EFFECT OF HALLOYSITE ALUMINOSILICATE CLAY NANOTUBE INCORPORATION INTO BONDING AGENTS ON SHEAR BOND STRENGTH TO HUMAN DENTIN

by

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INTRODUCTION

Due to an increased demand for esthetics and minimally invasive tooth preparations, tooth-colored resin restorations have become very popular worldwide. However, failure in obtaining a strong and durable interfacial bond between the restoration and the tooth substrate results in marginal discoloration, postoperative sensitivity, secondary caries, and pulpal pathology.[1] It has been reported that the average replacement time of tooth-colored resin restorations is only 5.7 years.[2] Replacing defective dental restorations costs about 5 billion dollars per year in the US alone.[3] As a result, research has focused on the development of these restorations and achieving a good bond has become a fundamental goal in adhesive dentistry.

At the tooth-restoration interface, the adhesive interface is considered the weakest area with the lowest elastic modulus of an adhesive restoration,[4,5] and thus the stresses concentrating in it during the shrinkage of the polymerizing composite resin or during occlusal loading can exceed the inherent strength of this weakest layer to create microcracks, defects, or catastrophic failure.[6,7]

In polymers, particle incorporation has shown a reinforcing effect by crack deflection and local plastic deformation around the particle.[8] With intention to improve physical and mechanical properties, fillers have been incorporated into dental adhesives.[9-15] The filler particles are used in dental adhesives to enhance the adhesive bond strength to dentin by increasing the elastic modulus of the adhesive layer and decreasing polymerization shrinkage.[13-15] Many studies have been published recently that incorporated fillers with different types, sizes, shapes, and characteristics into dental

adhesives and reported significant increase in bond strength to dentin and enhancement of adhesive layer mechanical properties.[9, 12-15]

Halloysite aluminosilicate clay nanotubes (HNTs), also named clay nanotubes, have an external diameter of 50 nm, with a 15-nm lumen, and length of 800 nm.[16] HNTs have many advantages that make them good candidates to be used as reinforcing agents for improving the properties of dental adhesives. HNTs are biocompatible,[17] hydrophilic, inexpensive, abundantly available and durable, with high mechanical strength and a viable nanoscale container for loading, storage, and controlled release of biologically active molecules. They are also a natural product that will not add risk to the environment as other nanomaterials do.[16]

Current adhesive systems interact with the tooth substrate by two different mechanisms, either the removal of the smear layer (the etch-and-rinse technique) or the modification of the layer (the self-etch technique).[18-21] The difference between the two techniques is represented by the use of a separate etching acid for etch-and-rinse systems that is later rinsed away.[18] Conversely, the self-etch systems do not require a separate etching. Etchant is combined within the bonding agent so that the etching of the tooth surface and the infiltration of the monomer happen simultaneously with the resulting dissolution of the smear layer rather than complete removal.[19-21] Currently, the classification of dental adhesive is mainly based on the number of the steps constituting the adhesive system.[22] Based on whether primer and bonding are separate or combined in one bottle, etch-and-rinse dental adhesive systems can be either two- or three-step. Similarly, self-etch dental adhesives can be either one- or two-step systems based on

whether the etching/primer agent is separated from the adhesive or combined with it to be used as a single application.

The two-step etch-and-rinse systems are commonly used and well-accepted in North America and Europe.[23] Recently, one-step self-adhesive systems (all-in-one systems) have been emerging in the market and promoted by the manufacturers to provide faster and easier handling by the clinician. However, there are reports that showed inferiority in the bond performance of one-step self-adhesive systems in comparison with the two-step adhesive systems[24,25] and the bond strength varied among products.[26,27]

Therefore, in this study, the two-step etch-and-rinse adhesive system represented by a commercial product (ONE-STEP®, Bisco, USA), and the one-step self-etch adhesive system represented by a commercial product (Xeno IV, Dentsply-Caulk, Milford, DE, USA) were selected and halloysite aluminosilicate clay nanotubes were incorporated in the concentrations of 0 wt%, 5 wt%, 10 wt%, and 20 wt%. These concentrations are based on preliminary data by Dr. Bottino's Research Group, which showed that up to 30 wt% incorporation of halloysite aluminosilicate clay nanotubes into the adhesive of Adper Scotchbond Multi-Purpose (3M, ESPE) increased the shear bond strength to human dentin. Also, they found that incorporating 30 wt% resulted in deterioration in microhardness and degree of conversion.

OBJECTIVE

The objective of this study was to evaluate the effect of incorporating halloysite aluminosilicate clay nanotubes either into the two-step etch-and-rinse adhesive system

(ONE-STEP®, Bisco, USA) or into the self-etch/one component adhesive system (Xeno IV, Dentsply-Caulk, Milford, DE, USA) on dentin shear bond strength.

HYPOTHESES

Null Hypotheses

The null hypotheses that were tested: 1) the incorporation of Halloysite aluminosilicate clay nanotubes would not increase adhesive bond strength to dentin; 2) there would be no effect of filler concentration on bond strength to dentin.

Alternative Hypotheses

The alternative hypotheses that were tested: 1) the incorporation of halloysite aluminosilicate clay nanotubes would increase adhesive bond strength to dentin; 2) The bond strength to dentin would be increased with increased filler concentration up to a threshold level.

REVIEW OF LITERATURE

In 1995 Miyazaki et al.[13] investigated the influence of silica (SiO_2) filler particle incorporation into bonding agents on shear bond strength to bovine dentin, and the optimum filler level for an experimental bonding agent by the temperature change during curing. Bonding agents were loaded with microfiller contents of 0 wt%, 10 wt%, 20 wt%, 30 wt%, 40 wt%, 50 wt%, 60 wt%, and 70 wt%. For each test group, 10 samples were prepared and stored at 37 C° in water for 24 h, and then the bond strength tested in shear mode. During the bonding agents' exothermic polymerization reaction, the time to reach peak temperature and the peak temperature were recorded. The results of this study showed that bond strength to dentin and the temperature change were greatly affected by the filler content level. The highest value of dentin bond strength was observed with 10 wt% filler level and significantly decreased with filler levels more than 30 wt%. With the higher filler content levels, peak temperature decreased and the time required to reach peak temperature increased. The peak temperatures for the bonding agents with filler levels above 40 wt% did not appear within the 30-s light curing time used for the bonding agent in the bond strength tests, which indicates that the polymerization reaction might not have been completed.

Kim et al.[10] in 2005 evaluated the microtensile bond strength, the degree of conversion, and the flexural strength of an ethanol-based one-bottle dentin adhesive loaded with hydrophilic nanofillers. Four groups of dentin adhesives containing 12-nm hydrophilic fumed silica at 0 wt%, 0.5 wt%, 1.0 wt%, and 3.0 wt% were evaluated, and transmission electron microscopy (TEM) was used to examine the distribution of the nanofillers. The results showed that the microtensile bond strength showed insignificant

increases when up to 1.0 wt% of the nanofillers were added. However, the microtensile bond strength decreased when 3.0 wt% of the nanofillers were added. The degree of conversion was not affected by the nanofiller content. The flexural strength increased with increasing nanofiller content. The TEM image revealed that the nanofillers aggregated into large clusters on the dentin surface when 3.0 wt% of the nanofillers were added, which explained the decrease in the microtensile bond strength.

In 2006 Lee et al.[11] assessed the effect of filler addition on the bonding parameters of dentin bonding adhesives bonded to human dentin. Two total-etch bonding systems with no-filler and filler-added versions were studied for the bond strength, displacement at debonding, stiffness of debonding, and energy absorbed during debonding of resin composites to human dentin. The results showed that the filler addition did not influence the bond strength, the displacement at debonding, and the energy to debonding.

In 2009 evaluation of radiopaque adhesives containing Ta_2O_5/SiO_2 nanoparticles was reported by Schulz et al.[28] They investigated the dispersion of flame-made Ta_2O_5/SiO_2 nanoparticles in methacrylic matrices and the influence of particle content on viscosity of the suspension, the shear bond strength to enamel and dentin, distribution of aggregate size and radiopacity. The results of this study indicated that flame-made Ta_2O_5/SiO_2 nanoparticles can be incorporated into dental adhesives as they form very stable suspensions. Even after incorporating radiopaque particles up to 20 wt%, viscosity remained low. The shear bond strength of these radiopaque particle-containing adhesives to enamel and dentin was not significantly different from the particle-free adhesive; also, there was no difference in shear bond strength between adhesive with functionalized

nanoparticles that surface treated with γ-methacryloxypropyltri-methoxysilane and adhesive with non-functionalized nanoparticles.

In 2009 Akasaka et al.[29] investigated the effect of coating dentin with carbon nanotubes (CNTs) on the tensile bond strength of dentin adhesives. Even though they just coated the dentin surface with carbon nanotubes and did not incorporate them into dental adhesive, micro-tensile bond strength testing showed that there was no significant difference between the CNT-coated and non-coated specimens. The results led to the conclusion that the advantages of CNTs, such as their effect on nucleation of hydroxyapatite,[30] strengthening composite materials[31] and providing protection against bacteria,[32] can be utilized without adversely affecting the bond strength of dental adhesives to dentin.

The effect of silica nanofiller loading of adhesive resins has been reported by Conde et al.[9] in 2009. Silica nanofillers were added into a formulated HEMA/Bis-GMA/TEGDMA-based adhesive in weight percentages (wt%) of 0 wt%, 1 wt%, 3 wt%, 5 wt% and 10 wt%. Adper Scotchbond Multi-Purpose (SBMP) adhesive system was also used as a commercial reference. Then, the effects of the filler content on the cohesive strength, Weibull modulus, and degree of conversion of an experimental adhesive system were evaluated. The results of this study showed that cohesive strength tended to increase with increasing silica nanofiller content, but a significant increase in cohesive strength was observed only when 10 wt% of the nanofillers were added, compared with the Scotchbond Multi-Purpose adhesive system (SBMP). Moreover, the experimental adhesives had significantly higher degree of conversion (DC) than the SBMP. The analysis of Weibull modulus revealed no significant difference between groups in

structural reliability. The results of this study indicated that improving the cohesive strength of the adhesive can be achieved by 10% nanofiller loading by weight without adversely affecting the structural reliability or the DC.

Lohbauer et al.[12] in 2010 evaluated zirconia nanoparticles prepared by laser vaporization as fillers for dental adhesives. They incorporated zirconia nanoparticles (20 nm to 50 nm) into the primer or into the adhesive of the SBMP adhesive system at 5 wt%, 10 wt%, 15 wt% and 20 wt% and tested to evaluate its effect on bond strength to dentin. Micro-tensile bond strength testing and transmission electron microscopy (TEM) indicated that incorporation of zirconia nanoparticles into the SBMP system increased bond strength to dentin by reinforcing the interfacial adhesive layer. The bond strength increased with increasing concentration of nanofiller incorporation into the primer solution. In particular, nanofiller incorporation at high concentrations (20 wt%) showed greater bond strength when incorporated in the primer than in the adhesive solution.

