Composite

Removal of Occlusal Enamel

Figure 1. Occlusal enamel is removed to expose a uniform layer of dentin.

Adhesive and Composite Application

Figure 2. **Adhesive are added to the surface of dentin and photo-cured.** Following adhesive application composite resin is added and photo-cured in 1mm increments to obtain a total of 4-5mm of composite resin.

Sectioning of Tooth into Specimen Sticks

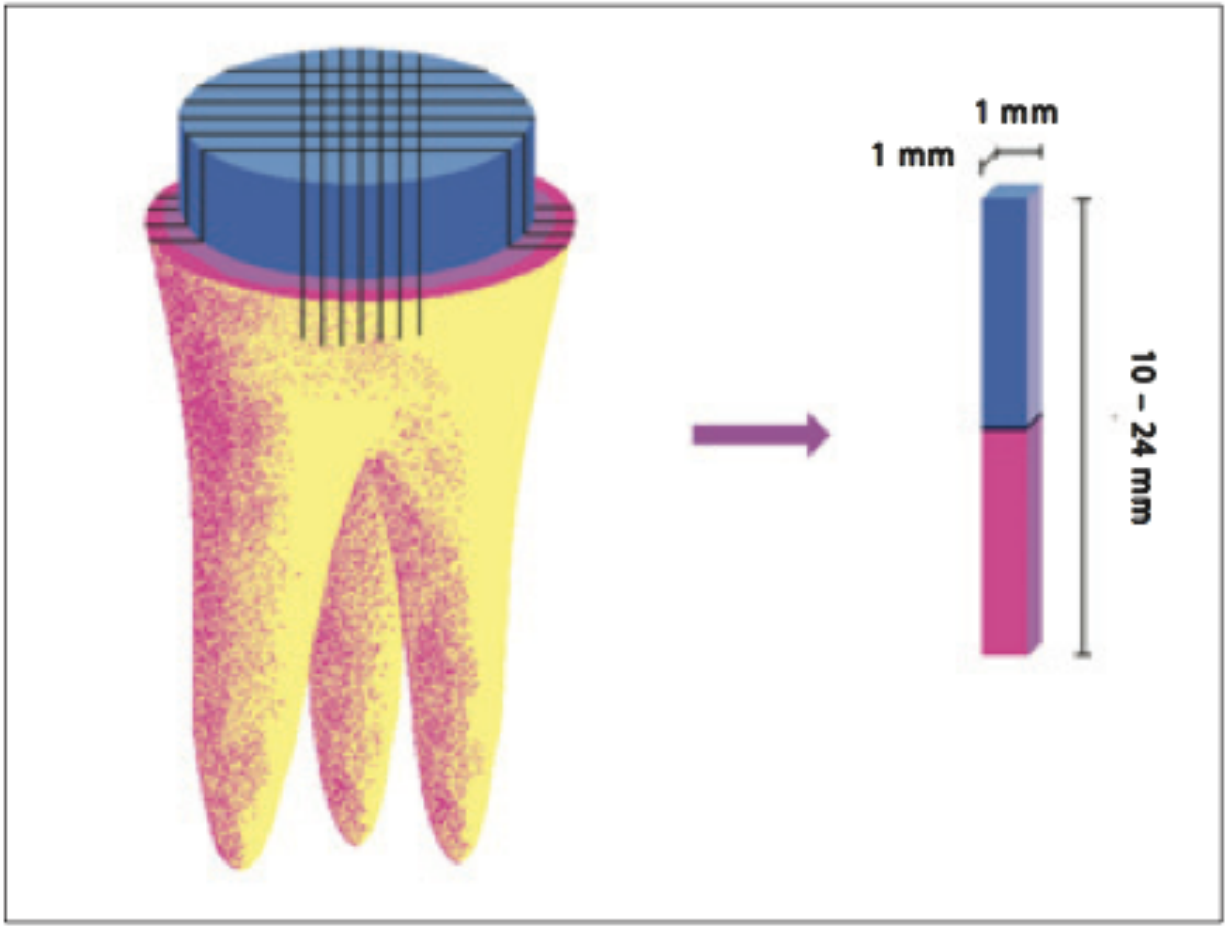

Figure 3: **Schematic representation of sectioning the tooth and composite resin into specimen sticks.**

