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Effect of partially demineralized dentin beneath the hybrid layer on dentin–adhesive interface micromechanics

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ABSTRACT

Objective: To investigate the presence of non-infiltrated, partially demineralized dentin (PDD) beneath the hybrid layer for self-etch adhesive systems, and its effect on micromechanical behavior of dentin–adhesive interfaces (DAIs). This in-vitro laboratory and computer simulation study hypothesized that the presence of non-infiltrated PDD beneath the hybrid layer does not influence the mechanical behavior of the DAI of self-etch adhesive systems.

Methods: Fifteen sound third molars were restored with composite resin using three adhesive systems: Scotchbond Multipurpose (SBMP), Clearfil SE Bond (CSEB) and Adper Promp L-Pop (APLP). The thickness and length of all DAIs were assessed using scanning electron microscopy, and used to generate three-dimensional finite element models. Elastic moduli of the hybrid layer, adhesive layer, intertubular dentin, peritubular dentin and resin tags were acquired using a nano-indenter. Finite element software was used to determine the maximum principal stress. Mixed models analysis of variance was used to verify statistical differences ($P < 0.05$).

Results: Elastic moduli and morphology were found to differ between the adhesive systems, as well as the presence and extension of PDD.

Significance: Both self-etch adhesive systems (APLP and CSEB) had PDD. The DAI stress levels were higher for the one-step self-etch adhesive system (APLP) compared with the etch-and-rinse adhesive system (SBMP) and the self-etch primer system (CSEB).

1. Introduction

Alterations in the mechanical properties and morphology of dentin beneath the hybrid layer (HL) have been reported when using etch-and-rinse adhesives (Hashimoto et al., 2000a, 2000b; Katz et al., 2001; Sauro et al., 2012; Schulze et al., 2005). These alterations are mainly associated with the depth of demineralization promoted by phosphoric acid conditioning of the dentin substrate (Bouillaguet et al., 2001), collapse of the collagen fibril network (Pashley et al., 1993), and discrepancy between dentin demineralization and efficiency of adhesive infiltration (Oliveira et al., 2004).

However, areas with nanoleakage (Carrilho et al., 2005; Reis et al., 2007a; Tay et al., 2002) or with partially demineralized dentin (PDD) beneath the hybrid layer (Oliveira et al., 2004) have been detected for self-etch adhesive systems (Carvalho et al., 2005). These defects are likely to be a consequence of the discrepancy between self-etch adhesive demineralization and the efficiency of monomer infiltration, or due to incomplete polymerization of monomers in the deepest regions of the hybrid layer (Schulze et al., 2005; Yuan et al., 2007).

Morphological alterations and mechanical properties of the supposedly altered dentin beneath the hybrid layer have only been studied for etch-and-rinse adhesives and mildly acidic, two-step

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self-etch primers (Katz et al., 2001; Oliveira et al., 2004). Little information is available regarding the mechanical properties of dentin following one-step self-etch adhesive systems, which are known to be more acidic and aggressive to the dental substrate, have greater potential for demineralization, and form more complex dentin–adhesive interfaces (DAIs) (Wang and Spencer, 2004). In addition, the influence of PDD on stress behavior and distribution in DAI structures has not been evaluated, to date, for self-etch adhesive systems.

This study investigated the presence of PDD beneath the hybrid layer for self-etch adhesive systems, and its effect on the micromechanical behavior of DAIs. This in-vitro laboratory and computer simulation study hypothesized that the presence of PDD beneath the hybrid layer does not influence the mechanical behavior of the DAI of self-etch adhesive systems.

2. Methods

2.1. Sample preparation

Fifteen human third molars (Protocol # 2009-02142) were used to obtain DAI morphology and mechanical properties. All teeth were stored in Hank's balanced salt solution (HBSS), and used within 3 months of extraction (Habelitz et al., 2002).

