Published in final edited form as:
Dent Mater. 2020 May ; 36(5): 687–697. doi:10.1016/j.dental.2020.03.007.

Chlorhexidine-modified nanotubes and their effects on the polymerization and bonding performance of a dental adhesive

Sara Kalagi[a,b], **Sabrina A. Feitosa**[c], **Eliseu A. Münchow**[d], **Victor M. Martins**[c], **Ashley E. Karczewski**[c], **N. Blaine Cook**[a], **Kim Diefenderfer**[a], **George J. Eckert**[d], **Saulo Geraldeli**[e], **Marco C. Bottino**[f,*]

[a]Department of Cariology, Operative Dentistry & Dental Public Health, Indiana University School of Dentistry (IUSD), Indianapolis, IN, USA.

[b]Department of Restorative Dental Sciences, College of Dentistry, King Saud bin Abdulaziz University for Health Sciences, Riyadh, KSA.

[c]Department of Biomedical and Applied Sciences, Division of Dental Biomaterials, IUSD, Indianapolis, IN, USA.

[d]Department of Conservative Dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS 90035-003, Brazil.

[e]Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, USA.

[f]Department of General Dentistry, School of Dental Medicine, East Carolina University, Greenville, North Carolina, USA.

[g]Department of Cariology, Restorative Sciences and Endodontics, University of Michigan School of Dentistry, Ann Arbor, MI, USA.

Abstract

Objectives: The purpose of this study was to synthesize chlorhexidine(CHX)-encapsulated aluminosilicate clay nanotubes (Halloysite®, HNTs) and to incorporate them into the primer/adhesive components of an etch-and-rinse adhesive system (SBMP; Scotchbond Multipurpose, 3M ESPE) and to test their effects on degree of conversion, viscosity, immediate and long-term bonding to dentin. [53,54]

Methods: CHX-modified HNTs were synthesized using 10% or 20% CHX solutions. The primer and the adhesive components of SBMP were incorporated with 15 wt.% of the CHX-encapsulated HNTs. Degree of conversion (DC) and viscosity analyses were performed to characterize the modified primers/adhesives. For bond strength testing, acid-etched dentin was treated with one of the following: SBMP (control); 0.2%CHX solution before SBMP; CHX-modified primers +

---

[*]**Corresponding author:** Dr. Marco C. Bottino, University of Michigan, School of Dentistry, Department of Cariology, Restorative Sciences, and Endodontics, 1011 N. University Avenue, Ann Arbor, MI, 48109, USA, Tel: +1-734-763-2206; fax: +1-734-936-1597. mbottino@umich.edu (M.C. Bottino).

SBMP adhesive; SBMP primer + CHX-modified adhesives; and SBMP primer + CHX-free HNT-modified adhesive. The microtensile bond strength test was performed after immediate (24 h) and long-term (6 months) of water storage. Data were analyzed using ANOVA and Tukey (α=5%) and the Weibull analysis.

Results: DC was greater for the CHX-free HNT-modified adhesive, whereas the other experimental adhesives showed similar DC as compared with the control. Primers were less viscous than the adhesives, without significant differences within the respective materials. At 24 h, all groups showed similar bonding performance and structural reliability; whereas at the 6-month period, groups treated with the 0.2%CHX solution prior bonding or with the CHX-modified primers resulted in greater bond strength than the control and superior reliability.

Keywords

nanotube; Halloysite; aluminosilicate clay; microtensile; dentin; adhesives; bonding

1. Introduction

Continued concerns about the incorporation of mercury in amalgam alloys and the high demand for esthetic restorations [1] resulted in the widespread use of adhesively bonded resin composite restorations. Nonetheless, in spite of many advances in dental materials and bonding techniques, the resin-dentin interface remains susceptible to degradation due to the morphological structure and composition of dentin, as well as to the non-uniform infiltration of resin into dentin and suboptimal micromechanical interlocking after acid-etching [2]. An additional concern in dentin bonding relates to the degradation of the hybrid layer (HL) by host-derived endogenous matrix metalloproteinases (MMPs) found within mineralized dentin [3–5], which become uncovered and activated by pH changes during bonding procedures, digesting unprotected collagen due to insufficient resin infiltration and to the increase in collagenolytic and gelatinolytic activities within the resin-dentin interface [5, 6], ultimately causing the failure of the restoration's adhesion [7].

The application of MMP inhibitors to the demineralized collagen matrix on dentin after acid-etching has been suggested to preventing HL degradation [8]. Among these inhibitors, the role of chlorhexidine (CHX) as a non-specific MMP-inhibitor has been investigated with promising results [9, 10], since it is a commonly used dental antiseptic agent that has both a bacteriostatic action at low concentrations, and a bactericidal action at high concentrations [11]. Further, due to its substantivity, it can remain active up to 3 months, resulting in an immediate bactericidal action followed by a prolonged bacteriostatic action extending its efficacy [11]. CHX has a dose-dependent inhibition mechanism, causing MMP inactivation by protein denaturation when CHX is used in high concentration, while it interacts at low concentrations with the essential sulfhydryl groups and cysteine site of MMPs to inhibit its action [3]. Indeed, CHX has been demonstrated to present a positive role in the stabilization of resin-dentin bonds when it was used after acid etching and upon several concentrations (e.g., 0.02%, 0.1–0.5%, 1–2%) [12, 13]. However, applying a CHX solution after acid etching would increase the clinical steps of the bonding procedure, so that other studies have also attempted the incorporation of CHX into the etchant material [14] or into the primer [15] and adhesive [16–18] components, making the bonding protocol unaltered. Despite the foregoing approaches demonstrating effectiveness in the preservation of dentin bonds over time [19], it may be suggested that CHX would be available only during the immediate application of the modified materials. Thus, alternative methods of delivering continuous amounts of CHX would account for a greater bioactivity of this substance, perhaps prolonging its action and benefits to the formation of durable HLs. Moreover, considering that most dental adhesives are complex mixtures of hydrophilic and hydrophobic ingredients [2], the incorporation of an aqueous CHX solution into the material would probably create a heterogeneous solution, causing phase separation reactions that would possibly impair the physical stability of the CHX-modified material.

In recent years, aluminosilicate clay nanotubes ($Al_2Si_2O_5(OH)_4 \cdot nH_2O$), Halloysite®, HNT) have been investigated as a platform for drug delivery in dental adhesive systems [20–22]. CHX has already been successfully encapsulated into HNTs and was used to modify a commercial dental adhesive [23–25], showing effective inhibition of MMPs without impacting mechanical properties or cytotoxicity. Nevertheless, the primer component is the true material that aims to intertwine within acid-etched collagen fibrils, so that it would be of interest to have CHX available during the priming step [19]. To the best of our knowledge, there is no previous study focusing on this issue. Hence, the purpose of this study was first to synthesize CHX-encapsulated nanotubes and incorporate them into the primer and/or adhesive components of an etch-and-rinse adhesive system. Second, the study aimed to investigate the effects of CHX-encapsulated nanotube-modified materials on bonding performance to dentin after immediate and long-term water storage. The null-hypothesis was that the experimental materials would demonstrate similar bonding performance as compared to the non-modified control adhesive system, regardless of the presence and concentration of CHX.

