# EFFECT OF CHLORHEXIDINE-ENCAPSULATED NANOTUBE-MODIFIED ADHESIVE SYSTEM ON THE BOND STRENGTH TO HUMAN DENTIN

by

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الحمد لله الذي بنعمه تتم الصالحات

Praise be to God that his grace is good

TABLE OF CONTENTS

# LIST OF ILLUSTRATIONS

# INTRODUCTION

Continued concerns about the incorporation of mercury in amalgam alloys and the high demand for esthetic restorations[1] have resulted in the increased use of resin composite restorations since the early 1980s. Resin composites can be bonded directly to the tooth structure, i.e. enamel and dentin, by following steps that were first introduced by Buonocore in 1955[2] through a successful attempt to alter the tooth structure by an acid-etching procedure. Later in 1982, Nakabayashi reported that resins could infiltrate into acid-etched dentin to form a layer of resin-matrix reinforced by collagen fibrils named the hybrid layer.[3] This thin hybrid layer of resin and tooth structure (i.e. collagen fibrils and residual hydroxyapatite crystallites)[4] is important for sealing the dentin surface against leakage[5] and for maintaining resin-dentin bond strength after application of mechanical stresses.[6]

In spite of many advances in dental materials and bonding techniques, the resin-dentin interface remains susceptible to degradation as a result of the histologic structure and composition of the dentin complex, as well as the limitations of the infiltration of resin into dentin and suboptimal micromechanical interlocking after acid-etching, which can result in a non-uniform hybrid layer.[7] An additional concern is hybrid layer degradation by endogenous matrix metalloproteinases (MMPs), which are multiple-host-derived endogenous enzymes found within mineralized dentin as MMP-2, -8, -9.[8,9,10] MMPs become uncovered and activated by the acidic adhesives used during bonding procedures and digest unprotected collagen resulting from insufficient resin infiltration. MMPs also increase the collagenolytic and gelatinolytic activities in the bonded collagen matrix.[10,11] This process causes more dentinal fluid influx into the hybrid layer,

contributes to the breakdown of collagen fibrils[11] and eventually leads to the complete destruction of the hybrid layer, causing lower bond strengths and failure of the adhesive restoration.[12]

To overcome such processes, application of several MMP inhibitors to the demineralized collagen matrix on dentin after acid-etching has been suggested to be a successful approach to preventing degradation of hybrid layer.[13] Among these inhibitors, the role of chlorhexidine as a non-specific MMP-inhibitor has been investigated with promising results.[14]

Chlorhexidine (CHX) is a commonly used antiseptic agent in dentistry that has bacteriostatic action at low concentrations, and bactericidal action at high concentrations. Further, due to its substantivity[15] and cationic properties, it can remain attached to tissues, resulting in immediate bactericidal action followed by prolonged bacteriostatic action extending its efficacy.[16] CHX has a dose-dependent inhibition mechanism, causing MMPs inactivation by protein denaturation when CHX is used in high concentration, while it interacts at low concentrations with the essential sulfhydryl groups and cysteine site of MMPs to inhibit its action.[8]

Several *in-vitro*, as well as *in-vivo* studies have shown that the application of chlorhexidine after acid-etching preserves the durability and structural integrity of the hybrid layer for up to 6 months[14] and can prevent the degradation of the hybrid layer through the application of low concentration of CHX as 0.2 percent leading to the reduction in the loss of bond strength of aged specimens up to 12 months[17] and up to 2 years.[18] Furthermore, CHX-saturated matrix gets isolated from interstitial fluids by resin tags that occlude dentinal tubules, by adhesive resin coating collagen fibrils, and by the

overlying adhesive layer, which prolongs the retention of CHX and inhibition of MMPs.[19]

On the other hand, due to its positive charge, CHX can bind to the negatively charged hydroxyapatite crystals and form a precipitate that could be a physical barrier that limits the interaction between the adhesive systems with the hydroxyapatite, and that affects bonding strength.[20] Therefore, a new delivery method of CHX was needed.

Recent advances in nanoscience and dental materials including engineered resins incorporated with aluminosilicate ($Al_2 Si_2 O_5 (OH)_4 \cdot n H_2O$) clay nanotubes (Halloysite® HNT) have been introduced.[21,22] Halloysite nanotubes are cytocompatible[23] and have a negatively charged outer surface and a positively charged lumen. Further, due to their high capillary forces, they are capable to absorb several materials[24] (with small molecular size and positive charge) through vacuum-loading into the inner lumen of the nanotube.[25] Therefore, nanotubes can be used as a reservoir for encapsulation and controlled delivery for several therapeutic drugs with sustained release[25] and ultimately as a reinforcing agent for improving the dental adhesives mechanical properties.[26,27]

The first incorporation of HNT into the adhesive resin was reported by our group in 2013[28] as a novel strategy for modifying adhesives and for evaluating the effect of encapsulating several materials into these nanotubes. The study concluded, in agreement with other studies done by our group,[29,30] that the incorporation of HNT of less than 20 wt% did not jeopardize the physicomechanical properties of the modified adhesives. The findings also suggested the great potential these adhesives hold towards achieving a durable resin-dentin bond strength.

Furthermore, our group evaluated the effect of locally delivering doxycycline (DOX)[31,32] through its encapsulation within Halloysite nanotubes to inhibit MMPs. Although the studies reported the successful MMP-inhibition through a gradual release of DOX dosages,[31] the resin-dentin bond strength was not enhanced.[32]

Further investigation and testing of other MMP inhibitors such as CHX incorporated into the adhesive system, as well as modification of the primer by adding the CHX-encapsulated nanotube, would be recommended for the possibility of enhancing the longevity and durability of the hybrid layer. Therefore, the objective of the present study was to evaluate the effect of a chlorhexidine-encapsulated nanotube-modified primer and dentin adhesive on the microtensile resin bond strength (μTBS) to dentin.

Null Hypothesis

There would be no differences in resin-dentin microtensile bond strengths between CHX-encapsulated nanotube-modified primer and adhesive experimental groups and control groups after 6 months aging.

Alternative Hypothesis

There would be differences in resin-dentin microtensile bond strengths between CHX-encapsulated nanotube-modified primer and adhesive experimental groups and control groups after 6 months aging.

# LITERATURE REVIEW

In 1998 Tjaderhane et al.[33] investigated the ability and the potential function of human matrix metalloproteinases (MMP) to degrade the dentin matrix after demineralization in caries lesions. The results of this study provided critical evidence that the bacterial acids in the area of caries lesions could not degrade the dentin matrix solely by themselves. Rather, they caused demineralization and mineral loss as well as the activation of MMPs, which cause the dentin matrix degradation. Therefore, the study raised the concern about MMP activation by acids and the risk of dentin degradation and the need for an inhibition mechanism.

In 1999 Gendron et al.[8] evaluated the effect of CHX as an MMP inhibitor on MMP-2,8,9 mainly due to its substantivity and antimicrobial activity. The inhibitory effect of CHX was concentration-dependent; the study proposed that high concentrations of CHX would inactivate MMPs by protein denaturation, while the inhibition mechanism at low concentration is by cation chelation. CHX directly inhibited MMP-2,8,9 present within mineralized dentin and it demonstrated a new promising antiproteolytic activity.

Furthermore, in 2004 Pashley et al.[7] evaluated whether acid-etched dentin matrices can be degraded by dentin-derived proteolytic enzymes in the absence of bacterial colonization over time. In addition, the study evaluated if incompletely infiltrated collagen fibrils in acid-etched dentin specimens were susceptible to degradation, compared with control specimens over 24 hours, 90 days and 250 days, while specimens were stored in artificial saliva. The results indicated that collagenolytic activity was inhibited by 0.2-percent chlorhexidine as an advantage to prevent

incompletely infiltrated collagen from degradation by host-derived MMPs in dentin hybrid layers.

Hebling et al. in 2005[14] showed that CHX preserved the bond between the dentin and resin-based adhesive layer and arrested the degradation of the hybrid layer below the bonded interface, demonstrating significant improvement of the hybrid layer integrity over a 6-month aging period. In correlation, Carrilho et al. in 2007[12] evaluated the ability of chlorhexidine to inhibit MMPs and prevent the decrease in the microtensile bond strength. The application of CHX improved the durability of the hybrid layer and the dentin bond strength when compared with the control; yet a reduction in the overall bond strength was present, and the need for an optimal CHX-MMP inhibition through a new advanced vehicle was indicated.

For the long-term effect of chlorhexidine, a study by Breschi in 2009[17] investigated the effect of 0.2-percent and 2.0-percent chlorhexidine on microtensile bond strengths of two etch-and-rinse adhesive systems over a 12-month aging period. Teeth were selected and occlusal enamel and superficial dentin were removed perpendicular to the long axis of the tooth; polishing was done to standardize the smear layer. During the bonding procedure of each adhesive system and after acid-etching, three protocols were followed: 0.2-percent CHX application, 2.0 percent CHX application, no CHX application as a control. The results of this study indicated that the use of as low as 0.2 percent CHX on acid-etched dentin can prevent degradation of microtensile bond strength, inhibit bacterial growth, and inhibit MMP-mediated collagen degradation over 12 months aging.

Moreover, a similar study by Breschi et al. in 2010[18] investigated the bond

strength over a 2-year aging period and demonstrated the efficacy of CHX inhibition of MMPs even if used at a low concentration of 0.2 percent. Their results suggested that MMP inhibition offers a valuable tool for improving the durability of composite dentin bonding.

In 2010 Vergaro et al.[23] evaluated the uptake and cytocompatibility of Halloysite nanotubes, which are important for their potential application and incorporation into multiple materials used in dentistry. Halloysite nanotube is aluminosilicate clay with the structure of a hollow tubule of 50 nm external diameter and 15 nm internal lumen diameter. The study used two quantitative measurements performed with two specific neoplastic cell line model systems to evaluate the nanotubes' concentration and incubation time. The results demonstrated that Halloysite nanotubes exhibit a high level of biocompatibility and very low cytotoxicity and therefore can be used safely and incorporated into resin-based dental materials for controlled drug delivery.

Bottino et al. in 2013[28] incorporated Halloysite nanotubes into a commercial three-step etch-and-rinse bonding system for the first time as a novel attempt for modifying adhesives and evaluating the effect of encapsulating several materials into these nanotubes on the dentin bond strength. Experimental adhesives with different HNT concentrations (5 wt%, 10 wt%, 15 wt%, 20 wt% and 30 wt%) were prepared and several properties were evaluated as shear bond strength, microhardness and degree of conversion. The incorporation of HNT up to 20 wt% did not jeopardize the physicomechanical properties of the modified adhesive and promoted the great potential these adhesives hold towards achieving a durable resin-dentin bond strength.