Microtensile Testing of Specimen Sticks

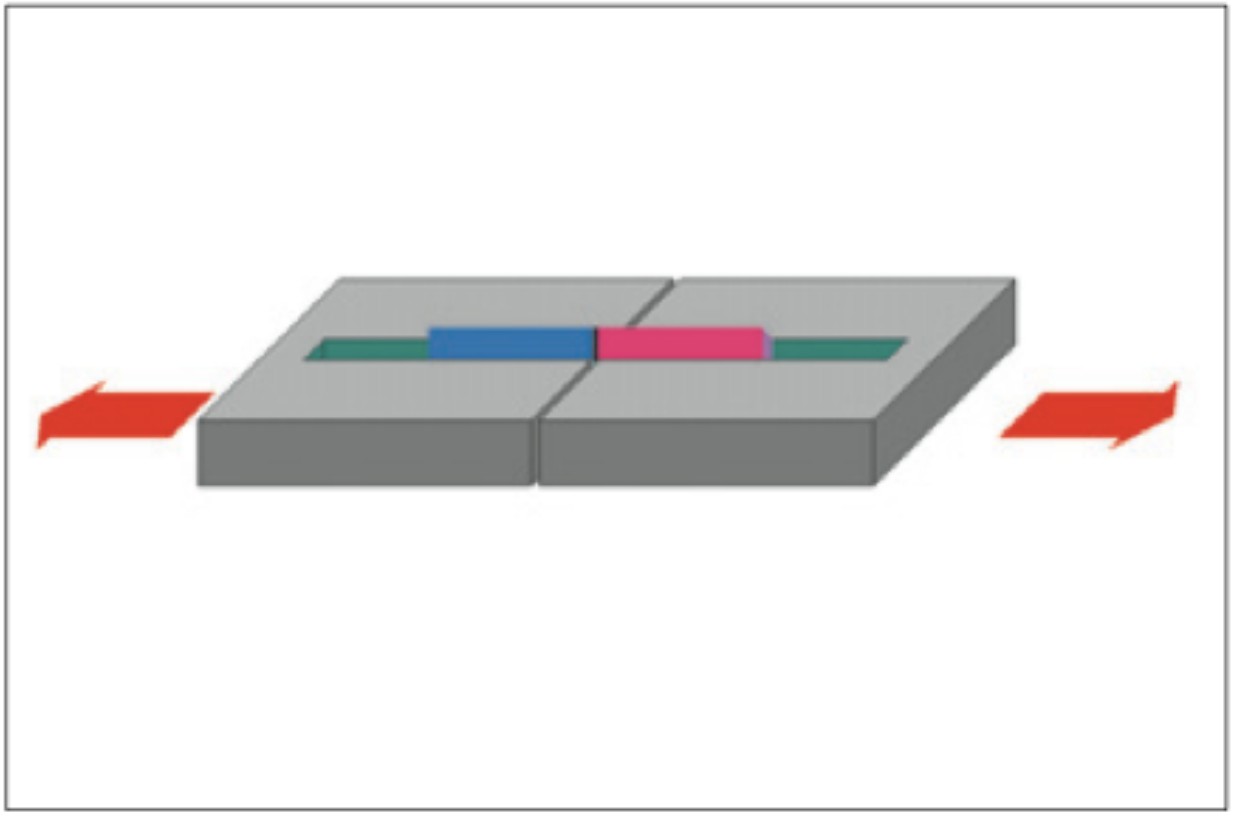

Figure 4: **Schematic representation of microtensile testing of specimen sticks.** Blue depicts the composite resin end of the specimen stick. Red depicts the dentin end of the specimen stick. The central area between blue and red represents the adhesive interface between composite resin and dentin. Tensile forces are applied 180 degrees to this interface until failure. Maximal tensile forces at the instant of failure are measured in mPa and represents the microtensile bond strength of the specimen being tested.

