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# Nanotube-modified dentin adhesive—Physicochemical and dentin bonding characterizations

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## ARTICLE INFO

### Article history:

Received 26 February 2013

Received in revised form

23 July 2013

Accepted 27 August 2013

### Keywords:

Halloysite

Nanotube

Adhesives

Dentin

Bond strength

SEM

Resin–dentin interface

## ABSTRACT

**Objective.** The aim of this study was to investigate the effect of aluminosilicate clay nanotubes (Halloysite, HNT) incorporated into the adhesive resin of a commercially available three-step etch and rinse bonding system (Adper Scotchbond Multi-Purpose/SBMP) on dentin bond strength, as well as the effect on several key physicochemical properties of the modified adhesive.

**Methods.** Experimental adhesives were prepared by adding five distinct HNT amounts (5–30 wt.%) into the adhesive resin (w/v) of the SBMP dentin bonding system. Bond strength to human dentin, microhardness, and degree of conversion (DC) of the modified adhesives were assessed.

**Results.** From the shear bond strength data, it was determined that HNT incorporation at a concentration of 30 wt.% resulted in the highest bond strength to dentin that was statistically significant ( $p = 0.025$ ) when compared to the control. Even though a significant increase in microhardness ( $p < 0.001$ ) was seen for the 30 wt.% HNT-incorporated group, a significantly lower DC ( $p < 0.001$ ) was recorded when compared to the control.

**Significance.** It was concluded that HNT can be incorporated up to 20 wt.% without jeopardizing important physicochemical properties of the adhesive. The modification of the SBMP dentin bonding agent with 20 wt.% HNT appears to hold great potential toward contributing to a durable dentin bond; not only from the possibility of strengthening the bond interface, but also due to HNT intrinsic capability of encapsulating therapeutic agents such as matrix metalloproteinase (MMP) inhibitors.

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## 1. Introduction

Adhesive dentistry has advanced tremendously over the past twenty years [1–5]. Needless to say, bonding of resin

composite restorations to enamel using the acid-etch technique advocated by Buonocore [6] has been considered a safe and predictable restorative procedure [1,3,5,7]. Regrettably, the achievement of a reliable and durable bond to dentin still poses a great challenge to the field, in part due to the high

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<http://dx.doi.org/10.1016/j.dental.2013.08.211>

organic content levels existing in dentin (e.g., collagen) when compared to enamel [2,4,5,7]. Nevertheless, another major contributor to resin–dentin bond degradation relates to the well-known hydrolytic process experienced by underpolymerized hydrophilic resins and the deterioration of water-rich, resin–sparse collagen matrices by matrix metalloproteinases (MMPs) and cysteine cathepsins [3–5,8,9].

In order to hinder resin–dentin bond degradation and therefore improve the longevity of resin-based restorations, numerous adhesive chemistries and bonding strategies have been proposed [3–5,8,9]. Among them, recent advances in nanoscience and nanomaterials processing have supported the use of an ample variety of fillers in the form of nanorods, nanoparticles, whiskers and nanotubes as reinforcing agents in adhesive systems targeting not only improved material properties (i.e., adhesive) but also the enhancement of bonding to dentin [2,10–14]. Noteworthy, the size scale of these fillers and the concomitant increased surface area yield a unique combination of mechanical properties through the reinforcement of the polymer matrix via localized plastic deformation around the fillers and crack deflection [15,16]. Furthermore, it has been suggested that these nanofillers can infiltrate into the dentinal tubules, decreasing polymerization shrinkage, stiffening the adhesive layer, and ultimately strengthening the adhesive interface via micromechanical bonding [12,13].

A series of studies investigating the mechanical properties enhancement of engineered resins incorporated with aluminosilicate ( $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$ ) clay nanotubes (Halloysite, HNT) have been reported [17–20]. Overall, the results from those studies led to the present work for two major reasons. First, aluminosilicate clay nanotubes [21–24] have many advantages (e.g., biocompatibility, hydrophilicity, and high mechanical strength) that make them a good candidate to be used as a reinforcing agent for improving resin-based dental adhesive properties. Second, these clay nanotubes have been demonstrated to act as biologically safe reservoirs for encapsulation and controlled delivery of a wide variety of therapeutic drugs [23,25], which in adhesive dentistry could potentially serve as a carrier of MMP inhibitors, that could in turn positively contribute to minimizing and/or eliminating resin–dentin bond degradation [4,8,9]. Currently, there appears to be no reports on the use of Halloysite in adhesive dental resins. The purpose of this study was to investigate the effect of incorporating Halloysite in the adhesive resin of a commercially available three-step etch and rinse bonding system on dentin bond strength. Physical properties of the HNT-incorporated adhesives such as microhardness and degree of conversion were also evaluated. The null hypotheses tested were that HNT incorporation would neither increase bond strength to dentin nor negatively impact adhesive properties such as degree of conversion and microhardness.

