Original Article

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Bonding to wet or dry deproteinized dentin: microtensile bond strength and confocal laser micromorphology analysis

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Abstract

Aim: To investigate the influence of deproteinization and moisture condition (wet vs. dry) on the bond strength and micromorphology of resin-dentin bonding interfaces. **Methods:** Dentin surfaces were etched with 37% phosphoric acid for 15 s and rinsed with water. Four groups (n = 10) were tested: WET: dentin was left visibly moist; DRY: dentin was dried with compressed air; WET-D: dentin was deproteinized for 60 s using 10% NaOCl solution and left moist; DRY-D: dentin was deproteinized and dried. Prime&Bond 2.1 adhesive was applied and the teeth were restored with composite resin. Microtensile test was carried out after 24 h, and failure modes classified under magnification. Data were subjected to two-way ANOVA and Tukey's test ($P < 0.05$). The bonding micromorphology was analyzed by confocal laser scanning microscopy. **Results:** The group DRY showed significantly lower bond strength ($P < 0.05$) than the other groups, which were similar to each other ($P > 0.05$). Adhesive failures were predominant. Analysis of micromorphology showed formation of a collagen-resin hybrid layer only for the non-deproteinized groups. Adhesive penetration into the dentinal tubules was deeper for the DRY-D compared to the WET-D group. **Conclusion:** The bond strength was not dependent on the moisture condition and a more homogeneous hybridization was obtained when dentin was deproteinized.

Keywords: bonding agents, dentin, microscopy, confocal, sodium hypochlorite, bond strength.

Introduction

When dentin is acid-etched, the moisture condition of the exposed collagen network is critical for achieving optimal bond strengths[1-2]. Dehydration causes shrinkage and collapse of the unsupported mesh, inhibiting efficient wetting and penetration by the bonding solution[3]. Incomplete resin infiltration leaves an exposed, non-infiltrated zone beneath the hybrid layer[4]. It has been suggested that this exposed collagen network is susceptible to hydrolytic degradation over the course of time[5]. However, pooled moisture must also be avoided, as excess water can dilute the bonding agent and impair the adhesive procedure[6].

Due to the clinical difficulty in maintaining the appropriate moisture level on the etched dentin, alternatives for reducing the technique sensitivity of bonding strategies have been investigated. Gwinnett et al.[7] suggested that the adhesive efficiency relies on resin diffusion into the partially demineralized dentin at the basal portion of the substrate rather than on micromechanical interlocking with the collagen fibrils. According to Vargas et al.[8], the collagen

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layer inhibits penetration of the resin monomers into the dentin, leaving collagen fibrils unprotected and susceptible to degradation. Therefore, it has been suggested that adhesion to the mineral substrate of the dentin after deproteinization with sodium hypochlorite (NaOCl) could produce a more stable bond, as the unprotected collagen areas would be eliminated[9-11].

NaOCl is a non-specific proteolytic agent that effectively removes organic compounds at room temperature[12]. Scanning electron microscopy studies of demineralized dentin treated with NaOCl showed that the collagen network is removed to reveal an eroded, rough mineral surface with numerous lateral branches, larger than normal tubular orifices[13-15]. Previous studies have reported an increase in bond strength to etched dentin when the bonding agent was applied after deproteinization[8,13,16]. Nonetheless, little is known about the interaction between dentin deproteinization and the moisture condition of the substrate during the bonding procedures.

The aim of this *in vitro* study was to evaluate the influence of deproteinization and moisture condition (wet *vs.* dry) on the bond strength of an acetone-based bonding agent to etched dentin. The micromorphology of the bonding interfaces created using the different bonding strategies was also evaluated. The hypothesis tested was that collagen removal would increase the bond strength irrespective of the dentin moisture condition.

Material and methods

The study was approved by the Research Ethics Committee of the Dental School of the University of Passo Fundo, Brazil (Protocol 856/2005). Extracted sound human third molars were immersed in distilled water at 4ºC and used within 4 months after extraction[17].