Similarly, Alkatheeri et al. in 2015[30] evaluated the effect of incorporation of

Halloysite nanotubes into a commercial two-step etch-and-rinse and a one-step self-etch adhesive on dentin shear bond strength. HNT incorporated at 5-percent, 10-percent, and 20-percent HNT into each adhesive system, and shear bond strength testing was performed to compare experimental groups with control groups with no HNT added after 24 hours' storage in deionized water at 37°C.

Incorporation of HNT up to 20 wt% into the two-step etch-and-rinse adhesive and up to 10 wt% into the one-step self-etch adhesive increased the dentin shear bond strength. Scanning electron microscopy (SEM) of the resin-dentin interface demonstrated the infiltration of HNT-modified adhesives into the dentinal tubules and the formation of resin tags similar to the control adhesives.

Meanwhile, a study done by Feitosa et al. in 2014[31] was taking a step forward into encapsulating nanotubes with MMP inhibitors and modifying adhesive systems. In the study, the nanotubes were encapsulated with doxycycline (DOX) and incorporated into a commercial adhesive system, and changes in degree of conversion (DC) and microtensile bond strength between experimental and control groups were evaluated. The cytotoxicity and biological activity of the DOX-modified adhesives were assessed to ensure the release of DOX at sub-antimicrobial levels against *Streptococcus mutans* through agar diffusion assays. The curing times were increased (10 s, 20 s, 40 s), but there was no significant difference in the degree of conversion between the modified adhesives compared with the control. No cytotoxicity was reported, and a significant growth inhibition of *S. mutans* suggested the successful encapsulation of DOX into the experimental adhesives. On the other hand, there was no significance difference in microtensile bond strength, suggesting that the resin-dentin bond strength durability did

not improve.

Another study by Feitosa et al. in 2018[34] evaluated chlorhexidine as an MMP inhibitor and modified a commercial adhesive system with chlorhexidine-loaded nanotubes at two different concentrations (CHX 10% and 20 %). Changes in the degree of conversion, Knoop hardness, water sorption, solubility, antimicrobial activity, cytotoxicity, and anti-matrix metalloproteinase activity were evaluated.

The study concluded there were no detrimental differences in degree of conversion when light curing for 20 seconds, Knoop microhardness, water sorption, and cytotoxicity when the CHX-HNT-modified adhesives were compared with the unmodified commercial adhesive. The CHX-loaded nanotube-modified adhesive presented sufficient evidence of the CHX release due to *S. mutans* and *L. casei* growth inhibition. The study recommended further *in-vivo* studies to confirm the role of CHX in bond durability.

In summary, the application of chlorhexidine as an MMP inhibitor directly after acid-etching has the ability to arrest the degradation and improve the integrity of the hybrid layer. Furthermore, the modification of adhesive systems with nanotubes didn't compromise their physicomechanical properties and held a great potential for encapsulation of MMP inhibitors such as CHX. The CHX-HNT modified adhesive system was evaluated for its physicochemical and biological properties, but not for the resin-dentin bond durability.

Therefore, the objectives of the present study were to modify a commercial adhesive system with its respective primer, which was done for the first time; to modify the system's dentin adhesive, and to evaluate the effect of this chlorhexidine-

encapsulated nanotube-modified adhesive system on the microtensile resin bond strength (μTBS) to dentin.

# MATERIALS AND METHODS

## FABRICATION OF CHX-ENCAPSULATED NANOTUBE-MODIFIED ADHESIVE AND PRIMER

### Chlorhexidine Digluconate Solution

Chlorhexidine digluconate solution (CHX) 20% in $H_2O$ (Sigma-Aldrich, St. Louis, MO, USA) was used to be encapsulated into the Halloysite® aluminosilicate clay nanotubes (HNT, Dragonite 1415JM, Applied Minerals, New York, NY, USA) at two different concentrations: 10 wt% and 20 wt%. In order to obtain CHX 10%, the commercial CHX material was dissolved in distilled water (2.5 ml CHX / 2.5 ml $H_2O$).

### Halloysite® Aluminosilicate Clay Nanotubes Encapsulation with Chlorhexidine

HNT powder was sieved using a 45-μm sieve (Test Sieve, ASTM E-11 Standard, 325MeshSz, Hogentogler & Co, USA) in order to obtain 45-μm HNT particles. For the encapsulation process, 1.25 g of HNT and 5 ml of CHX solution (10% and 20%) were added together; solutions were vortexed (Maxi Mix Plus Vortexer, Thermolyne, USA) for 20 seconds and sonicated for 2 hours as a first step for encapsulation. Then, tubes were vortexed again for 20 seconds and put in the vacuum (VWR Vacuum Oven, USA) without the cover under 25 mmHg pressure for 1 hour.

The materials were then mixed using the vortex for 20 seconds and the end-to-end mixer (Roto-Rack Tube Rotator, Fisher Scientific, USA) for 2 hours. The vacuum was used again for 18 hours; the tubes were vortexed for 20 seconds and put into the end-to-

end mixer for 2 hours, and then placed into the vacuum for 1 hour, and vortexed for 20 seconds (Figure 1).

Lastly, the tubes were centrifuged at two cycles of 3500 rpm (Centrifuge 5804 R, Eppendorf, USA) for 10 minutes each (5min/tube) at room temperature. Between the two cycles, tubes were vortexed for 20 seconds, placed into the vacuum for 1 hour, and vortexed for 20 seconds; and excess liquid was removed from each tube each time.

The encapsulation process was completed, and the mixed materials were stored at 37ºC in an incubator (HeraTherm Oven, TheromScientific, USA) for 7 days to dry with the tubes uncovered. After drying, the HNT-encapsulated with CHX (10% and 20%) materials were sieved at 45 µm. The HNT+CHX10% and HNT+CHX20% powders were obtained and put in amber jars separately and labeled.

## Experimental Chlorhexidine-Encapsulated Nanotube Dentin Adhesive/Primer Fabrication

A commercial three-step etch-and-rinse dentin adhesive system (Adper™ Scotchbond™ Multi-Purpose Primer [PR] and Adper™ Scotchbond™ Multi-Purpose Adhesive [ADH], 3M ESPE, St. Paul, MN, USA) was used based on a previous study.[30]

Briefly, the dried HNT-encapsulated with CHX (10% and 20%) powders were incorporated into the primer and/or adhesive according to Table I (HNT; PR+CHX10%; PR+CHX20%; ADH+CHX10%; ADH+CHX20%). An amount of 150 mg of HNT powder (15 HNT wt.%) was weighed and added for each 1 ml of primer/adhesive pipetted using a special 1000 µl Pos-D pipette (MR 1000, Mettler Toledo, Columbus, OH, USA), and a total of 3 ml for each experimental material was prepared (450mg/3ml) (Figure 2). Materials were mixed using a mechanical mixer in an amber jar for 1 minute

and put onto a stirring plate (VWR Standard Multi-Position Stirrer, USA) for 24 hours on a low speed after adding stirring bars. All the materials' manipulation and preparation and testing were done under constant temperature and filtered light system.

## EXPERIMENTAL ADHESIVE CHARACTERIZATION

### Degree of Conversion (DC)

DC was measured to determine if the CHX-encapsulated nanotube incorporation affected the adhesive polymerization. Disk-shaped adhesive specimens (7 mm × 0.24 mm; n = 3 for each group i.e. ADH; HNT; ADH+CHX10% and ADH+CHX20%) were prepared and cured for 10 seconds (following the manufacturer's instructions) from the top only. A light-emitting diode curing system (DEMI LED, Kerr, Orange, CA, USA) was used during the study with an output intensity of 1100 mW/cm$^2$. The intensity of the light curing unit was measured periodically using a calibrated handheld radiometer (Cure Rite Visible Curing Light Meter, DENTSPLY Caulk, USA).

The DC was evaluated with Fourier transform infrared spectroscopy (FT/IR-4100, Jasco, USA) in attenuated total reflection mode as previously described by Bourbia et al.[35] Three initial readings were taken for each uncured (unpolymerized) adhesive and three readings/sample were taken at different sites for each of the cured samples as shown in Figure 3. The absorbance bands at 1637 cm$^{-1}$ (methacrylate group, C = C) and 1607 cm$^{-1}$ (ester group, C = O) were used to calculate the DC (expressed in %), according to the following equation:

$$\text{DC (\%)} = 1 - \frac{\text{Cured (area under1637 / area under 1607)}}{\text{Uncured (area under1637 / area under 1607)}} \times 100$$

### Viscosity

The viscosity of the experimental primers and adhesives was determined using a viscometer (DV-II Viscometer, Brookfields, USA) to be compared with the control Adper™ Scotchbond™ Multi-Purpose Primer and adhesive. The viscometer was securely mounted to the laboratory stand, leveled and zeroed with no cone or cup attached and 0-percent torque displayed.

After calibration according to the manufacturer's instructions, the material to be tested was added to the cone plate and subjected to shear with a small-diameter spindle (CPA-52Z) under constant temperature of 25.3°C of the viscometer, and room temperature of 21.3°C and 26-percent humidity. According to the type of spindle, 0.5 ml was pipetted into the cone plate and measured. The procedure was repeated 3 times per material and all the parameters (cP, RPM, SS, SR, Torque%) were recorded at each time under the specific speed suitable for each material tested.

## DENTIN BONDING PROCEDURE

The project was submitted to and approved by the local Institutional Review Board of Indiana University under the IRB protocol number of 1711224202.

### Specimen Preparation

One hundred and five human non-carious molars with no restorations, defects, or fractures were selected, autoclaved for 20 minutes in a water cycle, and cleaned of any remaining debris using a piece of 2 x 2 gauze. A 3.3mm space from the cemento-enamel junction (CEJ) was measured using a digital caliper (SS, Marathon, USA) and marked as shown in Figure 4.

Teeth were individually mounted in clear acrylic resin (Lecoset 7008 kits/acrylic (medium), Leco Corporation, Saint Joseph, MI, USA) mixed in a liquid and powder form into reusable plastic molds (Leco Corporation, Saint Joseph, MI, USA). To ensure the teeth were aligned parallel to their long axes and perpendicular to the CEJ line, a dental surveyor was used to keep the teeth held and aligned while the acrylic was fully setting (Figure 5).