## 2. Materials and methods

### 2.1. Materials and experimental adhesive formulation

Halloysite aluminosilicate clay nanotubes (HNTs, Dragonite-1415JM<sup>®</sup>) were donated by Applied Minerals (New York, NY,

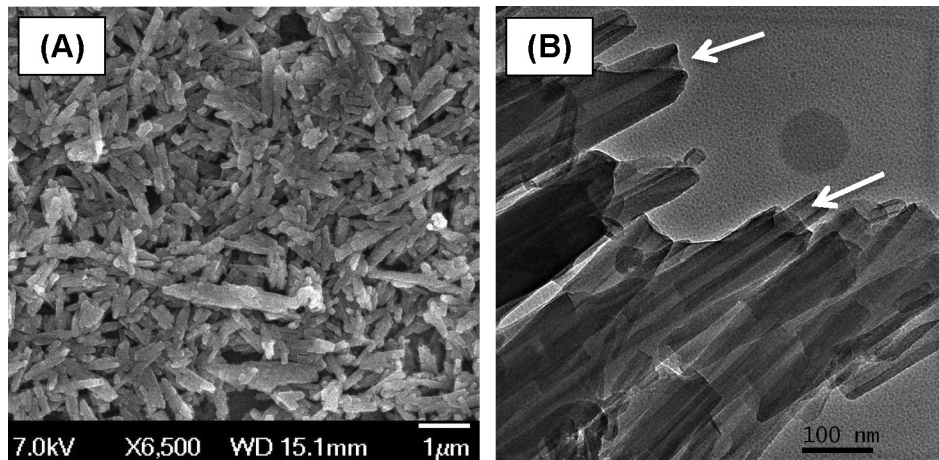
USA). Previous to dentin bonding agent modification (i.e., the adhesive resin of Adper Scotchbond Multi-Purpose/SBMP), an aqueous dispersion of HNTs was prepared to confirm the surface morphology as well as the hollow structure of the nanotubes by scanning (FE-SEM, JEOL, JSM6701-F, Tokyo, Japan) and transmission electron microscopy (TEM, JEM-2010, JEOL, Tokyo, Japan), respectively [26]. On an Al stub for HNT imaging, a predetermined approximate 0.5 mL volume of the HNT aqueous dispersion was added, allowed to dry and then sputter-coated with Au before SEM evaluation. Similarly, one 10  $\mu\text{L}$  drop of the dispersion was added to a holey carbon TEM grid (Cu 200 mesh, SPI Supplies, West Chester, PA, USA), allowed to air-dry and imaged at 100 kV [26]. Representative FE-SEM and TEM images of the HNTs are shown in Fig. 1. HNTs displayed an overall uniform rod-like tubular structure with an estimated mean length in the submicron range (Fig. 1A). The HNT hollow structure was confirmed by TEM (Fig. 1B).

As previously mentioned, the experimental adhesives were prepared by adding five distinct HNT amounts (5, 10, 15, 20 and 30 wt.%) accurately (five-decimal-places) weighed using an analytical balance (Mettler Toledo, Ohio, OH, USA) into the adhesive resin (w/v) of the SBMP dentin bonding system. Briefly, HNTs were incorporated into a predetermined adhesive resin volume (w/v), mixed with a motorized mechanical stirrer using the companion conical micro pestle adapter (Roti-Speed, Roth, Karlsruhe, Germany) and then sonicated (Ultrasonic cleaning L&R 2014, Mfg Co., Kearney, NJ, USA) for 1 h to further enhance the dispersion [12] of HNTs within the adhesive resin. Detailed information of the commercial dentin bonding system (SBMP) used is given in Table 1.

### 2.2. Dentin bonding, shear testing, failure mode and resin–dentin interface evaluation

Shear bond testing has been considered one of the most simple methods for testing adhesives bond strength in vitro [27]. Here, we used this test to screen for significant deleterious impact on the dentin bond strength achieved through HNT incorporation into the adhesive resin of a commercially available three-step etch and rinse bonding system (SBMP).

Seventy two caries-free human third molars were immersed in 0.5% chloramine solution after soft tissue removal and then stored in distilled water in a refrigerator at 4 °C. Teeth within six months of extraction were used under an approved IRB (Institutional Review Board, Indiana University-Purdue University Indianapolis) protocol (NS1004-03). Each tooth's occlusal third was removed with a low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA) mounted on a precision cutting machine (Isomet 1000, Buehler Ltd., Lake Bluff, IL). Teeth were individually embedded in Teflon molds using acrylic resin (Bosworth Fastray, powder lot# D681092139209, liquid lot# D68109213950C, Bosworth Company, Skokie, IL, USA). Following that, a flat mid-coronal dentin surface was obtained using a finishing procedure with SiC abrasive papers 240–600 grit under running water to ensure a standardized and uniform smear layer. The teeth were then randomly distributed into six groups ( $n = 12$ ), namely the control (G1-0 wt.%, no HNT incorporation) and five experimental groups (G2-5 wt.%, G3-10 wt.%, G4-15 wt.%, G5-20 wt.% and G6-30 wt.%).



