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Doxycycline-Encapsulated Nanotube-Modified Dentin Adhesives

ABSTRACT

This article presents details of fabrication, biological activity (*i.e.*, anti-matrix metalloproteinase [anti-MMP] inhibition), cytocompatibility, and bonding characteristics to dentin of a unique doxycycline (DOX)–encapsulated halloysite nanotube (HNT)–modified adhesive. We tested the hypothesis that the release of DOX from the DOX-encapsulated nanotube-modified adhesive can effectively inhibit MMP activity. We incorporated nanotubes, encapsulated or not with DOX, into the adhesive resin of a commercially available bonding system (Scotchbond Multi-Purpose [SBMP]). The following groups were tested: unmodified SBMP (control), SBMP with nanotubes (HNT), and DOX-encapsulated nanotube-modified adhesive (HNT+DOX). Changes in degree of conversion (DC) and microtensile bond strength were evaluated. Cytotoxicity was examined on human dental pulp stem cells (hDPSCs). To prove the successful encapsulation of DOX within the adhesives—but, more important, to support the hypothesis that the HNT+DOX adhesive would release DOX at subantimicrobial levels—we tested the antimicrobial activity of synthesized adhesives and the DOX-containing eluates against *Streptococcus mutans* through agar diffusion assays. Anti-MMP properties were assessed via β -casein cleavage assays. Increasing curing times (10, 20, 40 sec) led to increased DC values. There were no statistically significant differences ($p > .05$) in DC within each increasing curing time between the modified adhesives compared to SBMP. No statistically significant differences in microtensile bond strength were noted. None of the adhesives eluates were cytotoxic to the human dental pulp stem cells. A significant growth inhibition of *S. mutans* by direct contact illustrates successful encapsulation of DOX into the experimental adhesive. More important, DOX-containing eluates promoted inhibition of MMP-1 activity when compared to the control. Collectively, our findings provide a solid background for further testing of encapsulated MMP inhibitors into the synthesis of therapeutic adhesives that may enhance the longevity of hybrid layers and the overall clinical performance of adhesively bonded resin composite restorations.

INTRODUCTION

Dental caries continues and will likely remain a global health issue for many years to come, affecting nearly every individual (Ferreira-Zandona *et al.*, 2012). Regrettably, no single material for direct tooth restorations has totally replaced the use of amalgam, even with concerns over mercury toxicity. Meanwhile, with the increased demand for more aesthetically pleasing restorations, light-cured resin composites have been used, even though they do not invariably last as long (Rekow *et al.*, 2013). Accordingly, one high-priority research focus of the National Institute of Dental and Craniofacial Research

(2014) is on the innovations in materials for long-lasting and nontoxic direct tooth restorations. Despite remarkable advances in adhesive science and dentin bonding knowledge, research data continue to report a significant decrease in resin-dentin bond strength over time (Breschi *et al.*, 2008; Liu *et al.*, 2011; Pashley *et al.*, 2011; Tjäderhane *et al.*, 2013). Simply speaking, the failure of resin restorations is believed to be due in part to collagen fibril degradation in the dentin at the adhesive resin-dentin interface (*i.e.*, hybrid layer) by matrix metalloproteinases (MMPs; Mazzoni *et al.*, 2011, 2013).