Isomet Low-Speed saw

Figure 5: **Isomet Low Speed Saw**

Bisco MTBS Jig

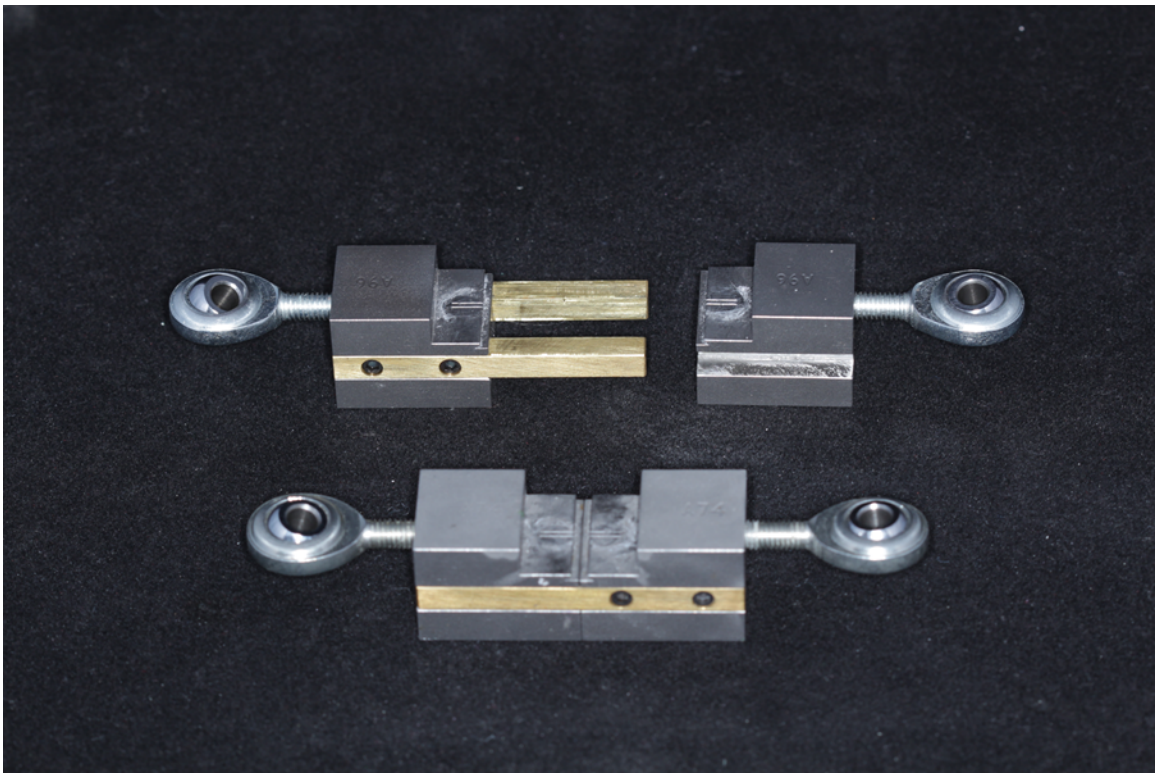

Figure 6: **Bisco MTBS Jig. The jig provides vertical alignment of the specimen stick during tensile loading.**

Instron 5943

Figure 7: Instron 5943.

Sample Size Calculation

- Sample Size was based on the Power Analysis as described by James Aden and ISR Statistical and Epidermiology 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.

Results

A chi- square test using Monte Carlo simulation (10,000 replicates) failed to find a significant relationship between the adhesive type and method of fracture at 24 hours, $\chi^2 = 8.25$, $p = 0.08$; at 16 weeks, $\chi^2 = 3.11$, $p = 0.54$; or at 32 weeks, $\chi^2 = 6.29$, $p = 0.10$. For the Clearfil SE group a significant relationship was found between time and method

of fracture, $\chi^2 = 4.67$, $p = 0.03$. A greater proportion of cohesive dentin/composite fractures occurred at the 24-hour time point than after 24 hours. No significant relationships were found between time and method of fracture for the Clearfil SE + 0.2% CHX or PUB SE groups.

Table 1 shows the mean MTBS at maximum load for each of the time points and adhesive type. There was a significant main effect of time on the MTBS at max load, $F(2,434) = 14.16$, $p < 0.001$. Additionally, there was a significant main effect of adhesive type on the MTBS at max load, $F(2,434) = 45.31$, $p < 0.001$. Tukey HSD post hoc tests showed that the MTBS at max load was similar between PUB Se and Clearfil SE + 0.2% CHX, Mdiff = -3.95, 95% CI [-8.34, 0.45]. However, differences did indicate higher MTBS for Clearfil SE + 0.2% CHX compared to Clearfil SE, Mdiff = -16.92, 95% CI [-21.20, -12.63]; as well as higher for PUB SE compared to Clearfil SE, Mdiff = -12.97, 95% CI [-17.44, -8.49]. Thus Clearfil SE demonstrated the lowest MTBS at max load.