In 2010 Sadat-Shojai et al.[14] evaluated the use of hydroxyapatite (HAp) nanorods as novel fillers for improving the properties of dental adhesives. In this study they synthesized fibrous hydroxyapatite nanorods by a hydrothermal method and incorporated them into an experimental one-bottle dentin adhesive at 0 wt%, 0.2 wt%, 0.5 wt%, 1 wt%, 2 wt%, and 5 wt% and homogenized by sonication. The adhesive systems containing different nanorod contents were tested for flexural strength, flexural modulus, diametral tensile strength, and the micro-shear bond strength to the dentin of human premolars. The mode of failure was also determined after micro-shear testing by using scanning electron microscopy (SEM). The results revealed that diametral tensile strength and flexural strength tended to increase when 0.2 wt% to 0.5 wt% . HAp nanorods were

incorporated to the adhesive systems, while flexural modulus remained unaffected. Also, the 0.2 wt% filler content showed the highest microshear bond strength. Evaluation of debonded surfaces under SEM indicated that most specimens had an adhesive-dentin interface failure.

Solhi et al.[15] in 2012 published a study about a novel dentin adhesive system in which polymethacrylic acid grafted nanoclay was used as a nanofiller and how it improved the bond strength and the mechanical properties. In this study, the fillers were synthesized and incorporated into experimental adhesive in 0.2 wt%, 0.5 wt%, 1 wt%, 2 wt% and 5 wt% dispersed in the adhesive solution by sonication. Then, the experimental adhesives were used to test the microshear bond strength to human dentin, and a commercial adhesive (Adper Single Bond 2, 3M, ESPE, USA) was used as control group. The mechanical properties also evaluated including flexural strength, flexural modulus, and diametral tensile strength. The result showed that microshear bond strength, diametral tensile strength, and flexural strength were significantly increased when 0.5 wt% of polymethacrylic acid grafted nanoclay nanofillers were incorporated. Flexural modulus increased with higher nanofiller contents.

METHODS AND MATERIALS

The shear bond strengths of two commercial adhesive systems bonded to dentin after incorporation of halloysite aluminosilicate clay nanotubes (HNTs) in different wt% were tested.

HALLOYSITE ALUMINOSILICATE CLAY NANOTUBE INCORPORATION INTO THE ADHESIVE SYSTEM

Halloysite aluminosilicate clay nanotubes (Sigma Aldrich, St. Louis, MO) were incorporated into the commercial adhesive systems, namely a two-step etch-and-rinse (ONE-STEP®, Bisco, USA) and a one-step self-etch (Xeno IV, Dentsply-Caulk, Milford, DE, USA) in 0 wt%, 5 wt%, 10 wt%, 20 wt.%. Five hundred microliters of the adhesive solutions were pipetted into dark amber Eppendorf tube and weighed using five-decimal accuracy electronic scale. The nanofillers were weighed in the same scale and added based on the weight percentages relative to the weight of the adhesive solution. The nanofillers were immediately added to the adhesive solution and mechanically mixed with a motorized stirrer (Roti-Speed hand piece with conical micro pestle adapter (Roth, Karlsruhe, Germany) (Figure 1) (5000 rpm) in the dark amber Eppendorf tube and immediately wrapped with aluminum foil to prevent light exposure and premature polymerization. Additionally, the resin adhesive mixtures were sonicated (Ultrasonic system, L&R-2014) for 1 h to increase filler dispersion. Then, the adhesive solutions were used immediately after sonication to bond the dentin specimens for shear bond strength testing as described later in this section.

For the etch-and-rinse adhesive system, the tested groups were as follows:

1. ER -control: Adhesive was used directly from the bottle.

2. ER-experimental control: Adhesive was subjected to mixing process with 0 wt% of HNTs.

3. ER-5: Adhesive was incorporated and mixed with 5 wt% of HNTs.

4. ER -10: Adhesive was incorporated and mixed with 10 wt% of HNTs.

5. ER-20: Adhesive was incorporated and mixed with 20 wt% of HNTs.

For self-etch adhesive system, the tested groups were as follow:

1. SE-control: Adhesive was used directly from the bottle.

2. SE-experimental control: Adhesive was subjected to mixing process with 0 wt% of HNTs.

3. SE-5: Adhesive was incorporated and mixed with 5 wt% of HNTs.

4. SE-10: Adhesive was incorporated and mixed with 10 wt% of HNTs.

5. SE-20: Adhesive was incorporated and mixed with 20 wt% of HNTs.

SPECIMEN FABRICATION AND SHEAR BOND STRENGTH TESTING

One hundred and twenty extracted non-carious and non-restored human molar teeth stored in 0.1-percent thymol solution were used. The occlusal surface of the crown of each tooth was ground to expose dentin using a wheel polishing machine with wet 180-grit silicon carbide paper (300 rpm). The absence of enamel was verified using a stereomicroscope (X45). Samples were stored in distilled water and then randomly allocated into 10 groups. The dentin surfaces were placed flat down on a Mylar sheet. Plastic cylinders (approximately 15 mm to 6 mm internal diameter and 20 mm to 25 mm tall) were placed over and around each tooth. The teeth were mounted in the cylinders by using self-curing acrylic resin (Figure 2). Acrylic resin was mixed and poured into the

cylinders until it completely covered the tooth and filled the cylinder. After the acrylic resin set, the exposed dentin was wet-finished with 400- and 600-grit silicon carbide papers to produce a standardized smear layer. In the control groups, the commercial adhesive systems without incorporation of halloysite aluminosilicate nanotubes were applied on the dentin surface according to the manufacturer's instructions. For the two step etch-and-rinse adhesive system (ONE-STEP®, Bisco), the dentin surface was etched with UNI-ETCH® (32-percent phosphoric acid) for 15 s, rinsed with water for 10 s and blotted dried with Kim wipes to leave the dentin moist. Then, two coats of ONE-STEP® adhesive were applied with a fully saturated disposable brush tip, gently air dried for 10 s to evaporate solvent, then light cured for 10 s. For the one-step self-etch adhesive system (Xeno IV, Dentsply-Caulk), no separate acid etchant was used. The adhesive was applied and rubbed on dentin surface for 15 s, then repeated by applying another coat and rubbing for 15 s followed by air drying and thinning for approximately 5 s and light cured for 10 sec. Experimental groups were prepared in the same way after nanotube incorporation. Then, resin composite (Z100™ Restorative, 3M ESPE) buttons were placed on top of the adhesive using a bonding jig (Ultradent Inc.) (Figure 3) with a cylindrical mold of 2.38 mm in diameter and approximately 2 mm in height, followed by light curing using a Demi light-curing unit (Kerr, Danbury, CT, USA). The output of the curing light was monitored using a Demetron radiometer to assure a >600 mW/cm^2 light output. Excess adhesive on the dentin surface and around the resin-bonded area was carefully removed using a surgical #15 blade. Prepared specimens were stored at 37ºC in water for 1 day before testing in a universal testing machine (MTS) using a notched, semi-circular shaped edge (Ultradent Inc.) (Figure 5) at a crosshead speed of 1.0 mm/min. The shear bond

strength was obtained on the computer with the software program (Test-Works 4.0, MTS Systems Corporation, St. Paul, MN). Shear bond strength (in MPa) was calculated by dividing the peak load by the bonding area. The values were recorded for statistical analysis.

FAILURE MODE EXAMINATION

Debonded specimens were examined under a stereomicroscope at X45 magnification to evaluate the fracture pattern and the failure mode was classified as follows:

- Adhesive failure at the dentin material interface.
- Cohesive failure within the dentin surface or within the restorative material.
- Mixed failure partially adhesive and partially cohesive.

RESIN-DENTIN INTERFACE EVALUATION

Four molars were used to obtain 4 dentin slabs with a thickness of 2 mm from middle dentin using a water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) (Figure 6), then a standard smear layer was created on the occlusal surface by wet finishing with 400- and 600-grit silicon carbide paper (300 rpm). The experimental adhesive groups that showed numerically the highest shear bond strength from each adhesive system (SE-5 and ER-10) were selected for resin-dentin interface evaluation and compared with their commercial control counterparts. Experimental adhesives were prepared and applied on dentin slabs as previously described in the shear bond test, and then a 1-mm thick layer of a flowable resin composite (Tetric® EvoFlow,

Ivoclar/Vivadent, Schaan, Liechtenstein) was applied (Figure 7). So, four slabs were prepared (ER-control, ER-10, SE-control, and SE-5) and stored at 37ºC in deionized water for 1 day. Then, slabs were sectioned perpendicular to the bonded interface using a water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain two 3-mm width slabs (Figure 8). The two slabs from each group were placed with cross-sectional interfaces down flat over an adhesive tape on the base of mounting cylinders (Buehler, USA) (Figure 9), and then mounted in self-cure epoxy resin (EpoxiCure, Buehler, USA). The cylinders were lubricated with Vaseline and then epoxy resin was mixed and poured into the cylinders until it completely covered the specimens and filled the cylinder. After setting, the mounted specimens were pushed out of the cylinders, and then exposed cross-sectional interfaces of the restorative material/adhesive/dentin were wet-finished using a wheel polishing machine with 400-, 600-, 800- and 1200-grit silicon carbide paper (300 rpm). This was followed by polishing using the same wheel polishing machine sequentially with 30-µm, 9-µm, and 3-µm diamond discs (Apex® Diamond Grinding Discs DGD, Buehler Ltd, USA) (Figure 10) and by cleaning in deionized water with an ultrasonic device between each diamond polishing disc polish for 5 min. At the end, specimens were sonicated in ethanol for 5 min to remove any remaining polishing debris, and then thoroughly dried.[33,34] The polished surfaces were then demineralized with 6 N HCl for 30 s,[33-35] followed by deproteinization in 5.0-percent sodium hypochlorite for 5 minutes.[36,37] Then, they were rinsed with deionized water and dried. After drying, the specimens were sputter-coated with gold for 90 s and examined using scanning electron microscopy (SEM).

STATISTICAL METHODS

The effects of adhesive system and nanofiller content on shear bond strength were evaluated using two-way analysis of variance (ANOVA. Pair-wise comparisons between groups were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5 percent. The percentage of specimens with adhesive failure was compared among the groups using logistic regression.

RESULTS

Incorporation of halloysite aluminosilicate nanotubes into either the two-step etch-and-rinse adhesive system or into the one-step self-etch adhesive system significantly increased the shear bond strength to dentin compared with the experimental control groups ($p < 0.05$). Compared with commercial control groups, only the self-etch adhesive system with 5 percent (HNTs) showed significant increase in shear bond strength, while the etch-and-rinse adhesive system showed an increase that was not significant.