**Fig. 1 – Representative FE-SEM (A) and (B) TEM micrographs of the as-received aluminosilicate clay nanotubes (Halloysite, Dragonite-1415JM<sup>®</sup>, Applied Minerals, New York, NY). Note the overall uniform rod-like tubular structure and the unique hollow (white arrows) structure with approximately 100–150 nm in width and an estimated length in the range 300–1500 nm.**

For all groups (G1–G6), the exposed dentin surface was etched with 35% phosphoric acid for 15 s, rinsed copiously with distilled water for 30 s and gently blot dried with low-linting wipes (Kimwipes, Kimberly-Clark Professional, Roswell, GA, USA) according to the wet bonding technique as per the manufacturer's recommendations. Next, the primer was applied with a microbrush and gently air-dried for 10 s. To ensure a homogeneous application, the primer was applied until a shiny surface was observed. Finally, the HNT-incorporated or the unfilled adhesive (G1-control) were applied to the primed dentin surface with a microbrush in agitating motions for 10 s and was gently dried for 5 s. A mild air blow was performed to help remove the excess solvent and then the adhesive cured for 20 s using a halogen curing unit (Optilux 501, Demetron/Kerr, Orange, CA, USA) with an output intensity of 720 mW/cm<sup>2</sup>. Next, a dental resin composite (Z100<sup>TM</sup>, shade A2, lot# N290515) button (2.38 mm in diameter × 2 mm in thickness) was incrementally built on the bonded dentin surfaces using a dedicated jig (Ultradent, South Jordan, UT, USA) for shear bond testing and cured for 40 s in accordance with the manufacturer instructions. Specimens were stored in distilled water at 37 °C. After 24 h from bonding procedures, the specimens were individually placed in a stainless steel base (Ultradent, South Jordan, UT, USA) and loaded by a custom notched fixture (Ultradent, South Jordan, UT, USA)

in compression using a universal testing machine (MTS Sintech Renew 1123, Eden Prairie, MN, USA) at a crosshead speed of 1 mm/min. Shear bond strength (SBS, in MPa) was calculated by dividing the maximum load (in N) at the time of fracture by the bonded area (in mm<sup>2</sup>). The failure modes were assessed by means of light microscopy at 40× magnification and categorized as follows: adhesive (at the dentin–resin interface), cohesive (exclusively within the dentin substrate or composite), or mixed (at the dentin–resin interface including cohesive failure of one of the substrates). Randomly selected specimens representing each of the evaluated groups were sputter-coated with Au and imaged using a scanning electron microscope (SEM, JSM-5310LV, JEOL, Tokyo, Japan).

To investigate the resin–dentin interface of the HNT-incorporated adhesives, additional caries-free human third molars were used to obtain dentin slabs (ca. 2 mm in thickness) from mid-coronal dentin with a low-speed diamond saw (Buehler) mounted on a precision cutting machine (Isomet 1000, Buehler) [28–32]. Bonding procedures were performed as previously mentioned in our dentin bonding protocol. In brief, the HNT-incorporated adhesives and the control groups were prepared and similarly applied to the smear-layer standardized dentin. Then a 1-mm thick flowable resin composite layer (Tetric<sup>®</sup> EvoFlow, lot# P80394, Ivoclar-Vivadent, Schaan, Liechtenstein) was built and light-cured

**Table 1 – Chemical composition of the commercial adhesive system used in the study.<sup>a</sup>**

Adper Scotchbond Multi-Purpose (SBMP)	Manufacturer	Batch no.	Components
1. Etchant	3M/ESPE, USA	N190139	35% H <sub>3</sub> PO <sub>4</sub>
2. Primer		N195379	HEMA, polyalkenoic acid polymer, water
3. Adhesive		N311862	Bis-GMA, HEMA, tertiary amines, photo-initiator

Abbreviations: bis-GMA: bisphenol A diglycidyl ether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate.

<sup>a</sup> According to the manufacturer.

according to the manufacturer instructions. Bonded samples were stored at 37 °C in deionized water for 24 h. Bonded samples were sectioned perpendicular to the bonding interface using a low-speed water cooled diamond saw [28,30,31]. Samples were mounted in self-cure epoxy resin (EpoxiCure®, Buehler). Finally, the exposed cross-sectional interfaces of the resin/adhesive/dentin were wet-finished with SiC papers (400–1200 grit) followed by polishing using diamond impregnated papers 30, 9, and 3 μm (Apex® Diamond Grinding Discs DGD, Buehler Ltd, USA). Samples were ultrasonically cleaned in distilled water for 5 min in between each of the diamond polishing steps and then in ethanol for additional 5 min to remove any remaining polishing debris [28–32]. Finally, the polished surfaces were then demineralized with 6N HCl for 30s, followed by deproteinization in 5% sodium hypochlorite for 5 min, rinsed with deionized water and air-dried prior to Au sputtering for SEM imaging (JSM-6390, JEOL, Tokyo, Japan).