Preparation of specimens

The roots of 40 teeth were embedded in self-curing acrylic resin. The occlusal enamel was removed using a low speed water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA) to expose a flat area in medium coronal dentin. The dentin surfaces were wet-polished with 600-grit SiC abrasive papers for 30 s using an automatic polisher (Metaserv 2000; Buehler) to standardize the smear layer[17]. All dentin surfaces were etched with 37% phosphoric acid gel (Dentsply Caulk, Milford, DE, USA) for 15 s and rinsed with water for 15 s. The teeth were randomly divided into four groups (n = 10), according to the dentin treatment after acid-etching and rinsing: WET: the dentin surface was dried with a cotton pellet, leaving the dentin visibly moist; DRY: the dentin surface was dried for 15 s using compressed air, leaving the dentin visibly dry; WET-D: the dentin surface was deproteinized for 60 s using 10% NaOCl solution, rinsed with water for 15 s, and dried with a cotton pellet, leaving the dentin visibly moist; DRY-D: the dentin surface was deproteinized for 60 s using 10% NaOCl solution, rinsed with water for 15 s, and dried for 15 s using compressed air, leaving the dentin visibly dry.

The acetone-based, single-bottle adhesive system Prime&Bond 2.1 (Dentsply Caulk) was applied to all groups, according to the manufacturer's directions. A first layer of the bonding agent was applied using a microbrush and left undisturbed for 20 s, and then another layer was applied and gently air-dried for 5 s. After light-activation for 20 s using a quartz-tungsten-halogen curing unit (XL2500; 3M ESPE, St. Paul MN, USA) with irradiance ~600 mW.cm^{-2}, 4 mm height blocks of resin composite (Supreme; 3M ESPE) were built up in 4 increments, which were light-activated for 20 s each. All bonding procedures were performed by only one operator. Specimens were stored in distilled water at 37ºC for 24 h.

Microtensile bond strength (μTBS) test

After storage, the specimens were sectioned to the long axis of the tooth into 1 mm-thick slices using the low speed water-cooled diamond saw. The bonding interface of each slice was trimmed to create an hourglass shape as previously described[18], with trimmed cross-sectional area of approximately 1 mm^2. Each specimen was fixed to the grips of a microtensile device and tested in tension on a mechanical testing machine (DL2000; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm.min^{-1} until failure. After testing, the fractured specimens were carefully removed from the testing device with a scalpel blade and the cross-sectional area at the site of fracture was measured to the nearest 0.01 mm using a digital caliper (Starret, Itu, SP, Brazil). The cross-sectional area was used to calculate bond strength values in MPa. The number of teeth tested was 10 for all groups, as the tooth was considered the experimental unit for the statistical analysis. However, during the trimming procedures, some slabs were lost. Therefore, for each specimen from all groups, 2-4 hourglass-shaped specimens were obtained and the average was recorded as the μTBS value for each tooth. Data were subjected to two-way ANOVA followed by Tukey's test (P < 0.05). The fractured specimens were analyzed under optical microscopy at 200× magnification. The modes of failure were classified as adhesive failure or mixed failure involving bonding agent and dentin.

Confocal laser scanning microscopy (CLSM) analysis

A mass of 0.5% of Rhodamine B fluorescent dye was added to the bonding agent prior to application to the dentin surfaces. For each bonding condition, two dentin slices (2 mm in thickness) were obtained, and the same bonding procedures were used. The fluorescent dye was added to the bonding solution only for the CLSM analysis, not for the bond strength measurements. Therefore, the dye did not have any influence on the bond strength test. The specimens were sectioned longitudinally using the low speed water-cooled diamond saw and embedded in polyester resin. The surfaces were wet-polished with 600 and 1200-grit SiC papers and 3 μm alumina paste, and ultrasonically cleansed for 10 min. A CLS microscope (TCS-SP2; Leica, Heidelberg, Germany) was used to obtain images of the bonded interfaces, focusing on the thickness of the bonding agent, formation of a collagen-resin hybrid layer, and penetration of the bonding solution into the dentinal tubules. The protocol used to obtain the images was described elsewhere[19].

