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

Ariene A. Leme, Cristina M.P. Vidal, Lina Saleh Hassan, Ana K. Bedran-Russo *

Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, Chicago, IL 60612, USA

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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 the dentin matrix on the wettability and the long-term reduced modulus of elasticity (E_r) of 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 assessed 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. 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 E_r in the hybrid layer and underlying dentin of control groups. 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.

1. Introduction

The dentin–resin interface is a complex multilayered structure anchored by the adhesive layer, the hybridlayer and underlying dentin substrate. Bond strength was shown to decrease in dentin–resin 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 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 (Vidal et al., 2014).

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–resin interface has been reported (Dos Santos et al., 2011a, 2011b; Bedran-Russo et al., 2012). However, changes to the physical properties, such as surface hydration as a result of agents interactions with the dentin matrix has not been investigated. Changes to the physical propertiesof dentin matrix may have an impact in resin-infiltration and the dentin–adhesive interface components.

In this study, the effects of surface biomodification strategies on the dentin hydrophilicity and modulus of elasticity of individual components of the dentin-resin interface (adhesive layer, hybrid

* Correspondence to: Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, 801 South Paulina Street, Room 531, Chicago, IL 60612, USA. Tel.: +1 312 413 9581; fax: +1 312 996 3535.
E-mail address: bedran@uic.edu (A.K. Bedran-Russo).

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layer, underlying dentin) were studied, as well as the effects of 30-month artificial saliva storage on the degradation of the 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-resin 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 was 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., Shaumburg, 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/cm^2 (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 $CaCl_2$, 0.05 mM $ZnCl_2$, 0.3 mM NaN_3 (Tezvergil-Mutluay et al., 2010).

2.5. Interfacial nanomechanical properties

Dentin-resin 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, 2011b). 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 and 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., 2012).

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 (α=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 α=0.05.

3. Results

3.1. Contact angle

The average contact angle valuesvaried between 14.6° (baseline/control) and 24.7° (surface treatments). All priming solutions significantly increased the water-surface contact angle (Fig. 1) of phosphoric acid etched dentin (p=0.004 for PACs, p=0.001 for EDC/NHS and

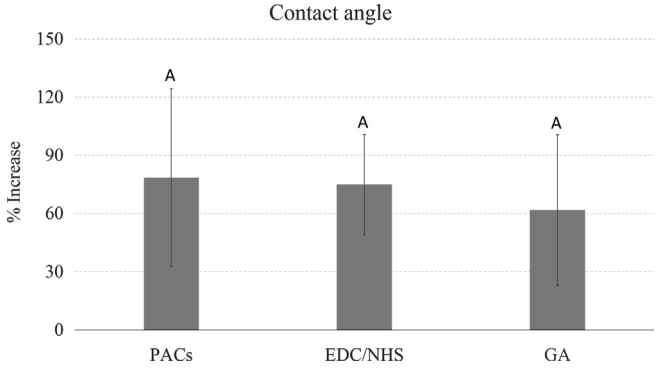

Fig. 1. Percentage increase of the water contact angle measured before (baseline) and after application of priming solutions onthe demineralized dentin surface. PACs: oligomeric proanthocyanidins; EDC/NHS: carbodiimide hydrochloride/N-Hydroxysuccinimide; and GA: glutaraldehyde.