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Potential role of surface wettability on the long-term stability of dentin bonds after surface biomodification

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Abstract

Degradation of the adhesive interface contributes to the failure of resin composite restorations. The hydrophilicity of the dentin matrix during and after bonding procedures may result in an adhesive interface that is more prone to degradation over time. This study assessed the effect of chemical modification of dentin matrix on the wettability and the long-term reduced modulus of elasticity (E_r) of the adhesive interfaces. Human molars were divided into groups according to the priming solutions: distilled water (control), 6.5% Proanthocyanidin-rich grape seed extract (PACs), 5.75% 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/1.4% n-hydroxysuccinimide (EDC/NHS) and 5% Glutaraldehyde (GA). The water-surface contact angle was verified before and after chemical modification of the dentin matrix. The demineralized dentin surface was treated with the priming solutions and restored with One Step Plus (OS) and Single Bond Plus (SB) and resin composite. The E_r of the adhesive, hybrid layer and underlying dentin was evaluated after 24 h and 30 months in artificial saliva. The dentin hydrophilicity significantly decreased after application of the priming solutions. Aging significantly decreased the E_r in the hybrid layer and underlying dentin of control groups. The E_r of GA groups remained stable over time at the hybrid layer and underlying dentin. Significant higher E_r was observed for PACs and EDC/NHS groups at the hybrid layer after 24 h. The decreased hydrophilicity of the modified dentin matrix likely influence the immediate mechanical properties of the hybrid layer. Dentin biomodification prevented substantial aging at the hybrid layer and underlying dentin after 30 months storage.

Keywords

Dentin; Type I collagen; Adhesive interfaces; nano-indentation; wettability

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Conflict of interest

The authors declare no conflict of interest.

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1. Introduction

The dentin-adhesive interface is a complex multilayered structure anchored by the resin layer, the dentin-resin hybrid-layer and underlying dentin substrate. Bond strength was shown to decrease in dentin-adhesive interfaces after three and four years aging (Hashimoto et al., 2000; De Munck et al., 2003), likely due to the development of early stage flaws with progressive growth. Poor resin infiltration and incomplete enveloping of the dentin matrix leaves exposed collagen at the adhesive interface (Spencer et al., 2010), where chemical and biological degradation of adhesive components and biological tissue (i.e. collagen) occurs due to the environmental conditions and material susceptibility (Ferracane 2006; Shokati et al., 2010).

The composition of contemporary adhesive systems is driven by the intrinsic properties of the substrate. Because dentin is a wet substrate, hydrophilic and amphiphilic molecules are needed as a bridge between the moist dentin and the highly hydrophobic restorative composite. However, the hydrophilic components are known to produce an adhesive layer that is permeable to water after polymerization and susceptible to hydrolysis of the polymeric chains (Parthasarathy et al., 2012). Recent *in vitro* studies have demonstrated reduced hydrophilicity of the hybrid layer and improved restoration interfaces when ethanol replaces water in the tissue (Pashley et al., 2007; Sadek et al., 2010). While dehydration of the tissue with ethanol is not clinically feasible; decreased swelling of dentin matrices has been observed with plant-derived chemical agents (Castellan et al., 2010). Specifically proanthocyanidin-rich plant derived agents can interact with collagen-based tissue and induce non-enzymatic collagen-cross-linking.

In addition to natural occurring agents, synthetic chemicals can also induce non-enzymatic collagen cross-linking (Bohin et al., 2014). The *in vitro* benefits of these chemical agents for the dentin-adhesive interface has been reported (Dos Santos et al., 2011a;b; Bedran-Russo et al., 2012). However, changes to the physical properties, such as the surface hydration as a result of their interactions with the dentin matrix has not been investigated. They may have an impact in resin-infiltration and therefore the properties of the dentin-adhesive interface components.

In this study, the effects of dentin biomodification strategies on the dentin hydrophilicity and modulus of elasticity of individual components of the dentin-adhesive interface (adhesive layer, hybrid layer, underlying dentin) were studied, as well as the effects of 30-month artificial saliva storage on the degradation of the dentin-adhesive interface components. The hypotheses under study were that (1) dentin biomodification will affect the wettability of the demineralized dentin, (2) in addition to chemical modifications to the collagen, the reduced hydrophilicity will result in improved mechanical properties of the dentin-adhesive interface components and (3) the deleterious effects of long-term artificial saliva storage would be reduced by the use of chemical agents as surface primers.