2. Materials and methods

2.1. Synthesis of the CHX-encapsulated nanotubes

A commercially available chlorhexidine (CHX) digluconate aqueous solution (20% in H_2O, SigmaAldrich, St. Louis, MO, USA) was used to modify aluminosilicate clay nanotubes (HNT, Halloysite®, Dragonite 1415JM, Applied Minerals, New York, NY, USA). The CHX solution was used as-received (CHX20%) or diluted in distilled water to obtain a 10% CHX solution (CHX10%). The encapsulation process was performed as demonstrated elsewhere [24, 26]. Briefly, HNT powder was sieved using a 45-μm sieve (Test Sieve, ASTM E-11 Standard, 325MeshSz, Hogentogler & Co. Inc., Columbia, MD, USA) and was then mixed (Maxi Mix Plus Vortexer, Barnstead Thermolyne Co, Dubuque, IA, USA) with one of the two CHX solutions (1.25 g of sieved HNT powder for each 5 mL of CHX solution). The HNT+CHX mixture was sonicated for 2 h to enhance dispersion, mixed using an end-to-end mixer (Roto-Rack Tube Rotator, Thermo Fisher Scientific, Waltham, MA, USA), and then submitted to vacuum and drying at 37 °C (VWR Vacuum Oven, Despatch Industries, Minneapolis, MN, USA). The mixed materials were stored at 37ºC in an incubator (HeraTherm Oven, Thermo Fisher Scientific) for 7 days to let them dry. After drying, the CHX-loaded nanotubes were sieved at 45 μm, resulting in CHX-encapsulated HNT powders, which were stored separately in amber vials at room temperature until use.

2.2. Modification of a dental adhesive with the CHX-encapsulated HNTs

A commercial three-step etch-and-rinse adhesive system (Adper™ Scotchbond™ Multi-Purpose [SBMP], 3M ESPE, St. Paul, MN, USA) was used in this study [27]. Both the Primer and the Adhesive materials of that system were incorporated with the CHX-encapsulated HNTs at the 15 wt.% level (i.e., 150 mg of the CHX-encapsulated HNTs per 1 mL of primer/adhesive). Thus, two modified primers (P_CHX10% and P_CHX20% groups) and two modified adhesives (A_CHX10% and A_CHX20% groups) were prepared. Unmodified aliquots of the original Primer and Adhesive were used to serve as control (SBMP group). Also, an aliquot of the Adhesive was incorporated with CHX-free HNTs to serve as an additional and internal control (HNT group). All materials were stirred overnight before their use.

2.3. Characterization of the as-received and HNT-modified materials

Degree of conversion (DC) of the commercial and experimental adhesives was measured to determine whether the incorporation of HNTs would impair their polymerization. Disk-shaped specimens (7 mm × 0.24 mm; n = 3 for each adhesive) were prepared and cured for 10 seconds using a light-emitting diode curing system (DEMI LED, Kerr, Orange, CA, USA) with an output intensity of 1100 mW/cm^2. The intensity of the light curing unit was measured periodically using a calibrated handheld radiometer (Cure Rite Visible Curing Light Meter, DENTSPLY Caulk, Milford, DE, USA). The DC was evaluated with Fourier transform infrared spectroscopy (FT/IR-4100, JASCO, Easton, MD, USA) in attenuated total reflection mode, within the range of 1750 and 1550 cm^{-1}, at an 8 cm^{-1} resolution and a mirror speed of 2.8 mm/s [28]. Three initial readings were taken for each uncured (unpolymerized) adhesive and three readings were taken at different sites for each of the cured samples. The absorbance bands at 1637 cm^{-1} (methacrylate group, C = C) and 1607 cm^{-1} (ester group, C = O) were used to calculate the DC (expressed in %), according to the following equation [29]:

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

The viscosity of the as-received and the experimental primers and adhesives was determined using a viscometer (DV-II Viscometer, Brookfield, Middleboro, MA, USA). Six milliliters of each sample (n = 5) were introduced into the adapter and coupled to the spindle (CPA-52Z), then accelerated from 0 to 15 rpm at a constant temperature of 25.3°C and 26% humidity. All parameters (torque constant = 0.09373; spindle multiplier constant = 9.83; spindle shear rate constant = 2) were recorded under the specific speed suitable for each material tested.

2.4. Bonding performance of the HNT-modified materials

One hundred and five sound human molars were selected (Institutional Review Board of Indiana University, IRB protocol #1711224202, Indianapolis, IN, USA) and autoclaved for 20 min; after that, the remaining debris were removed using a gauze. A 3.3 mm distance below the cemento-enamel junction (CEJ) was measured using a digital caliper (SS, Marathon, USA), marked and used as the limit for the embedding process; the teeth were then individually embedded in acrylic resin (Lecoset 7008, Leco Corporation, Saint Joseph, MI, USA) using a dental surveyor to ensure the teeth were aligned parallel to their long axes and perpendicular to the CEJ line. The occlusal third of each tooth was removed to expose mid-coronal dentin surface using a low-speed diamond disk mounted on a cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling. A standardized smear layer was created on flat mid-coronal dentin surface with 600-grit SiC paper (Leco Corporation) using a polishing machine (Spectrum System 1000, Leco Corporation) [25]. Last, the teeth were randomly assigned to seven groups (n=15) according to the bonding protocol and/or combination of bonding agents used: SBMP – only the commercial SBMP adhesive system was applied to dentin (positive control); HNT – dentin was treated with the commercial SBMP primer followed by the CHX-free nanotube-modified adhesive; P_CHX10% and P_CHX20% – dentin was treated with the respective CHX-modified primers combined with the commercial SBMP adhesive; A_CHX10% and A_CHX20% – dentin was treated with the commercial SBMP primer combined with the respective CHX-modified adhesives; and CHX0.2% – dentin was treated with a 0.2% CHX solution prior to the application of the SBMP adhesive system.

Prior bonding, dentin surface of each tooth was cleaned with oil- and fluoride-free pumice paste (Nada pumice paste, Preventive Technologies Inc., Indian Trail, NC, USA) for 10 s using polishing brushes, followed by water rinse with distilled water and air drying for 10 s. All teeth were acid-etched with 35% phosphoric acid (Scotchbond™ Universal Etchant, 3M ESPE) using a microbrush for 15 s, water rinsed for 15 s and gently air-dried for 5 s with absorbent paper points. All teeth were treated following the same protocol used in the application of the SBMP primer and adhesive (manufacturer's instructions). To that end, the primer was applied with a microbrush for 5 s and air-dried for 5 s, whereas the adhesive was applied with a microbrush, air-dried for 5 s, and light-cured (DEMI LED, Kerr, USA) for 10 s. For the CHX0.2% group, 100 μl of 0.2% CHX solution was rubbed into the acid-etched dentin surface using a microbrush and left undisturbed for 1 min [30]. The SBMP primer and adhesive were applied, as previously described.

All tooth samples were restored with two 2-mm increments of resin composite (Filtek™ One Bulk Fill Restorative, 3M ESPE, St. Paul, MN, USA; Lot #N890621, N90143, N917600, N910792) contained by a Tofflemeire matrix band and retainer, then light-cured (DEMI LED, Kerr) for 20 s from the top surface. After matrix removal, the resin composite was light-cured for 10 s from all sides according to the manufacturer's instructions. The samples were stored in distilled water at 37°C for 24 h. Next, the tooth samples were cut into beams (1×1 mm^2) using a diamond disc (Isomet Diamond Wafering Blades, Buehler, Lake Bluff, IL, USA) mounted on a low speed cutting saw machine (Isomet 1000, Buehler) under water cooling. The collected specimens were assigned randomly and equally in number as possible into subgroups according to the following aging conditions: immediate – the specimens were stored in distilled water for 24 h at 37°C before testing; and long-term – the specimens were stored in distilled water for 6 months at 37°C. In the latter subgroup, the distilled water was replaced by fresh water at every 2 weeks. After each aging condition, the specimens of each group (n = 20) were carefully attached to a metallic device for the microtensile bond strength (μTBS) test with cyanoacrylate gel (Super Glue, Gel Control, Loctite) and tested using a Universal Testing Machine (MTS Sintech Renew 1123, Eden

Prairie, MN, USA) at a crosshead speed of 1 mm/min. For the data analysis, the tooth was considered the unit and the data were the average values of specimens per tooth [31].