### 2.3. Knoop microhardness (KHN)

Disk-shaped (10 mm in diameter × 1.0 mm) specimens (n = 5) of the adhesive for all the groups were fabricated using a custom made silicone mold (Exaflex® putty, lot#1208281, GC America Inc., Alsip, IL) and stored at 37 °C for 24 h. The specimens were subjected to a microhardness test (M-400, Leco Corp., St. Joseph, Michigan USA) using a Knoop diamond indenter at a 50 gf load and 15 s dwell time. Eight readings at different locations (at least 1 mm apart) were obtained from each specimen. The diagonal lengths were measured immediately after each indentation. The values were converted to KHN number (kg/mm<sup>2</sup>).

### 2.4. Degree of conversion (DC)

DC for the formulated HNT-incorporated adhesives, as well as the control, was evaluated at RT using a Fourier Transform infrared spectrometer (Jasco 4100, Jasco Corp., Tokyo, Japan) in attenuated total reflection (ATR) mode. Two absorbance bands at 1637 cm<sup>-1</sup> (methacrylate group, C=C) and 1715 cm<sup>-1</sup> (ester group, C=O) were used to calculate the DC. The scans were run twice per specimen (n = 10). The average was used for analysis based on a previously established protocol [33]. The degree of conversion was calculated by the following equation [33]:

$$DC = \left( 1 - \frac{(\text{Absorbance } 1637 \text{ cm}^{-1} / \text{Absorbance } 1715 \text{ cm}^{-1}) \text{ of polymerized sample}}{(\text{Absorbance } 1637 \text{ cm}^{-1} / \text{Absorbance } 1715 \text{ cm}^{-1}) \text{ of unpolymerized sample}} \right) \times 100\%$$

### 2.5. Statistical analysis

One-way ANOVAs were performed to identify significant effects of Halloysite incorporation. Bonferroni's multiple comparison procedure was used to compare the groups for differences in shear bond strength, microhardness and degree of conversion. The level of significance was set at  $\alpha = 0.05$ .

## 3. Results

The mean SBS values (in MPa) and standard deviations (SD), as well as the bond failure mode (%), are shown in Table 2.

**Table 2 – Mean SBS, standard deviation and failure mode.**

Groups	SBS (MPa)	Failure mode (%)		
		AD	MX	CH
G1-control	21.8 ± 5.9 <sup>a</sup>	66.7	33.3	0
G2-5 wt.%HNT	24.0 ± 6.9 <sup>a,b</sup>	16.7	83.3	0
G3-10 wt.%HNT	23.6 ± 5.4 <sup>a,b</sup>	41.7	58.3	0
G4-15 wt.%HNT	27.1 ± 7.8 <sup>a,b</sup>	33.3	66.7	0
G5-20 wt.%HNT	29.8 ± 6.1 <sup>a,b</sup>	25.0	66.7	8.3
G6-30 wt.%HNT	30.5 ± 6.5 <sup>b,c</sup>	83.3	16.7	0

Means with same superscript letters are not significantly different ( $p < 0.05$ ).

Oneway ANOVA revealed a significant difference ( $p < 0.001$ ) in mean SBS due to Halloysite (HNT) incorporation. Indeed, the multiple comparisons indicated that HNT incorporation at a concentration of 30 wt.% into the adhesive resin (G6) resulted in the highest SBS (30.5 ± 6.5 MPa) to dentin and was statistically significant ( $p = 0.025$ ) when compared to the control (G1). Analysis of the debonded specimens using optical microscopy revealed mostly mixed failures for the HNT-incorporated adhesives up to a certain level (20 wt.%), while the unmodified adhesive revealed mostly adhesive failure. The incorporation of high nanofiller amounts (G6-30 wt.%) led to an increase in adhesive failure. Fig. 2 displays representative SEM images of failure surfaces from nanotube-modified and control groups. Representative SEM micrographs of the resin–dentin interface for the 20 wt.% HNT-incorporated adhesive and the control groups are presented in Fig. 3. Hybrid layer and resin tag formation were seen in both the control (Fig. 3A and B) and the 20 wt.% HNT-incorporated adhesives (Fig. 3C and D). Overall, shorter and apparently rougher resin tags were seen for HNT-containing (Fig. 3C and D) adhesive when compared to the control (Fig. 3A and B), suggesting the presence of HNTs.