2. Materials and methods

2.1. Teeth selection

Extracted sound human molar teeth were used following protocol approval by the University of Illinois at Chicago Institutional Review Board Committee (#2009-0198). The occlusal surface of 56 teeth were ground flat using #180, 320 and 600 grit SiC paper (Buehler, Lake Bluff, IL, USA) to expose the superficial dentin. Teeth were randomly divided into the various experimental groups according to the dentin matrix biomodification strategy and adhesive system.

2.2. Preparation of dentin priming solutions

Biomodification of the demineralized dentin surface was carried out for 10 min with the priming solutions containing: 6.5% oligomeric proanthocyanidins (PACs, MegaNatural™ Gold Grape Seed Extract, California, USA); 5.75% 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC, Thermo Scientific Pierce, Rockford, IL, USA), 1.4% N-Hydroxysuccinimide (NHS, Thermo Scientific Pierce) and 5% Glutaraldehyde (GA, Fisher Scientific, Fair Lawn, NJ, USA). The priming solutions were diluted in distilled water and had the pH adjusted to 7.2 (Bedran-Russo et al., 2008). Distilled water was used as a control group.

2.3. Surface contact angle

The water contact angle was evaluated on flat dentin surfaces of 21 human molars. The occlusal enamel was removed and the smear layer on the dentin surface was standardized with abrasive disks (SiC grits #320 and #600). The surface contact angle was evaluated under controlled temperature (23 ± 1 °C) and humidity (greater than 30%), at two time-points: (1) after etching with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA) for 15 s and; (2) after dentin modification with the chemical agents for 10 min. The biomodification agents were applied following the same protocol as used for the restorative procedures. A standard contact angle goniometer model 200-F4 (Ramé-hart instrument co., Succasunna, NJ, USA) was used to dispense one 5 µl drop of deionized water on the dentin surface. The distance from the tip and the dentin surface was kept constant for all assays. The water contact angle was calculated from the image obtained by the coupled CCD camera using the software DropImage (Ramé-hart instrument co.).

2.4. Restorative procedure

Two commercially available bonding systems with different chemistry were applied following the manufacturer's instructions except for the additional application of the priming solutions. Dentin surface was demineralized with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE) and rinsed with water for 15 s. Priming time was set to 10 min. For control group, distilled water was used as priming solution without bioactive agents. Surfaces were washed thoroughly and the adhesive systems One Step Plus (OS – Bisco Inc., Schaumburg, IL, USA) and Single Bond Plus (SB – 3M ESPE, St. Paul, MN, USA), were applied to the dentin surface for 15 s, air dried and light cured for 40 s (Optilux 501, Kerr Corp., Orange, CA, USA).

Resin composite (Filtek Supreme, 3M ESPE) was used to build up a 5 mm height restoration in 3 increments. Each increment was light cured for 40 s with intensity of 600 mw/cm² (Optilux 501, Kerr Corp.), verified by the radiometer coupled to the light curing unit.

Resin-dentin beams with 1 × 1 mm cross-sectional area were obtained using an Isomet precision saw (Buehler) after 24 h storage in artificial saliva at 37 °C. The resin-dentin beams were randomly selected for immediate test and the remaining stored in artificial saliva for 30 months. Artificial saliva was replaced every 2 weeks and it was prepared using 5 mM Hepes, 2.5 mM CaCl₂, 0.05 mM ZnCl₂, 0.3 mM NaN₃ (Tezvergil-Mutluay et al., 2010).

2.5. Interfacial nanomechanical properties

Beams were embedded in epoxy resin (Buehler) and allowed to cure for 8 h at room temperature. The specimens were gloss polished with silicon carbide paper grits 400, 600, 800 and 1200 (Buehler), followed by 9, 6, 3, 1 µm diamond suspension and 0.05 µm alumina suspension polish MasterPrep (Buehler). Specimens underwent ultrasonic cleaning between each suspension for 320 s. The polishing procedures were carried out immediately before the testing (Kinney et al., 1999).

The reduced modulus of elasticity (E_r) of the bonded interface components was evaluated using a customized Triboindenter and a Berkovich fluid cell tip with 100 nm radius (Hysitron Inc, Minneapolis, MN). Prior to testing, a calibration function was carried on a standard quartz sample with known modulus of elasticity ($E_r = 69.6$ GPa) and hardness ($H = 9.36$ GPa). Specimens were attached to a metal disc using cyanoacrylate glue (Scotch, 3M, St. Paul, MN, US) and positioned on the stage. The indentations were performed in a standard trapezoidal load function with 5 s loading and unloading times to a maximum load of 1000 µN, and a hold period of 2 s (Dos Santos et al., 2011a, b). Nine indents were made in each specimen, being three in each component of the bonding interface: adhesive layer (AL), hybrid layer (HL) and underlying dentin (UD). Indentations were carried out with the specimens fully immersed in HBSS (Hank's balanced salt solution, Lonza Group Ltd., Basel, Switzerland) and a minimum distance of 10 µm was respected between each indentation.