For the etch-and-rinse adhesive, the experimental control had significantly lower shear bond strength than control ($p = 0.0038$), 5 percent ($p = 0.0114$), 10 percent ($p= 0.0002$), and 20 percent ($p = 0.0015$), but there were no other differences among filler contents. The self-etch, 20-percent, and experimental control groups had significantly lower shear bond strength than the control ($p \leq 0.007$), the 5-percent group ($p \leq 0.008$), and 10-percent group ($p \leq 0.001$). The 10-percent and control groups had significantly lower shear bond strength than the 5-percent group ($p \leq 0.036$). The etch-and-rinse had significantly lower shear bond strength than the self-etch for control ($p = 0.0177$), the 5-percent group ($p < 0.0001$), the 10-percent group ($p = 0.0394$), and the experimental control ($p = 0.0109$); but the two systems did not have a significantly different shear bond strength for the 20-percent group ($p = 0.08$) (Table II and Table IV) (Figures 14 and 15).

The adhesive system did not have a significant effect on failure mode ($p = 0.39$). For the etch-and-rinse, the 5-percent group had a significantly lower percentage of specimens with adhesive failure than the 10-percent group ($p = 0.0197$), the 20-percent

group ($p = 0.0033$), and the experimental control ($p = 0.0451$). For the self-etch, the control had a significantly lower percentage of specimens with adhesive failure than the 10 percent ($p = 0.0478$) and 20-percent ($p = 0.0478$) (Table III) (Figures 16 and 17).

Resin-dentin interface SEM evaluation showed that 10 wt% HNTs filled etch-and-rinse adhesive (Figures 11 and 12) has a thicker adhesive layer, thick and long resin tags, and signs of presence of HNTs within resin tags such as an apparently rougher surface. Additionally, SEM images revealed the presence of agglomerated HNTs on resin tags. The SEM images of 5 wt% HNTs filled self-etch adhesive showed increase in number of short resin tags compared with the control adhesive.

FIGURES AND TABLES

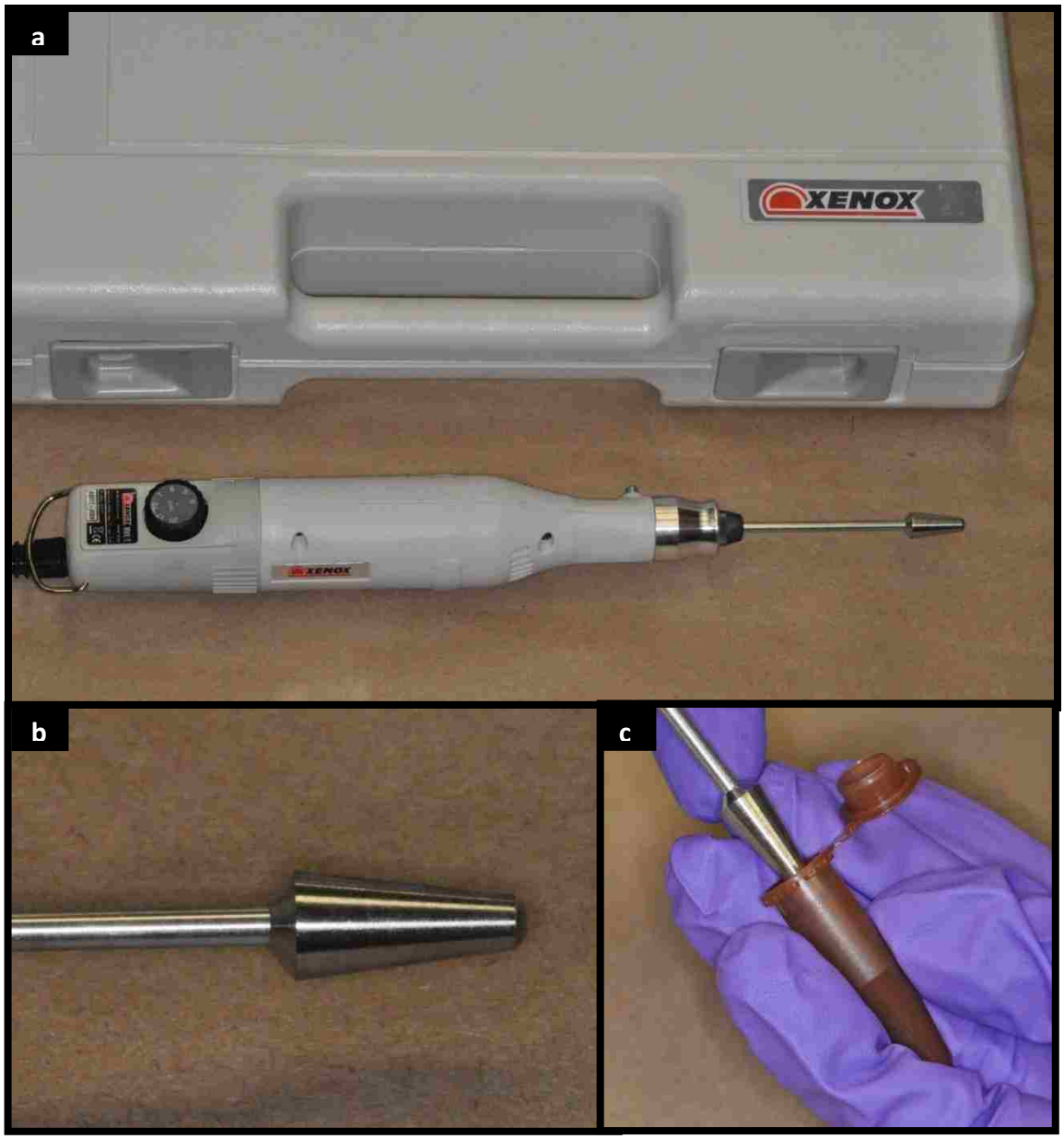

FIGURE 1. A) Roti-Speed hand piece, Roth, Karlsruhe, Germany. B) Conical micro pestle adapter. C) The size of the conical micro pestle adapter compared with the Eppendorf tube and the way it was positioned and inserted to mix the adhesive solution.

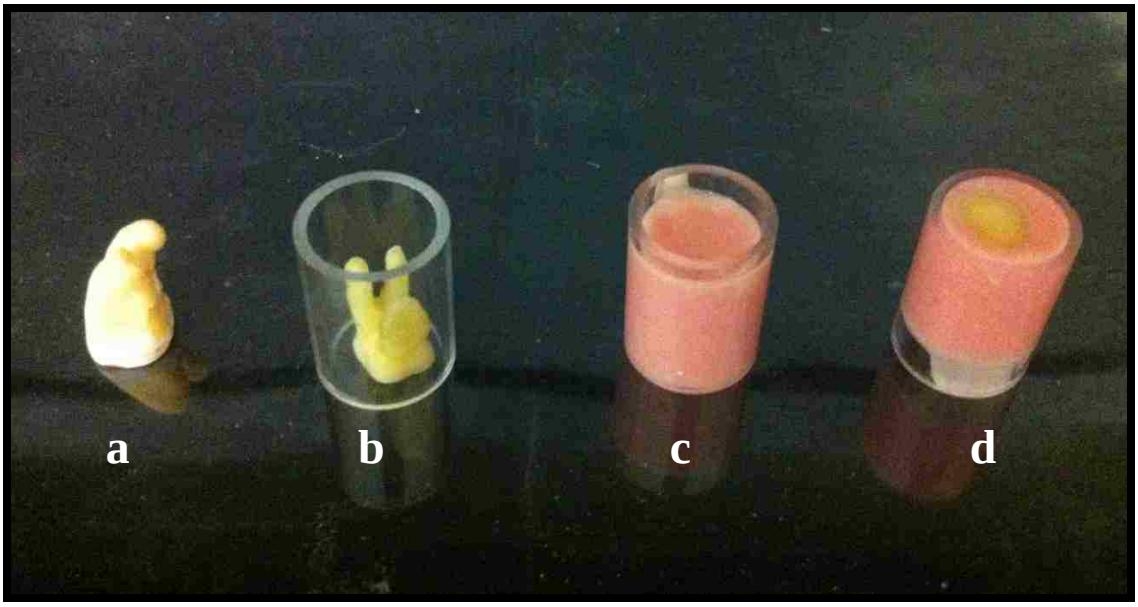

FIGURE 2. Sequence of steps for mounting teeth in acrylic resin. A) Tooth placed with dentin surfaces flat down on a Mylar sheet. B) Plastic cylinder placed over and around the tooth. C) Acrylic resin poured into the cylinders until it completely covered the tooth. D) After the acrylic resin set.

FIGURE 3. Bonding jig (Ultradent Inc.).