Next, the occlusal third of each tooth was removed to the marked line to expose mid-coronal dentin surface using a low-speed diamond disk (Isomet Diamond Wafering Blades, Buehler, Lake Bluff, Illinois, USA) mounted on a cutting machine (Isomet 1000, Buehler, Lake Bluff, Illinois, USA), under water cooling during cutting (Figure 6). A flat mid-coronal dentin surface was created with Silicon Carbide sanding discs (600 grit, Leco Corporation, Saint Joseph, MI, USA) using a polishing machine (Spectrum System 1000, Leco Corporation, Saint Joseph, MI, USA) for 10 seconds with runny water to standardize the smear layer.[31]

Then, teeth were randomly assigned to 7 experimental groups and labeled (n = 15) (Table I): Unmodified primer and adhesive (ADH, control); unmodified primer and adhesive and CHX (0.2%CHX); unmodified primer and adhesive with HNTs (HNT, control); unmodified primer and adhesive with CHX-encapsulated HNTs (ADH+CHX10%, ADH+CHX20%); and primer with CHX-encapsulated HNTs (PR+CHX10%, PR+CHX20%) and unmodified adhesive. Teeth were stored in distilled water-filled glass containers at 37°C in an incubator, ready for the bonding procedure after 24 hours.

Bonding Procedure

Before bonding, dentin surfaces were cleaned with pumice paste without oil and fluoride (Nada pumice paste, PreventTech) for 10 seconds using polishing brushes (One Gross Latch Prophylaxis Polishing Brushes, DentAmerica), rinsed with distilled water for 10 seconds and air-dried (VWR Air Duster Spray) for 10 seconds.

The bonding procedure started with the application of 35-percent phosphoric acid ($H_3PO_4$) (Scotchbond™ Universal Etchant, 3M ESPE, USA) with a microbrush (Disposable Microbrush Applicators, Schein) and left undisturbed for 15 seconds, then thoroughly rinsed for 15 seconds and gently dried for 5 seconds with absorbent paper (Kimtech wipes, Kimberly-Clark Professional).

All groups (except the 0.2-percent CHX group) were treated following the same protocol in the application of the modified or unmodified primers and adhesives. The primer was applied with a microbrush for 5 seconds and air-dried for 5 seconds, while the adhesive was applied with a microbrush and air-dried for 5 seconds, then light-cured (DEMI LED, Kerr, USA) for 10 seconds, according to the manufacturer's instructions and according to the data collected from DC.

For the CHX group, 100 µl of 0.2-percent CHX was rubbed into the etched dentin surface using a microbrush and left undisturbed for 1 minute,[36] followed by the primer and adhesive application as previously described.

All specimens were restored with resin composite (Bulk Fill Filtek Resin Composite, 3M ESPE, USA) for 2 mm with the help of the Tofflemire matrix band (SS, Henry Schein) and retainer and light cured (DEMI LED, Kerr, USA) for 20 seconds occlusally. Then, the matrix band and retainer were removed and the resin composite was

cured for 10 seconds from all sides according to the manufacturer's instructions. The intensity of the light curing unit was checked before any procedure using a calibrated handheld radiometer (Cure Rite Visible Curing Light Meter, DENTSPLY Caulk, USA).

After the bonding procedure was completed, all specimens were stored in a 37°C incubator in a distilled water-filled glass container for each group (n=15) (Tables I, II; Figures 7, 8).

Cutting and Aging Conditions

After 24 hours, specimens were cut into beams (1.0 × 1.0 mm) using a diamond disc (Isomet Diamond Wafering Blades, Buehler, Lake Bluff, Illinois, USA) mounted on a low-speed cutting saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA) under water cooling.

The mounted teeth were inserted into the metal tool and stabilized in the cutting machine, and cut vertically into slices 1.3 mm apart to compensate for the 0.3 thickness of the diamond disk, flipped 90° and cut again into slices 1.3 mm apart from each other as shown in Figure 9. The final cut was done at the CEJ to ensure separation of the beams from the tooth, and beams were collected carefully and measured. Beams with the dimensions of 1 x 1 mm (± 0.1) were accepted and others were discarded.

The collected accepted beams were assigned randomly and equally in number as possible into subgroups according to the aging conditions as follows: 1) 24 h (i.e., the beams were kept in distilled water at 37℃ for 24 hours before testing); 2) 6 months (i.e., the beams were kept at 37°C in distilled water for 6 months before testing; the distilled water was changed every 2 weeks, to avoid contamination and fungal growth) (Figure 10).

### Microtensile Bond Strength and Failure Analysis

After the determined aging time, beams were carefully attached to a specific metallic device for the microtensile bond strength (μTBS) test with cyanoacrylate gel (Super Glue, Gel Control, Loctite), avoiding the bonding area and at the same distance from it (Figure 11); an accelerator was used to speed up the drying of the gel.[37] The μTBS test was performed using a Universal Testing Machine (MTS Sintech Renew 1123, Eden Prairie, MN, USA) at a crosshead speed of 1 mm/min (Figure 12).

Each beam/tooth's measurements (width and thickness) were recorded and inserted into the machine during testing, and peak stresses in Mpa were recorded and saved.

For the data analysis, the tooth was considered the unit and the data were the average values of beam per tooth for each aging condition.[37]

### Failure Mode

The fractured interfacial surfaces were examined under a light microscope (X40 magnification). Failure modes were classified as: Adhesive failure, Mixed failure, Cohesive failure in dentin, or Cohesive failure in resin composite.[31]

## STATISTICAL ANALYSIS

Data from DC and viscosity tests were analyzed using one-way ANOVA. The data from microtensile bond strength testing were analyzed by pair-wise comparisons using the Sidak method to control the overall significance level at 5.0-percent for each aging time separately.

Weibull-distribution survival analysis was used to compare the differences in the microtensile bond strength results among the 7 groups after 24 hours and 6 months separately, and to compare the differences in the microtensile bond strength results between the two aging times for each group separately. Differences between the groups for failure type (Adhesive, Mixed, Cohesive) were summarized for each aging time. The stress value (MPa) required to produce failure was used as time to event, and was summarized by group for each aging time.

# RESULTS

Test results of degree of conversion and viscosity were analyzed using One-way ANOVA to identify the differences between groups, as shown in Table III. DC analysis revealed no significant difference among adhesive groups: ADH, HNT, ADH+CHX10%, ADH+CHX20% ($p > 0.05$). However, there were significant differences in viscosity among groups ($p < 0.05$). ADH group was significantly lower than ADH+CHX10%, ADH+CHX20% and HNT group. ADH group was significantly higher than PR, PR+CHX10% and PR+CHX20% group. In addition, there were no significant differences among the experimental adhesive groups (HNT, ADH+CHX10%, ADH+CHX20%) or between the experimental primer groups (PR+CHX10% and PR+CHX20%).

Weibull-distribution survival analysis was used to compare the differences in the microtensile bond strength (μTBS) among the 7 groups with testing after 24 hours and 6 months separately, and to compare the differences in the μTBS for the two testing times for each group specifically. Test results of stress value (MPa) of each group for each aging time revealed no significant difference between groups after 24 hours (Figure 13). However, after 6 months, modified primer groups (PR+CHX10%, PR+CHX20%) and 0.2%CHX group showed significant differences in μTBS compared with control groups (ADH, HNT) and modified adhesive groups (ADH+CHX10%, ADH+CHX20%) in the same aging time testing (Figure 14). When comparing the μTBS at 24 hours and 6 months, there were no significant differences among the groups except for the ADH+CHX20% group where MPa values were higher after 24 hours than 6 months ($p = 0.0487$) as shown in Table IV and Figure 15. Differences among the groups for type of

failure (adhesive, mixed, or cohesive) are summarized by group for each aging time (Table V).

# FIGURES AND TABLES

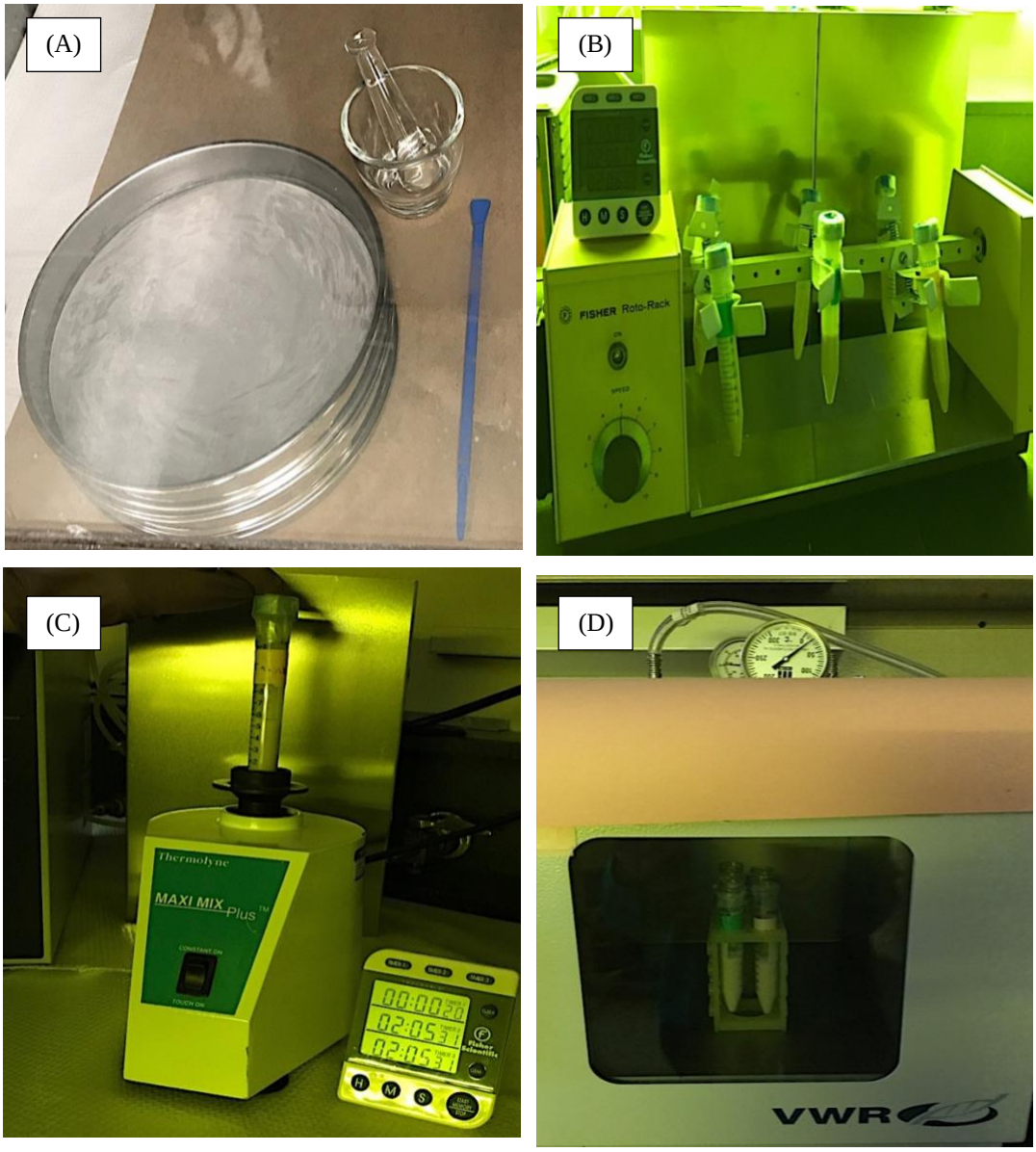



FIGURE 1. Part of the encapsulation process. (A) 45 μm powder sieve (ASTM E-11 Standard, 325MeshSz, Hogentogler & Co, USA); (B) Vortex (Maxi Mix Plus Vortexer, Thermolyne, USA); (C) End-to-end mixer (Roto-Rack Tube Rotator, Fisher Scientific, USA); (D) Vacuum (VWR Vacuum Oven, USA).