After exposure of the dentin surface 3 mm from the enamel–cementum junction, (Isomet 2000; Buehler, Lake Bluff, IL, USA), three non-restored teeth were used to characterize the morphology of dentin. One slab (2 mm thick) of each tooth was obtained by cross-sectioning the tooth perpendicular to the dentin tubules (Isomet 2000).

For the remaining teeth ($n=12$), a standard smear layer was created using silicon carbide paper #600 (Buehler) (Tay and Pashley, 2001). Three adhesive systems were used in accordance with the manufacturers' recommendations, followed by composite resin placement ($n=4$): ScotchBond Multipurpose (SBMP; 3M ESPE, St. Paul, MN, USA), Clearfil SE Bond (CSEB; Kuraray Noritake Dental Inc., Kurashiki, Japan) and Adper Prompt L-Pop (APLP; 3M ESPE). The restored teeth were cross-sectioned mesio-distally with a diamond disc in order to obtain at least four slabs (~2 mm thick) containing the DAI. Two slabs of each tooth were used for scanning electron microscopy (SEM) and two slabs of each tooth were used for nano-indentation.

2.2. Scanning electron microscopy

SEM was used to obtain the morphological characteristics of the DAI for each adhesive system tested, including the thickness of the hybrid layer and the adhesive layer, and the length of resin tags. The non-restored dentin specimens ($n=3$) were used to measure the number and diameter of dentin tubules, and the radius of peritubular dentin. These data were used to build the finite element models.

All restored and non-restored specimens were prepared for SEM in accordance with previous studies, including fixation, dehydration, drying, mounting and polishing (Kaaden et al., 2003; Perdigao et al., 1995).

The restored specimens were ultra-sonicated in 100% ethanol for 5 min, dried, demineralized in 6N HCl for 30 s, and protein was denatured in 2% NaOCl for 10 min (Duarte et al., 2009). After drying, all the specimens (restored and non-restored) were coated with gold using a sputter coater (Emitech K650; Emitech Products Inc., Houston, TX, USA) at 10 mA for 4 min. The slabs were observed under an SEM (Hitachi S-3500 N; Hitachi Science System Ltd., Ibaraki, Japan) at an accelerating voltage of 5 kV and a working distance of 10 mm (2000 × and 4000 ×). Three images were obtained from each slab (left, center and right), and linear measurements were taken using PCI 5.5 Quartz software (Quartz Imaging Corporation, Vancouver, Canada).

2.3. Nano-indentation

The two restored dentin slabs that had not been prepared for SEM were embedded in epoxy resin, ground with silicon carbide papers of decreasing abrasiveness (600–1200 grit), and polished with diamond solution (9, 6, 3, 1 and 0.5 μm) under cloth paper.

The imaging and indentation processes were undertaken using a Berkovich fluid cell diamond three-sided pyramid probe in a nano-indenter (950TI; Hysitron, Minneapolis, MN, USA), and tests were performed in wet conditions (HBSS) (Dos Santos et al., 2011; Habelitz et al., 2002).

A loading profile was developed with a peak load of 300 μN for dentin and 100 μN for the hybrid layer and adhesive layer, at a rate of 60 μN/s and 20 μN/s, respectively, followed by a holding time of 10 s and an unloading time of 2 s. For each indentation, the reduced elastic modulus was calculated from each load-displacement curve, and the elastic modulus was obtained (Anchieta et al., 2014; Oliver and Pharr, 1992).

Prior to indenting the region of interest, the interface was scanned with an in-situ scanning probe microscope with the Berkovich indenter to verify appropriate positioning. Ten indentations were made in the DAI (hybrid layer and adhesive layer) on each slab. Indentations were made horizontally following the adhesive layer and hybrid layer. For intertubular dentin, the first 10 indentations were made below the hybrid layer, moving away from the interface in a single column. Indentations were made in a straight line, at intervals of ~1 μm, from the hybrid layer to the dentin with a load of approximately 300 μN (Fig. 1). A large indent of 2000 μN in the adhesive served as a fiduciary mark. Five additional indentations were made horizontally, far from the DAI, in order to assess intact intertubular dentin (Fig. 1).