Additionally, Tukey HSD post hoc tests showed no significant difference between 16 and 32 weeks on MTBS at max load, Mdiff = -3.09, 95% CI [-1.34, 7.52]. Differences were found between 24 hours and 16weeks, Mdiff = 6.91, 95% CI [2.57, 11.24] and 32 weeks, Mdiff = 10.00, 95% CI [5.63, 14.37]. In other words, MTBS at max load was greatest at 24hours followed by16 weeks and 32 weeks respectively.

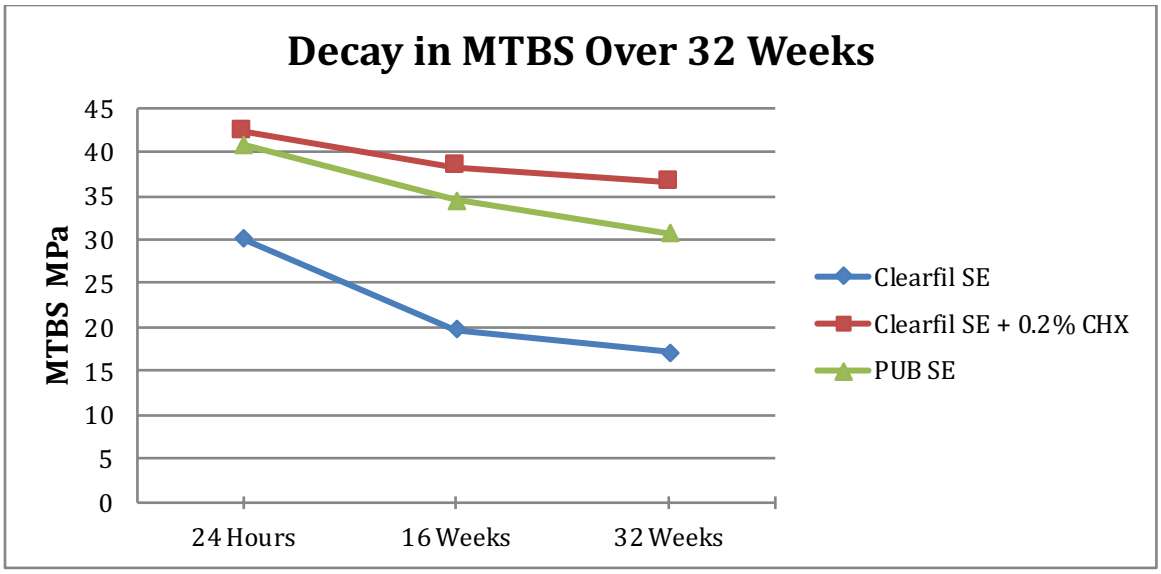

Figure 8, decay bond strength by test group and time

Group	Time	Mean	Std. Deviation	N
Clearfil SE	24 Hours	30.05	13.13	50
	16 Weeks	19.81	15.37	48
	32 Weeks	17.23	15.48	50
Clearfil SE + 0.2% CHX	24 Hours	42.35	17.72	60
	16 Weeks	38.35	16.12	52
	32 Weeks	36.57	14.55	48
PUB SE	24 Hours	40.84	18.69	45
	16 Weeks	34.48	17.76	46
	32 Weeks	30.7	13.92	44

Table1. Summary of bond strength by test group and time.

Discussion

Composite restorations are becoming more popular do to their esthetic appearance compared to alternatives such as amalgam. However, unlike amalgam, which relies on a mechanical retention within the preparation, composites require an adhesive bond. Bonding to enamel has proven more predictable and successful compared to dentin bonding.

Bonding to dentin, which has significantly histologic variation and greater water content when compared to enamel, is further complicated by the presence of MMPs. MMPs are a family of proteases involved in tissue remodeling and wound healing and have been identified as playing a significant role in the degradation of dentin collagen fibrils at bonded dentin surfaces. Previous research has evaluated CHX's ability to inactivate dentinal MMPs and preserve the initial MTBS.