In light of calculating the bond strength variation (ΔμTBS) of the resin-dentin bonds after 6 months of water storage, the following formula was used [32]: $\Delta\mu TBS\ (\%) = 100 - [(\mu TBS\_6\ months \times 100)/\mu TBS\_24\ hours]$, where μTBS_6 months and μTBS_24 hours represent the average bond strength values obtained at long-term and immediate testing periods, respectively. While a negative ΔμTBS value indicates the occurrence of bond strength reduction over time, a positive value would represent an increase in resin-dentin bonds.

2.5. Fracture analysis

The fractured interfacial surfaces were examined under a light microscope (40× magnification). Failure modes were classified as "adhesive", "mixed", "cohesive in dentin", and "cohesive in resin composite" [25]. The resin–dentin interface of randomly selected specimens were sputter coated with gold-palladium (Denton Vacuum) and imaged with a scanning electron microscope (SEM, JSM-6390LV, JEOL). Images were submitted to qualitative evaluation.

2.6. Statistical analysis

Data from DC and viscosity tests were analyzed using one-way ANOVA. Microtensile bond strength data were analyzed by pair-wise comparisons using the Sidak method to control the overall significance level at 5% for each aging time separately. Weibull-distribution survival analysis was used to compare the differences in the microtensile bond strength results among the seven groups after 24 h and 6 months separately, and to compare the differences in the microtensile bond strength results between the two aging times for each group separately. Differences between the groups for failure type (Adhesive, Mixed, or Cohesive) were summarized for each aging time. The stress value (MPa) required to produce failure was used as time to event and was summarized by group for each aging time.

3. RESULTS

3.1. Degree of conversion and viscosity

Table 1 shows the results for the degree of conversion and viscosity of the materials tested in the study. The experimental adhesive modified with the CHX-free HNTs acquired greater conversion of monomers as compared to the control adhesive ($p<0.001$). Conversely, the CHX-modified adhesives demonstrated similar degree of conversion when compared to the control and to the HNT adhesive ($p\geq0.074$). Concerning the viscosity of the materials, all the primers were considerably less viscous than the adhesives ($p<0.001$). However, the presence of the CHX-encapsulated HNTs did not change the overall viscosity of the materials ($p\geq0.063$).

3.2. Bonding performance to dentin

The bond strength results are presented in Table 2. The factors "bonding agent" and "aging period" were not significant ($p>0.05$), although their interaction was statistically significant (p≤0.001). At the immediate period testing, the unmodified SBMP adhesive system demonstrated the lowest bonding as compared to the control and t≥0.095), except for the bonding protocol using the more concentrated CHX-modified primer, which produced lower bonds than the CHX0.2% group (p=0.030). At the long-term period testing, the greatest bonds were observed within the groups treated with the 0.2% CHX solution, as well as with CHX-modified primers that demonstrated greater bonds than the control adhesive and the CHX-free HNT-modified adhesive ≤0.015). The P_CHX20% group demonstrated similar bond strengths as compared to the groups treated with the CHX-≥0.232). (~22.5%) and A_CHX20% (~14%) groups (p≤0.043), whereas for the groups treated with the CHX -modified primers the bonds were increased p≤0.032), ranging from approximately 16.6% to 20.1% of gain in adhesion. The control showed ~15.4% of bond strength reduction, although it was not statistically significant (p=0.089). Bonds obtained with the A_CHX10% and CHX0.2% groups were stable after aging (p ≥0.252), resulting in only 6.6% and 8.4% of bond strength variation overtime, respectively.

3.3. Fracture analysis

At the immediate testing, adhesive failures were more frequently observed for the HNT and CHX-modified adhesive groups, whereas the other groups failed mostly within the cohesiveness of resin composite (Figure 1). Representative micrographs of the most frequent fracture mode obtained for each group tested in the study can be observed in Figure 2. At the long-term testing, adhesive failures occurred mostly for the groups treated with the CHX-modified materials (i.e., both the primers and the adhesives). The SBMP control group as well as the group treated with the 0.2%

CHX solution showed an equilibrium between mixed and cohesive in resin composite failures. Last, approximately 33% of cohesive failures within dentin occurred for the HNT group, the greatest percentage of this fracture mode in the study. A fractured/non-leveled dentin substrate can be clearly observed in Figure 2 (SEM image for the HNT group at 6 months).

3.4. Weibull analysis

The results for the Weibull analysis performed in this study are shown in Table 3 and Figure 3. The Weibull modulus and characteristic strength at the 24 h period did not differ among the groups (p>0.05), but at the long-term period there were significant differences (p<0.05) for the characteristic strength of the groups. The groups treated with the CHX-modified primers and the 0.2% CHX solution resulted in greater characteristic strength than the other groups, which did not differ among each other. The Weibull modulus was similar among all the groups tested after 6 months of water aging. Despite the absence of statistical differences in the Weibull modulus of the groups, a superior reliability can be expected for the resin-dentin bonds obtained with the use of the CHX-encapsulated HNT-modified primers and with the 0.2% CHX solution prior bonding (Figure 3), since these groups displayed an apparent greater survival probability of their bonds.

4. DISCUSSION

The modification of low viscosity resin-based materials such as dental adhesives with nanotubes is not new in dentistry, with several studies demonstrating significant improvements in the physico-mechanical behavior of the modified material [23–27, 33]. However, it seems to exist a concentration threshold in the incorporation of nanotubes into the resin, especially in terms of change of color (e.g., the material may become greyish [34]) and the possibility of non-uniform dispersion of nanotubes within the matrix [35]. Indeed, the darker and the more saturated the HNT-containing material, the more affected would become its polymerization and the acquisition of mechanical properties. In our study, the addition of 15 wt.% of HNTs did not impair the degree of conversion of the adhesive, thus indicating the adequate amount of nanotubes used and their homogeneous distribution as fillers. This finding corroborates a previous study in which an HNT-modified adhesive incorporated in the 20 wt.% level resulted in similar conversion of monomers as compared with the HNT-free control [23]. Also, the study by Satish et al. [33] suggests that HNTs possess a superior loading capacity as compared to other types of nanotubes (e.g., carbon nanotubes, graphene), probably due to their unique hollow structure and overall uniform rod-like tubular morphology. As shown in Table 1, the HNT adhesive without CHX presented greater degree of conversion than the unmodified control, suggesting that the presence of nanotubes has somewhat contributed to an enhanced polymerization reaction for the adhesive. One may consider that the slightly greater viscosity of the HNT adhesive, which was ~21% more viscous than A_SBMP, reduced polymer chain termination and the mobility of the polymer radicals, increasing the free-radical propagation and monomers' conversion (Trommsdorff effect) [36]. No less important, HNTs have electrophilic characteristics that may cause a potential effect on the dynamic equilibrium of the activation-deactivation cycle during atom-transfer of the radical polymerization, as suggested elsewhere [37, 38]. Indeed, the strong electronegativity of oxygen atoms makes the outer surface of HNTs negatively charged, allowing the HNT to act as an effective sensing channel that would facilitate electron transport and interactions between molecules [33], and consequently, facilitating monomer conversion.

Despite the CHX-modified adhesives exhibited a slightly greater viscosity than the control, the degree of conversion of the foregoing groups was statistically similar, thus indicating that the presence of CHX has perhaps reduced the electrophilic state of the HNTs, thus preventing the gain in the conversion of monomers. This is possibly supported by the better dispersion ability of HNTs in nonpolar polymers (resin adhesives) as compared to polar systems, such as a dental primer that has been demonstrated in the study by Yu et al. [39]. Alternatively, one may also consider that the chloride composition of CHX may change the electrophilic characteristic of the HNTs [40], although without impairing the polymerization of the adhesive.