2.4. Finite element analysis

A micromodel of dentin restored with composite resin ($41 \times 41 \times 82\ \mu m^3$) (Fig. 2) was built using solid modelling software (SolidWorks 2010; SolidWorks Corporation, Concord, MA, USA), in accordance with previously published data, with appropriate tensile loading (0.03 N), boundary conditions (Anchieta et al., 2007, 2011; Junior et al., 2012; Martini et al., 2009) and cross-sectional area (Fig. 2) (Lin et al., 2011; Phrukkanon et al., 1998) taken into account.

The morphological characteristics and dimensions of the DAI for each adhesive system were obtained from SEM analysis, and are shown in Table 1. All materials were considered to be linear, isotropic and homogenous. Elastic modulus (E) and Poisson's ratio (υ) were used in accordance with Table 2. The elastic modulus of peritubular dentin obtained from the nano-indentation test was 30 ($\pm$3.6) GPa, and the Poisson ratio was 0.3 (Le et al., 2001). Composite resin had an elastic modulus of 30 GPa and a Poisson ratio of 0.3 (Junior et al., 2012). The morphological characteristics and dimensions of the DAI for each adhesive system were used to build geometrical models, as follows:

- SBMP – 4-μm-thick hybrid layer, funnel-shaped resin tags with length of 30 μm and base thickness of 3.6 μm, 15-μm-thick adhesive layer and 5-μm-thick PDD layer;
- CSEB – similar to SBMP, but with 0.85-μm-thick hybrid layer, funnel-shaped resin tags with length of 16 μm and base thickness of 2.5 μm, 10-μm-thick adhesive layer and 1.5-μm-thick PDD layer; and
- APLP – similar to SBMP, but with 3-μm-thick hybrid layer, funnel-shaped resin tags with length of 25 μm and base thickness of 3 μm, 6-μm-thick adhesive layer and 3-μm-thick PDD layer (Table 1 and Fig. 3).

The thickness of the PDD layer was subdivided into 1-μm layers, and the elastic modulus was obtained through nano-indentation in order to reproduce a graded structure. Two other models (CSEB-0 and APLP-0) were created to verify the influence of the PDD layer. These comprised the exact same features of the CSEB and APLP models, correspondingly, but did not include a PDD layer.

Quadratic tetrahedral elements were used for high-quality finite element mesh generation, which was driven by the convergence of analysis (6%). On average, the models presented 1,148,776 elements and 5,077,374 nodes. The bases of all models were fixed in the x, y, and z axes, and loading conditions were applied to each model. For numerical analysis, finite element software (ANSYS Workbench 14.0; Swanson Analysis System, Canonsburg, PA, USA) was used to acquire maximum principal stress (σ_{max}). The loading definition details are presented in Appendix A (Fig. 2).

2.5. Data analysis

Preliminary analysis evaluated distributional assumptions. The elastic moduli of the hybrid layer and adhesive layer were compared using mixed model analysis of variance (ANOVA) ($P<0.05$). For dentin, the mechanical behavior of each adhesive system was compared at increasing distances from the hybrid layer using a mixed model ANOVA; first, with fixed factors of adhesive system (three levels) and distance (linear trend); second, with fixed factors of adhesive system and squared distance (quadratic trend); and third, with fixed factors of adhesive system, linear trend, quadratic trend and their two-way interactions. All models included a random intercept to account for multiple observations from the same samples. The model fit was summarized using Aikaike's Information Criterion, and $P<0.05$ was considered to indicate significance. Statistical Package for the Social Sciences Version 21 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

Descriptive analyses were used to describe results from SEM and finite element analysis.

3. Results

The DAI linear measurements from SEM [mean and standard deviation (SD)] for each adhesive system are presented in Table 1, and Fig. 3 shows the features of each adhesive system interface. The mean number of dentin tubules counted inside a square of 40 μm^2 was 36 (SD 1.3), and the peritubular dentin radius was