



Different Concentrations of Ethanol as Dentin Pre-treatment to Bonding of an Etch-and-rinse Adhesive

Diferentes Concentrações de Etanol como Pré-Tratamento da Dentina na adesão de um Adesivo Convencional

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Abstract

Objective: to evaluate the bond strength (BS) to dentin pre-treated with ethanol in single application for 20 s after etching and before application of a two-step etch-and-rinse adhesive and to analyze the morphological features at the resin-dentin interface. **Methods:** twenty four third molars were collected and randomly assigned into four groups (n=5) according to the ethanol concentration: control (traditional water-wet technique), 50vol%, 70vol% and 100vol%. In experimental groups, ethanol was applied for 20 s and gently air-dried for 5 s. Bonding procedures were performed and resin composite was built up incrementally. Bonded teeth were sectioned into 0.8mm² sticks on average. These specimens were submitted to microtensile bond strength test (μ TBS). One-way ANOVA and post hoc Tukey's test were applied to analyze statistical data ($\alpha=0.05$). Light microscopy (LM) assisted by Masson's trichrome staining was used to observe the features of resin/dentin interfaces. **Results:** control BS was not different from ethanol 50vol% ($p>0.05$). However, the pre-treatment using ethanol 70vol% and 100vol% showed increase on BS ($p<0.001$ and $p=0.003$ respectively) with no difference between these two groups. LM Showed a discrete decrease in denuded collagen fibrils to teeth treated with 70vol% and 100vol%. **Conclusion:** the pre-treatment of dentin using ethanol may increase the initial BS of resin/dentin interface when applied using 70vol% or 100vol% ethanol. Ethanol 70vol% is also able to decrease the thickness of the resin-sparse collagen fibrils.

Key words: Dentin bonding. Ethanol-saturated dentin. Microtensile bond strength.

Resumo

Objetivo: avaliar a resistência de união (RU) à dentina pré-tratada com etanol em aplicação única por 20 s após condicionamento e antes da aplicação de um adesivo convencional de dois passos e analisar as características morfológicas na interface resina-dentina. **Métodos:** vinte e quatro terceiros molares foram coletados e distribuídos aleatoriamente em quatro grupos (n = 5) de acordo com a concentração de etanol: controle (técnica tradicional - úmida), 50%, 70% e 100%. Em grupos experimentais, o etanol foi aplicado por 20 s e gentilmente seco ao ar por 5 s. Procedimentos de colagem foram realizados e a restauração em resina composta foi construída de forma incremental. Os espécimes foram seccionados em palitos com média de 0,8 mm² e submetidos ao teste de resistência à microtração (μ TBS). One-way ANOVA e teste post hoc de Tukey foram aplicados para analisar os dados ($\alpha = 0,05$). Microscopia ótica (MO) auxiliada pela coloração tricrômica de Masson foi usada para observar as características das interfaces resina-dentina. **Resultados:** a RU do controle não foi diferente do etanol a 50% ($p > 0,05$). No entanto, o pré-tratamento utilizando etanol a 70% e 100% apresentou aumento na RU ($p < 0,001$ e $p = 0,003$ respectivamente) sem diferença entre esses dois grupos. MO apresentou uma diminuição discreta nas fibrilas de colágeno desnudadas nos dentes tratados com 70% e 100%. **Conclusão:** o pré-tratamento da dentina usando etanol pode aumentar a RU inicial da interface resina-dentina quando aplicado nas concentrações de 70% ou 100%. O etanol a 70% também é capaz de diminuir a espessura das fibrilas de colágeno-resina esparsas.

Palavras-chave: Cone-beam computed tomography. Endodontics. Root fracture. Dental radiograph.

INTRODUCTION

Resin-dentin bonds are less durable than resin/enamel bonds^{1,2,3}. In fact, this is due to the fact that dentin bonding relies on organic components which are not remarkable in enamel bonding^{4,5,6,8}. Even though the moisture after etching is essential for successful bonding using etch-and-rinse adhesives, it also affects the long-term bonding stability^{9,10}. Although immediate bond strengths of contemporary adhesives have been acceptable and in some cases relatively high^{6,7,11,12}, substantial decreases occurred after several sorts of aging^{2,8,15,14}.