The total amount of HNTs incorporated into the SBMP primer/adhesive (15 wt.%) did not change their overall viscosity, which would be essential in order to allow the HNT-modified materials to function as properly as the original unmodified adhesive system. According to the study by Bhattacharya [41], once the HNT compound gets hydrated by the resin it may acquire increased interfacial or surface area, thus enhancing its ability to interact with the polymer system. However, this superficial interaction is only based on ion-dipole bonding due to the presence of aluminosilicate ($Al_2Si_2O_5$) compounds in the outer surface of HNTs [41], which allow for the maintenance of viscosity in the same fashion as the unmodified adhesive. In the case of forming covalent or hydrogen bonding, the incorporation of HNTs into the resin adhesive would probably increase molecular interactions, significantly increasing viscosity of the system, which is not the requisite to an adequate bonding agent. Concerning the SBMP primer, it showed an approximately 3.8 times lower viscosity than the SBMP adhesive, although this is indeed expected since the primer is a water-based (~ 50 wt.% of water) solution that aims to infiltrate the acid-etched dentin with hydrophilic resin monomers, thereby enabling formation of a deeply structured hybrid layer [2]. Remarkably, the HNT-modified primers exhibited nearly 6 times lower viscosity than their respective adhesive counterparts, which may be related to the presence of CHX. Considering the inert state of HNTs [40], it seems that both the hydrophilic nature of CHX as well as its cationic composition contributed for the apparent better wettability of the primer, which is also suggestive of a proper interaction with the solvent component; and for the formation of some hydrogen bonding with the ingredients of the adhesive, resulting in a cross-linked and moderately viscous system. However, these assumptions deserve further investigation.

The main goal of this study was to successfully modify the primer and adhesive components of a commercial adhesive system (SBMP) with CHX-encapsulated HNTs and to verify whether this approach would account for the improvement of the resin-dentin bonds over time. As shown in Table 2, the bonding protocol combining the CHX-modified primers and the unmodified SBMP adhesive resulted in greater bonds after prolonged water storage, showing from ~17 to 20% of gain in the adhesion, which was a different result from the other bonding protocols tested in the study. It is already known that during dentin bonding following an etch-and-rinse approach the first critical event associated to significant MMPs activation and ultimately bond strength degradation is the acid-etching step of the substrate, since the exposure of the collagen fibrils instantly may activate bound MMPs, starting collagen degradation. Taking this into consideration, the priming step would become the most reliable moment for reinforcement of the hybrid layer and the inhibition of any activated MMPs, which was indeed accomplished by our findings. There were only three bonding protocols tested here that resulted in bond strength increase after water aging: one that applied a 0.2% CHX solution directly to the etched dentin prior bonding, which is a well-recognized method used in daily practice [2, 30]; and the two other protocols that used the experimental CHX-modified primers. CHX was probably released from the HNTs during priming step, and due to the prevention of MMPs activation, resin infiltration was adequately obtained, resulting in high-quality hybrid layers. The degradation of dental resin bonds is a complex process that involves the break of intermolecular bonds existing within the hybrid layer, i.e., degradation of both collagen and resin monomer cross-links, creating free zones that would thereafter undergo water uptake, and consequently, hydrolysis [2, 5]. Once this foregoing process is prevented or somewhat diminished, the hygroscopic and hydrolytic phenomena of degradation could be considerably delayed, preserving the adhesive properties of the restoration.

The group bonded using the SBMP control demonstrated a ~15% reduction in the resin-dentin bonds after 6 months of water storage, corroborating other studies [42, 43]. Despite the lack of statistical difference, it may be understood that hydrolysis occurs within the hybrid layer of this group, and is probably due to water uptake. One may consider that MMP-mediated collagen degradation is more likely to occur in this group, since there was not any MMP inhibitor, such as CHX, that is available during application of the SBMP system. Nevertheless, statistically speaking, the resin-dentin bonds were not any different after 6 months of water storage. Perhaps if the study investigated a longer period of time (e.g., one year or longer), a greater and statistically significant reduction in bond strength results could have been observed. In the same fashion, the bonds obtained by the application of the CHX-free HNT-modified adhesive were not prevented to undergo degradation, resulting in the greatest bond strength reduction of the study (22.5%). It has been suggested that the fragile superficial interaction between HNTs and the resin adhesive was somewhat affected by the water uptake derived from water storage, causing a slow but constant swelling of the interfacial resin matrix, giving space for the plasticization (i.e., softening) of the resinous system [44]. Simply speaking, it is probable that the HNT itself is not responsible for the bond strength reduction observed in this group, but, instead, disruption of the weak interchain interactions between its surface and the resin matrix, allowing for hygroscopic and hydrolytic phenomena to occur [45], and consequently, weakening of the adhesive layer and degradation of the resin-dentin bonds following a much faster scenario than the other groups. One may consider that functionalization of the nanotubes would make their superficial interactions with the resin matrix less susceptible to hydrolysis, as demonstrated elsewhere [46, 47]. According to the study by Sabatini et al. [45], surface modification of HNTs with aminosilanes may prevent diffusion of water molecules in the system, thus enhancing its overall durability and resistance to hydrolysis. However, functionalization of the HNTs was not considered in this study since they were mostly intended for modification of the primer component, which is a solvent-containing material that would directly induce severe hydrolysis onto the functionalized sites of the HNTs, although future studies would benefit from investigating the effects of surface functionalization of nanotubes on nanotube-modified dental primers and adhesives.

The null-hypothesis tested in this study was that the experimental materials would demonstrate similar bonding performance as compared to the non-modified control adhesive system, regardless of the presence and concentration of CHX. This null-hypothesis should be accepted only for the immediate period investigated, in which all groups treated with the HNT-modified materials demonstrated similar bond strength values to the control. On the other hand, the null-hypothesis should be rejected when considering the long-term period, since the groups treated with the CHX-modified primers resulted in greater resin-dentin bonds as compared to the control. It seems that time is a necessary variable to be considered during bonding experiments, probably because bond strength degradation is a time-dependent event. Therefore, in the short-term analysis, all the experimental materials tested here could be adequate for clinical applicability, but when considering bonding durability, the protocol combining the CHX-modified primers (at both concentrations tested) with the SBMP bonding agent may be more interestingly used as a strategy to improve dental bonds after aging. Future research focused on the structural characterization (e.g., SEM of the hybrid layers, nanoleakage challenge) of the resin-dentin interfaces prepared with the synthesized CHX-encapsulated HNT-modified materials are paramount to gain a better understanding of this straightforward strategy on adhesive bonding procedures.

The occurrence of failures within the cohesiveness of resin composite was high at the immediate period testing, denoting that the maximum polymerization state and mechanical properties of the bulk-fill resin composite may be acquired at a delayed time-point. Indeed, defects derived from the placing of the resin composite during preparation of specimens may not be diminished by water swelling (i.e., like a "healing" process of the restoration) during the first days of water storage, thus explaining the considerably greater occurrence of this type of failure at the short-term period. Notwithstanding, cohesive failures were less frequently observed at the long-term period, suggesting that the cohesiveness of the restoration was improved with water aging. Adhesive failures occurred most at the long-term period, suggestive of the expected degradation of the hybrid layer after prolonged water storage. As shown in Figure 1 and in the SEM micrographs (Figure 2), the groups which most failed at the adhesive interface were those treated with the HNT-modified materials, regardless of the presence and concentration of CHX. One study performed by Akasaka et al. [48] demonstrated that when nanotubes are applied to dentin, they may selectively coat the surface and adhere to the exposed collagen fibrils, thus increasing mechanical interlocking of bonding agents with dentin. It is indeed possible to observe in Figure 2 that when dentin was treated with the HNT-modified materials, the fractured specimens appeared mostly covered with primer/adhesive, partially or totally covering the tubules or the peritubular dentin. It is possible to assume that the HNTs have strengthened the hybrid layer due to the interlocking effect, leading to the occurrence of irregularly-oriented adhesive failures, although still related to adequate bond strength values (Table 2). Despite this assumption, one should also infer that a better mechanical interlocking would occur when the HNTs are incorporated into the primer component, since they would be in direct contact with the collagen fibrils during the priming of dentin; conversely, the HNTs found in the modified adhesives may have only roughly reached the dentin tubules and collagen network, thus minimizing their coating effect on dentin and the consequent strengthening effect, producing a greater occurrence of adhesive failures [49]. As shown in Figure 2, the dentin tubules (i.e., adhesive failure) were more representatively observed when dentin was treated with CHX.