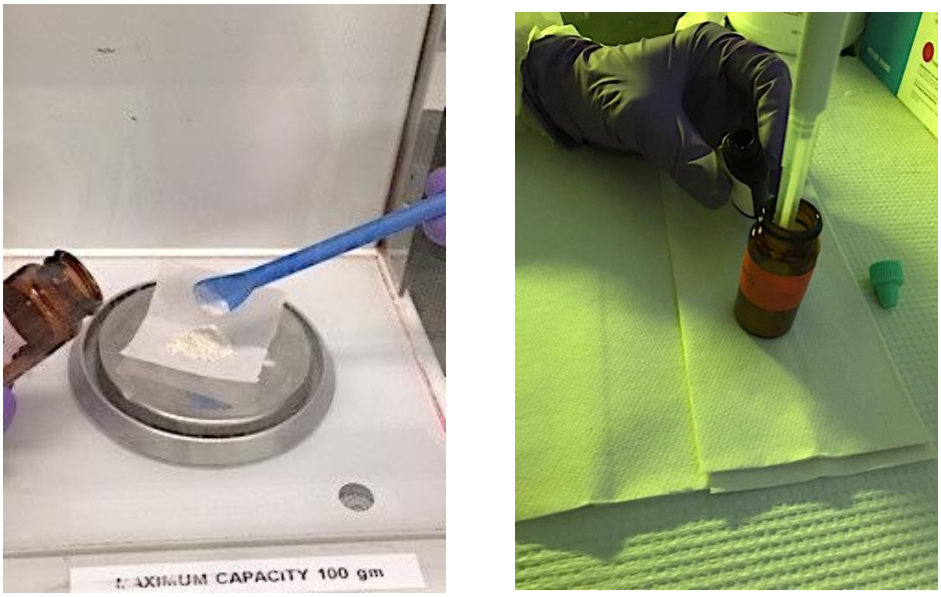



FIGURE 2. Dried HNT-encapsulated with CHX powder weighed and incorporated into the adhesive.

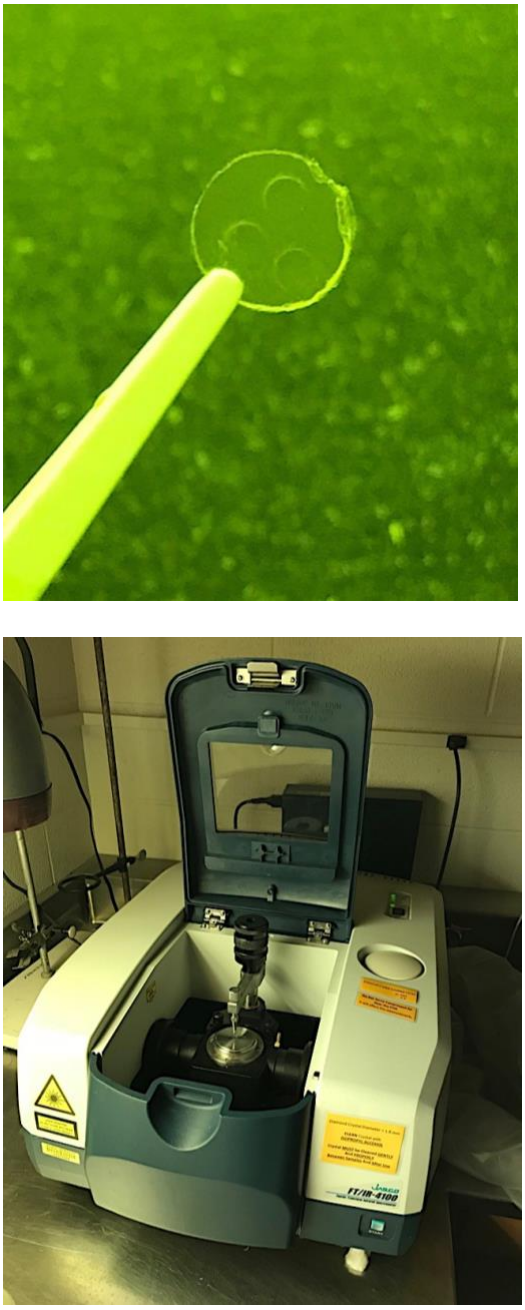



FIGURE 3. Degree of conversion. (Top) Disk-shaped adhesive specimen (7 mm × 0.24 mm) prepared and cured for 10 seconds from the top only. (Bottom) Fourier transform infrared spectroscopy (FT/IR-4100, Jasco, USA) for the degree of conversion evaluation in attenuated total reflection mode. Three initial readings were taken for each uncured adhesive and three readings/sample were taken at different sites for each of the cured samples.

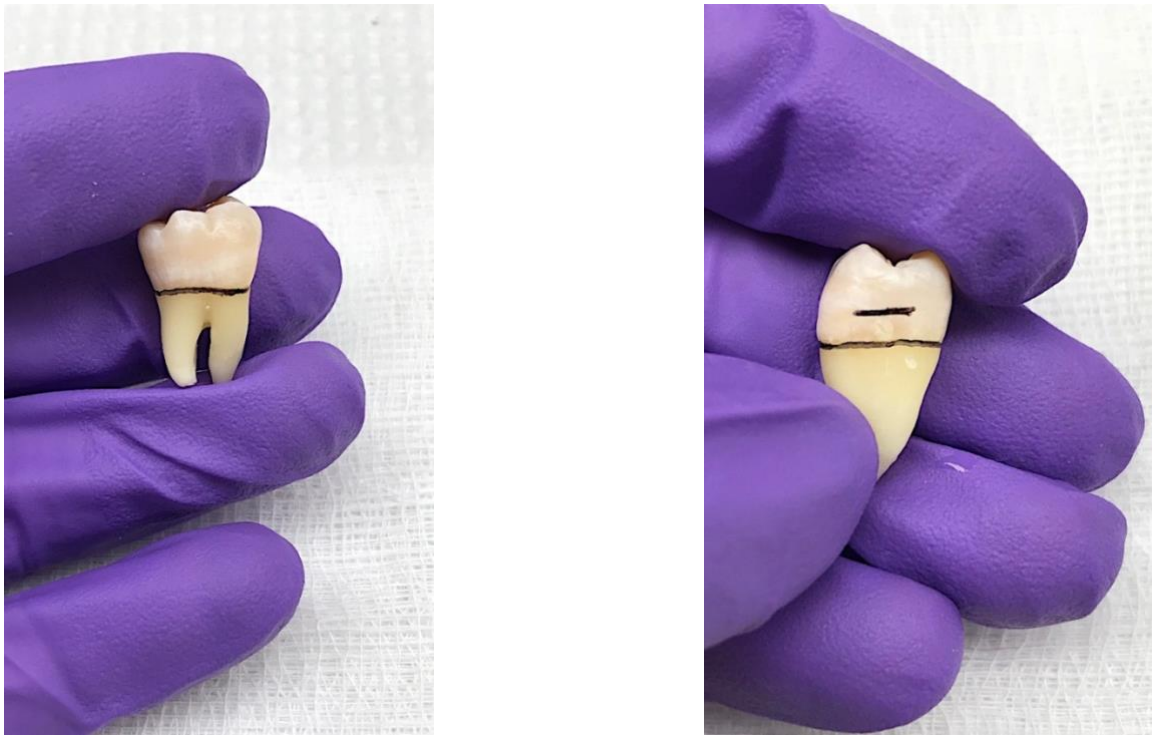
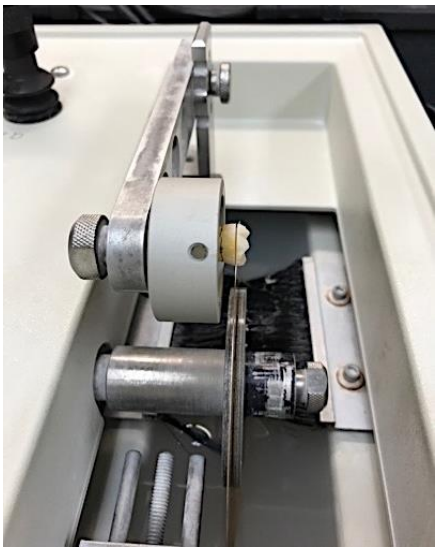


FIGURE 4. Tooth measurement and marking. A 3.3 mm space from the cemento-enamel junction (CEJ) measured using a digital caliper (SS, Marathon, USA) and marked.


FIGURE 5. Tooth mounting in clear acrylic resin (Lecoset 7008 kits/acrylic (medium), Leco Corporation, Saint Joseph, MI, USA) mixed in a liquid and powder form into a reusable plastic mold (Leco Corporation, Saint Joseph, MI, USA). Tooth inserted parallel to its long axes and perpendicular to the CEJ line using a dental surveyor.