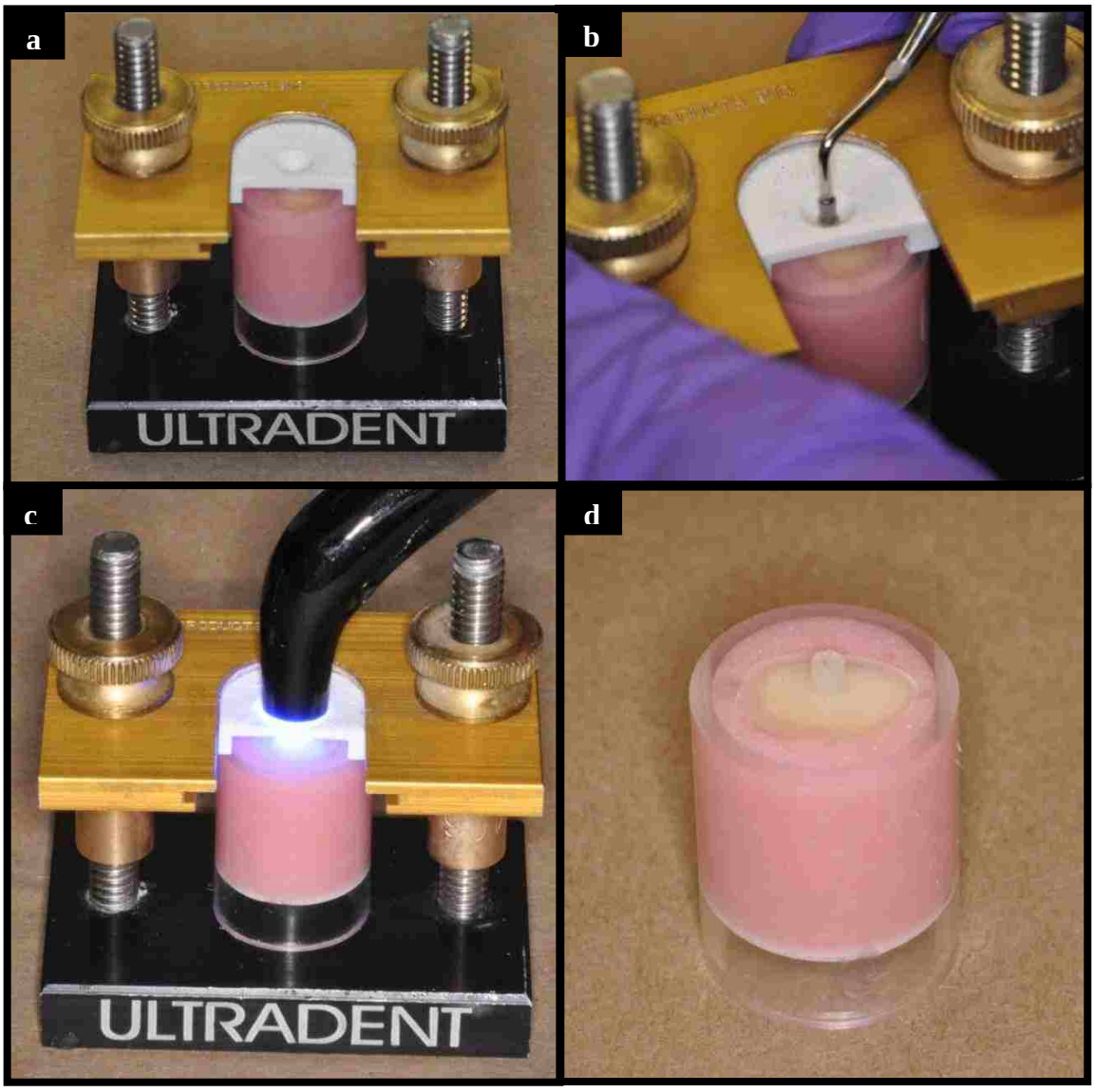

FIGURE 4. Sequence of steps for bonding procedure using Ultradent bonding jig. A. Specimen mounted in Ultradent bonding jig after application of adhesive on dentin surface. B. Resin composite packed into the cylindrical mold. C. Light curing. D. The resulted specimen ready to be tested for shear bond strength.

FIGURE 5. A. Universal testing machine (MTS Sintech Renew 1123, Eden Prairie, MN). B. Notched, semi-circular shaped edge (Ultradent Inc.).

FIGURE 6. Low-speed cutting saw (Isomet 1000, Buehler, Lake Bluff, IL).

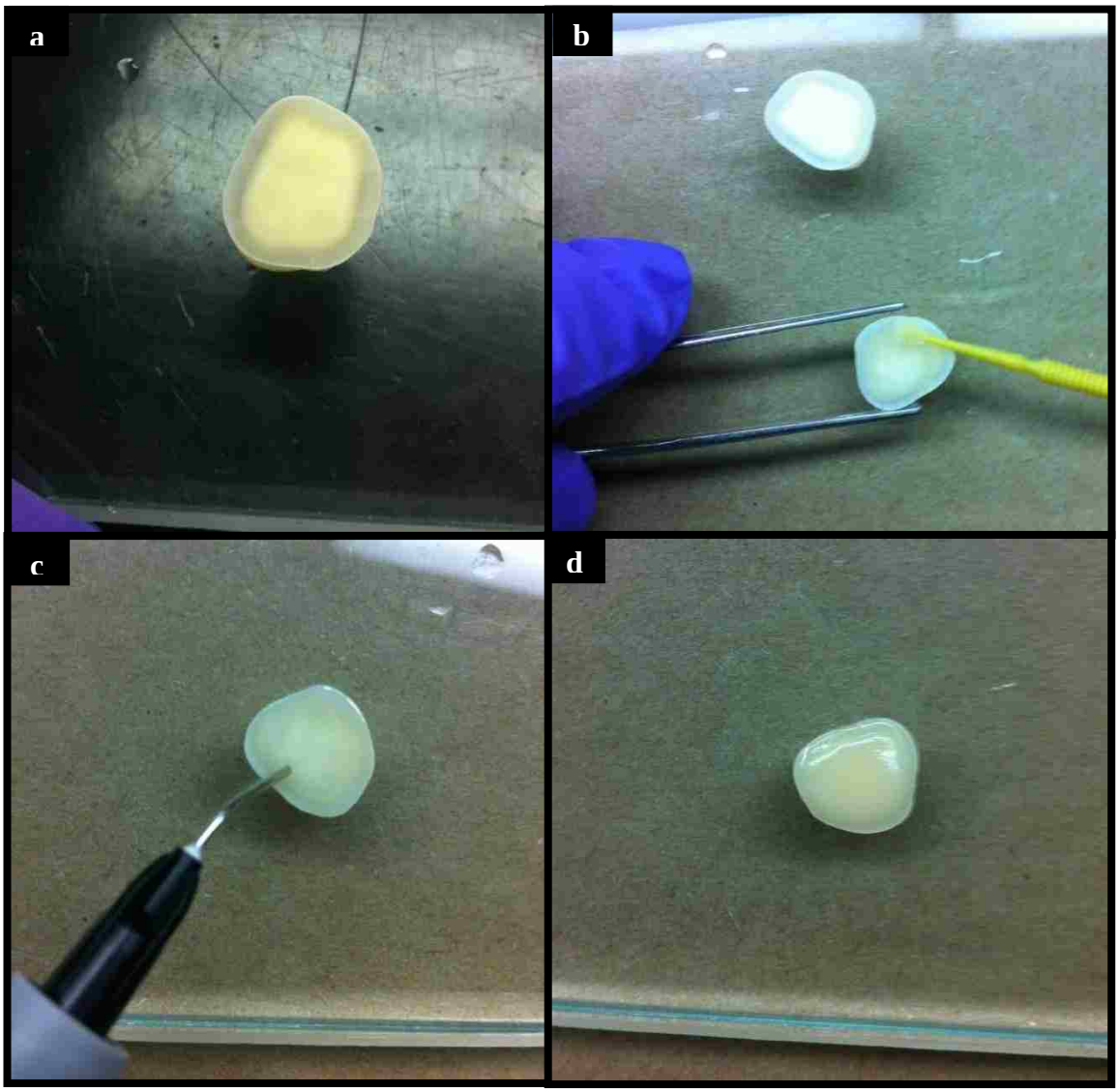

FIGURE 7. Dentin slabs and bonding procedure. A. Dentin slab after polishing placed on glass slab. B. Adhesive application. C. Flowable resin composite applied on top of light cured adhesive. D. The resulting specimen (dentin, adhesive and resin composite).

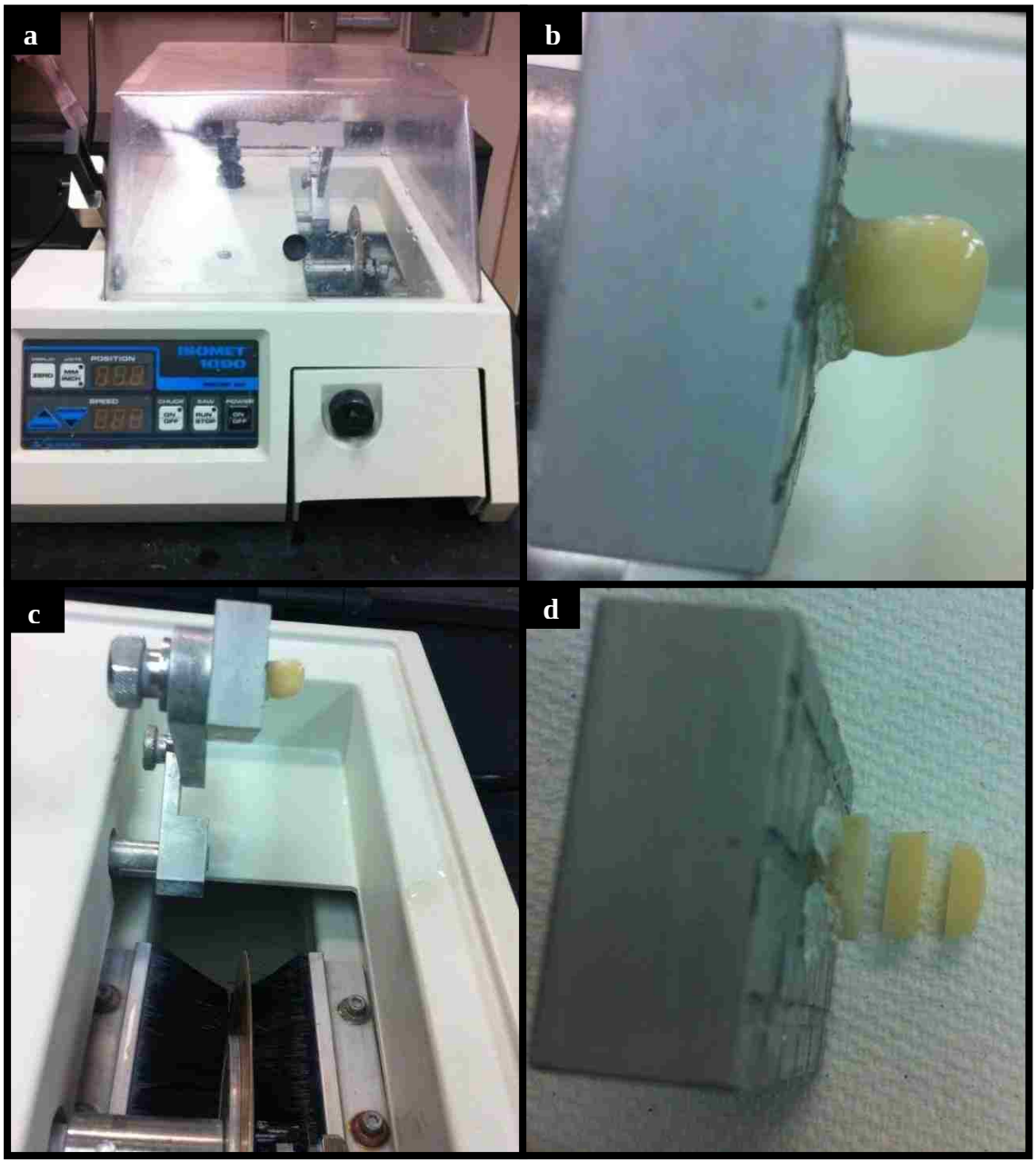

FIGURE 8. Dentin slabs cutting. A. Low-speed cutting saw (Isomet 1000, Buehler, Lake Bluff, IL). B. Dentin slab glued to metal holder. C) Sectioning perpendicular to the bonded interface. D) Two cuttings were made to obtain two 3-mm width slabs and the glued part was discarded.