The reliability of the resin-dentin bonds was also investigated in this study in order to verify whether one bonding protocol would demonstrate better structural consistency when comparing to the other protocols tested. According to Table 3 and Figure 3, the Weibull modulus did not differ among the groups at both periods investigated, suggesting that all experimental materials are as reliable as the control. The Weibull modulus is a parameter that describes the variability of the strength of a material and its likelihood to break at a stress much lower than a mean experimental value [50, 51]. This parameter is commonly used in the evaluation of materials containing a filler phase due to its direct relationship to toughness and brittleness characteristics [52]. Despite our experimental materials were not comprised of regular fillers (e.g., glass fillers, silica), the HNTmodified primers/adhesives would demonstrate a heterogeneous phase, so that the Weibull analysis contributed for the elucidation that HNT incorporation do not impair the structure of the modified materials, making their suitable for clinical application. Concerning the characteristic strength parameter, which corresponds to the stress level giving a 63.2% probability of failure [53, 54], groups were all similar at the 24 h period, but at the long-term the groups treated with the CHX0.2% protocol as well as with the CHX-modified primers resulted in greater strength than the other groups. This is probably related to the presence of CHX that was released and directly rubbed onto the collagen fibrils, allowing for the formation of adequate hybrid layers with apparent better resistance to hydrolysis. Also, the possibility of functioning in the inhibition of MMPs over time may guarantee preservation of the resin-dentin bonds, as suggested in Figure 3, wherein the foregoing groups (especially CHX0.2% and P_CHX10%) showed a slightly greater tendency to support stress when compared to the control and groups treated with the HNT-modified adhesives (see that the former groups maintained a 1.0 survival probability at greater stress than the other groups). Therefore, it seems that the CHX-modified primers prepared in our study could become a suitable therapeutic adhesive system with confirmed benefits in the adhesion of dental restorations. Future studies should focus on encapsulating nanotubes with other bioactive compounds in order to modify dental primers' composition, aiming the stabilization and strengthening of dental bonds.

In conclusion, and within the limitations of this study (e.g., only 6 months of water storage and a lack of using interfacial analyses to characterize the hybrid layers after aging), we demonstrated for the first time the potential application of modified dental primers with CHX-encapsulated nanotubes in the preservation of resin-dentin bonds over a 6-month time period. Furthermore, the modification of a primer or adhesive was an advantageous approach that did not impair the polymerization, viscosity, and bonding performance of the materials, showing a promising long-term effect on resin-dentin bonds. The concentration threshold of CHX to be encapsulated into the nanotube needs further investigation to elucidate its effects on the stabilization of dental bonds.

References

[1]. Moraschini V, Fai CK, Alto RM, Dos Santos GO. Amalgam and resin composite longevity of posterior restorations: A systematic review and meta-analysis. J Dent. 2015;43:1043–50.

[2]. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27:1–16. [PubMed: 21112620]

[3]. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol. 1999;6:437–9. [PubMed: 10225852]

[4]. Tjäderhane L PH, Sulkala M, Wahlgren J, Salo T. The expression of matrix metalloproteinases (MMPs) in human odontoblasts In: Proceedings of the International Conference on Dentin/Pulp Complex, 2001 Ishikawa T, Takahashi K, Maeda T, Suda H, Shimono M, Inoue T, editors Chicago, IL: Quintessence Publishing Co, Ltd. 2002:pp. 45–51.

[5]. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res. 2011;90:953–68. [PubMed: 21220360]

[6]. MazzonA, PashleH, NishitanY, BreschL, MannellF, TjäderhanL, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006;27:4470–6.

[7]. Carrilho MR, Carvalho RM, de Goes MF, di Hipólito V, Geraldeli S, Tay FR, et al. Chlorhexidine preserves dentin bond in vitro. J Dent Res. 2007;86:90–4. [PubMed: 17189470]

[8]. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24:90–101. [PubMed: 17442386]

[9]. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005;84:741–6. [PubMed: 16040733]

[10]. Maravić T, Comba A, Cunha SR, Angeloni V, Cadenaro M, Visinitini E, et al. Long-term bond strength and endogenous enzymatic activity of a chlorhexidine-containing commercially available adhesive. J Dent. 2019;84:60–6.

[11]. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. Minerva Stomatol. 2012;61:399–419. [PubMed: 22976567]

[12]. Chang YE, Shin DH. Effect of chlorhexidine application methods on microtensile bond strength to dentin in Class I cavities. Oper Dent. 2010;35:618–23.

[13]. Dionysopoulos D Effect of digluconate chlorhexidine on bond strength between dental adhesive systems and dentin: A systematic review. J Conserv Dent. 2016;19:11–6. [PubMed: 26957786]

[14]. Stanislawczuk R, Reis A, Loguercio AD. A 2-year in vitro evaluation of a chlorhexidine-containing acid on the durability of resin-dentin interfaces. J Dent. 2011;39:40–7.

[15]. Silva Sousa AB, Vidal CMP, Leme-Kraus AA, Pires-de-Souza FCP, Bedran-Russo AK. Experimental primers containing synthetic and natural compounds reduce enzymatic activity at the dentin-adhesive interface under cyclic loading. Dent Mater. 2016;32:1248–55. [PubMed: 27524231]

[16]. Castellan CS, Pereira PN, Grande RH, Bedran-Russo AK. Mechanical characterization of proanthocyanidin-dentin matrix interaction. Dent Mater. 2010;26:968–73. [PubMed: 20650510]

[17]. EkambaraM, YiK, MatinlinnP, KinM, TaR. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. J Dent. 2014;42:709–19.

[18]. Sabatini C, Pashley DH. Aging of adhesive interfaces treated with benzalkonium chloride and benzalkonium methacrylate. Eur J Oral Sci. 2015;123:102–7. [PubMed: 25639285]

[19]. Munchow EA, Bottino MC. Recent advances in adhesive bonding - The role of biomolecules, nanocompounds, and bonding strategies in enhancing resin bonding to dental substrates. Curr Oral Health Rep. 2017;4:215–27. [PubMed: 29177123]

[20]. Alamri H, Low IM, Alothman Z. Mechanical, thermal and microstructural characteristics of cellulose fibre reinforced epoxy/organoclay nanocomposites. Composites Part B: Engineering. 2012;43:2762–71.

[21]. Chen Q, Zhao Y, Wu W, Xu T, Fong H. Fabrication and evaluation of Bis-GMA/TEGDMA dental resins/composites containing halloysite nanotubes. Dent Mater. 2012;28:1071–9. [PubMed: 22796038]

[22]. Vergaro V, Abdullayev E, Lvov YM, Zeitoun A, Cingolani R, Rinaldi R, et al. Cytocompatibility and uptake of halloysite clay nanotubes. Biomacromolecules. 2010;11:820–6.

[23]. Feitosa SA, Münchow EA, Al-Zain AO, Kamocki K, Platt JA, Bottino MC. Synthesis and characterization of novel halloysite-incorporated adhesive resins. J Dent. 2015;43:1316–22.