(A)


(B)



(C)

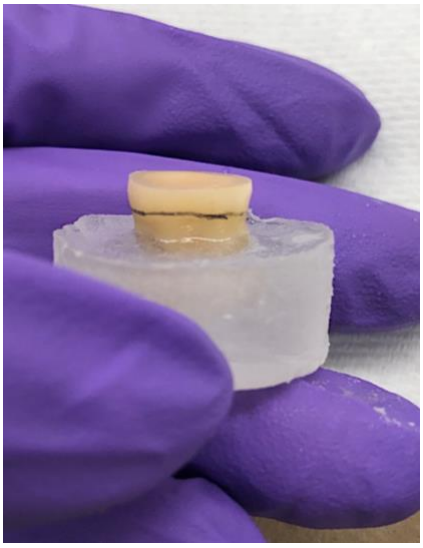


FIGURE 6. Tooth occlusal third cutting. (A) Cutting machine (Isomet 1000, Buehler, Lake Bluff, Illinois, USA). (B) Occlusal third removal to the marked line using a low-speed diamond disk (Isomet Diamond Wafering Blades, Buehler, Lake Bluff, Illinois, USA) under water cooling during cutting. C) A flat mid-coronal dentin surface created.


FIGURE 7. A three-step etch-and-rinse dentin adhesive system. Adper™ Scotchbond™ Etchant; Adper™ Scotchbond™ Multi-Purpose Primer; Adper™ Scotchbond™ Multi-Purpose Adhesive (3M ESPE, St. Paul, MN, USA).

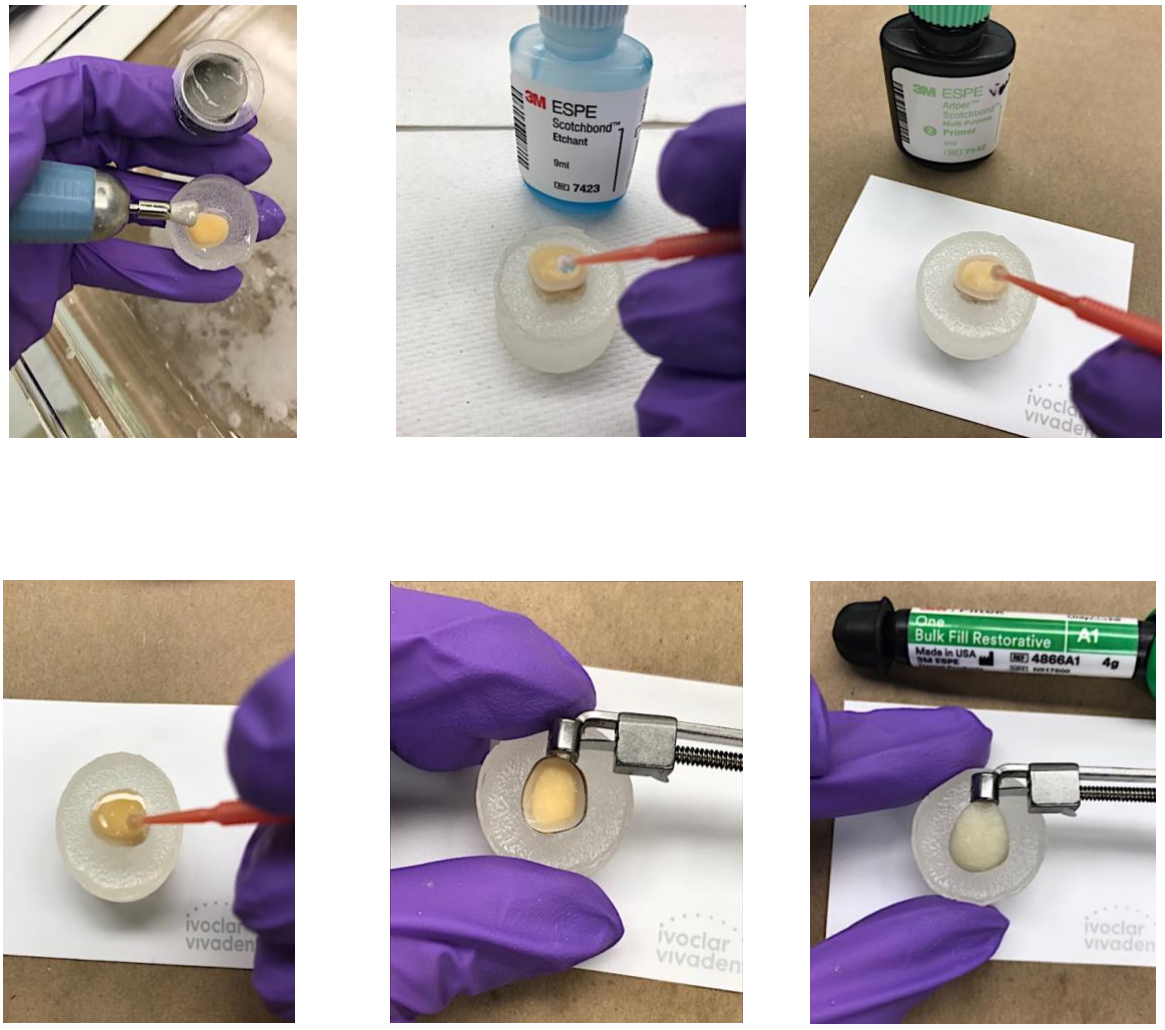



FIGURE 8. The bonding procedure. Application of Adper™ Scotchbond™ Etchant; Adper™ Scotchbond™ Multi-Purpose Adhesive Primer; Adper™ Scotchbond™ Multi-Purpose Adhesive (3M ESPE, St. Paul, MN, USA). Specimen restored with resin composite (Bulk Fill Filtek Resin Composite, 3M ESPE, USA) for 2 mm with the help of the Tofflemire matrix bands (SS, Henry Schein).

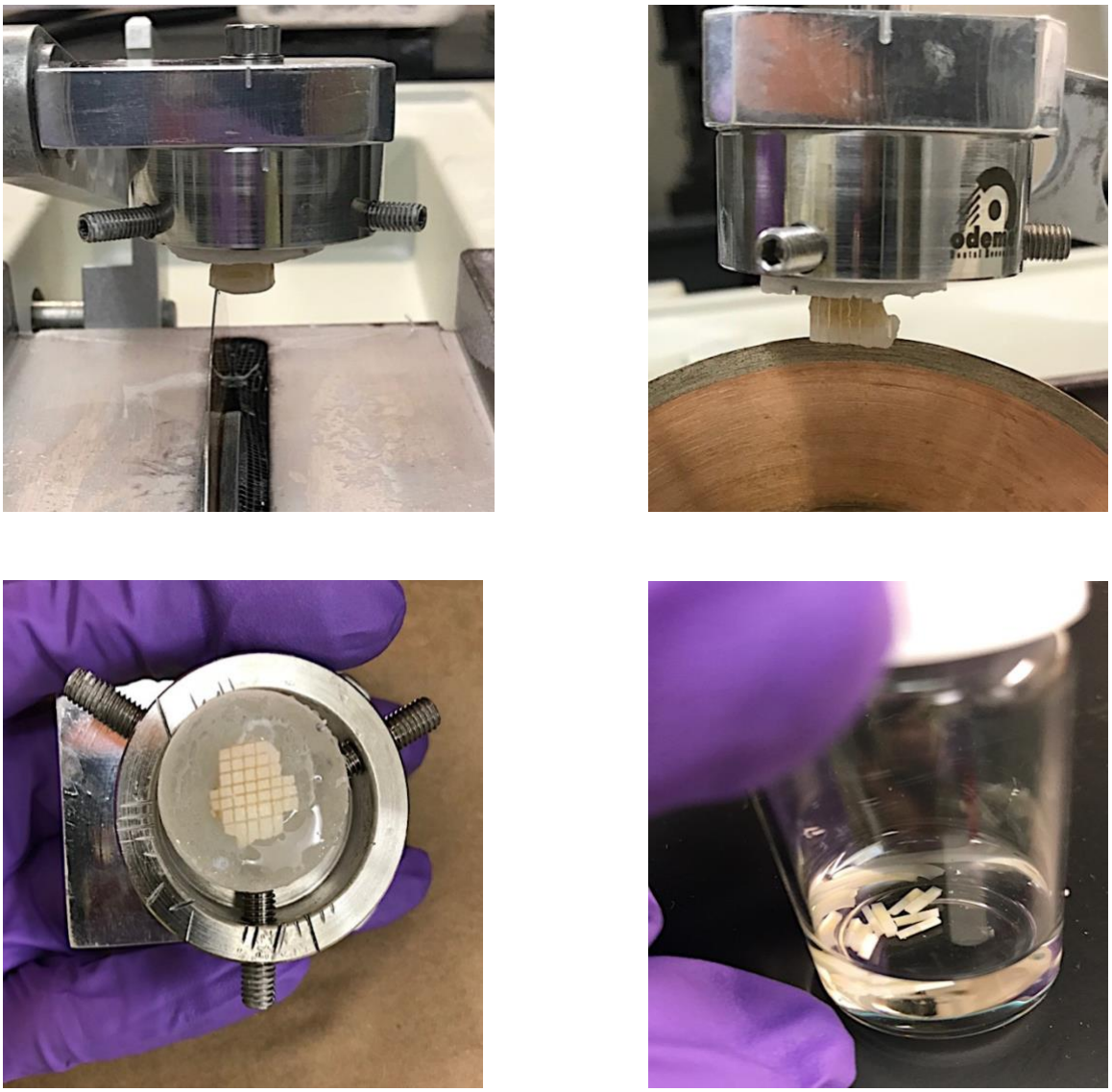


FIGURE 9. Specimen cut into beams (1.0 × 1.0 mm) cut using a diamond disc (Isomet Diamond Wafering Blades, Buehler, Lake Bluff, Illinois, USA) under water cooling. Tooth cut vertically into slices 1.3 mm apart to compensate for the 0.3 thickness of the diamond disk, flipped 90° and cut again into slices 1.3 mm apart from each other.

FIGURE 10. Autoclaved distilled water changed for all groups every two weeks.

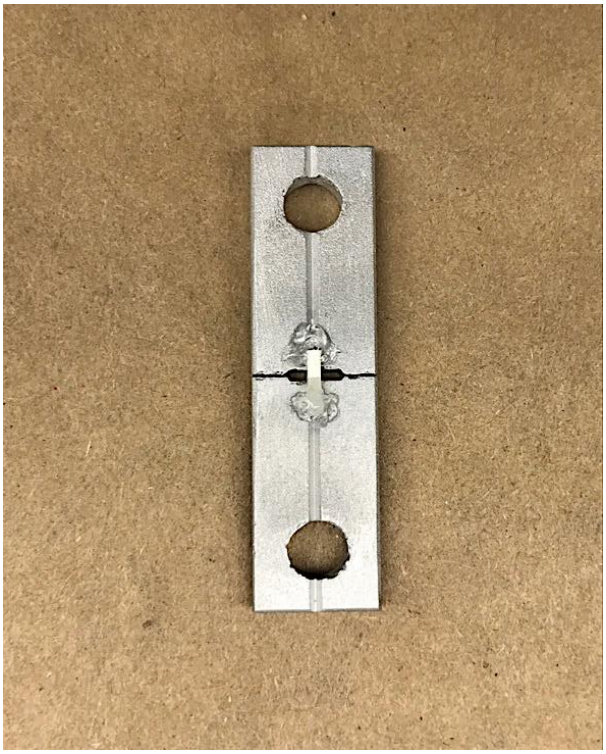


FIGURE 11. Beam attached to the specific metallic jig for the microtensile bond strength (μTBS) test with cyanoacrylate gel (Super Glue, Gel Control, Loctite), avoiding the bonding area and at the same distance from it. The μTBS test was performed using a Universal Testing Machine (MTS Sintech Renew 1123, Eden Prairie, MN, USA) at a crosshead speed of 1 mm/min.