Resin-dentin bonding uses a partially demineralized dentin collagen matrix as the scaffold for resin infiltration, to produce a hybrid layer that couples the adhesives, the resin composite and the underlying mineralized dentin⁸. However, the permeation of resin monomers within exposed collagen fibrils is not complete^{8,15} and one of the reasons for this to occur is the presence of residual water from the etch-and-rinse technique¹⁶. Water is responsible for maintaining the collagen fibrils (exposed after demineralization) optimally expanded

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for the infiltration of the co-monomer blend and formation of the hybrid layer¹⁷. Nevertheless, excessive water also impairs the infiltration of hydrophobic monomers and precludes the polymerization of the resin facilitating the degradation of the bond over time^{5,9,18}. Furthermore, the resin-sparse collagen fibrils are more susceptible to hydrolytic and enzymatic degradation^{10,19}. Therefore, the control of water content (dentin wetness) after etching is important to create a reliable resin-dentin bond^{1,3,8,20,21}.

In order to assist the removal of residual water and improving the infiltration of hydrophobic monomers into exposed collagen fibrils, as well as to allow the formation of a homogeneous hybrid layer (HL), it has been proposed to saturate with ethanol the etched dentin prior to the application of the two-step etch-and-rinse adhesive, the so-called “ethanol-wet-bonding”²². This technique involves the application of a gradual and increasing concentration of ethanol, starting with 50vol%, 70vol%, 80vol%, 95vol% and finally 100vol%²²⁻²⁶. Such process is required to allow complete exchange of water by ethanol within the partially demineralized dentin^{21,27}. These additional clinical steps are justified to obtain a more adequate infiltration of hydrophobic monomers of the adhesive²⁶ as well as less water content which could result in higher bond strength and more durable resin-dentin interfaces^{23-25,28}. However, this technique is considered clinically unattainable as it is laborious, time consuming and technique sensitive. In an attempt to turn the ethanol pre-treatment clinically viable, an alternative and simplified technique has been suggested, in which seven applications of 100vol% ethanol are conducted, showing promising results^{23-25,29,30}.

Although this simplified technique presented high bond strength, it is still time consuming and unattractive to be used clinically. It would be interesting to observe the effects of ethanol pre-treatment in a single application during a shorter period prior to the adhesive application, in order to make the technique clinically acceptable.

Table 1. Materials used in the study.

| Comercial brand | Main components | Mode of application | Manufacturer |
|---------------------|---|---|--------------|
| Adper Single Bond 2 | Ethanol, bis-GMA, HEMA, GDMA, polycarboxylic acid copolymer, UDMA, water ter, CQ, EDMAB, DPIHFP | Apply two layers of the adhesive resin, gently air-stream for 5 s and light-cure for 10 s | 3M/ESPE |
| Scotchbond Etchant | 35% phosphoric acid, water, silica | Apply a layer for 15 s and wash for 30 s. | 3M/ESPE |
| Filtek Z350 XT | Bis-GMA, UDMA, Bis-EMA, TEGDMA resins, zirconium, silica | Apply increments of 2 mm and light-cure for 20 s | 3M/ESPE |

Microtensile Bond Strength (μ TBS)

Following storage in distilled water at 37 oC for 24 h, each restored tooth was longitudinally sectioned in both “x” and “y” directions, across the bonded interface using a diamond blade

Therefore, the aim of this study was to evaluate the bond strength and morphological features of resin-dentin bonds created using ethanol pre-treatment after the etching procedures by means of a single ethanol application for 20 s. The null hypothesis tested is that ethanol saturated dentin with 50vol%, 70vol% and 100vol% does not interfere on BS.