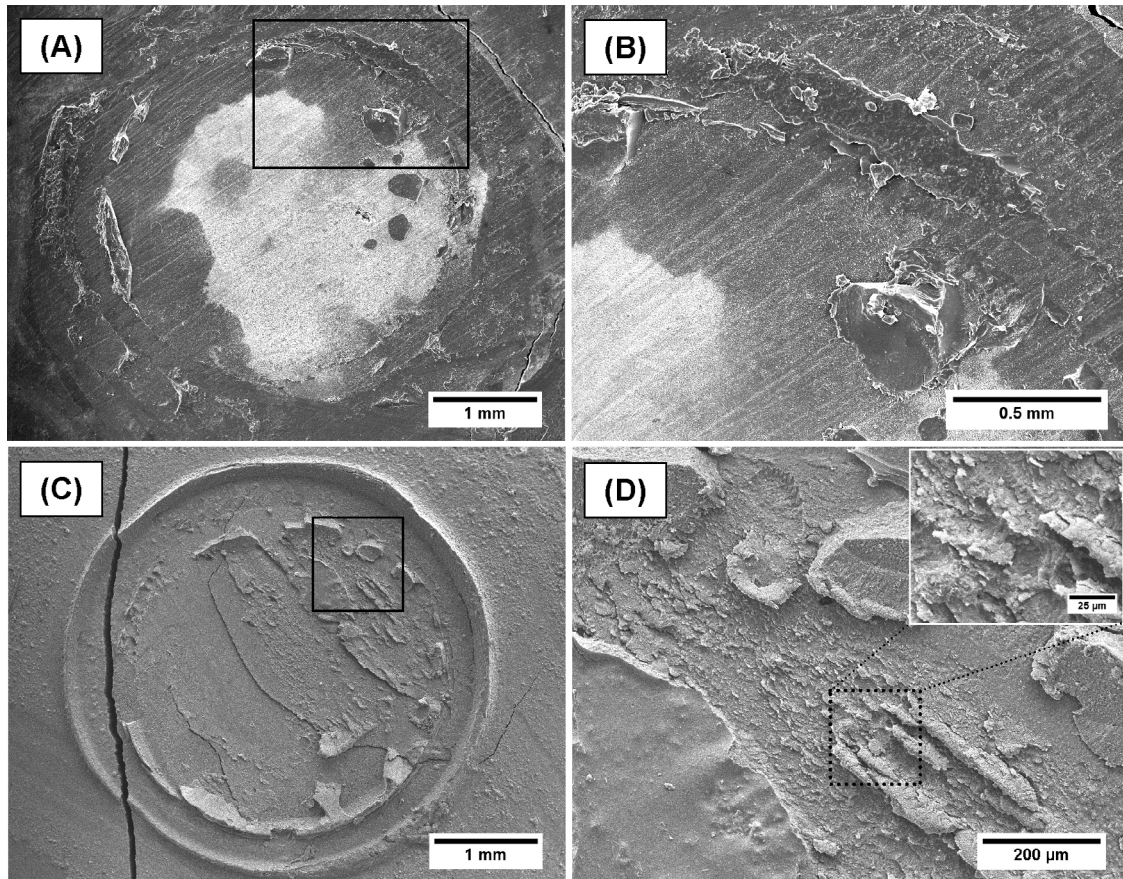
One-way ANOVA revealed that both Knoop microhardness (KHN) and degree of conversion (DC) were significantly affected by incorporating filler ( $p < 0.001$ ). However, no statistically significant differences were detected among the experimental groups G2-G5 when compared to the control ( $p > 0.05$ ) (Fig. 4). More importantly, even though a significant increase in microhardness ( $p < 0.001$ ) was seen for

the 30 wt.% HNT-incorporated group (G6), a significantly lower DC ( $p < 0.001$ ) was recorded when compared to the control (G1).

## 4. Discussion

Over the past decade, several research groups have attempted to improve the resin–dentin bond strength through the incorporation of a wide range (e.g., carbon nanotubes, zirconia nanoparticles, among others) of fillers [10–14]. In this study, we aimed to investigate the effect of aluminosilicate clay nanotubes (HNT) incorporated into the adhesive resin of a commercially available three-step etch and rinse bonding system (SBMP) on dentin bond strength, as well as the effect on