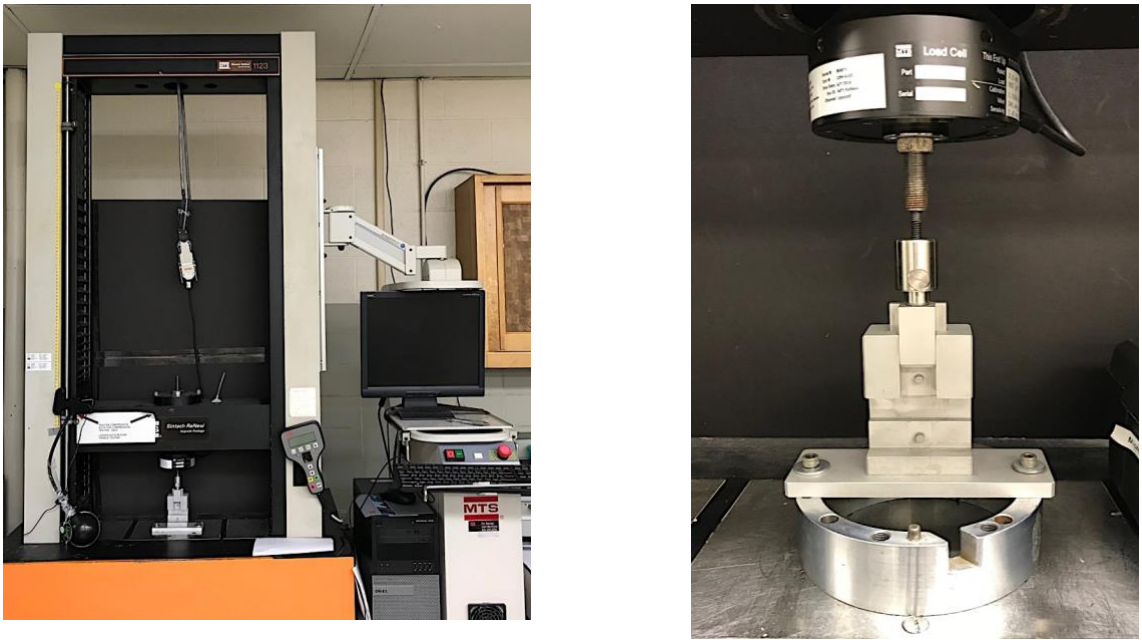



FIGURE 12. A Universal Testing Machine (Sintech Renew 1123, Eden Prairie, MN, USA) used for the microtensile bond strength testing at a crosshead speed of 1 mm/min.

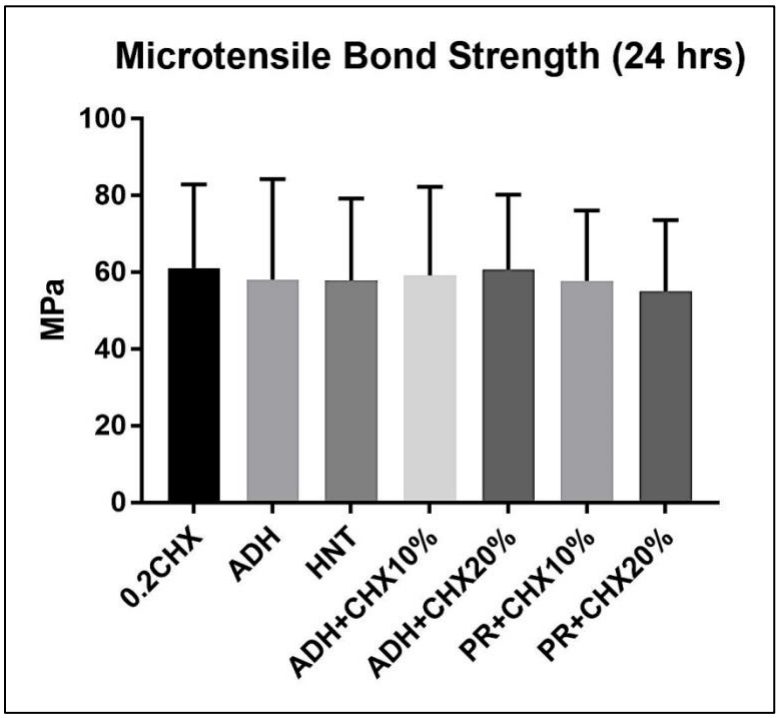



FIGURE 13. Paired comparison results from Weibull-distribution survival analysis for microtensile bond strength testing after 24 hours. Abbreviations: CHX: Chlorhexidine; ADH: Adhesive; HNT: Halloysite® nanotubes; PR: Primer.The absence of letters means no significant difference ($p > 0.05$) between groups. Microtensile bond strength test results of stress value (MPa) for each group revealed no statistical significant difference between groups in 24 hours.

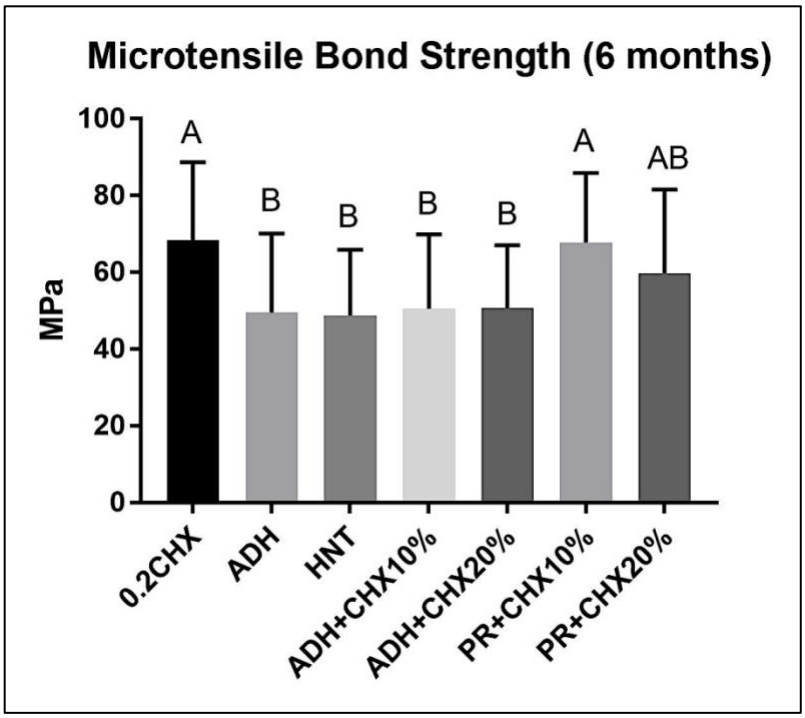



FIGURE 14. Paired comparison results from Weibull-distribution survival analysis for microtensile bond strength testing after 6 months. Abbreviations: CHX: Chlorhexidine; ADH: Adhesive; HNT: Halloysite® nanotubes; PR: Primer. Different letters indicate statistical differences ($p < 0.05$). The absence of letters means no significant difference between groups. After 6 months testing, modified primer groups (PR+CHX10%, PR+CHX20%) and 0.2%CHX group showed a significant difference in microtensile bond strength compared to control groups (ADH, HNT) and modified adhesive groups (ADH+CHX10%, ADH+CHX20%) in the same aging time testing.