METHODS

Tooth preparation

Twenty four recently extracted, caries free human third molars were used under a protocol approved by an institutional Research Ethics Committee Institution (Process #107/10). The teeth were stored in 0.01% thymol solution at 4°C and were used within 2 months after extraction. After being copiously rinsed in running water, cleaned, and pumiced, tooth crowns were cut flat using a low-speed diamond saw under water irrigation (Isomet, Buehler; Lake Bluff, USA), and a standardized smear layer was produced on the exposed coronal dentin using 600-grit wet silicon carbide paper mounted in a polishing machine (Aropol 2V – Arotec; São Paulo, SP, Brazil). The teeth were randomly assigned into four groups (n=5) according to the ethanol concentration: control (water), 50vol%, 70vol% and 100vol%. Each tooth was etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE; St Paul, MN, USA) for 30 s and rinsed thoroughly with distilled water for 15 s. The excess of water was removed from the surface with absorbent paper (Mellita; São Paulo, SP, Brazil). In experimental groups, the different concentrations of ethanol were applied for 20 s in a single application. Thereafter, the adhesive and resin composite (Z350 XT 3M/ESPE St. Paul, MN, USA) applications were performed according to the manufacturer’s instructions. Each resin composite increment was light cured for 20 s with 600 mW/cm², by light-curing unit (VALO - Ultradent - South Jordan, UT, USA). The details of all materials used are described in Table 1.

(Isomet 1000, Buehler Ltd., Lake Bluff, USA) to obtain sticks with cross-sectional areas of approximately 0.9 mm². Each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokio, Japan) and fixed to a test apparatus (Bencor Multi-T Device - Danville Engineering, San Ramon, CA, USA) using

cianoacrylate glue (Zapit, Dental Ventures of America, Corona, CA) and stressed to failure with tensile force in a universal testing machine (Instron 4411, Canton, MA, USA) at a cross-head speed of 1 mm/min and data were collected in MPa. The bond strengths from the same bonded tooth were averaged and the mean was used as a statistical unit. The fractured beams were analyzed using a stereomicroscope (Stemi 2000-C, Carl Zeiss Jena GmbH, Germany) at 50x magnification and the failure mode classified as cohesive in dentin (CD), cohesive in resin (CR), adhesive (A) or mixed (M).

Light Microscopy – Masson's trichrome

One tooth from each group were sectioned serial 1 mm thick resin-dentin slabs, which were fixed in a glass holder with cyanoacrylate glue (Super Bonder flex gel – Henkel Ltd., Düsseldorf, Germany) and polished with SiC papers on increasing fine grits (800, 1000, 1200 and 2500) under running water (Buehler, Lake Bluff, IL, USA) reducing the slabs to about 150 µm in thickness. After polishing, the specimens were treated with Masson's trichrome staining technique, as previously described³¹. This staining technique has high affinity for cationic elements normally found in mineralized type I collagen, resulting in blue color. The acid etching of dentin causes the removal of these cationic elements and exposes collagen fibers showing a red pigmentation. These exposed collagen fibrils, showed by LM images, represented by a thin red colored layer at the HL, is called "red zone". Using this microscopic technique, lower incidence of red zones in the interface indicates less denuded collagen fibrils^{5,11}. The composite resin usually stains in beige color. After all staining procedures, the specimens were covered with a glass coverslip and analyzed using light microscopy (LM) with a 400x magnification (Olympus BH-2, Tokyo, Japan). h' Trichrome was performed qualitatively.

Statistical Analysis

SPSS 17.0 (SPSS, Chicago, IL, USA) software was used to perform the statistical analysis. One Way ANOVA was applied to analyze the µTBS data (MPa) complemented by *post hoc* Tukey' multiple comparison test with significance level set at $\alpha=5\%$. Premature failures were noted but not included in the statistical analysis.

RESULTS

Microtensile Bond Strength - µTBS

One-way ANOVA test showed a statistically significant difference on BS between groups ($p<0.001$). Tukey multiple comparison post hoc test showed no difference between control and ethanol 50vol% ($p>0.05$), although pre-treatment with ethanol 70vol% and 100vol% showed statistically higher BS ($p<0.001$ and $p=0.003$, respectively), with no difference between these two treatments ($p>0.05$). Mixed failures (M) were the most common fracture pattern observed in all groups. Mean bond strength and failure mode distribution are summarized in Table².