[24]. Feitosa SA, Palasuk J, Geraldeli S, Windsor LJ, Bottino MC. Physicochemical and biological properties of novel chlorhexidine-loaded nanotube-modified dentin adhesive. J Biomed Mater Res B Appl Biomater. 2019;107:868–75. [PubMed: 30199597]

[25]. Feitosa SA, Palasuk J, Kamocki K, Geraldeli S, Gregory RL, Platt JA, et al. Doxycycline-encapsulated nanotube-modified dentin adhesives. J Dent Res. 2014;93:1270–6. [PubMed: 25201918]

[26]. Bottino MC, Batarseh G, Palasuk J, Alkatheeri MS, Windsor LJ, Platt JA. Nanotube-modified dentin adhesive--physicochemical and dentin bonding characterizations. Dent Mater. 2013;29:1158–65. [PubMed: 24054334]

[27]. Alkatheeri MS, Palasuk J, Eckert GJ, Platt JA, Bottino MC. Halloysite nanotube incorporation into adhesive systems-effect on bond strength to human dentin. Clin Oral Investig. 2015;19:1905–12.

[28]. Münchow EA, Zanchi CH, Ogliari FA, Silva MG, de Oliveira IR, Piva E. Replacing HEMA with alternative dimethacrylates in dental adhesive systems: evaluation of polymerization kinetics and physicochemical properties. J Adhes Dent. 2014;16:221–8. [PubMed: 24683593]

[29]. Almeida SM, Meereis CTW, Leal FB, Carvalho RV, Boeira PO, Chisini LA, et al. Evaluation of alternative photoinitiator systems in two-step self-etch adhesive systems. Dent Mater. 2020;36:e29–e37. [PubMed: 31831181]

[30]. PerotC, KamozakB, GutierreC, TaR, PuccR. Effect of matrix metalloproteinase-inhibiting solutions and aging methods on dentin bond strength. J Adhes Dent. 2015;17:347–52.

[31]. Feitosa SA, Corazza PH, Cesar PF, Bottino MA, Valandro LF. Pressable feldspathic inlays in premolars: effect of cementation strategy and mechanical cycling on the adhesive bond between dentin and restoration. J Adhes Dent. 2014;16:147–54. [PubMed: 24027771]

[32]. Lee J, Sabatini C. Glutaraldehyde collagen cross-linking stabilizes resin-dentin interfaces and reduces bond degradation. Eur J Oral Sci. 2017;125:63–71. [PubMed: 27862353]

[33]. Satish S, Tharmavaram M, Rawtani D. Halloysite nanotubes as a nature’s boon for biomedical applications. Nanobiomedicine (Rij). 2019;6:1849543519863625.

[34]. Zhang F, Xia Y, Xu L, Gu N. Surface modification and microstructure of single-walled carbon nanotubes for dental resin-based composites. J Biomed Mater Res B Appl Biomater. 2008;86:90–7. [PubMed: 18098184]

[35]. Nuvoli D, Alzari V, Sanna R, Scognamillo S, Alongi J. Synthesis and characterization of graphene-based nanocomposites with potential use for biomedical applications. J Nanopart Res. 2013;15:1512.

[36]. Oguri M, Yoshida Y, Yoshihara K, Miyauchi T, Nakamura Y, Shimoda S, et al. Effects of functional monomers and photo-initiators on the degree of conversion of a dental adhesive. Acta Biomater. 2012;8:1928–34.

[37]. Degrazia FW, Leitune VC, Takimi AS, Collares FM, Sauro S. Physicochemical and bioactive properties of innovative resin-based materials containing functional halloysite-nanotubes fillers. Dent Mater. 2016;32:1133–43. [PubMed: 27393637]

[38]. Luo R, Sen A. Rate enhancement in controlled radical polymerization of acrylates using recyclable heterogeneous Lewis acid. Macromolecules. 2007;40:154–6.

[39]. Yu L, Wang H, Zhang Y, Zhang B, Liu J. Recent advances in halloysite nanotube derived composites for water treatment. Environm Sci: Nano. 2016;3:28–44.

[40]. Liu M, Jia Z, Jia D, Zhou C. Recent advances in research on halloysite nanotubes-polymer nanocomposite. Prog Polym Sci. 2014;39:1498–525.

[41]. Bhattacharya M Polymer nanocomposites-A comparison between carbon nanotubes, graphene, and clay as nanofillers. Materials (Basel). 2016;9.

[42]. LY, HX, RuaJ, ArolD, JC, WeiD, et al. Bonding durability, antibacterial activity and biofilm pH of novel adhesive containing antibacterial monomer and nanoparticles of amorphous calcium phosphate. J Dent. 2019;81:91–101.

[43]. Zhang K, Cheng L, Wu EJ, Weir MD, Bai Y, Xu HH. Effect of water-ageing on dentine bond strength and anti-biofilm activity of bonding agent containing new monomer dimethylaminododecyl methacrylate. J Dent. 2013;41:504–13.

[44]. Van Landuyt KL, De Munck J, Mine A, Cardoso MV, Peumans M, Van Meerbeek B. Filler debonding & subhybrid-layer failures in self-etch adhesives. J Dent Res. 2010;89:1045–50. [PubMed: 20631093]

[45]. Sabatini V, Taroni T, Rampazzo R, Bompieri M, Maggioni D, Meroni D, et al. PA6 and Halloysite nanotubes composites with improved hydrothermal ageing resistance: Role of filler physicochemical properties, functionalization and dispersion technique. Polymers (Basel). 2020;12:211.

[46]. Dafar MO, Grol MW, Canham PB, Dixon SJ, Rizkalla AS. Reinforcement of flowable dental composites with titanium dioxide nanotubes. Dent Mater. 2016;32:817–26. [PubMed: 27059772]

[47]. Yadav R, Kumar M. Dental restorative composite materials: A review. J Oral Biosci. 2019;61:78–83. [PubMed: 31109861]

[48]. Akasaka T, Nakata K, Uo M, Watari F. Modification of the dentin surface by using carbon nanotubes. Biomed Mater Eng. 2009;19:179–85.

[49]. Degrazia FW, Leitune VCB, Visioli F, Samuel SMW, Collares FM. Long-term stability of dental adhesive incorporated by boron nitride nanotubes. Dent Mater. 2018;34:427–33. [PubMed: 29217312]

[50]. Quinn JB, Quinn GD. A practical and systematic review of Weibull statistics for reporting strengths of dental materials. Dent Mater. 2010;26:135–47. [PubMed: 19945745]

[51]. Warangkulkasemkit S, Pumpaluk P. Comparison of physical properties of three commercial composite core build-up materials. Dent Mater J. 2019;38:177–81. [PubMed: 30381634]

[52]. Rosa de LacerdL, BossardM, Silveira MitterhofeJ, Galbiatti de CarvalhF, CarlL, PivE, et al. New generation bulk-fill resin composites: Effects on mechanical strength and fracture reliability. J Mech Behav Biomed Mater. 2019;96:214–8.

[53]. Bona AD, Anusavice KJ, DeHoff PH. Weibull analysis and flexural strength of hot-pressed core and veneered ceramic structures. Dent Mater. 2003;19:662–9. [PubMed: 12901992]

[54]. Vidhawan SA, Yap AU, Ornaghi BP, Banas A, Banas K, Neo JC, et al. Fatigue stipulation of bulk-fill composites: An in vitro appraisal. Dent Mater. 2015;31:1068–74. [PubMed: 26104806]

HIGHLIGHTS

- Degree of conversion was greater for the CHX-free nanotube-modified adhesive.
- Nanotube-modified primers were less viscous than the adhesives.
- At 24 h, all groups showed similar bonding performance and structural reliability.
- At 6-months, CHX-modified primers resulted in greater bond strength than the control.