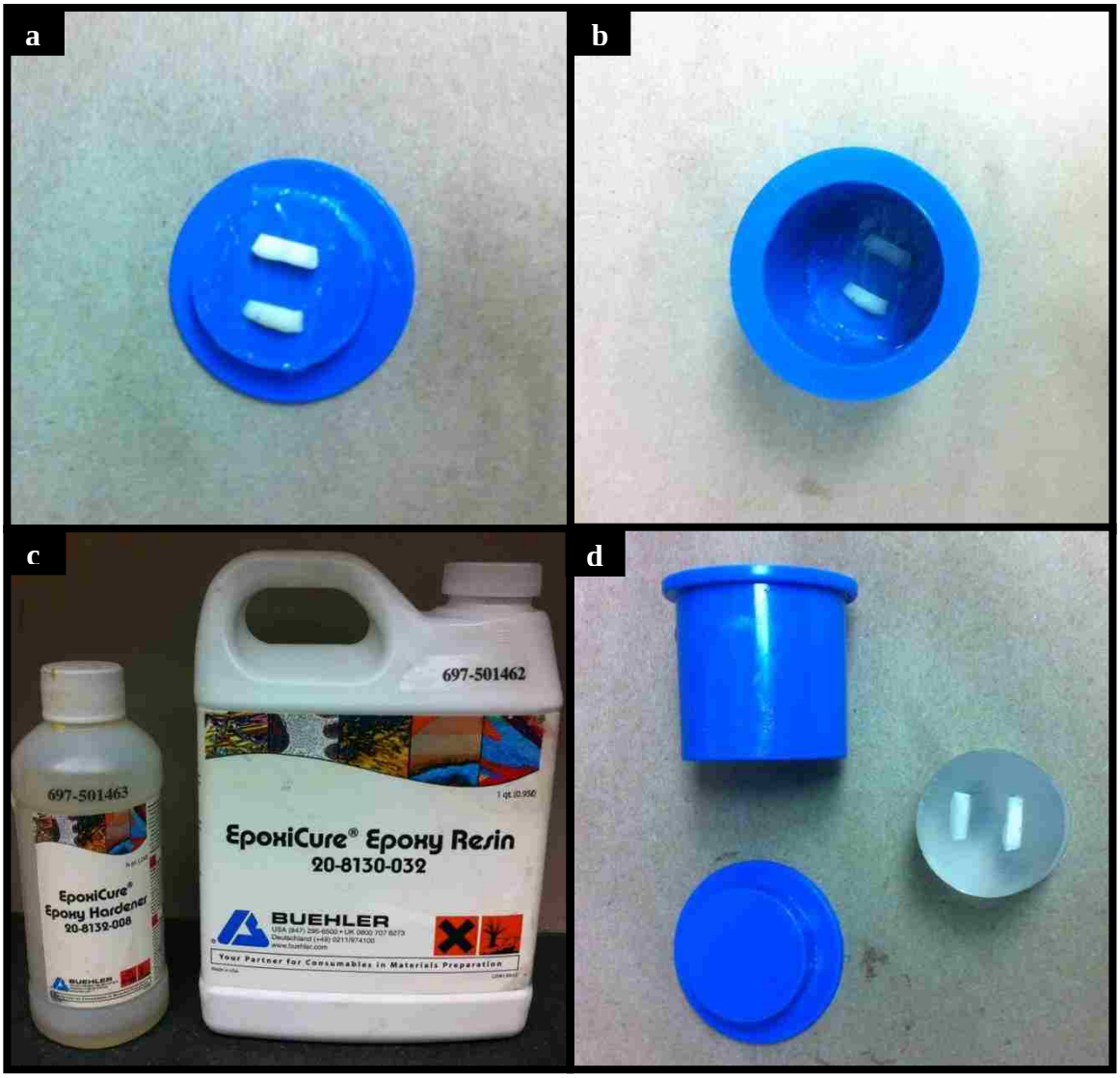

FIGURE 9. Specimens Mounted in epoxy resin using reusable mounting cylinders (Buehler, USA). A. The two slabs were placed with cross-sectional interfaces flat down over an adhesive tape on the base of mounting cylinders. B. The mounting cylinders reassembled. C. Self-cure epoxy resin (EpoxiCure, Buehler, USA) was mixed and poured into the cylinders. D. The mounted specimens were pushed out of the cylinder.

FIGURE 10. A. Apex® Diamond Grinding Discs DGD, Buehler Ltd, USA.
B. Specimen with resin bonded interface against the polishing disc.

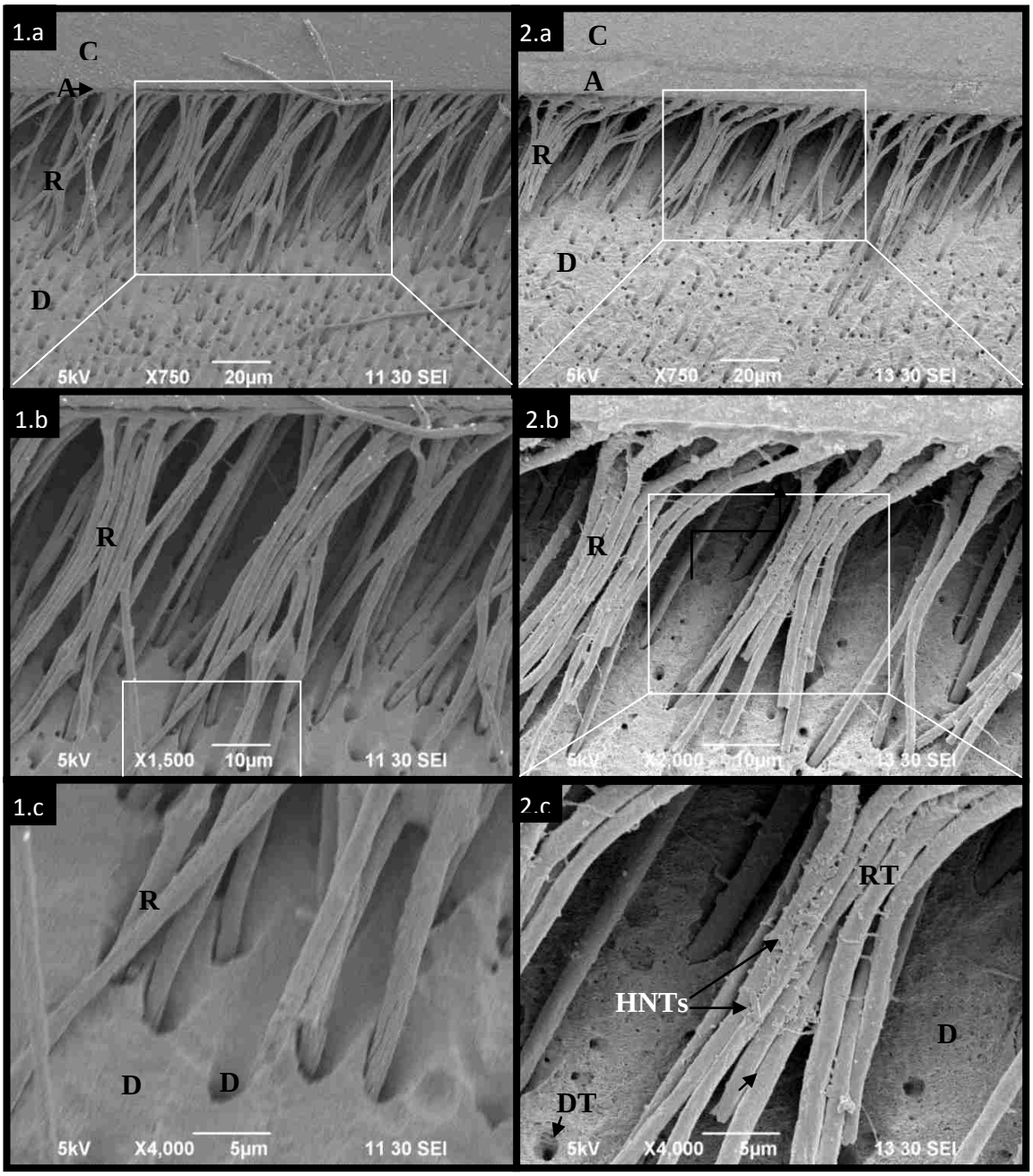

FIGURE 11. Resin-dentin interface SEM images of the etch-and-rinse adhesive system, control on left (1.a, b, c), 10 wt.% HNTs filled adhesive on right (2.a, b, c). C= composite resin, A = adhesive layer, RT = resin tags, D = dentin, DT = dentinal tubules. The images of 10 wt.% HNTs filled etch-and-rinse adhesive showed thicker adhesive layer (2.a), thick and long resin tags (2.b) with signs of presence of HNTs within resin tags such as an apparently rougher surface (arrow in 2.b). Note: the presence of agglomerated HNTs on resin tags (2.c).