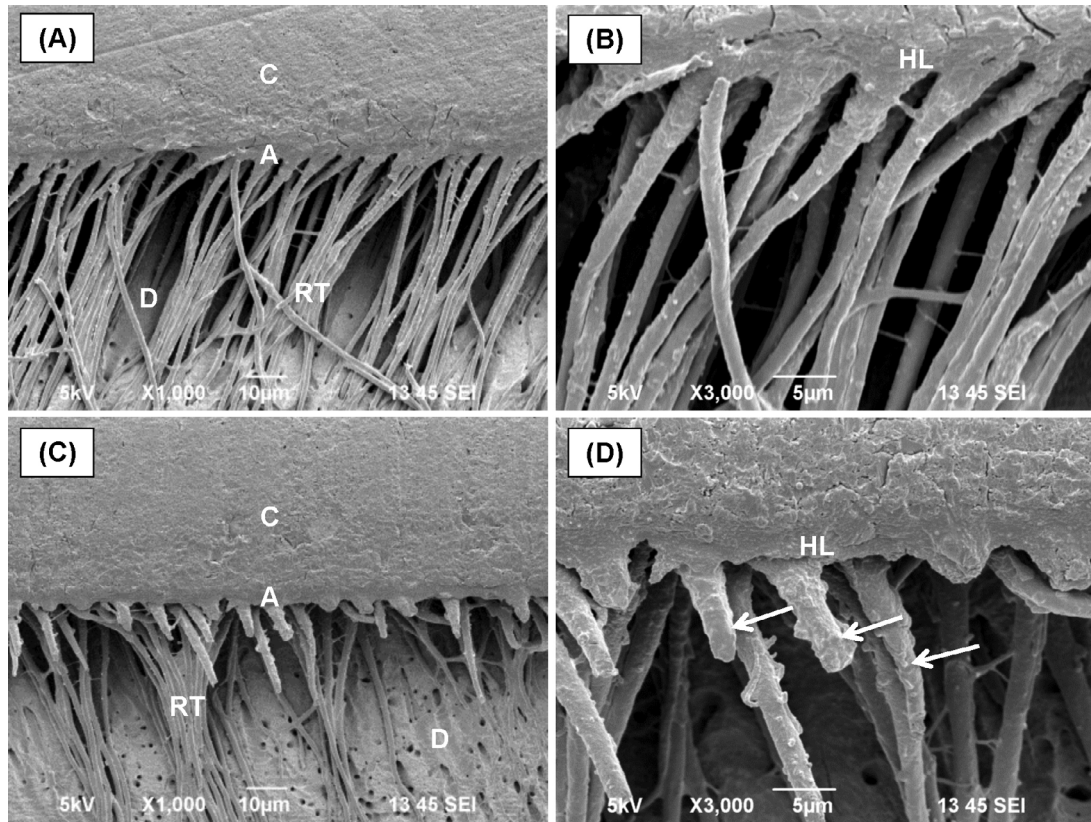
**Fig. 2 – Representative SEM micrographs of the fractured surfaces of (A and B) the unfilled control adhesive and (C and D) 20 wt.% HNT-modified adhesive.**

several key physicochemical properties of the modified adhesive.

To the best of our knowledge, this is the first report on incorporating HNT into the adhesive resin of a commercial unfilled bonding agent. The collected findings led to the rejection of both hypotheses since HNT incorporation led to a significant change in resin–dentin bond strength, as well as in the degree of conversion and microhardness. Primarily, the present study demonstrated that HNT incorporation into the adhesive resin of SBMP significantly increased the SBS to dentin compared to the control group (no HNT). Even though other testing methods could have been used to assess the bond strength to human dentin, avoiding the drawbacks associated with the macroshear test such as inhomogeneous distribution of stress over the load application area and occurrence of failure in the dentinal substrate at much lower stresses than the substrate strength [13], our SBS findings proved to be a reliable method at least for the first preliminary screening. Importantly, our SBS data agree with those of previous studies, which demonstrated that nanofiller incorporation in adhesive resin tends to increase the bond strength to dentin [11,12]. Worth mentioning, as suggested previously, a significant decrease in bond strength when using these filled adhesives could possibly be associated with loading amounts above a threshold limit, potentially due to filler agglomeration, poor infiltration through the interfibrillar spaces and the creation of

defects/voids within the hybrid layer [12,34,35]. Nonetheless, SEM micrographs of the resin–dentin interface for the HNT-incorporated adhesive revealed significant resin infiltration into dentinal tubules and resin tag formation (Fig. 3C and D). This might be explained by the HNT's hydrophilic nature that facilitated the penetration of resin into wet primed dentin even with a potentially higher viscosity. Even though the resin tags were less uniform and shorter, the 20 wt.% HNT-incorporated adhesive did not affect the shear bond strength to dentin (Fig. 3C and D).

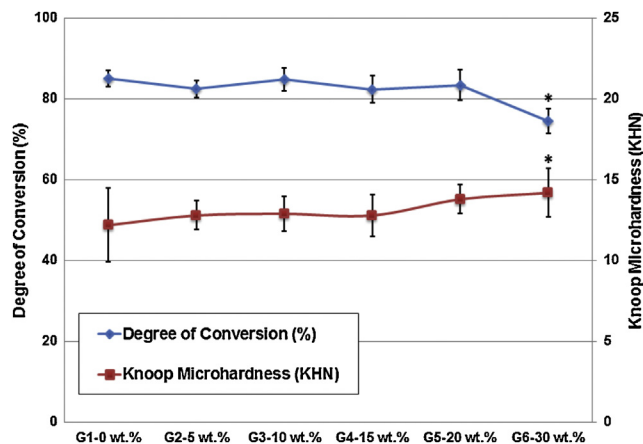
Regarding the physicochemical properties measured, one-way ANOVA revealed that HNT incorporation significantly ( $p < 0.001$ ) affected Knoop microhardness and degree of conversion. However, no statistically ( $p > 0.05$ ) significant differences were detected among the experimental groups G2–G5 when compared to the control (Fig. 4). More importantly, though a significant increase in microhardness ( $p < 0.001$ ) was seen for the 30 wt.% HNT-incorporated group (G6), a significantly lower DC ( $p < 0.001$ ) was recorded when compared to the control (G1). Even though one would anticipate to see an increase in mechanical properties as a function of curing effectiveness, our data corroborates with several other studies in the literature, in which DC did not correlate with the mechanical properties of the resin [36,37]. In this study, the incorporation of HNT at 30 wt.% into the adhesive seemed to have contributed to a less effective



**Fig. 3** – Representative SEM micrographs of the resin–dentin interfaces for the control (A and B) (no HNTs) and the (C and D) 20 wt.% HNT-incorporated SBMP adhesives. C = composite resin, A = adhesive layer, RT = resin tags, and D = dentin. Note relatively shorter and apparently rougher resin tags for the 20 wt.% HNT-incorporated adhesive when compared to the control suggesting the presence of HNTs.

monomer conversion when compared to the control group (Fig. 4).