Bonding agents with CHX already incorporated, such as Peak Universal Bond, claim to have simplified the process while maintaining the increased MTBS over time.

MTBS was not significantly different between samples where CHX was previously incorporated to the bonding agent and samples where CHX was freshly incorporated at 16 or 32 weeks. Both groups with CHX displayed a higher MTBS over time when compared to the bonding agent without CHX. These findings are promising for the inclusion of CHX into more bonding agents and for the advancement of dentin bonding. Future research

should continue to extend the study length to provide a greater understanding of CHX's ability to maintain a high MTBS over greater periods of time.

One limitation of this study was time. While three testing points over 32 weeks provided a statistically significant trend, it is hoped that composite restorations last longer than 32 weeks and future data collection should reflect that.

Conclusions

There was no statistical difference in MTBS at max load between PUB SE and Clearfil SE + 0.2% CHX. Both Clearfil SE + 0.2% CHX and PUB SE demonstrated a higher max load over time when compared to Clearfil SE. Within the limitations of this study, it can be concluded that the use of a 2% CHX incorporated bonding agent may stabilize the bond strength of composite to dentin over a 32 week period of time. If this trend is found to persist at a clinically significant degree it may change both our bond agents and the way we think of composite restorations. While the improved bond durability of PUB supports the incorporation of chlorhexidine into bonding protocols or agents, furth research is needed.

Appendix:

Breakage_Type* Group * Time Crosstabulation

Time				Group			Total
				Clearfil SE	Clearfil SE + 0.2% CHX	PUB SE	
24 Hours	Breakage_Type	Adhesive	Count	43	50	41	134
			% within Breakage_Type	32.1%	37.3%	30.6%	100.0%
		Cohesive Dentin	Count	3	8	0	11
			% within Breakage_Type	27.3%	72.7%	0.0%	100.0%
		Cohesive Composite	Count	4	2	4	10
			% within Breakage_Type	40.0%	20.0%	40.0%	100.0%
	Total		Count	50	60	45	155
			% within Breakage_Type	32.3%	38.7%	29.0%	100.0%
16 Weeks	Breakage_Type	Adhesive	Count	47	48	44	139
			% within Breakage_Type	33.8%	34.5%	31.7%	100.0%
		Cohesive Dentin	Count	0	3	1	4
			% within Breakage_Type	0.0%	75.0%	25.0%	100.0%
			Count	1	1	1	3

		Cohesive Composite	% within Breakage_Type	33.3%	33.3%	33.3%	100.0%
	Total		Count	48	52	46	146
			% within Breakage_Type	32.9%	35.6%	31.5%	100.0%
32 Weeks	Breakage_Type	Adhesive	Count	48	43	42	133
			% within Breakage_Type	36.1%	32.3%	31.6%	100.0%
		Cohesive Dentin	Count	0	4	0	4
			% within Breakage_Type	0.0%	100.0%	0.0%	100.0%
		Cohesive Composite	Count	2	1	2	5
			% within Breakage_Type	40.0%	20.0%	40.0%	100.0%
	Total		Count	50	48	44	142
			% within Breakage_Type	35.2%	33.8%	31.0%	100.0%
Total	Breakage_Type	Adhesive	Count	138	141	127	406
			% within Breakage_Type	34.0%	34.7%	31.3%	100.0%
		Cohesive Dentin	Count	3	15	1	19
			% within Breakage_Type	15.8%	78.9%	5.3%	100.0%
		Cohesive Composite	Count	7	4	7	18
			% within Breakage_Type	38.9%	22.2%	38.9%	100.0%
	Total		Count	148	160	135	443

	% within Breakage_Type	33.4%	36.1%	30.5%	100.0%

Time		Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)		
					Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24 Hours	Pearson Chi-Square	8.253[f]	4	.083	.077[b]	.071	.082
	Likelihood Ratio	10.907	4	.028	.048[b]	.043	.052
	Fisher's Exact Test	8.467			.060[b]	.055	.065
	Linear-by-Linear Association	.145[g]	1	.703	.777[b]	.769	.785
	N of Valid Cases	155					
16 Weeks	Pearson Chi-Square	3.202[d]	4	.525	.536[b]	.527	.546
	Likelihood Ratio	4.097	4	.393	.536[b]	.527	.546
	Fisher's Exact Test	3.112			.536[b]	.527	.546
	Linear-by-Linear Association	.130[e]	1	.718	.758[b]	.749	.766
	N of Valid Cases	146					
32 Weeks	Pearson Chi-Square	8.408[h]	4	.078	.060[b]	.056	.065
	Likelihood Ratio	9.289	4	.054	.093[b]	.088	.099
	Fisher's Exact Test	6.293			.095[b]	.090	.101