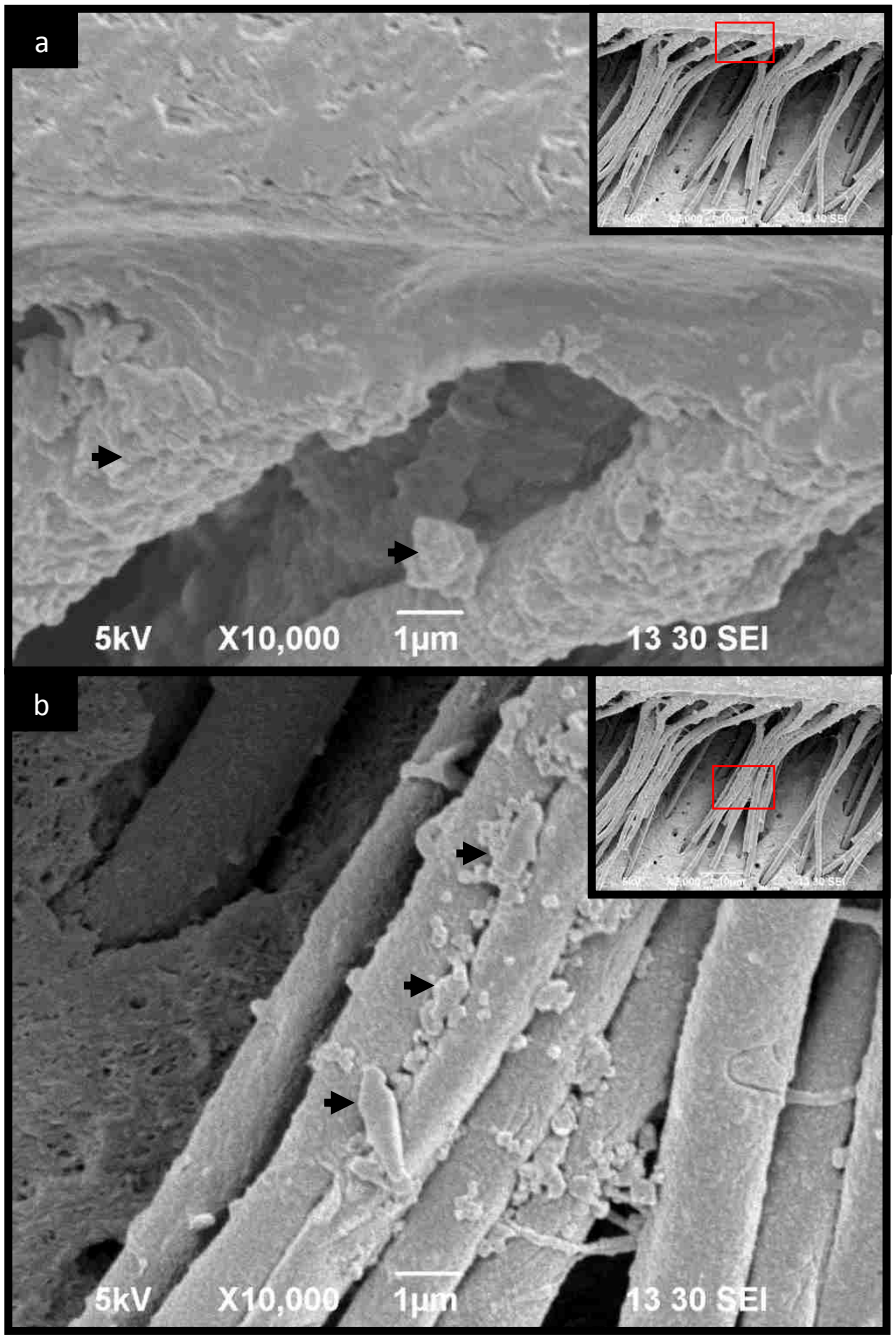

FIGURE 12. High magnification SEM images for the etch-and-rinse adhesive with 10 wt. HNTs; shows HNT agglomeration on resin tags.

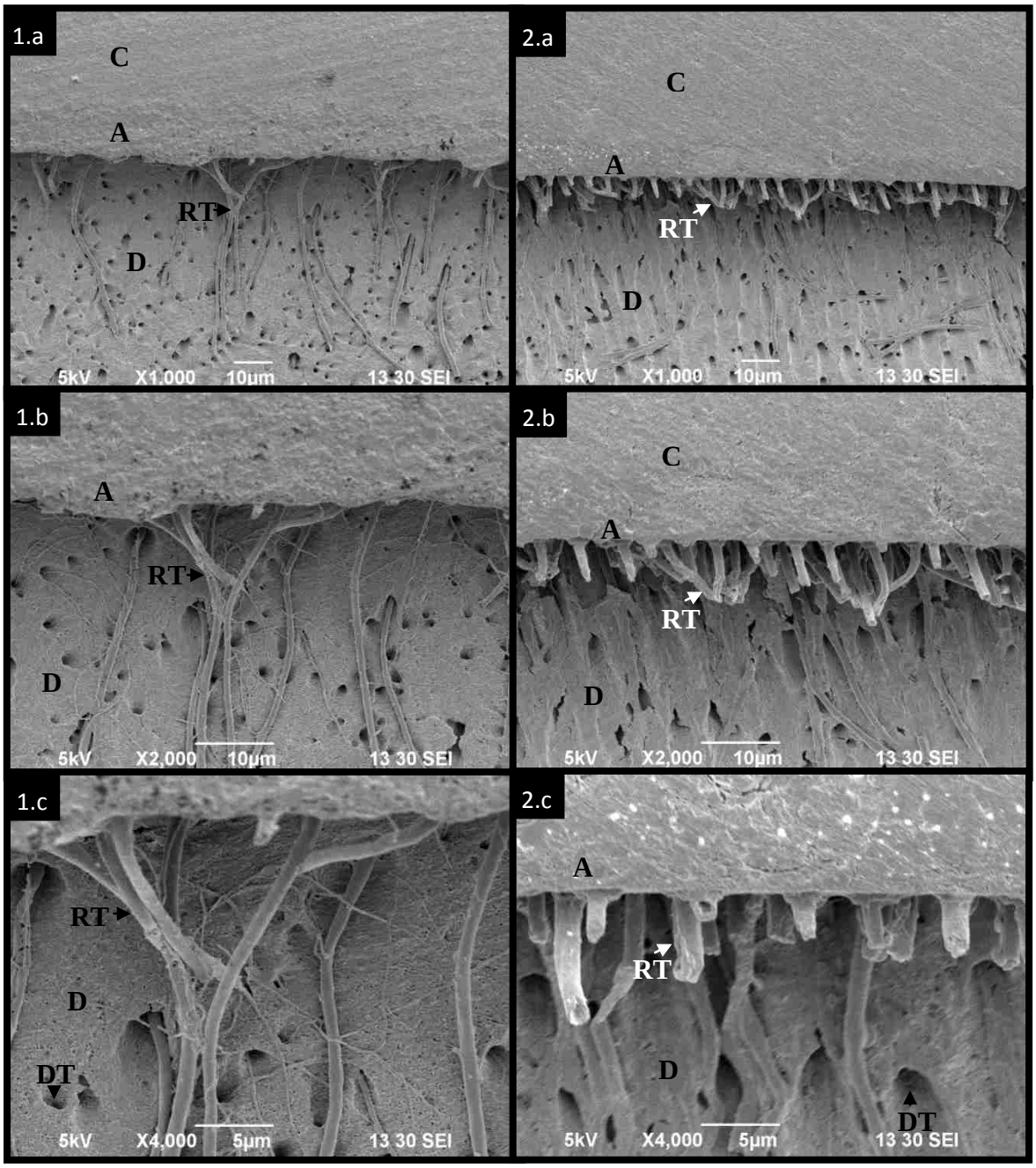

FIGURE 13. Resin-dentin interface SEM images of the self-etch adhesive system, control on left (1.a, b, c), 5 wt% HNTs filled adhesive on right (2.a, b, c). C = composite resin, A = adhesive layer, RT = resin tags, D = dentin, DT = dentinal tubules. The SEM images of 5 wt% HNTs filled self-etch adhesive showed increase in number of short resin tags compared with the control adhesive.

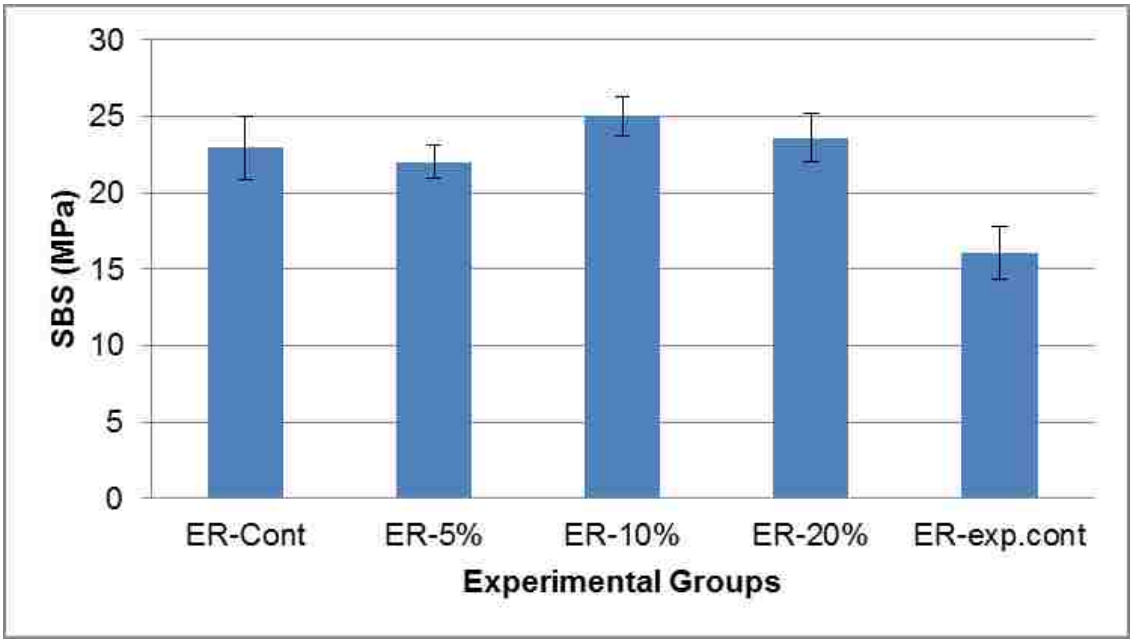

FIGURE 14. Mean bond strength with standard error for etch-and-rinse experimental adhesives.

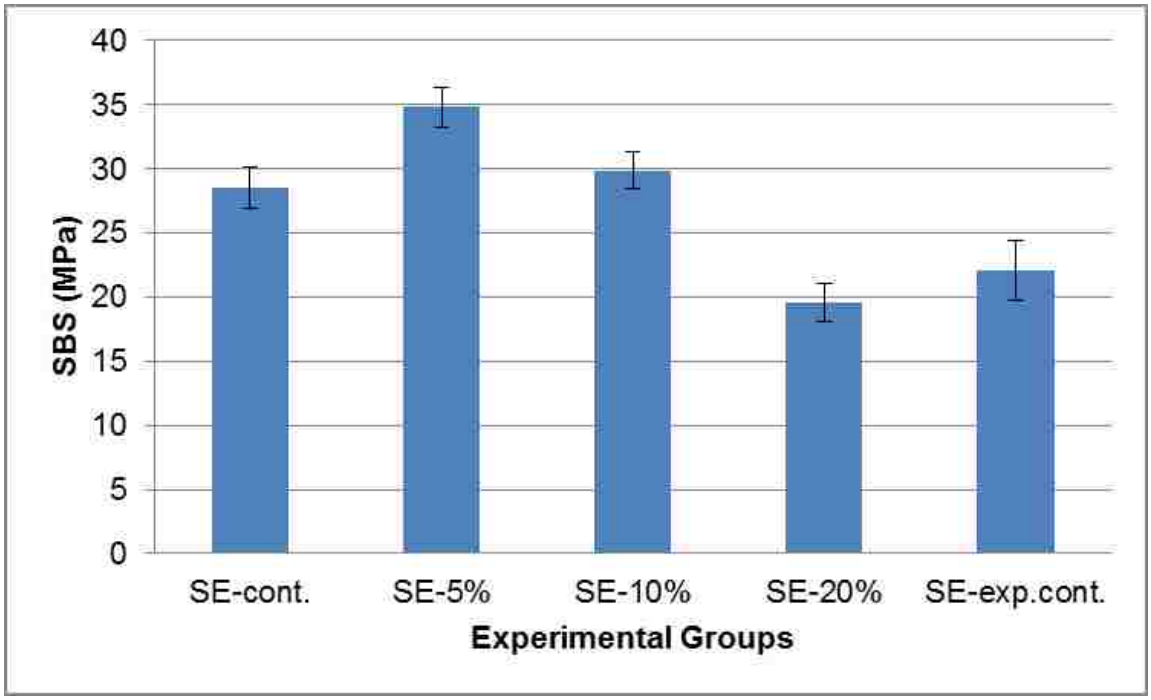

FIGURE 15. Mean bond strength with standard error for the self-etch experimental adhesives.