E_r was calculated based on the load-displacement curves according to the following relationships (Oliver, Pharr, 1992):

$$E_r = S \left(\frac{\sqrt{\pi}}{2\sqrt{A}} \right)$$

Where, S is obtained from the slope in the initial segment of the unloading curve and A is the projected contact area between the indenter tip and the specimen at maximum load. The contact area is calculated from the contact depth for a Berkovich tip. For the underlying dentin, indentations were performed in the intertubular region (Sauro et al., 2012) as it may governs the elastic behavior of dentin (Kinney et al., 1999) and the collagen fibrils are concentrated in this area (Bertassoni et al., 2012b).

2.6. Statistical analysis

The statistical analyses were performed with SPSS-22 (IBM Corp., NY, US). A two-way ANOVA with repeated measures test was used to analyze the dentin surface wettability before and after application of the priming solutions ($\alpha = 0.05$). The average E_r was calculated from each component of the dentin-adhesive interface and statistically evaluated by a two-way ANOVA and Tukey's post-hoc test, with $\alpha = 0.05$.

3. Results

3.1. Contact angle

The contact angle varied between 13.9° (baseline) and 24.8° (surface treatments). The priming solutions increased the water-surface contact angle (Figure 1) of phosphoric acid etched dentin ($p = 0.004$ for PACs, $p = 0.001$ for EDC/NHS and $p = 0.005$ for GA). No statistically significant differences were observed among the groups treated with PACs, EDC/NHS and GA ($p > 0.05$).

3.2. Nanomechanical properties

Adhesive layer—The reduced modulus of elasticity data is shown in Figure 2. The effects of surface modification and storage were analyzed within each adhesive system. The E_r of the adhesive layer was significantly affected by the dentin surface modification as well as the storage time. The changes were adhesive dependent. Specifically, PACs significantly increased the immediate E_r of OS ($p = 0.003$). The E_r of the adhesive layer was stable over time, and PACs and GA treatment significantly increased E_r of SB after 30 months ($p = 0.004$ and $p = 0.008$, respectively).

Hybrid layer—Although no differences were observed among the chemical dentin treatments, PACs and EDC/NHS treatment significantly increased E_r when compared to control group ($p = 0.002$ and $p = 0.044$, respectively). The 30 months artificial saliva storage has a deleterious effect on the properties of the hybrid layer, except for OS/GA, OS/PACs, SB/GA and SB/PACs. E_r of the hybrid layer significantly decreased after 30 months storage for control groups ($p = 0.004$ for OS and $p = 0.03$ for SB).

Underlying dentin—Except for OS/PACs ($p = 0.000$), there were no statistical differences in the E_r of the underlying dentin at 24 h ($p > 0.05$). The E_r of GA-treated groups remained stable over time ($p > 0.05$). The most significant drop in E_r was observed for the control groups (OS $p = 0.003$ and SB $p = 0.001$). PACs and EDC treated groups also exhibited a decrease in E_r after aging, but the values were statistically higher than the control groups.

4. Discussion

Degradation of the resin-dentin interface remains one of the major shortcomings to the service life of dental restorations and therefore strategies to reduce degradation have been widely investigated. There are two major findings in this study: (1) the decrease in dentin surface hydrophilicity contributes to the enhanced mechanical properties of the adhesive and hybrid layers at the dentin-adhesive interface, and (2) the dentin surface biomodification

effects are long-lasting and decelerate degradation of the interface components after 30 months storage; mostly remarkable at the underlying dentin.

It is known that dentin hydrophilicity increases after acid etching (Rosales-Leal et al., 2001). To facilitate the infiltration within hydrophilic surfaces such as demineralized dentin, organic solvents and hydrophilic monomers are included in the adhesive resins (Van Landuyt et al., 2007). However, moisture in the tissue during resin infiltration results in heterogeneity of the polymeric chain (Ye et al., 2007; Yiu et al., 2005), phase-separation and a more prone-to-degradation polymer is likely to occur when the adhesive systems are polymerized in presence of water (Abedin et al., 2014). In this study, it was shown that dentin matrix biomodification with the priming solutions decreased the hydrophilicity of the demineralized dentin. The cross-linking agents may have changed the dynamics of water within the dentin matrix, by establishing molecular interactions with collagen, resulting in changes on the water content (Fathima et al., 2010). Lower water within the dentin matrix is likely to facilitate the infiltration and polymerization of the adhesives resulting in increased mechanical properties of the adhesive layer and HL.