Results

μTBS test

Results for μTBS for all groups are shown in Table 1. The statistical analysis showed significant differences for the dentin treatment (P = 0.002), but not for the moisture condition (P = 0.125). However, the interaction between the two factors was significant (P = 0.025). Significantly higher μTBS values ($P \leq 0.009$) were observed for the groups WET and DRY-D compared to the group DRY. No significant differences were detected between the wet and dry moisture conditions for the deproteinized samples (P = 0.588), and no significant differences were detected between the dentin treatments when adhesion was obtained using the wet-bonding technique (P = 0.465). Results for the failure analysis are also shown in Table 1. A predominance of adhesive

Table 1. Means (standard deviations) for microtensile bond strength and failure mode distribution.

	Bond strength (MPa)		Failure modes (% A – M*)	
Dentin treatment	WET	DRY	WET	DRY
Non-treated	15.3 (7.6) ^{A,a}	8.2 (3.2) ^{B,b}	85 – 15	91 – 9
Deproteinized dentin	17.8 (8.0) ^{A,a}	19.2 (7.2) ^{A,a}	81 – 19	76 – 24

Means followed by different uppercase letters in the same line and lowercase letters in the same column are significantly different at $P < 0.05$. *Percentage of adhesive (A) and mixed (M) failures.

failures was observed for all groups. However, the group DRY-D presented higher percentage of mixed failures than the other groups.

CLSM analysis

Figure 1 shows representative CLSM images for all bonding strategies. Irrespective of the moisture condition, the specimens not exposed to NaOCl showed evidence of hybridization[11], characterized by encapsulation of the inter-tubular collagen mesh by the adhesive resin forming a collagen-resin hybrid layer beneath the adhesive layer. Hybridization was more clearly visible for the WET group compared to the DRY group. The WET group also showed deeper penetration of the bonding agent into the dentinal tubules than the DRY group. For the non-deproteinized samples, a distinct layer of concentrated bonding agent was observed in the bottom of the adhesive layer, characterized by a highlighted thin line right above the hybrid layer. On the other hand, no hybrid layer formation and bonding agent concentration were observed for the deproteinized groups, irrespective of the dentin moisture condition. A thicker layer of bonding agent and deeper penetration into the dentinal tubules was observed for the DRY-D compared to the WET-D group.

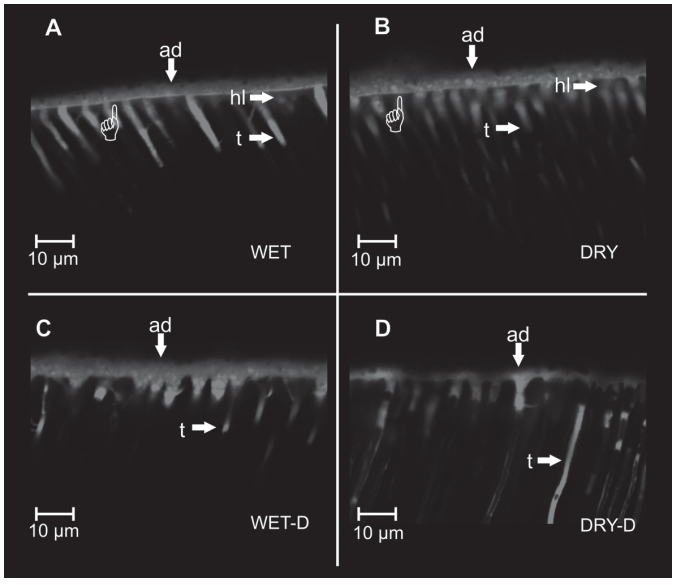

Fig. 1 CLSM images of the resin-dentin bonding interfaces (ad = adhesive; hl = hybrid layer; t = resin tag). The specimens not exposed to NaOCl (A, B) showed formation of a collagen-resin hybrid layer; a distinct layer of adhesive concentration above the hybrid layer (pointers) was also observed. The WET group showed deeper penetration of the bonding agent into the dentinal tubules than the DRY group. No hybrid layer formation or bonding agent concentration was observed for the deproteinized groups (C, D). The DRY-D group showed a thicker layer of bonding agent and deeper penetration of the bonding solution into the dentinal tubules compared to the WET-D group.