In recent years, several approaches have been proposed to prolong the durability and overall clinical success of adhesively bonded restorations. For instance, the use of MMP inhibitors, such as the application of chlorhexidine (Carrilho *et al.*, 2007; Liu *et al.*, 2011; Mazzoni *et al.*, 2011) and galardin (Breschi *et al.*, 2010), after acid etching has been investigated. Meanwhile, doxycycline (DOX), a tetracycline derivative, at subantimicrobial dosages has displayed a valuable therapeutic role of MMP inhibition in periodontal disease (Choi *et al.*, 2004). Through a materials-based strategy, our group recently reported (Bottino *et al.*, 2013a) on the potential benefits of modifying adhesive resins using biocompatible, hydrophilic, and high mechanical strength aluminosilicate clay nanotubes (*i.e.*, halloysite nanotubes [HNTs]; Lvov *et al.*, 2013). Notably, several recent studies have highlighted the potential of using HNT as safe reservoirs for drug encapsulation and sustained release (Qi *et al.*, 2010, 2013). Accordingly, the synthesis of DOX-encapsulated nanotube-modified dentin adhesive could serve as a carrier for the sustained release of DOX at the adhesive layer for MMP inhibition and overall improvement of dentin bond durability. We tested the hypothesis that the release of DOX from the DOX-encapsulated nanotube-modified adhesive can inhibit MMP activity. Furthermore, changes in degree of conversion (DC) and microtensile bond strength (μ TBS) to dentin were evaluated to ensure that modification with HNTs or DOX-encapsulated HNTs would not compromise these characteristics of a commercial reference adhesive.

MATERIALS & METHODS

Fabrication of DOX-Encapsulated Nanotube-Modified Adhesive

DOX (Sigma, St. Louis, MO, USA)—a Food and Drug Administration-approved MMP inhibitor (Emingil *et al.*, 2004)—was dissolved at 10% (w/v) in phosphate buffered saline (PBS; pH 7.2, Fisher Scientific, Pittsburgh, PA, USA) at 50°C for 2 hr under stirring conditions (Fisher Thermix, model 310T, Fisher Scientific). The encapsulation process was modified from a previously reported procedure (Qi *et al.*, 2010). Briefly, aluminosilicate ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$) clay nanotubes (HNTs; Dragonite 1415JM, Applied Minerals Inc., New York, NY, USA) were sieved (45- μm sieve), mixed with the DOX solution (1 g/10 mL), and sonicated for 2 hr to enhance dispersion. Vacuum (25 inHg) was applied and maintained for 1 hr to remove any air between and within the HNTs (Qi *et al.*, 2010). The solution was then stirred for 1 hr, and vacuum was reapplied. Finally, the HNT+DOX solution was dried in an incubator

at 37°C for 5 d. On the basis of our findings that HNT can be incorporated up to 20% (w/v) without jeopardizing physicochemical and bonding properties of the adhesive (Bottino *et al.*, 2013a), we fabricated adhesives incorporated with 15% (w/v) of HNT, encapsulated or not with DOX. The dried HNT+DOX powder was sieved before incorporation (150 mg of DOX-encapsulated nanotubes per 1 mL of adhesive) into the adhesive resin of a commercial bonding system (Adper Scotchbond Multi-Purpose [SBMP]; 3M ESPE, St. Paul, MN, USA) by mixing with a stirrer and a conical micropestle followed by sonication to further enhance HNT dispersion (Bottino *et al.*, 2013a). It is important to note that the experimental adhesives were fabricated with foil-covered light-proof (amber) microcentrifuge tubes, because of DOX photosensitivity (Smith and Leyden, 2005). All specimens were fabricated in a constant-temperature room (23°C) with a filtered lighting system to minimize unintentional polymerization.

Degree of Conversion

DC was used to determine whether adhesive polymerization could be affected by DOX incorporation. Disc-shaped specimens (7×0.24 mm, $n = 5$) were fabricated and light cured for 10 sec (manufacturer's recommendation), 20 sec, and 40 sec. A light-emitting diode curing system (DEMI LED, Kerr, Orange, CA, USA) with an output irradiance of *circa* 1,300 mW/cm² was used and periodically monitored (Managing Accurate Resin Curing, MARC-RC Calibrator, BlueLight analytics Inc., Halifax, Nova Scotia, Canada).