Consistent with the TEM micrograph presented in Fig. 1B, the HNT hollow structure shows promise for the encapsulation and sustained release of macromolecules and/or therapeutic drugs of matching diameter. Recently, Qi et al. [25] reported on the fabrication of nanofibrous drug-loaded



**Fig. 4** – Degree of conversion (%) and Knoop microhardness for the different evaluated groups. \*means  $p < 0.001$  compared to the control.

scaffolds by combining the drug loading capability of biocompatible Halloysite nanotubes and electrospinning technique. Tetracycline hydrochloride was vacuum-encapsulated within HNTs, mixed with a poly(lactic-co-glycolic acid) solution and then electrospun into composite drug-loaded nanofibers [25]. Overall, the results of the study revealed that drug encapsulation into HNTs decreases the initial drug burst release while enhancing the mechanical properties of the nanocomposite scaffold [25].

Taken together, the modification of SBMP dentin bonding agent with Halloysite appears to hold high potential toward achieving a durable bond to dentin not only for the possibility of strengthening the bond interface but also due to the intrinsic capability of holding distinct molecules and therapeutic agents such as MMP inhibitors. We have established an optimized HNT concentration that could lead to a stronger dentin–adhesive interface without adversely impacting some of the adhesive resin properties such as microhardness and degree of conversion.

## 5. Conclusion

In conclusion, HNT incorporation (5–30 wt.%) into the SBMP adhesive resin had no detrimental influence on dentin bond strength, however due to a significant reduction in DC when compared to the unfilled adhesive, the present study indicated



that HNT can be incorporated up to 20 wt.% without jeopardizing important physicochemical properties of the adhesive. Now the focus will be on exploiting the potential encapsulation of several MMP inhibitors within the HNT lumen, and therefore the modification of not only the adhesive resin but also the primer of the dental adhesive, targeting the increase of resin–dentin bond longevity.

## Acknowledgement

The authors are grateful to Mr. Andre Zeitoun (Applied Minerals Inc., New York, NY) for the generous donation of the Halloysite clay used in this project.

**Disclosure.** There is a pending patent request under the name of the Indiana University Purdue University Indianapolis that refers to the proposed innovation.