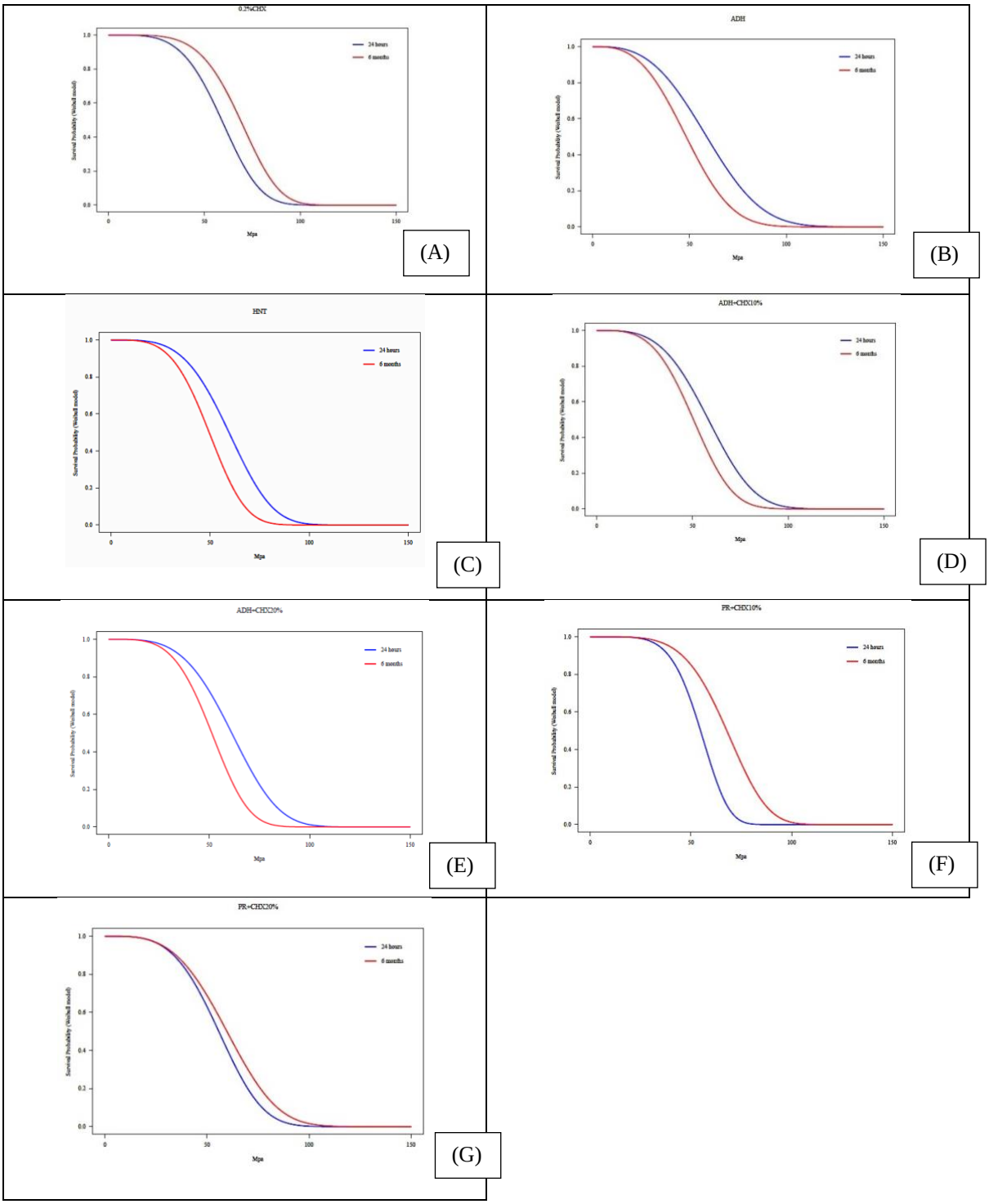



FIGURE 15. Survival plots for each experimental group: (A) 0.2%CHX; (B) ADH; (C) HNT; (D) ADH+CHX10%; (E) ADH+CHX20%; (F) PR+CHX10%; (G) PR+CHX20%. Each plot illustrates the relation between the survival probability (Weibull model) and microtensile bond strength test value (MPa) in 24 hours (–) and 6 months (–).

TABLE I

Experimental groups description and aging time

| Experimental Group | | Beam Aging |
|---|---|---|
| 0.2%CHX | 0.2wt.%CHX rubbed into dentin before PR and SBMP | 24h / 6 months |
| ADH | PR + SBMP (Control) | 24h / 6 months |
| HNT | PR + (SBMP+ 15wt.%HNT) (Control) | 24h / 6 months |
| ADH+CHX10% | PR+ (SBMP+ 15wt.%HNT encapsulated with CHX10%) | 24h / 6 months |
| ADH+CHX20% | PR + (SBMP+ 15wt.%HNT encapsulated with CHX20%) | 24h / 6 months |
| PR+CHX10% | (PR+ 15wt.%HNT encapsulated with CHX10%) + SBMP | 24h / 6 months |
| PR+CHX20% | (PR+ 15wt.%HNT encapsulated with CHX20%) +SBMP | 24h / 6 months |

Abbreviations: CHX: Chlorhexidine; ADH: Adhesive; HNT: Halloysite® nanotubes; PR: Primer (Adper™ Scotchbond™ Multi-Purpose Primer); SBMP: Adper™ Scotchbond™ Multi-Purpose Adhesive. A commercial three-step etch-and-rinse dentin adhesive system (Adper™ Scotchbond™ Multi-Purpose Adhesive Primer and Adper™ Scotchbond™ Multi-Purpose Adhesive) was used in this study. Chlorhexidine digluconate solution (20% in $H_2O$) was encapsulated into the Halloysite® aluminosilicate clay nanotubes powder at two different concentrations 10% and 20%. Chlorhexidine-encapsulated nanotubes (10% and 20%) were then incorporated into the primer and/or adhesive to achieve the experimental groups (n = 15).

TABLE II

Chemical composition, manufacturer and lot number of materials used in this study*

| Material | Manufacturer | Composition | Lot # |
|---|---|---|---|
| Chlorhexidine | Sigma-Aldrich, USA | 20% Chlorhexidine digluconate solution in $H_2O$ | 8893J |
| Adper™ Scotchbond™ Etchant | 3M ESPE, USA | 35% $H_3$ $PO_4$ | N872800 |
| Adper™ Scotchbond™ Multi-Purpose Primer | 3M ESPE, USA | HEMA, Polyalkenoic acid polymer, Water | N901524<br>N876945 |
| Adper™ Scotchbond™ Multi-Purpose Adhesive | 3M ESPE, USA | Bis-GMA, HEMA, Tertiary amines, Photo-initiator | N900742 |
| Bulk Fill Filtek Resin Composite | 3M ESPE, USA | AUDMA, UDMA and DDDMA, Silica/zirconia and ytterbium trifluoride filler | N890621<br>N901433<br>N917600<br>N910792 |

Abbreviations:
HEMA: 2-hydroxyethyl methacrylate;
Bis-GMA: bisphenol A diglycidyl ether dimethacrylate;
AUDMA: Aromatic dimethacrylate;
UDMA: Urethane dimethacrylate;
DDDMA (1, 12-Dodecanediol dimethacrylate).
* According to the manufacturer.

TABLE III

One-way ANOVA test results for degree of conversion (DC %) and viscosity (cP) with standard deviation values (SD)

| Group | DC (%) Mean (SD) | Viscosity (cP) Mean (SD) |
|---|---|---|
| ADH | 61.3 (2.6) | 641.88 (77.27) [A] |
| HNT | 74.1 (7.9) | 777.02 (94.56) [B] |
| ADH+CHX 10% | 68.5 (5.6) | 786.23 (78.36) [B] |
| ADH+CHX 20% | 70.1 (6.7) | 786.23 (42.56) [B] |
| PR | * | 168.92 (55.37) [C] |
| PR+CHX 10% | * | 127.97 (23.46) [C] |
| PR+CHX 20% | * | 133.09 (17.73) [C] |

Abbreviations: ADH: Adhesive; HNT: Halloysite® nanotubes; CHX: Chlorhexidine; PR: Primer.* Primers were not evaluated for degree of conversion. Different letters indicate statistically significant differences. The absence of letters means no significant difference between groups. DC analysis revealed no significant difference between adhesive groups; ADH, HNT, ADH+CHX10%, ADH+CHX20% ($p > 0.05$). However, there were significant differences in viscosity among groups ($p < 0.05$).

TABLE IV

Weibull-distribution survival analysis results comparison between 24 hours and 6 months for each group for microtensile bond strength testing

| Group | Results | P value |
|---|---|---|
| 0.2%CHX | * | 0.2992 |
| ADH | * | 0.1377 |
| HNT | * | 0.0943 |
| ADH+CHX10% | * | 0.2440 |
| ADH+CHX20% | 24 hours > 6 months | 0.0487 |
| PR+CHX10% | * | 0.1051 |
| PR+CHX20% | * | 0.4614 |

Abbreviations: CHX: Chlorhexidine; ADH: Adhesive; HNT: Halloysite® nanotubes; PR: Primer.* No significant difference ($p > 0.05$) between groups. When comparing the microtensile bond strength testing results after 24 hours and 6 months, there was no significant difference between the groups except for the ADH+CHX20% group where MPa values were higher in 24 hours than 6 months ($p < 0.05$).

TABLE V

Data summary for type of failure for each group

| Group | 24 hours | | | 6 months | | |
|---|---|---|---|---|---|---|
| | *ADH | MIX | COH | ADH | MIX | COH |
| 0.2%CHX | 18 (34.0%) | 14 (26.4%) | 21 (39.6%) | 15 (24.6%) | 18 (29.5%) | 28 (45.9%) |
| ADH | 13 (16.3%) | 24 (30.0%) | 43 (53.8%) | 31 (32.0%) | 33 (34.0%) | 33 (34.0%) |
| HNT | 31 (33.0%) | 17 (18.1%) | 46 (48.9%) | 43 (38.4%) | 26 (23.2%) | 43 (38.4%) |
| ADH+CHX 10% | 43 (53.1%) | 19 (23.5%) | 19 (23.5%) | 41 (48.2%) | 31 (36.5%) | 13 (15.3%) |
| ADH+CHX 20% | 49 (47.1%) | 35 (33.7%) | 20 (19.2%) | 65 (62.5%) | 22 (21.2%) | 17 (16.3%) |
| PR+CHX 10% | 19 (40.4%) | 15 (31.9%) | 13 (27.7%) | 29 (44.6%) | 10 (15.4%) | 26 (40.0%) |
| PR+CHX 20% | 18 (24.0%) | 23 (30.7%) | 34 (45.3%) | 24 (27.9%) | 28 (32.6%) | 34 (39.5%) |

Abbreviations: CHX: Chlorhexidine; ADH: Adhesive; HNT: Halloysite® nanotubes; PR: Primer.
* ADH: Adhesive failure; MIX: Mixed failure; COH: Cohesive failure. Differences between the groups for type of failure (Adhesive, Mixed, or Cohesive) were summarized by group for each aging time.

# DISCUSSION

Halloysite clay nanotubes (HNT) have been recently introduced and incorporated into dental adhesives for reinforcement and as a reservoir for encapsulation for several drugs.[26,27] The incorporation of HNT (up to 20 wt.%) into adhesive resins was done in several recent studies with no deterioration, regarding mechanical properties, to the resin matrix.[28,29,30] Besides, HNTs have shown great potential for encapsulation with several matrix metalloproteinase (MMP) inhibitors such as doxycycline and chlorhexidine, which could protect the hybrid layer from the action of active MMPs and improve the resin-dentin bond strength.[14,15,19] This study was the first attempt, to the best of our knowledge, to encapsulate CHX under two different concentrations (10% and 20%) into HNT and incorporate it into a commercial primer/PR and adhesive/ADH for μTBS testing.

The present study showed that the incorporation of HNT and HNT encapsulated with CHX (10% and 20%) did not affect the degree of conversion (DC) of the modified adhesives when specimens were cured for 10 seconds, as recommended by the adhesive manufacturer. This result suggests a homogenous distribution of the nanotubes into the dental adhesives matrix, which was achieved by the proper mix of the material using a mechanical mixer right before any procedure. This data is in agreement with previous researches that showed that the HNT had no deleterious impact on the DC.[29,31]

Regarding viscosity, among the adhesives, ADH presented significantly lower viscosity when compared with the groups ADH+CHX10%, ADH+CHX20%, and HNT, suggesting that the addition of nanotubes led to an increase in the viscosity of the experimental adhesives. During bonding procedures, dentin adhesives with high viscosity

can result in a thicker adhesive layer,[38] which may affect the penetration of the adhesive into the dentinal tubules and eventually the resin-dentin bond strength.[39,40]

Interestingly, in the present study, the bond strength (μTBS) was not impacted by the viscosity of the material, meaning that no statistically significant difference was detected between the ADH and ADH-HNT-CHX modified dentin adhesives. On the other hand, the experimental primers (PR+CHX10% and PR+CHX20%) presented no statistical significant differences in viscosity when compared with the control primer, without HNT. For the PR+CHX10% group, the results of the bond strength after 6 months were statistically higher when compared with all the adhesive groups and similar to the group treated with chlorhexidine solution (0.2%CHX). Therefore, the results of viscosity and microtensile together suggested that the use of the primers as a vehicle to deliver chlorhexidine encapsulated into the dentin tubules can be promising.