Prepared specimens were stored for 24 hr in the dark under dry conditions. DC was evaluated with Fourier transform infrared spectroscopy (FTIR; Jasco 4100, Jasco Corp., Tokyo, Japan) in attenuated total reflection mode. The area under 2 absorbance bands at 1,637 cm⁻¹ (methacrylate group C=C) and 1,715 cm⁻¹ (ester group C=O) was used in the following equation to calculate the DC (Zhang and Wang, 2012; Bottino *et al.*, 2013a). The scans were run twice per specimen ($n = 5$) and the average used for analysis (Zhang and Wang, 2012):

$$\text{DC (\%)} = \left(1 - \frac{\text{Cured (area under } 1637 \text{ cm}^{-1} / \text{area under } 1715 \text{ cm}^{-1})}{\text{Uncured (area under } 1637 \text{ cm}^{-1} / \text{area under } 1715 \text{ cm}^{-1})}\right) \times 100$$

Dentin Bonding

Thirty-six caries-free human third molars were used within 6 mo of extraction under an approved (NS1004-03) local Institutional Review Board protocol (Indiana University). The occlusal third of each tooth was removed with a low-speed diamond disk (Buehler Ltd., Lake Bluff, IL, USA) mounted on a cutting machine (Isomet 1000, Buehler Ltd.). Following that, a flat midcoronal dentin surface was obtained with SiC papers (240-600 grit) to standardize the smear layer (Bottino *et al.*, 2013a). Teeth were then randomly assigned into 3 adhesive groups ($n = 12$): unmodified adhesive (SBMP, control), SBMP with HNTs (HNT), and SBMP with DOX-encapsulated HNTs (HNT+DOX). Prior to resin bonding, dentin surfaces were etched with 37% phosphoric acid, primed, bonded (according to the manufacturer's recommendations), polymerized (DEMI LED, Kerr, Orange, CA, USA), and

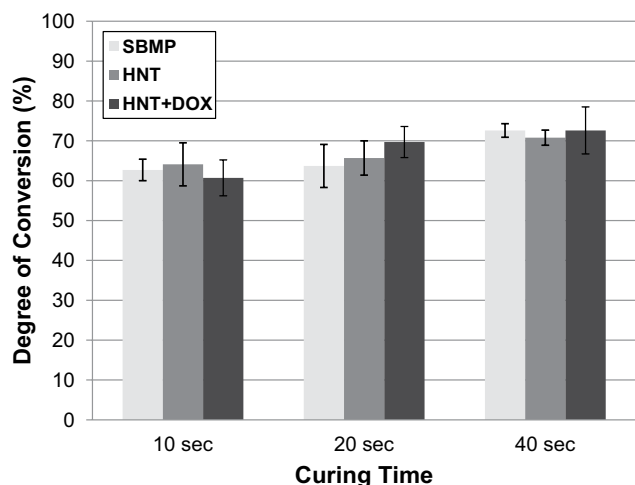


Figure 1. Graphic depicting the degree of conversion (DC) for control (SBMP) and experimental adhesives that were polymerized for 10, 20, and 40 sec. Increasing curing times led to a general increase in DC values: 60.7%-64.1% (10 sec), 63.7%-69.7% (20 sec), and 70.8%-72.6% (40 sec). There were no statistically significant differences ($p > .05$) in DC within each curing time between the modified adhesives compared to SBMP. DOX, doxycycline; HNT, halloysite nanotube; SBMP, Scotchbond Multi-Purpose.

restored with composite (~1-2 mm thick/increment; Z100, 3M ESPE). All specimens were stored in 37°C deionized water for 24 hr. Specimens were then cut into beams, 0.8 × 0.8 mm (Carrilho *et al.*, 2007). The beams were kept in 37°C artificial saliva for 24 hr and tested at a crosshead speed of 1 mm/min with a universal testing machine (MTS Sintech Renew 1123, Eden Prairie, MN, USA; Raposo *et al.*, 2012). Beams ($n = 152$) were tested for bond strength. Fracture surfaces were examined under a light microscope (40×) of 121 recovered beams (80% recovery). Failure type was classified as cohesive failure in dentin, cohesive failure in resin composite, adhesive failure, or mixed failure. Randomly selected fractured specimens were sputter coated with gold and imaged with a scanning electron microscope (SEM; JSM-5310LV, JEOL, Tokyo, Japan).