## REFERENCES

- [1] De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: methods and results. *Journal of Dental Research* 2005;84:118.
- [2] Van Landuyt KL, Snauwaert J, De Munck J, Peurnans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechtsa P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials* 2007;28:3757.
- [3] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, Dorigo ED. Dental adhesion review: aging and stability of the bonded interface. *Dental Materials* 2008;24:90.
- [4] Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *Journal of Dental Research* 2011;90:953.
- [5] Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. *Dental Materials* 2011;27:1.
- [6] Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research* 1955;34:849.
- [7] Loguercio AD, Moura SK, Pellizzaro A, Dal-Bianco K, Patzlaff RT, Grande RHM, Reis A. Durability of enamel bonding using two-step self-etch systems on ground and unground enamel. *Operative Dentistry* 2008;33:79.
- [8] Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjaderhane L, Visintini E, Cadenaro M, Tay FR, Dorigo EDS, Pashley DH. Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dental Materials* 2010;26:571.
- [9] Carrilho MRO, Geraldini S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. *Journal of Dental Research* 2007;86:529.
- [10] Conde MCM, Zanchi CH, Rodrigues SA, Carreno NLV, Ogliaeri FA, Piva E. Nanofiller loading level: influence on selected properties of an adhesive resin. *Journal of Dentistry* 2009;37:331.
- [11] Kim JS, Cho BH, Lee IB, Um CM, Lim BS, Oh MH, Chang CG, Son HH. Effect of the hydrophilic nanofiller loading on the mechanical properties and the microtensile bond strength of an ethanol-based one-bottle dentin adhesive. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2005;72B:284.
- [12] Lohbauer U, Wagner A, Belli R, Stoetzel C, Hilpert A, Kurland HD, Grabow J, Muller FA. Zirconia nanoparticles prepared by laser vaporization as fillers for dental adhesives. *Acta Biomaterialia* 2010;6:4539.
- [13] Sadat-Shojai M, Atai M, Nodehi A, Khanlar LN. Hydroxyapatite nanorods as novel fillers for improving the properties of dental adhesives: synthesis and application. *Dental Materials* 2010;26:471.
- [14] Akasaka T, Nakata K, Uo M, Watari F. Modification of the dentin surface by using carbon nanotubes. *Bio-Medical Materials and Engineering* 2009;19:179.
- [15] Thio YS, Argon AS, Cohen RE, Weinberg M. Toughening of isotactic polypropylene with CaCO<sub>3</sub> particles. *Polymer* 2002;43:3661.
- [16] Lin Y, Chen HB, Chan CM, Wu JS. The toughening mechanism of polypropylene/calcium carbonate nanocomposites. *Polymer* 2010;51:3277.
- [17] Alamri H, Low IM, Allothman Z. Mechanical, thermal and microstructural characteristics of cellulose fibre reinforced epoxy/organoclay nanocomposites. *Composites Part B: Engineering* 2012;43:2762.
- [18] Chen Q, Zhao Y, Wu WD, Xu T, Fong H. Fabrication and evaluation of Bis-GMA/TEGDMA dental resins/composites containing halloysite nanotubes. *Dental Materials* 2012;28:1071.
- [19] Deng SQ, Zhang JN, Ye L, Wu JS. Toughening epoxies with halloysite nanotubes. *Polymer* 2008;49:5119.
- [20] Ye YP, Chen HB, Wu JS, Ye L. High impact strength epoxy nanocomposites with natural nanotubes. *Polymer* 2007;48:6426.
- [21] Lvov YM, Shchukin DG, Mohwald H, Price RR. Halloysite clay nanotubes for controlled release of protective agents. *ACS Nano* 2008;2:814.
- [22] Shchukin DG, Sukhorukov GB, Price RR, Lvov YM. Halloysite nanotubes as biomimetic nanoreactors. *Small* 2005;1:510.
- [23] Veerabadran NG, Price RR, Lvov YM. Clay nanotubes for encapsulation and sustained release of drugs. *Nano* 2007;2:115.
- [24] Vergaro V, Abdullayev E, Lvov YM, Zeitoun A, Cingolani R, Rinaldi R, Leporatti S. Cytocompatibility and uptake of halloysite clay nanotubes. *Biomacromolecules* 2010;11:820.
- [25] Qi RL, Guo R, Shen MW, Cao XY, Zhang LQ, Xu JJ, Yu JY, Shi XY. Electrospun poly(lactic-co-glycolic acid)/halloysite nanotube composite nanofibers for drug encapsulation and sustained release. *Journal of Materials Chemistry* 2010;20:10622.
- [26] Bottino MC, Yassen GH, Platt JA, Labban N, Windsor LJ, Spolnik KJ, Bressiani AH. A novel three-dimensional scaffold for regenerative endodontics: materials and biological characterizations. *Journal of Tissue Engineering and Regenerative Medicine* 2013;(March), <http://dx.doi.org/10.1002/term.1712> [Epub ahead of print].
- [27] Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. *Dental Materials* 2010;26:E78.
- [28] Perdigao J, Lopes MM, Gomes G. In vitro bonding performance of self-etch adhesives. II. Ultramorphological evaluation. *Operative Dentistry* 2008;33:534.
- [29] Korkmaz Y, Gurgan S, Firat E, Nathanson D. Effect of adhesives and thermocycling on the shear bond strength of a nano-composite to coronal and root dentin. *Operative Dentistry* 2010;35:522.
- [30] Mortazavi V, Fathi M, Ataei E, Khodaeian N, Askari N. Shear bond strengths and morphological evaluation of filled and unfilled adhesive interfaces to enamel and dentine. *International Journal of Dentistry* 2012;2012:858459.
- [31] Hegde MN, Hegde P, Chandra CR. Morphological evaluation of new total etching and self etching adhesive system

- interfaces with dentin. *Journal of Conservative Dentistry: JCD* 2012;15:151.
- [32] Fu J, Kakuda S, Pan F, Hoshika S, Ting S, Fukuoka A, Bao Y, Ikeda T, Nakaoki Y, Selimovic D, Sano H, Sidhu SK. Bonding performance of a newly developed step-less all-in-one system on dentin. *Dental Materials Journal* 2013;32:203.
- [33] Zhang Y, Wang Y. The effect of hydroxyapatite presence on the degree of conversion and polymerization rate in a model self-etching adhesive. *Dental Materials* 2012;28:237.
- [34] Lee YK, Pinzon LM, O'Keefe KL, Powers JM. Effect of filler addition on the bonding parameters of dentin bonding adhesives bonded to human dentin. *American Journal of Dentistry* 2006;19:23.
- [35] Miyazaki M, Ando S, Hinoura K, Onose H, Moore BK. Influence of filler addition to bonding agents on shear bond strength to bovine dentin. *Dental Materials* 1995; 11:234.
- [36] Dickens SH, Cho BH. Interpretation of bond failure through conversion and residual solvent measurements and Weibull analyses of flexural and microtensile bond strengths of bonding agents. *Dental Materials* 2005; 21:354.
- [37] Koliniotou-Kubia E, Jacobsen PH. The effect of irradiation time on the physical properties of light-cured resins. *Clinical Materials* 1990;6:21.