	Linear-by-Linear Association	.023[i]	1	.879	.900[b]	.894	.906
	N of Valid Cases	142					
Total	Pearson Chi-Square	17.265[a]	4	.002	.001[b]	.000	.002
	Likelihood Ratio	17.452	4	.002	.002[b]	.001	.003
	Fisher's Exact Test	15.943			.001[b]	.001	.002
	Linear-by-Linear Association	.003[c]	1	.958	1.000[b]	1.000	1.000
	N of Valid Cases	443					

Time_recoded * Breakage_recoded * Group Crosstabulation

Group				Breakage_recoded		Total
				Adhesive	Other	
1	Time_recoded	24 hours	Count	43	7	50
			% within Time_recoded	86.0%	14.0%	100.0%
		greater than 24hrs	Count	95	3	98
			% within Time_recoded	96.9%	3.1%	100.0%
	Total		Count	138	10	148
			% within Time_recoded	93.2%	6.8%	100.0%
2	Time_recoded	24 hours	Count	50	10	60
			% within Time_recoded	83.3%	16.7%	100.0%
		greater than 24hrs	Count	91	9	100

			% within Time_recoded	91.0%	9.0%	100.0%
	Total		Count	141	19	160
			% within Time_recoded	88.1%	11.9%	100.0%
3	Time_recoded	24 hours	Count	41	4	45
			% within Time_recoded	91.1%	8.9%	100.0%
		greater than 24hrs	Count	86	4	90
			% within Time_recoded	95.6%	4.4%	100.0%
	Total		Count	127	8	135
			% within Time_recoded	94.1%	5.9%	100.0%

Chi-Square Tests

Group		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
1	Pearson Chi-Square	6.288[c]	1	.012		
	Continuity Correction[b]	4.672	1	.031		
	Likelihood Ratio	5.879	1	.015		
	Fisher's Exact Test				.031	.018
	Linear-by-Linear Association	6.246	1	.012		
	N of Valid Cases	148				
2	Pearson Chi-Square	2.106[d]	1	.147		
	Continuity Correction[b]	1.437	1	.231		
	Likelihood Ratio	2.042	1	.153		

	Fisher's Exact Test				.206	.116
	Linear-by-Linear Association	2.093	1	.148		
	N of Valid Cases	160				
3	Pearson Chi-Square	1.063[e]	1	.303		
	Continuity Correction[b]	.415	1	.519		
	Likelihood Ratio	1.006	1	.316		
	Fisher's Exact Test				.440	.253
	Linear-by-Linear Association	1.055	1	.304		
	N of Valid Cases	135				
Total	Pearson Chi-Square	8.410[a]	1	.004		
	Continuity Correction[b]	7.398	1	.007		
	Likelihood Ratio	7.979	1	.005		
	Fisher's Exact Test				.006	.004
	Linear-by-Linear Association	8.391	1	.004		
	N of Valid Cases	443				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.95.

b. Computed only for a 2x2 table

c. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.38.

d. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.13.

e. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.67.

Factorial ANOVA

Table 1. Tensile stress at Max Load Mpa

Group	Time	Mean	Std. Deviation	N
Clearfil SE	2 - 16 Weeks	19.81	15.37	48
	1 - 24 Hours	30.05	13.13	50
	3 - 32 Weeks	17.23	15.48	50
Clearfil SE + 0.2% CHX	2 - 16 Weeks	38.35	16.12	52
	1 - 24 Hours	42.35	17.72	60
	3 - 32 Weeks	36.57	14.55	48
PUB SE	2 - 16 Weeks	34.48	17.76	46
	1 - 24 Hours	40.84	18.69	45
	3 - 32 Weeks	30.70	13.92	44
Total	2 - 16 Weeks	31.04	18.18	146
	1 - 24 Hours	37.94	17.47	155
	3 - 32 Weeks	27.94	16.77	142
	Total	32.46	17.95	443