Discussion

The present results showed that the deproteinization increased the bond strength to the dry dentin, while no increase in bond strength was observed for the wet condition. Therefore, the tested hypothesis must be partially rejected. The CLSM analysis also showed no formation of collagen-resin hybrid layer for the deproteinized groups. Previous studies agree that the collagen network might not be required to achieve high bond strengths to dentin[1,7-8]. Deproteinized surfaces show a completely eroded, rough mineral surface with numerous lateral branches, larger than normal dentin orifices. These characteristics may explain the improved bonding performance to dry dentin after NaOCl treatment, as higher amount of monomers could diffuse into the irregularities for mechanical interlocking. The increased wettability of the collagen-depleted substrate may also facilitate inter- and intra-tubular resin infiltration[11].

Regarding the moisture conditions, the DRY group showed the lowest bond strength values. It is well-known that dehydration of the demineralized dentin leads to a collapse of the collagen network, impairing infiltration of the bonding agent into the mesh. The CLSM analysis, however, showed formation of a hybrid layer and resin tags even for the DRY group, indicating diffusion of the bonding solution into the exposed mesh. Nonetheless, the WET group showed a more clearly visible hybrid layer, and also deeper penetration of the bonding agent into the dentinal tubules, indicating the wet condition is important for bonding when no deproteinization is carried out. Indeed, no significant differences were detected between the dentin treatments when adhesion was obtained using the wet-bonding technique.

Another aspect to be highlighted is that no significant differences in μTBS were detected between the wet and dry moisture conditions for the deproteinized samples. Also, no layer of bonding agent concentration was detected when deproteinization was performed, probably due to the absence of the collagen mesh interfering with the infiltration of the bonding agent into the substrate. These characteristics provide evidence that the adhesion associated with collagen depletion might have advantageous characteristics. One characteristic is that the moisture condition may not affect the bond strength when deproteinization is carried out. In addition, the bonding agent diffuses better within the deproteinized substrate, forming a more homogeneous bonding layer that may be potentially less sensitive to hydrolytic degradation over the course of time[20].

The DRY-D group presented higher percentage of mixed failures than the other groups. The CLSM analysis provided evidence to explain this result. The group DRY-D showed the deepest penetration of the bonding agent into the dentinal tubules. In corroboration, Dayem[21] reported deeper penetration depth of a one-bottle adhesive through the acid-conditioned dentin after treatment with 10% NaOCl. During the μTBS test, the better mechanical interlocking of the adhesive with

the dentin may favor the generation of mixed failures involving both substrates. However, the deepest penetration into the dentinal tubules did not provide additional increase in bond strength. A probable explanation for this finding is that the deeper resin tags may have occupied the tubular liners without adhering to the tubular walls[22].

In conclusion, the bond strength to dentin became independent of the moisture condition and a more homogeneous hybridization (e.g. no adhesive concentration) was observed when deproteinization was carried out. These findings confirm that the collagen layer is not primordial for bonding, and its presence may impair the diffusion of monomers into the substrate. These results suggest that a clinical adhesive procedure combining collagen and moisture removal might be effective, as the control of the dentin moisture is a critical procedure and the deproteinization may compensate for the dehydration by removing the collapsible collagen fibrils. However, other bonding solutions should be tested, as the effect of NaOCl treatment might be material-dependent[23]. As the results of this study cannot be directly extrapolated to in vivo situations, clinical data are still required.

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