Overall, nanomechanical properties of the adhesive layer remained stable over time. Evidence to the higher susceptibility to degradation of the hybrid layer over the adhesive layer is supported by permeability and leakage studies (Suppa et al., 2005, Reis et al., 2007). Degradation of the resin components is linked to the hydrophilicity of dental resin monomers (i.e. hydroxyethyl methacrylate and triethylene glycol dimethacrylate) susceptible to ester bonds cleavages by water (Ito et al., 2005; Malacarne et al., 2006; Ferracane 2006). The notable increase in the E_r of the adhesive layer observed for PACs and GA treated specimens can be attributed to the higher density of cross-links as compared to EDC/NHS (Shepherd et al., 2013). The decrease in dentin matrix hydrophilicity may have contributed to improved polymerization and uniformity of the adhesive layer (Wang and Spencer 2005, Yiu et al., 2005, Sadek et al., 2010) and reduced degradation after 30 months (Figure 3).

The immediate E_r of the hybrid-layer was higher after dentin biomodification using PACs and EDC/NHS, in similar manner as described previously when agents were applied for 1 hour (Dos Santos et al., 2011a). EDC/NHS and PACs have been shown to increase collagen cross-linking (Bedran-Russo et al., 2012; Bedran-Russo et al., 2010) and inhibit enzymatic activity (La et al., 2009; Mazzoni et al., 2014; Tezvergil-Mutluay et al., 2012). After 30 months, significant decrease in the E_r was observed for all hybrid layers with exception to GA treated groups. The lowest E_r was observed for control groups (Figure 2). Noticeable stability was observed for GA groups, likely due to the stable nature of the induced covalent cross-links. The decrease of EDC/NHS and PACs groups are likely associated with the low density on induced cross-linking (Shepherd et al., 2013) and the instability of a large density of hydrogen bond (He et al., 2011), respectively.

Collagen degradation at the hybrid layer is well documented and occurs due to the ability of host-derived enzymes to breakdown collagen (Pashley et al., 2004). In this study, significant decrease in the E_r was observed also at the underlying dentin aged in artificial saliva. Therefore, it is likely that the enzymatic and water degrading ability is not limited to the

exposed collagen area in the hybrid layer (Pashley et al., 2004), but reaches a greater extension throughout the underlying undemineralized dentin. Lower dentin degradation was observed for all the treated groups and GA effectively prevented the decrease in E_r at the underlying dentin. This stability is due to the aldehydes great diffusion into biological tissues (Bigi et al., 2001) and ability to establish covalent collagen cross-links (Olde Damink 1995; Stenzel et al., 1974). The high cytotoxicity of GA limits its clinical application (Han et al., 2003); thus less toxic forms of aldehydes should be considered in future studies.

The differences in magnitude observed in the nano-mechanical properties among the three layers of the adhesive interface may affect the propagation of flaws and consequently fracture of restorations (Soappman et al., 2007, Mutluay et al., 2013). Aging of the bonded interfaces has been traditionally assessed by bond strength studies (De Munck et al., 2003; Hashimoto et al., 2000) and the use of nanoindentation expands the characterization of degradation at individual components of the adhesive interface. Biomodification using chemical agents prevented substantial aging of the hybrid layer and underlying dentin. Besides the ability to increase collagen stiffness, it is herein shown that the application of biomodification agents as priming solution changed the surface contact angle of acid-exposed collagen fibrils, and therefore the hydrophilicity of the tissue, likely facilitating water and organic solvent volatilization during the application of adhesive systems. As a result, a less hydrophilic dentin surface likely contributed to increased stability of resin-dentin interface components.

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Fig. 1.

Percentage increase of the water contact angle measured before (baseline) and after application of priming solutions on the demineralized dentin surface. PACs: oligomeric proanthocyanidins; EDC/NHS: carbodiimide hydrochloride/ N-Hydroxysuccinimide; and GA: glutaraldehyde.

Fig. 2.

Box graphs depict the reduced modulus of elasticity (E_r) data in GPa after 24 h and 30 months aging. Delta symbol (δ) indicates groups where statistically significant difference were observed among treatments for the same period. Asterisk (*) highlights statistically significant difference between times for the same group. OS – One Step Plus; SB – Adper Single Bond Plus; PACs – oligomeric proanthocyanidins; EDC/NHS - 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, N-Hydroxysuccinimide; GA – glutaraldehyde.