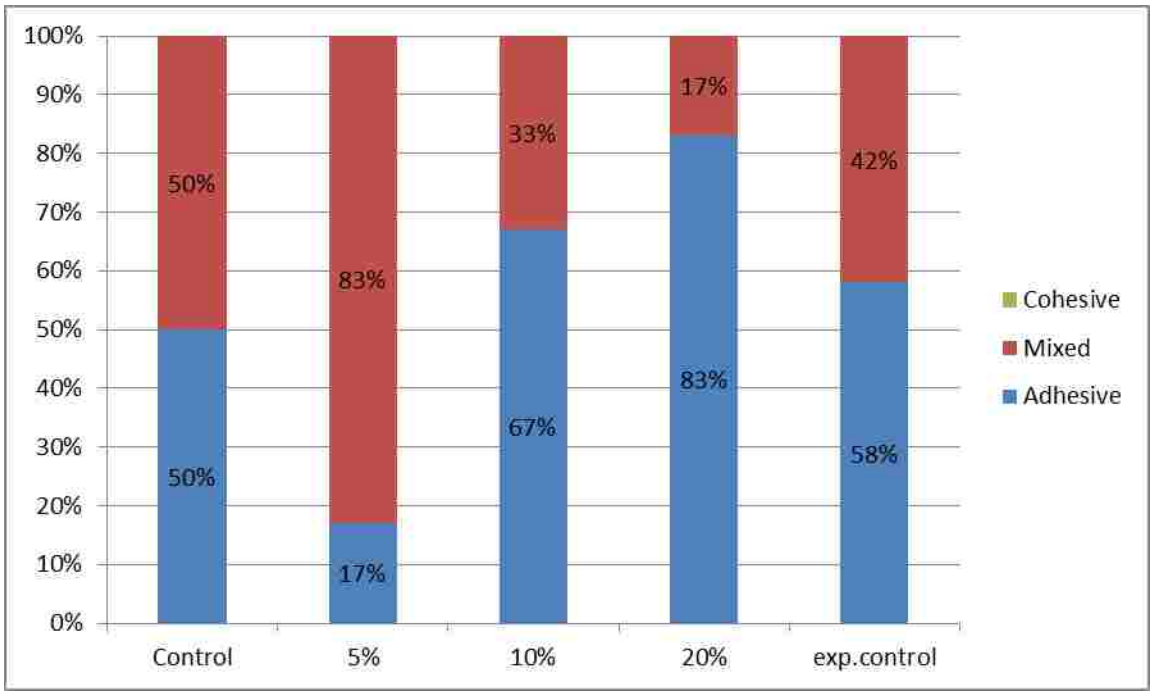

FIGURE 16. Failure mode in percentage % for the etch-and-rinse experimental adhesives.

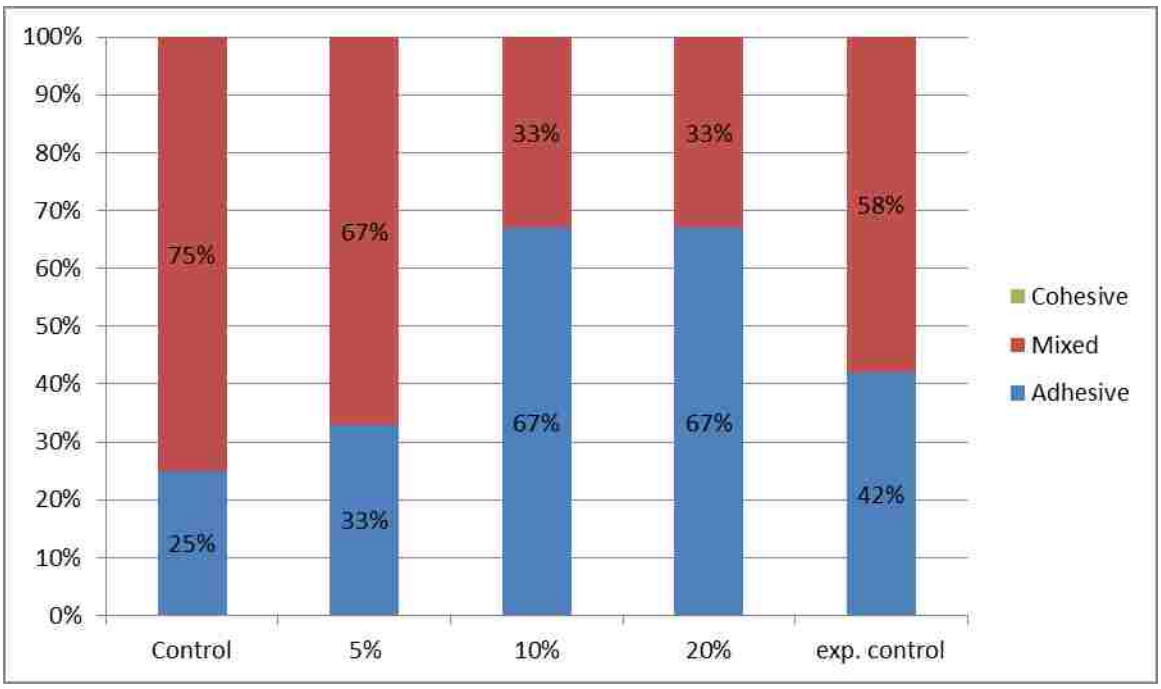

FIGURE 17. Failure mode in percentage % for the self-etch experimental adhesives.

TABLE I

Materials compositions

	COMPOSITIONS	BATCH NUMBER
ONE-STEP®, Bisco	– Acetone – Biphenyl dimethacrylate. – Hydroxyethyl methacrylate – Bis-GMA	1200012491
Xeno IV, Dentsply-Caulk	– Acetone – Urethane dimethacrylate resin – Dipentaerythritol penta-acrylate phosphate – Polymerizable dimethacrylate resin – Polymerizable trimethacrylate resin	120822
Z100™ Restorative, 3M, ESPE	– Silane treated ceramic – Triethylene glycol dimethacrylate – Bisphenol a diglycidyl ether dimethacrylate – 2-benzotriazolyl-4-methylphenol	N290515
Tetric® EvoFlow, Ivoclar/Vivadent, Schaan, Liechtenstein	– Bis-GMA, – UDMA, – Decandioldimethacrylat – Barium glass filler, – Ytterbiumtrifluoride, – Highly dispersed silica	P80394

TABLE II

Shear bond strength

System	Filler	N	Min	Max	Mean (SE)
ER	control	12	11.6	35.6	22.9 (2.1)[a]
ER	5%	12	17.5	28.8	22.0 (1.1)[a]
ER	10%	12	18.6	31.3	25.0 (1.3)[a]
ER	20%	12	16.4	36.9	23.6 (1.6)[a]
ER	exp control	12	7.6	23.5	16.1 (1.7)[b]
SE	control	12	18.6	35.3	28.5 (1.6)[c]
SE	5%	12	28.3	44.7	34.8 (1.6)[e]
SE	10%	12	19.7	39	29.9 (1.4)[c]
SE	20%	12	11.5	28.8	19.5 (1.5)[bd]
SE	exp control	12	13	36.1	22.1 (2.3)[d]

** Means with the same superscript letter are not significantly different at p = 0.05.

TABLE III

Failure mode

System	Filler	Adhesive	Mixed
ER	control	6 (50%)	6 (50%)
ER	5%	2 (17%)	10 (83%)
ER	10%	8 (67%)	4 (33%)
ER	20%	10 (83%)	2 (17%)
ER	exp control	7 (58%)	5 (42%)
SE	control	3 (25%)	9 (75%)
SE	5%	4 (33%)	8 (67%)
SE	10%	8 (67%)	4 (33%)
SE	20%	8 (67%)	4 (33%)
SE	exp control	5 (42%)	7 (58%)

TABLE IV

P-values for all pair-wise comparisons

Comparison	Shear Bond Strength	Failure Mode
ER-10% vs. SE-10%	0.0394	1.0000
ER-20% vs. SE-20%	0.0822	0.3534
ER-5% vs. SE-5%	0.0000	0.3534
ER-control vs. SE-control	0.0177	0.2129
ER-exp control vs. SE-exp control	0.0109	0.4164
ER-control vs. ER-5%	0.6992	0.0957
ER-control vs. ER-10%	0.3679	0.4102
ER-control vs. ER-20%	0.7746	0.0957
ER-control vs. ER-exp control	0.0038	0.6824
ER-5% vs. ER-10%	0.1992	0.0197
ER-5% vs. ER-20%	0.5014	0.0033
ER-5% vs. ER-exp control	0.0114	0.0451
ER-10% vs. ER-20%	0.5385	0.3534
ER-10% vs. ER-exp control	0.0002	0.6738
ER-20% vs. ER-exp control	0.0015	0.1899
SE-control vs. SE-5%	0.0080	0.6542
SE-control vs. SE-10%	0.5623	0.0478
SE-control vs. SE-20%	0.0002	0.0478
SE-control vs. SE-exp control	0.0065	0.3904
SE-5% vs. SE-10%	0.0362	0.1094
SE-5% vs. SE-20%	0.0000	0.1094
SE-5% vs. SE-exp control	0.0000	0.6738
SE-10% vs. SE-20%	0.0000	1.0000
SE-10% vs. SE-exp control	0.0011	0.2243
SE-20% vs. SE-exp control	0.2747	0.2243
ER-control vs. SE-5%	0.0000	0.4102
ER-control vs. SE-10%	0.0035	0.4102
ER-control vs. SE-20%	0.1451	0.4102
ER-control vs. SE-exp control	0.7124	0.6824
ER-5% vs. SE-control	0.0061	0.6172
ER-5% vs. SE-10%	0.0010	0.0197
ER-5% vs. SE-20%	0.2826	0.0197
ER-5% vs. SE-exp control	0.9857	0.1899
ER-10% vs. SE-control	0.1356	0.0478
ER-10% vs. SE-5%	0.0001	0.1094
ER-10% vs. SE-20%	0.0195	1.0000
ER-10% vs. SE-exp control	0.2055	0.2243
ER-20% vs. SE-control	0.0362	0.0081
ER-20% vs. SE-5%	0.0000	0.0197
ER-20% vs. SE-10%	0.0080	0.3534
ER-20% vs. SE-exp control	0.5128	0.0451
ER-exp control vs. SE-control	0.0000	0.1058
ER-exp control vs. SE-5%	0.0000	0.2243
ER-exp control vs. SE-10%	0.0000	0.6738
ER-exp control vs. SE-20%	0.1384	0.6738