Cell Culture and Cytotoxicity Test

Cytotoxicity of the nanotube-modified adhesives with and without DOX, as well as the SBMP (control), was evaluated on human dental pulp stem cells (see Appendix; Zhang *et al.*, 2013a, 2013b).

Biological Activity

To prove the successful encapsulation of DOX within the adhesives but, more important, to further support our hypothesis that the HNT+DOX adhesive would release DOX at subantimicrobial levels, we tested first the antimicrobial activity of synthesized adhesives and the DOX-containing eluates against *Streptococcus mutans* through agar diffusion assays (Bottino *et al.*, 2013b). Eight disk-shaped adhesive specimens (5 × 2 mm) per group were fabricated with a Teflon mold, followed by light curing for 10 sec. Specimens were kept at 37°C for 24 hr,

disinfected by ultraviolet light exposure (30 min/side), and then assigned to 2 distinct assays. Four specimens ($n = 4$ per group) were tested by directly placing the disks on blood agar plates (BAPs; Fisher Scientific) containing a freshly prepared lawn of *S. mutans* (UA159). *S. mutans* was cultured aerobically in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) for 24 hr in 5% CO₂ at 37°C. Then, 100 μL of the bacterial suspension was swabbed onto BAPs to create the lawn (Gregson *et al.*, 2012; Huang and Gregory, 2012). In a different assay, the remaining specimens were used to obtain aliquots containing the eluates (*i.e.*, the leachable compounds) from the adhesives. Subsequently, specimens were individually incubated in 10 mL (37°C) of PBS (pH 7.2), and 1-mL aliquots were removed after 1, 7, and 14 d. An equal amount of sterile PBS was added to replace the removed volume. Aliquots were stored at -20°C until tested. Each BAP containing a freshly prepared lawn of *S. mutans* was divided into zones; the adhesive disks or 10 μL of the obtained aliquots were placed into the center of each zone; and the plates were incubated in 5% CO₂ at 37°C. The inhibition zones (mm) were measured after 96 hr. Chlorhexidine (0.12%) and sterile water served as positive and negative controls, respectively (Bottino *et al.*, 2013b).

MMP-mediated casein cleavage assays were performed to evaluate the anti-MMP activity of the synthesized DOX-encapsulated nanotube-modified adhesive. Disk-shaped specimens (5 × 2 mm, $n = 3$) were placed into 12-well plates and incubated in 2 mL of 50 mM Tris with 0.2 M NaCl, 10 mM CaCl₂, and 1 μM ZnCl₂ (pH 7.4). On days 1, 7, and 14, aliquots (500 μL) containing the eluates from the adhesive groups (*i.e.*, HNT and HNT+DOX) were collected. An equal volume of the solution was added back to the wells to keep the volume constant. Aliquots were stored at -20°C until tested. MMP-1 purified from human gingival fibroblast-conditioned media by anticollagenase affinity chromatography was incubated with β-casein at 37°C (Windsor *et al.*, 1994, 1997) with or without the aliquots (200 μL, $n = 3$) in a total volume of 400 μL. Samples were periodically (0-120 min) removed, and the reaction was terminated by addition of 1,10-phenanthroline to a final concentration of 10 mM; then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer was added (1:1). Samples were resolved in 10% SDS-PAGE, stained with Coomassie blue, and analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Data were analyzed with 1-way analysis of variance—except the microtensile dentin bond strength data, in which a mixed-model analysis of variance was used. Tukey's test was used for *post hoc* comparisons. The level of significance was set at 0.05.