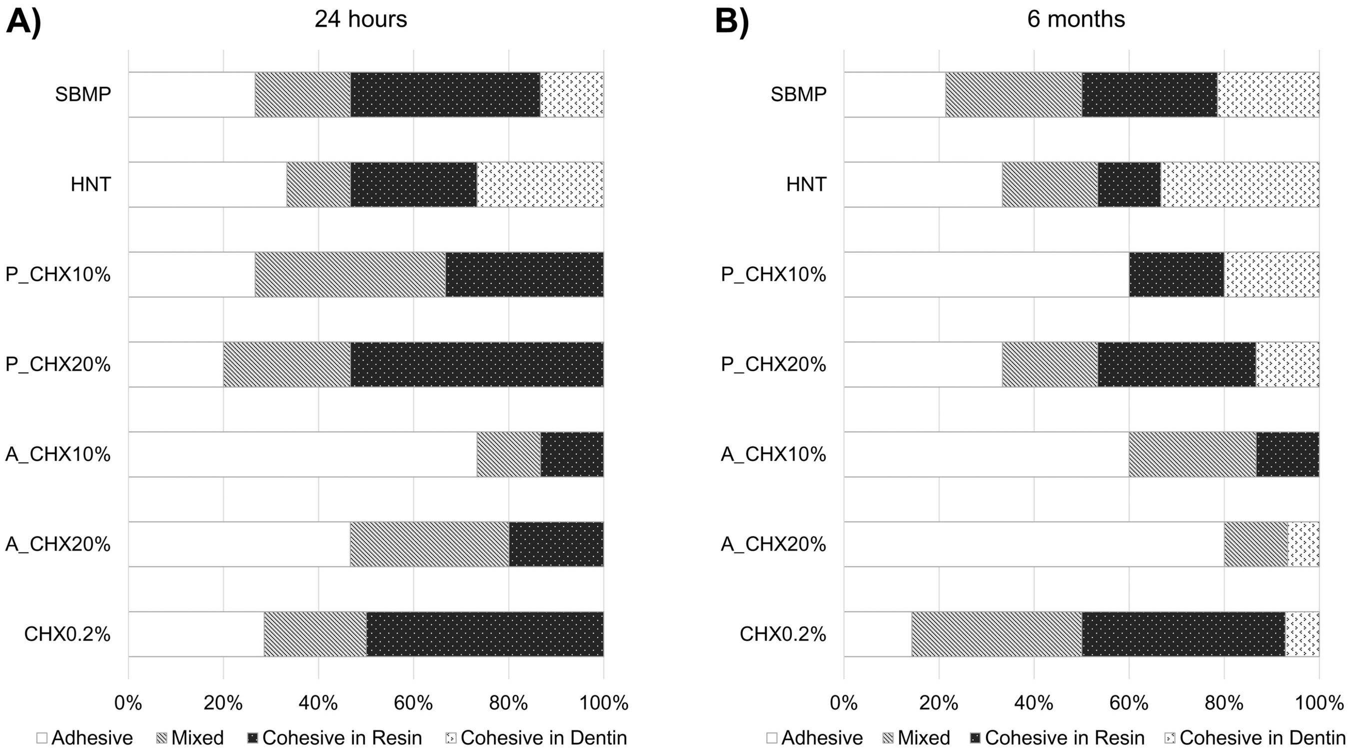

FIGURE 1.

Percentage of the failure modes obtained for each group after 24 hours and 6 months of water storage.

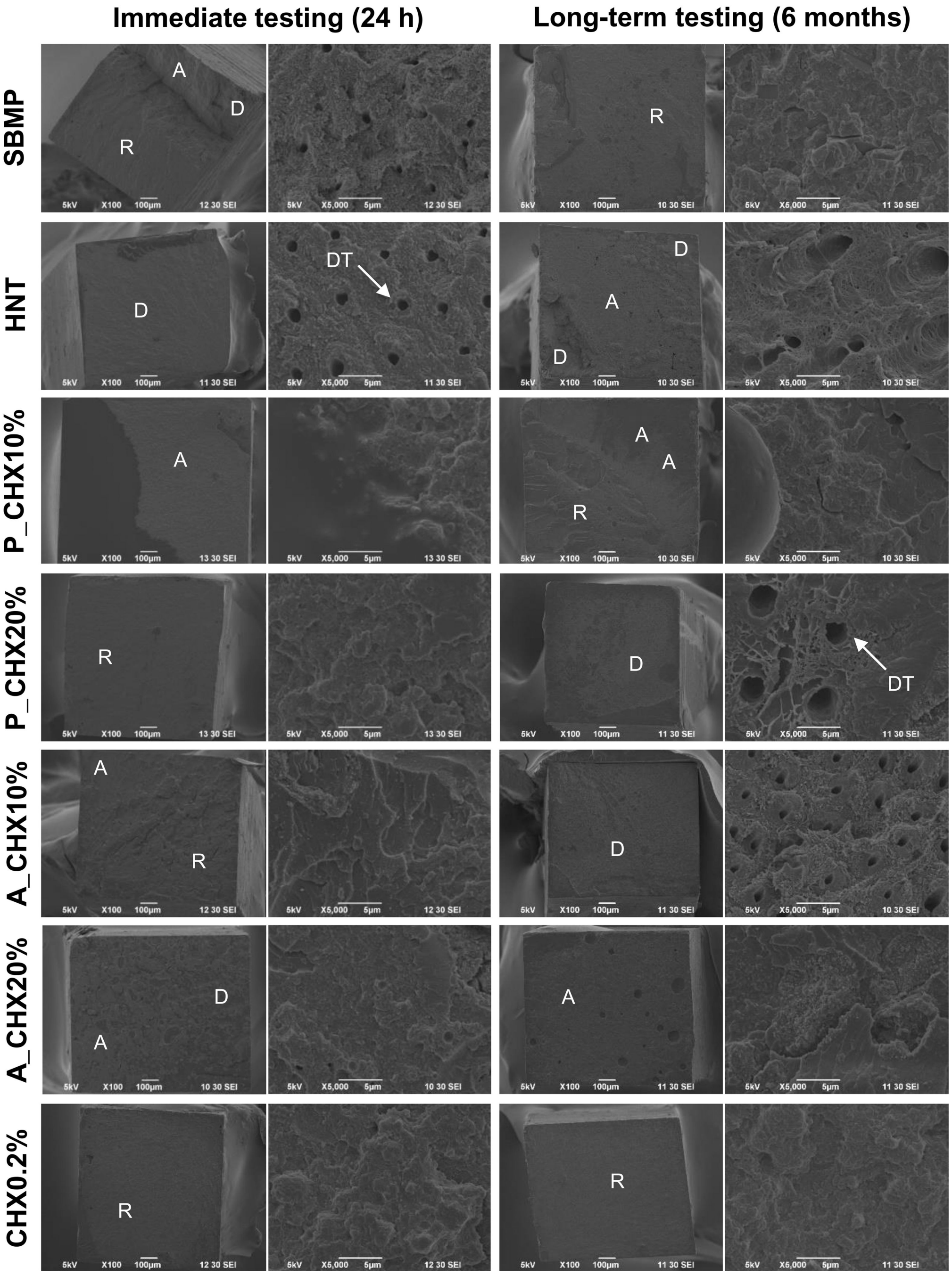

FIGURE 2.

Representative SEM micrographs (100× and 5,000× of magnification) for each group tested in the study at both periods tested (immediate and long-term). A: adhesive layer; D: dentin substrate; R: resin composite; DT: dentinal tubule.