The main concern about the long-term storage (6 months) was the presence of microorganisms already present in the dentin tubules. Their presence could lead to the activation of latent MMPs, competing with the latent MMPs activated after acid etching, and this process could be involved in the degradation of the hybrid layer and dentin collagen fibrils, which could therefore cause adhesive layer failure.[41] In the present study, the teeth were previously stored in a thymol solution to disinfect and to prevent them from further bacterial growth. Recent publications showed that after immersion in thymol for 24 hours, microorganisms would starve considering that they have no access to substrates that are essential for cell growth and replication.[42] However, a different study showed that thymol is the least successful agent for sterilizing extracted teeth when compared with 10-percent formalin, 5.25-percent sodium hypochlorite and autoclaving.[43]

In the present study, autoclaving of the teeth was performed because previous studies concluded there were no morphological changes,[44] or changes in the dentin bond strength,[45] observed in the dentin specimens after autoclaving.

Microshear and microtensile bond strength tests have been widely used to evaluate the bond strength to dentin.[46,47] Regarding the microshear test, the easy specimen preparation and the relatively simple protocol make the test appealing when compared with the microtensile test.[48] However, concerns regarding the stress distribution along the substrates and the high number of cohesive failures in the substrate have been raised.[48] On the other hand, for the microtensile test, stresses are better distributed throughout the variations in the structure of dentin during the test,[46] and it offers a versatility that cannot be achieved with any other testing method, providing excellent insight on the resin-dentin adhesion after aging.[49]

Therefore, μTBS testing was used in this study as the most reliable method to evaluate and compare the resin-dentin bond strength at small regions of the dental tissues,[49] despite its limitation as a labor-intensive and time-consuming test. The number of prematurely debonded beams per each tested group was also recorded, but not included in the statistical analysis. Only 3 beams were debonded during the 6-month aging, two beams for the ADH control group, and one for the experimental ADH+CHX20% group. All premature failures of beams that occurred during the cutting procedure were not considered in the statistical analysis. These failures occurred at time zero before the μTBS testing, and could cause masking of the bond strength values making them appear lower than the actual values.[17]

Differences in the μTBS for the two testing times (24 hours and 6 months) were analyzed between groups and for each group separately. The results showed no statistically significant differences among all the experimental groups 24 hours after the bonding protocol. These results were expected since no differences are anticipated directly after bonding to dentin, given that resin-dentin degradation is a time-dependent process.[50]

After 6 months aging, CHX-encapsulated nanotube-modified primers (PR+CHX10% and PR+CHX20%) and 0.2%CHX group demonstrated higher μTBS than control groups (ADH, HNT) and modified adhesive groups (ADH+CHX10%, ADH+CHX20%), which led to the rejection of the null hypothesis. However, PR+CHX10%, PR+CHX20%, and 0.2%CHX groups demonstrated no significant differences between them. These findings can be explained by the ability of the primer to directly penetrate the demineralized dentinal tubules after acid-etching and re-expand the collapsed dentin as well as to coat the wet dentinal collagen fibrils with a hydrophilic monomer like HEMA.[51] Besides, recent studies have reinforced the therapeutic opportunities modified primers can offer,[28] and the efficacy of incorporating 0.2–2 wt.% chlorhexidine, which binds to acid-etched dentin to inhibit MMPs and prolong resin-dentin bond strength durability.[52,53] These data support the advantage of incorporation of CHX into the primers and adhesives for sustained release over time,[34] and to eliminate the additional operator-related and time variables when CHX is rubbed directly onto acid-etched dentin, and to eventually improve the clinical performance of dentin adhesives.

Although the release of CHX-encapsulated in nanotubes was not quantitatively measured over an extended period of time, it is possible that the release was ongoing

throughout the 6-month period and contributed to the improved μTBS for the experimental primers in comparison with the adhesive control and experimental groups. However, it is speculated that the release of CHX-encapsulated nanotubes incorporated in the experimental adhesives may be contained in the resin matrix, which delays the release to a long-term period up to 1 year. Further study is needed to determine the nature and duration of CHX release and its influence on dentin bond strengths.

It is worth mentioning that, in all experimental groups in this study, the μTBS was not jeopardized in 24 hours and was maintained during the 6-month aging after bonding, except for the ADH+CHX20% group where the bond strength was higher in 24 hours than 6 months. This might be due to the high concentration of CHX encapsulation compared with CHX10% or incomplete encapsulation, which might have led to the accumulation of CHX in the resin matrix and prevented complete penetration into the tubules and thus affected the long-term durability of bond strength. Another interesting observation was that the μTBS values of the control ADH and HNT group as well as ADH+CHX10%, ADH+CHX20% groups showed similar results. This strongly suggests agreement with other studies ,[30,54] in that the addition of HNT and HNT encapsulated with CHX (10% and 20%) with 15 wt% did not prevent the infiltration of the primer and adhesive into the hybrid layer to form resin tags into the dentinal tubules;[30] therefore, a strong micromechanical interlocking was formed.[54]

# SUMMARY AND CONCLUSION

The objective of this study was to evaluate the effect of a chlorhexidine-encapsulated nanotube-modified primer and dentin adhesive on the microtensile resin bond strength to dentin for the possibility of enhancing the longevity and durability of the hybrid layer.

The null hypothesis stated that there would be no differences in resin-dentin microtensile bond strengths (μTBS) between CHX-encapsulated nanotube-modified primer and adhesive experimental groups and control groups after 6 months aging, was rejected because the CHX-encapsulated nanotube-modified primers (PR+CHX10% and PR+CHX20%) and 0.2%CHX group demonstrated higher μTBS than control groups (ADH, HNT) and modified adhesive groups (ADH+CHX10%, ADH+CHX20%).

In conclusion, and within the limitations of the present study, great potential was shown for modified dental primers with CHX-encapsulated nanotubes in preservation of the resin-dentin bond strength over a 6-month time period. Additionally, modification of dental primers and adhesives was a successful approach that did not compromise the characteristics or the mechanical properties of the materials and has a promising long-term effect on resin-dentin bond strength. Further testing should be considered to evaluate long-term durability of the resin-dentin μTBS and to assess the continuous release of CHX- encapsulated nanotubes.

## CLINICAL RELEVANCE

The incorporation of chlorhexidine-encapsulated nanotubes into dentin adhesive systems and specifically primers gives the advantage of preserving the hybrid layer from degradation, and of increasing the microtensile resin bond strength to dentin up to at least 6 months. Incorporation of CHX-encapsulated nanotubes holds a great potential to enhance the long-term durability of dentin bond strength and to decrease the failure of adhesive restorations.

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ABSTRACT

# EFFECT OF CHLORHEXIDINE-ENCAPSULATED NANOTUBE-MODIFIED ADHESIVE SYSTEM ON THE BOND STRENGTH TO HUMAN DENTIN

by

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Introduction: The resin-dentin interface undergoes degradation by endogenous matrix metalloproteinases (MMPs) after adhesive procedures. Application of several MMP inhibitors such as chlorhexidine (CHX) to the demineralized collagen dentin matrix after acid-etching has been suggested to be a successful approach to prevent degradation of the hybrid layer. Further, nanotubes (HNT) have been used as a reservoir for encapsulation and controlled delivery for several therapeutic drugs with sustained

release. Therefore, HNT can be encapsulated with CHX and incorporated into dentin adhesives for the possibility of enhancing the longevity and durability of the hybrid layer.

Objective: To evaluate the effect of a CHX-encapsulated nanotube-modified primer/PR and adhesive/ADH on the microtensile resin bond strength (μTBS) to dentin.

Materials and Methods: A commercial adhesive and its respective primer were modified by adding CHX-encapsulated nanotubes at two distinct concentrations (10 and 20 wt.%). The experimental adhesives were evaluated by degree of conversion (DC) and viscosity. Meanwhile, only viscosity was determined for the experimental primers. The prepared HNT-encapsulated with CHX (10 and 20 wt.%) powders were incorporated into the primer and/or adhesive according to the groups: ADH (control); HNT (control); 0.2% CHX; PR+CHX10%; PR+CHX20%; ADH+CHX10%; ADH+CHX20%. Human molars were selected and autoclaved; mid-coronal dentin surfaces were exposed for bonding purposes. Dentin surfaces were etched, followed by primer and adhesive application, and restored with a resin composite. After 24 hours, the teeth were sliced into beams for μTBS testing; beams collected for each tooth were equally assigned into two testing condition groups: 24 hours and 6 months. Microtensile bond strength was tested using a universal testing machine, and the types of failure were classified as adhesive, mixed, and cohesive failure. Data from DC and viscosity tests were analyzed using one-way ANOVA. Bond strength data were analyzed by pair-wise comparisons using the Sidak method to control the overall significance level at 5% for each aging time separately. Weibull-distribution survival analysis was used to compare the differences in the microtensile bond strength results among the groups after 24 hours and 6 months.

Results and Conclusion: DC analysis revealed no significant differences among adhesive groups. However, ADH group had a significantly lower viscosity than modified adhesive groups, and a significantly higher viscosity than modified primer groups. Test results of stress value (MPa) by each group for each aging time revealed no significant differences among groups after 24 hours. However, after 6-month storage, modified primer groups (PR+CHX10%, PR+CHX20%) and 0.2%CHX group showed a significant difference in μTBS compared to control groups (ADH, HNT) and modified adhesive groups (ADH+CHX10%, ADH+CHX20%) in the same aging time testing ($p < 0.05$). When comparing the μTBS after 24 hours and 6 months, there were no significant differences among the groups except for the ADH+CHX20% group, for which MPa values were higher after 24 hours than 6 months ($p = 0.0487$). In conclusion, this study has demonstrated the great potential of modified dental primers with CHX-encapsulated nanotubes in preservation of the resin-dentin bond strength over a 6-month time period. Additionally, modification of dental primers and adhesives was a successful approach that didn't compromise the characteristics or the mechanical properties of the materials and has a promising long-term effect on resin-dentin bond strength.

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