RESULTS

Increasing curing times led to a general increase in DC values: 60.7%-64.1% (10 sec), 63.7%-69.7% (20 sec), and 70.8%-72.6% (40 sec). There were no statistically significant differences ($p > .05$) in DC within each curing time between the modified adhesives compared to SBMP (Fig. 1). Mean μTBSs (Appendix Table) were 44.6 ± 15.2 MPa (HNT), 51.7 ± 23.3

MPa (SBMP), and 54.3 ± 19.1 MPa (HNT+DOX). Statistical analysis revealed no significant differences in μ TBS among the groups ($p = .07$). Optical microscopy of the fractured surfaces revealed predominantly mixed failures (52.78%-60.98%; Appendix Table). Representative SEM micrographs of the fractured surfaces are shown in Figure 2. At high magnification (Fig. 2D, 2F), rod-like structures suggest HNT presence (Fig. 2A, 2B).

Growth inhibition of *S. mutans* (12.0 ± 0.0 mm; Fig. 3) supported the successful encapsulation and release of DOX from the experimental adhesive (HNT+DOX). No growth inhibition was seen for DOX-free groups (*i.e.*, SBMP and HNT). Eluates from HNT+DOX adhesive specimens, incubated up to 14 d in PBS, did not inhibit bacterial growth (data not shown). Figure 3E shows cytocompatibility assay results on human dental pulp stem cells viability in response to different eluate concentrations from SBMP, HNT, and HNT+DOX groups. Human DPSC viability ranged from 92.0% to 107.8% (Fig. 3E). No statistically significant differences were detected among all the adhesive groups and dilutions tested when compared to the control ($p > .05$). MMP-mediated casein cleavage assays (Fig. 4) demonstrated that 14-d eluates from HNT+DOX adhesive inhibited 13.4% of the MMP-1 activity, compared to 48% by 0.1% DOX (positive control). Eluates from days 1 and 7 (HNT+DOX) demonstrated 0% inhibition, whereas eluates from HNT adhesive (negative control) ranged from 0% to 1.8% inhibition.

DISCUSSION

Over the past few years, several approaches have been proposed to inhibit MMP activity within the hybrid layer, such as a local single application of chlorhexidine or galardin onto demineralized dentin (Carrilho *et al.*, 2007; Breschi *et al.*, 2010; Stanislawczuk *et al.*, 2011). Our long-term goal is to be able to inhibit the degradation of collagen fibrils within the hybrid layer by taking advantage of the unique and novel strategy of using HNT as a reservoir for MMP inhibitors and cysteine cathepsins. Here we take the first step toward our long-term goal by proving the concept that MMP inhibitors such as DOX can indeed be incorporated into dentin bonding agents through encapsulation within HNTs.

To the best of our knowledge, this is the first report that associates nanotubes with an MMP inhibitor (*i.e.*, DOX) aiming to