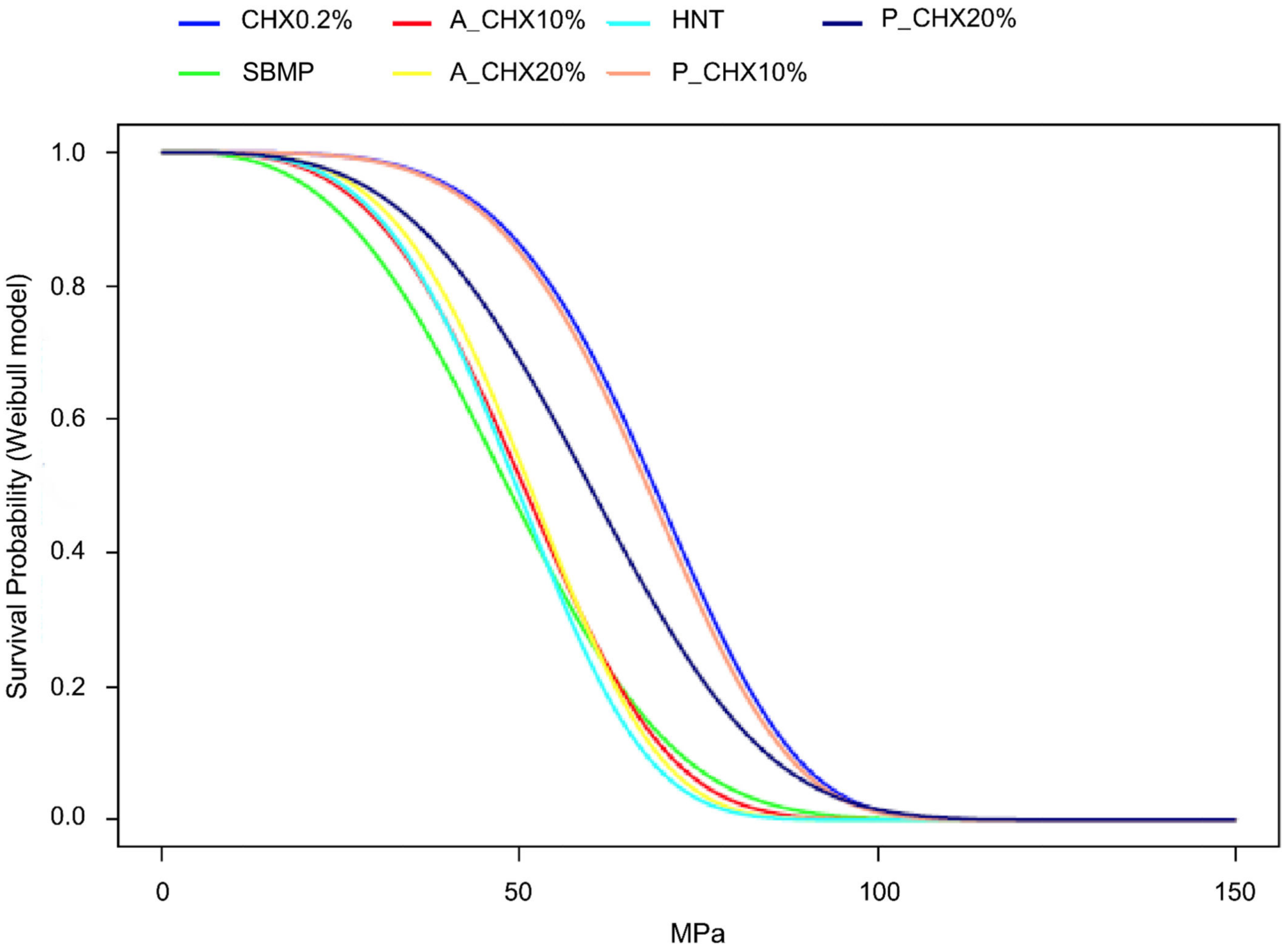

FIGURE 3.

Survival plots for each group tested. Each plot illustrates the relation between the survival probability (Weibull model) and microtensile bond strength values (in MPa). The bond strength values obtained in 24 h were averaged with those from the 6-months period.

Table 1.

Mean and standard deviation (SD) values for the degree of conversion (DC, in %) and viscosity (cP) of the commercial and experimental materials tested in the study.

Materials	**DC (n=3)**	**Viscosity (n=5)**
P_SBMP	*	168.9 (55.4)^{B}
P_CHX10%	*	128.0 (23.5)^{B}
P_CHX20%	*	133.1 (17.7)^{B}
A_SBMP	61.3 (2.6)^{B}	641.9 (77.3)^{A}
A_CHX10%	68.5 (5.6)^{AB}	786.2 (78.4)^{A}
A_CHX20%	70.1 (6.7)^{AB}	786.2 (42.6)^{A}
HNT	74.1 (7.9)^{A}	777.0 (94.6)

P_SBMP: commercial Primer; P_CHX: primer modified with the chlorhexidine-encapsulated nanotubes; A_SBMP: commercial Adhesive; A_CHX: adhesive modified with the chlorhexidine-encapsulated nanotubes; HNT: nanotube-modified adhesive without chlorhexidine.

[*] Primers were not evaluated for degree of conversion.

Distinct letters indicate statistically significant differences among the materials (p<0.05).

Table 2.

Mean and standard deviation (SD) values for the microtensile bond strength of groups tested after immediate (24 h) and long-term (6 months) water storage, and the bond strength variation (mean and SD) of the groups after aging (n=25).

Groups	**Microtensile bond strength (MPa)**		**Bond strength variation (%)**
	24 h	**6 months**	
SBMP	49.1 (12.6)^{B,a}	41.6 (10.6)^{C,a}	−15.4 (4.9)
HNT	60.8 (12.7)^{AB,a}	47.1 (7.5)^{C,b}	−22.5 (7.0)
P_CHX10%	57.4 (11.2)^{AB,b}	67.0 (10.6) ^{A,a}	+16.6 (5.8)
P_CHX20%	52.4 (9.7)^{B,b}	62.9 (15.7)^{AB,a}	+20.1 (6.3)
A_CHX10%	56.4 (10.9)^{AB,a}	52.7 (13.1)^{BC,a}	−6.6 (3.9)
A_CHX20%	60.6 (6.4)^{AB,a}	52.1 (8.0)^{BC,b}	−14.0 (5.0)
CHX0.2%	66.4 (8.8)^{A,a}	71.9 (14.7)^{A,a}	+8.4 (3.2)

Distinct superscript letters in the same column and lowercase letters in the same row indicate statistically significant differences among the groups and between the aging conditions, respectively ($p<0.05$).

Table 3.

Weibull modulus (m) and characteristic strength (σ_0) with 95% confidence intervals for the groups tested in the study (n=25) after 24 h and 6 months of water storage.

Groups	**24 h**		**6 months**	
	***m* (95% CI)**	**σ_0 (95% CI)**	***m* (95% CI)**	**σ_0 (95% CI)**
SBMP	3.0 (1.0 – 4.5) A	66.6 (52 – 69) A	3.0 (2.1 – 5.4) A	54.7 (45 – 58) B
HNT	3.9 (3.1 – 6.7) A	65.5 (56 – 74) A	3.9 (2.7 – 6.2) A	54.5 (49 – 58) B
P_CHX10%	5.5 (3.6 – 7.8) A	58.6 (51 – 68) A	4.8 (4.2 – 7.7) A	73.3 (69 – 77) A
P_CHX20%	3.8 (2.8 – 9.0) A	61.8 (57 – 69) A	3.5 (2.9 – 6.9) A	66.5 (60 – 78) A
A_CHX10%	3.5 (2.9 – 7.2) A	64.8 (54 – 68) A	3.6 (3.1 – 6.5) A	56.0 (52 – 59) B
A_CHX20%	3.8 (2.0 – 6.5) A	67.6 (58 – 73) A	4.0 (2.4 – 6.3) A	56.3 (52 – 59) B
CHX0.2%	4.2 (2.7 – 8.9) A	64.5 (55 – 70) A	4.8 (3.3 – 7.7) A	74.3 (68 – 84) A

Distinct letters indicate statistically significant differences among the groups ($p<0.05$).