Tests of Between-Subjects Effects

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	31523.521[a]	8	3940.440	15.417	.000	.221

Intercept	457634.609	1	457634.609	1790.506	.000	.805
Group	23162.236	2	11581.118	45.311	.000	.173
Time	7236.889	2	3618.445	14.157	.000	.061
Group * Time	818.332	4	204.583	.800	.525	.007
Error	110925.849	434	255.590			
Total	609236.318	443				
Corrected Total	142449.370	442				

a. R Squared = .221 (Adjusted R Squared = .207)

1. Group

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Clearfil SE	22.364	1.314	19.781	24.948
Clearfil SE + 0.2% CHX	39.089	1.269	36.594	41.583
PUB SE	35.340	1.376	32.635	38.045

2. Time

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Time	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound

2 - 16 Weeks	30.880	1.325	28.276	33.484
1 - 24 Hours	37.744	1.293	35.203	40.286
3 - 32 Weeks	28.169	1.344	25.528	30.809

3. Group * Time

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Group	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Clearfil SE	2 - 16 Weeks	19.813	2.308	15.277	24.348
	1 - 24 Hours	30.048	2.261	25.604	34.491
	3 - 32 Weeks	17.233	2.261	12.789	21.677
Clearfil SE + 0.2% CHX	2 - 16 Weeks	38.348	2.217	33.991	42.706
	1 - 24 Hours	42.348	2.064	38.291	46.405
	3 - 32 Weeks	36.570	2.308	32.035	41.105
PUB SE	2 - 16 Weeks	34.480	2.357	29.847	39.113
	1 - 24 Hours	40.838	2.383	36.154	45.522
	3 - 32 Weeks	30.703	2.410	25.966	35.440

Multiple Comparisons

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Clearfil SE	Clearfil SE + 0.2% CHX	-16.9156743*	1.82329115	.000	-21.2036567	-12.6276918
	PUB SE	-12.9690399*	1.90268542	.000	-17.4437403	-8.4943394
Clearfil SE + 0.2% CHX	Clearfil SE	16.9156743*	1.82329115	.000	12.6276918	21.2036567
	PUB SE	3.9466344	1.86833886	.088	-.4472904	8.3405592
PUB SE	Clearfil SE	12.9690399*	1.90268542	.000	8.4943394	17.4437403
	Clearfil SE + 0.2% CHX	-3.9466344	1.86833886	.088	-8.3405592	.4472904

Based on observed means.

The error term is Mean Square(Error) = 255.590.

*. The mean difference is significant at the .05 level.

Tensile_stress_at_Max_Load_Mpa

Tukey HSD[a,b,c]

Group	N	Subset	
		1	2
Clearfil SE	148	22.3989816	
PUB SE	135		35.3680215
Clearfil SE + 0.2% CHX	160		39.3146559
Sig.		1.000	.088

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 255.590.

a. Uses Harmonic Mean Sample Size = 146.957.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Multiple Comparisons

Dependent Variable: Tensile_stress_at_Max_Load_Mpa

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2 - 16 Weeks	1 - 24 Hours	-6.9060969*	1.84379438	.001	-11.2422984	-2.5698953
	3 - 32 Weeks	3.0923933	1.88428746	.230	-1.3390391	7.5238257
1 - 24 Hours	2 - 16 Weeks	6.9060969*	1.84379438	.001	2.5698953	11.2422984
	3 - 32 Weeks	9.9984902*	1.85711894	.000	5.6309522	14.3660282
3 - 32 Weeks	2 - 16 Weeks	-3.0923933	1.88428746	.230	-7.5238257	1.3390391
	1 - 24 Hours	-9.9984902*	1.85711894	.000	-14.3660282	-5.6309522

Based on observed means.

The error term is Mean Square(Error) = 255.590.

*. The mean difference is significant at the .05 level.

Tensile_stress_at_Max_Load_Mpa

Tukey HSD[a,b,c]

Time	N	Subset

		1	2
3 - 32 Weeks	142	27.9431642	
2 - 16 Weeks	146	31.0355575	
1 - 24 Hours	155		37.9416543
Sig.		.222	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 255.590.

a. Uses Harmonic Mean Sample Size = 147.470.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

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