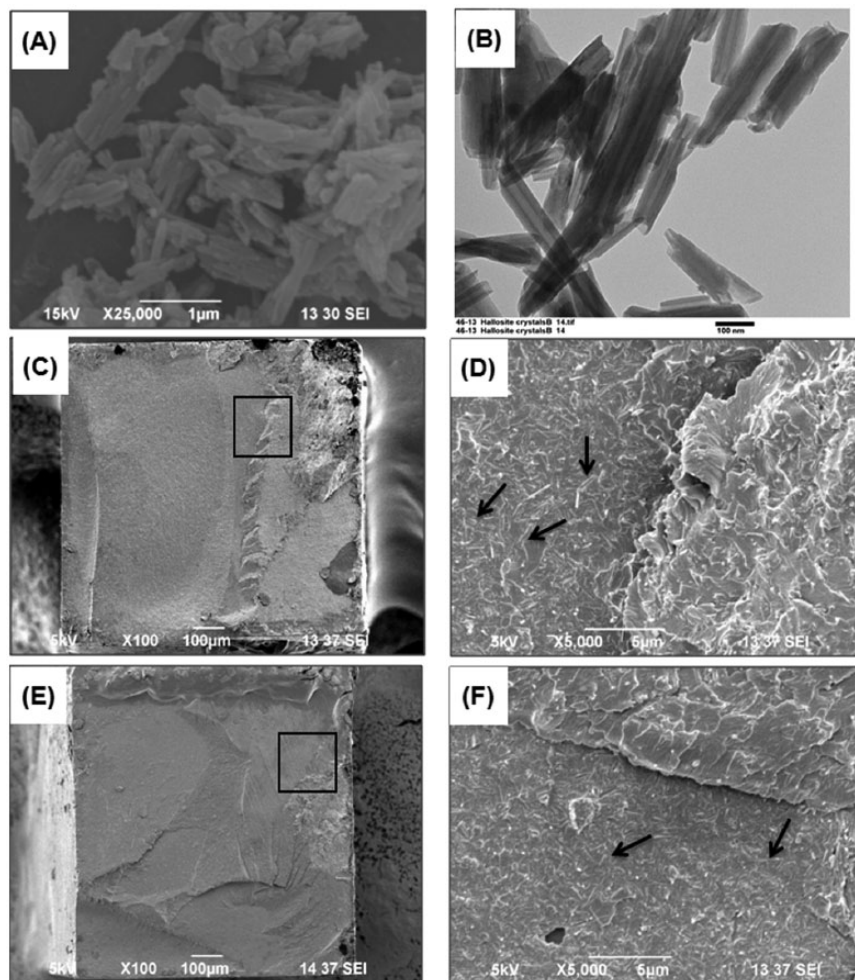


Figure 2. Micrographs of halloysite nanotubes (HNTs; Dragonite 1415JM, Applied Minerals Inc., New York, NY, USA): (A) scanning electron microscope (25,000 \times ; bar, 1 μ m) and (B) transmission electron microscope (98,000 \times ; bar, 100 nm). Representative scanning electron microscope micrographs of fractured surfaces: (C, D) SBMP with HNTs (100 \times , 5,000 \times) and (E, F) SBMP with doxycycline-encapsulated HNTs (100 \times , 5,000 \times). Arrows show rodlike structures approximately 1 to 2 μ m in length representing the HNTs incorporated into the experimental adhesive. SBMP, Scotchbond Multi-Purpose.

promote MMP inhibition through its release from the adhesive resin matrix. Our data clearly show that the amount of drug encapsulated into the nanotubes and consequently incorporated within the resin was high enough to inhibit the growth of *S. mutans* when in direct contact, as shown by the agar diffusion test. As anticipated, through the collection of eluates over a 14-d period, it was confirmed that the DOX concentration in those aliquots was not adequate to inhibit the growth of *S. mutans*. Importantly, at day 14, the subantimicrobial concentration was sufficient to inhibit MMP-1 activity. Taken together, the data led us to accept our hypothesis that DOX-encapsulated adhesives can, in fact, inhibit MMP activity through a gradual release of subantimicrobial DOX dosages.

Knowing that monomer conversion in dental adhesives plays a significant role in the attainment of stable and, more important, durable resin-dentin bonds (Reis *et al.*, 2004), not only did

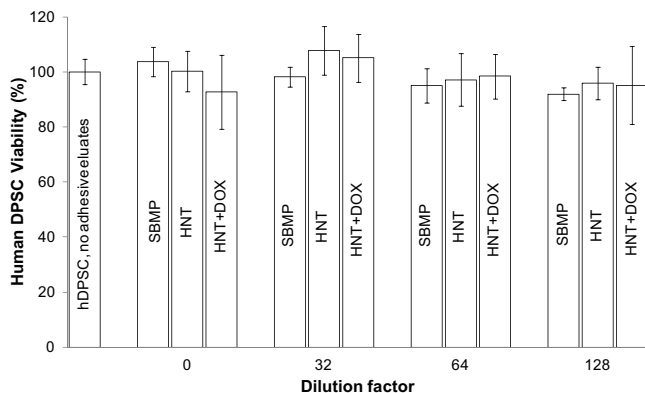
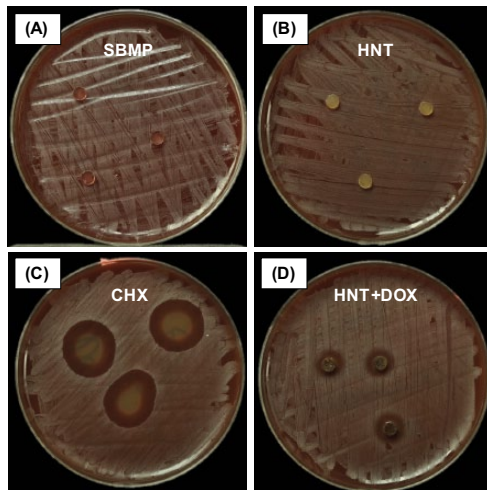