DISCUSSION

Filler incorporation into dental adhesives has been intended for enhancing their mechanical properties and increasing the elastic modulus of the adhesive layer that can resist stresses induced by composite resin polymerization shrinkage. Furthermore, it has been shown that it can improve the distribution of the occlusal load stresses,[38] and consequently increase the resin-dentin bond strength. The bond strength can be evaluated with different methods, among which shear bond strength is considered as a simple and acceptable method.[39, 40]

The current study showed that incorporation of halloysite aluminosilicate clay nanotubes into either the two-step etch-and-rinse adhesive system or into the one-step self-etch adhesive system significantly increased the shear bond strength to dentin compared with the experimental control groups with 0 wt% (HNTs). They were subjected to similar mixing process. Compared with the commercial control group, only the self-etch adhesive system with 5 wt% (HNTs) showed significant increase in shear bond strength, while the etch-and-rinse adhesive system showed an increase with highest mean value for the 10 wt% (HNTs) but this increase was not statistically significant. The decrease in shear bond strength of the experimental control groups compared with the commercial control groups can be related to the mixing process (dispensing in mixing tube, mechanical mixing, and sonication for 1 h), which can cause solvent evaporation. An adequate amount of solvent in dental adhesives is essential for achieving effective bonding to dentin.[41] Some studies have reported a reduction in the bond strength of dental adhesives with acetone solvent after repeated opening of the adhesive bottle and loss of

acetone by evaporation[42,43] or after delayed application.[44] The possibility of air entrapment during the mixing process also can result in weakening of the adhesive layer.

The results of increased bond strength to dentin by filler incorporation are supported by several studies,[9,12-15] while other studies have failed to show any influence of filler addition on bond strength to dentin.[10,11,28]

The current study showed a different effect of filler concentrations incorporated into the two adhesive systems on their shear bond strength to dentin. The two-step etch-and-rinse adhesive system showed no significant difference in bond strength between the different filler concentrations (5 wt%, 10 wt%, and 20 wt%) with an insignificant increase up to 10 wt%, then a decrease with 20 wt%. While the one-step self-etch adhesive system showed significant difference in bond strength between the different filler concentrations with significantly high bond strength for 5 wt%, it significantly decreased with increasing filler concentration up to 20 wt%.

The increase in bond strength can be related to overall strengthening of the adhesive layer by filler addition. And, it also can be related to strong micromechanical interlocking provided by infiltrated adhesive resin into the dentinal tubules forming resin tags with higher strength.[14] In our study, SEM images (Figures 11 and 12) showed resin tags with some evidence of HNTs present within the resin and into dentinal tubules. Also, SEM images of 5 wt% HNTs-filled self-etch adhesive showed an increase in the number of short resin tags compared with the control adhesive (Figure 13) which may explain the significant increase in shear bond strength. This increase in the number of short resin tags could be related to the hydrophilic property of HNTs that promoted more penetration of resin into wet dentin. The reduction in bond strength with higher filler concentration can

be related to the increased viscosity and the tendency of fillers to agglomerate when incorporated at a certain point with a higher percentage and form clusters on the dentin surface that can reduce the adhesive's penetration into the dentinal tubules and etched dentin, which results in voids within the adhesive layer[10,45] and reduced bond strength to dentin. [9]

Resin composition of the two-step etch-and-rinse adhesive system (ONE-STEP®, Bisco, USA), and the one-step self-etch adhesive system (Xeno IV, Dentsply-Caulk, Milford, DE, USA) are not similar. Therefore, it is difficult to evaluate the effect of filler incorporation into dentin adhesives that have different resin composition.[46] The self-etch adhesive system (Xeno IV, Dentsply-Caulk, Milford, DE, USA) contains (PENTA) phosphonated penta acrylate ester that is a self-etching primer with hydrophilic monomers that can bond to organic and inorganic structures,[47,48] which may explain the significant increase in shear bond strength compared to commercial control, which is not seen in the two-step etch-and-rinse adhesive system (ONE-STEP®, Bisco, USA). Furthermore, the significant decrease in shear bond strength of the self-etch adhesive system with higher filler concentration (20 wt%) can be explained by the effect of increased filler amount on decreasing the acidity of the adhesive, which may impact the self-etching property and consequently affect the bond strength.

When making bond strength specimens, experimental groups with 20 wt% filler concentration from both adhesive systems showed relatively high viscosity and difficulties in handling compared with other groups with less filler concentrations. It has been suggested that incorporation of functionalized nanoparticles can increase the miscibility of the adhesive mixture.[12] Schulz et al.[28] found that particles functionalization

can decrease the viscosity at higher concentrations, although it showed no significant difference in bond strength between adhesives with functionalized or non-functionalized nanoparticles. However, halloysite aluminosilicate clay nanotubes (HNTs) are hydrophilic and can easily be wet by common polymers. Using functional silanes may render the HNTs wetting unless they are incorporated into hydrophobic polymers, in which case the use of functional silanes can be indicated.[16] Therefore, in the current study, the HNTs were incorporated non-functionalized into adhesives because they contain hydrophilic monomers, and also to simplify production.

The current study showed that the mode of failure evaluated under the stereomicroscope was adhesive and mixed in all the groups with no cohesive failures. These results are in agreement with another study done by Sadat-Shojai et al.[14] that incorporated hydroxyapatite nanorods. In contrast, another study by Miyazaki, et al.[13] incorporated silica microfillers, and they showed a predominant cohesive failure. Braga et al.[40] reported the failure mode distribution for dentin shear bond tests observed in 37 studies recently published to be approximately 60 percent of the specimens failing adhesively along the bonded interface, while 21 percent presented mixed failures and 19 percent presented predominantly cohesive failure. The cohesive failure that is not found in the current study is not an indication of strong bonding; cohesive failure is explained by the test mechanics and the tested material brittleness.[40] Versluis et al.[49] confirmed by experimenting and by using a failure accumulation computer model that cohesive dentin failure tended to increase at lower crosshead speeds, when the point of load application displaced away from the bonded interface, and with thicker adhesive layers. However,

several literature reviews suggest that failure mode evaluation should be done under SEM with high magnification and not only visually or by stereomicroscope.[50,51]

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SUMMARY AND CONCLUSIONS

The objective of this study was to evaluate the effect of incorporating halloysite aluminosilicate nanotubes (HNTs) either into the two-step etch-and-rinse adhesive system (ONE-STEP®, Bisco) or into the one-step self-etch adhesive system (Xeno IV, Dentsply-Caulk, Milford, DE) on dentin shear bond strength

The first null hypothesis that said the incorporation of halloysite aluminosilicate clay nanotubes would not increase adhesive bond strength to dentin was partially rejected because the current study showed that incorporation of HNTs into the self-etch adhesive system at 5 wt% filler concentration showed significant increase in shear bond strength compared to the commercial control adhesive. And, it was partially accepted because the etch-and-rinse adhesive system did not show significant increase in shear bond strength to dentin.

The second null hypothesis that said there would be no effect of filler concentration on bond strength to dentin was also partially rejected because the self-etch adhesive system showed significant difference in shear bond strength between the groups with different concentrations that increased and reached a threshold level at 5 wt% filler concentration. Also, the hypothesis was partially accepted because the etch-and-rinse adhesive system did not show significant difference in shear bond strength to dentin between the groups with different concentrations.

In conclusion, within the limitations of this study, halloysite aluminosilicate clay nanotubes can increase bond strength to dentin when incorporated into the self-etch adhesive system at 5 wt% filler concentration. Also, HNTs can be incorporated up to 10

wt% filler concentration into both the self-etch and the etch-and-rinse adhesive systems without adversely affecting the bond strength to dentin or the handling properties. In addition, HNTs showed the ability to penetrate along with resin tags into dentinal tubules that could expand their applications.

CLINICAL RELEVANCE

The long-term durability of the bond in adhesive restorations still remains a challenge that could be partially solved with a stronger adhesive material, but here with HNTs, in addition to their potential to strengthen the bond, the unique property of loading and controlled release of materials could be utilized to solve some other challenges such as degradation of adhesive-dentin interfaces and improving bond durability. Further investigations of mechanical and physical properties of HNTs filled adhesive are recommended.

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ABSTRACT

EFFECT OF HALLOYSITE ALUMINOSILICATE CLAY NANOTUBE INCORPORATION INTO BONDING AGENTS ON SHEAR BOND STRENGTH TO HUMAN DENTIN

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In adhesive dentistry, obtaining a good bond is a fundamental goal. It has been suggested that filler addition to the adhesives would increase the bonding strength of the adhesive layer. Halloysite aluminosilicate nanotubes (HNTs) are biocompatible, hydrophilic, durable, and have high mechanical strength. These advantages make them good candidates to be used as reinforcing agents for improving the properties of dental adhesives.

The objective of this study was to evaluate the effect of incorporating HNTs into a commercial two-step etch-and-rinse adhesive system or one-step self-etch adhesive system on dentin shear bond strength.

HNTs were incorporated into the two commercial adhesive systems in 0 wt%, 5 wt%, 10 wt%, and 20 wt%. The commercial control adhesives and the experimental adhesives were used to bond occlusal dentin of 120 extracted human molar teeth and then tested for shear bond strength by a universal testing machine with a semi-circular edge at a crosshead speed of 1.0 mm/min. Debonded specimens were examined under light microscopy to evaluate the fracture pattern. Resin-dentin interface were evaluated under scanning electron microscopy (SEM) after bonding dentin slabs using commercial control adhesives and experimental adhesive that showed numerically highest shear bond strength from each adhesive system. Two-way ANOVA was used to evaluate the effects of adhesive system and nanofiller content on shear bond strength. Pair-wise comparisons between groups were made using Fisher's (LSD) ($p < 0.05$).

For the self-etch adhesive system, only incorporation of 5 wt% showed a significant increase in shear bond strength to dentin compared with the commercial control group. For the etch-and-rinse adhesive system, there was no significant difference in shear bond strength between HNTs filled adhesives groups and the commercial control group. Resin-dentin interface SEM evaluation showed nanotubes infiltrated into dentinal tubules.

In conclusion, incorporating the self-etch adhesive system with 5 wt% HNTs increased the bond strength to dentin. Incorporation of up to 10 wt% filler concentration into both the self-etch and the etch-and-rinse adhesive systems did not adversely affect

the bond strength to dentin or the handling properties. HNTs can penetrate along with resin tags into dentinal tubules, which could expand the use of their unique properties.

CURRICULUM VITAE

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Professional Organizations

The Academy of Operative Dentistry
Saudi Dental Society
The Saudi Commission for Health Specialties