Figure 3. Representative macrophotographs of agar diffusion test against *Streptococcus mutans* at 96 hr of incubation: (A) SBMP (Scotchbond Multi-Purpose), (B) HNT (SBMP with halloysite nanotubes), (C) CHX (0.12% chlorhexidine; positive control), and (D) HNT+DOX (SBMP with doxycycline-encapsulated HNTs). Mean inhibition zone of 0.12% chlorhexidine and HNT+DOX was 21.0 ± 1.0 mm and 12.0 ± 0.0 mm, respectively. No inhibition zone was observed from SBMP and HNT. (E) Cytotoxicity of adhesive resin eluates released from specimens tested on human dental pulp stem cells. No significant differences ($p = .225$) were found comparing all groups of study and dilutions tested.

we decide to evaluate the DC using the 10-sec curing time per the manufacturer's instructions, but we also tested the effects of 20- and 40-sec exposures. Overall, the DC data showed no statistically significant difference between the modified adhesives when compared to SBMP (control) when tested with the same curing time. Nonetheless, it is important to point out that even though DOX incorporation led to a slightly darker adhesive, it did not compromise resin monomer conversion. Therefore, from a clinical point of view, these modified adhesives could be potentially handled and used in the same fashion as the commercial one.

Regarding the bonding characteristics, although DOX-encapsulated nanotubes did not enhance resin-dentin bond strength, it also did not jeopardize it, as no differences were seen among all groups ($p = .07$). SEM images (Fig. 3D, 3F) confirmed the presence of HNT within the adhesive layer. One might speculate over the potential difficulty associated with

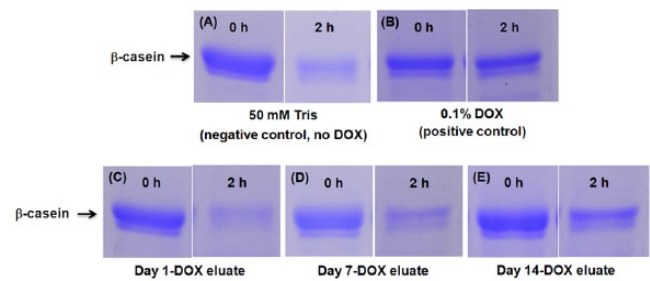


Figure 4. Representative images of Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of β -casein cleavage assays. (A) Negative control: 50 mM Tris with 0.2 M NaCl, 10 mM CaCl_2 , and $1 \mu\text{M}$ ZnCl_2 (pH 7.4). (B) Positive control: 0.1% doxycycline (DOX) solution (Sigma). DOX eluates: (C) day 1, (D) day 7, and (E) day 14. As depicted, more β -casein is present in panel E compared to the negative control (50 mM Tris) at 2 hr, which is not seen in day 1 and 7 aliquots.

ensuring an effective infiltration of these aluminosilicate clay nanotubes (halloysite) into the collagen-rich layer of the demineralized dentin to be able to perform the intended task—that is, release of MMP inhibitors such as DOX. Although prior research has shown the inability of spherical colloidal silica nanoparticles to infiltrate into the hybrid layer (Tay *et al.*, 1999), it is important to note that the general characteristics of the HNT crystals include dimensions of 50 to 200 nm in diameter, 0.5 to 1.5 μm in length, and an aspect ratio (L/D) of 20 (Lvov *et al.*, 2013), which could enhance their infiltration ability. Transmission electron microscopy studies need to be conducted to show and/or confirm the distribution of the nanotubes across the adhesive interface. Importantly, our group recently reported on the presence of HNT within resin tags according to cross-section SEM imaging of dentin/HNT-modified adhesive specimens (Bottino *et al.*, 2013a). Nonetheless, as previously highlighted, the presence of HNT within the adhesive layer might serve as a reservoir for the sustained release and subsequent diffusion of DOX and play a key role in prolonging the bond durability. To that end, the other half of the beams collected in this study are being aged (6 mo) in artificial saliva at 37°C. We anticipate, on the basis of the observed MMP inhibition capability of the DOX-modified adhesive, to see a significantly higher μTBS associated with the HNT+DOX group when compared with SBMP (control).

Results from the agar diffusion assays confirmed the successful incorporation of DOX into the nanotube-modified adhesive. HNT+DOX disks that contacted the BAP inhibited *S. mutans* growth. Conversely, the eluates obtained after incubation in PBS of the DOX-encapsulated adhesives for 2 wk exhibited no inhibition. This led to our assumption that the concentration of DOX released from the specimens did not reach the minimum inhibitory concentration (250-500 $\mu\text{g/mL}$; Al-Ahmad *et al.*, 2014). In other words, the eluates obtained from DOX-encapsulated specimens might contain subantimicrobial level of DOX.

To prove the concept of subantimicrobial levels of DOX, we further tested the DOX-containing adhesive eluates on the activity of MMP-1 using casein cleavage assays. According to the

literature, only MMP-1, MMP-13, and MMP-8 are true collagenases that can cleave native type I collagen effectively. One should note that, along with MMP-2 and MMP-9, MMP-1 has been found in dentin, although at a lower concentration (van Strijp *et al.*, 2003; Wang *et al.*, 2012). Although MMP-2 and MT1-MMP can cleave native type I collagen, they are not as efficient as MMP-1 in digesting type I collagen (Song *et al.*, 2006). The casein cleavage assay can examine inhibition of DOX-encapsulated modified dentin adhesive eluates on MMP-1 activity. The 14-d sample demonstrated 13.4% inhibition of MMP-1 activity, suggesting a gradual release of DOX from the nanotube-modified adhesives. Notably, the DOX mechanism of inhibition through the chelation of zinc present in the catalytic domain is the same for all MMPs. Therefore, if DOX inhibits MMP-1 as demonstrated in this work, it will certainly have a high chance of inhibiting other MMPs as well. Nonetheless, it is essential to expand the time frame over which eluates are collected and to obtain the exact amount of DOX released over time through analytic instrumentation (*e.g.*, high-performance liquid chromatography).

Taken together, these findings led to our conclusion that DOX-containing eluates indeed had subantimicrobial properties and the ability to inhibit MMPs. Although DOX has been successfully used to control the progression of periodontal disease by systemic delivery at a subantimicrobial level (*i.e.*, Periostat, 20 mg, orally, 2×/d) and localized delivery at antimicrobial levels (Atridox, 10% DOX hyclate, Atrix Laboratories, Fort Collins, CO, USA; Choi *et al.*, 2004), this is the first report in adhesive dentistry focused on the strategy of locally delivering a subantimicrobial level of DOX through its encapsulation within nanotubes to inhibit MMP.

In conclusion, our results establish the successful encapsulation and release of DOX from the nanotube-modified dentin adhesive. Furthermore, the results from the cell viability assays further support the use of DOX-encapsulated nanotube-modified dentin adhesive in the clinical setting. Collectively, our findings provide a solid background for further testing of encapsulated MMP inhibitors into the synthesis of therapeutic adhesives that may enhance the longevity of hybrid layers and the overall clinical performance of adhesive bonded resin composite restorations.

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