Table 2. Bond Strength and distribution of Fracture Mode.

| Dentin pre-treatment | µTBS* | Failure Mode | | | |
|----------------------|------------------------------|--------------|----|----|----|
| | | CD | CR | M | A |
| Water (control) | 34,8 ± 9,8 (45) ^b | 9 | 17 | 65 | 9 |
| 50 vol% | 36,1 ± 9,4 (59) ^b | 12 | 15 | 62 | 11 |
| 70 vol% | 41,5 ± 8,8 (53) ^a | 10 | 12 | 71 | 7 |
| 100 vol% | 40,9 ± 8,4 (57) ^a | 13 | 11 | 69 | 7 |

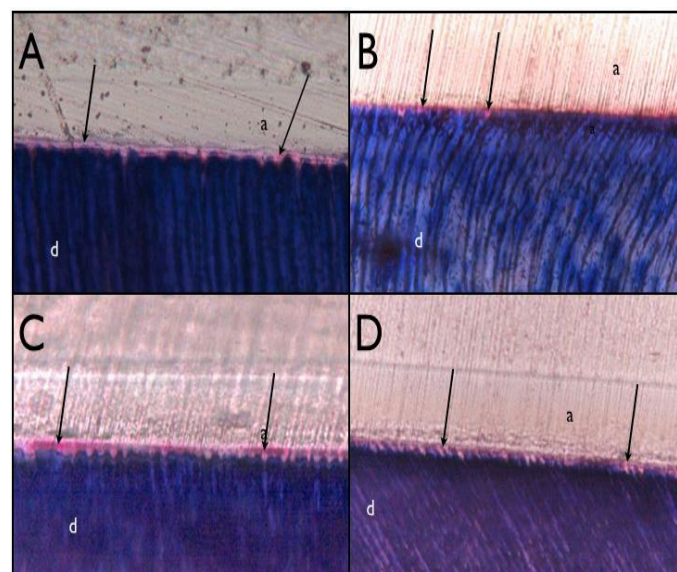
* Bond Strength values are means ± Standard Deviations (beams/group). Different superscripts indicate statistically differences ($p<0.05$).

**Failure modes pattern: CD - cohesive in dentin; CR - cohesive in resin; M - mixed; A - adhesive.

Light microscopy - LM

Representative light microscopy (LM) images of the adhesive interface showed presence of denuded collagen fibrils in all groups, demonstrated by red zones representing non-encapsulated/exposed collagen, able to react with the stain (Fig. 1 - arrows). A thicker red zone (resin-sparse collagen) can be seen along the whole adhesive interface of control and 50vol% groups (Fig. 1A and 1B) suggesting less ability of these techniques in replacing the residual water by polymers. When dentin was treated with 70vol% and 100vol% ethanol, images showed less red zones indicating better infiltration of monomers during the hybrid layer formation, (Fig. 1C and 1D).

Figure 1. Light micrographs Masson's Trichrome of resin-dentin interface.



DISCUSSION

In the present study, it could be observed that dentin pre-treatment using 50vol% ethanol applied for 20 s showed no statistical significant difference on BS ($p>0.05$) in comparison with the traditional water-wet technique (control group) additionally, based on morphological features of the resin-dentin interfaces imaged by LM (Fig. 1A and 1B) we could suggest that the effectiveness in replacing water was the same for these groups. Contrariwise, 70vol% and 100vol% ethanol presented a significant increase on BS (Table 2) compared with control group ($p<0.05$). The null hypothesis was rejected once 70vol% and 100vol% ethanol increased BS despite 50vol% presented similar BS to control (water). The microscopical survey showed that dentin pre-treatment using both 70vol% and 100vol% ethanol achieved higher resin infiltration than using 50vol% ethanol or water-wet bonding, as demonstrated by the less amount of red zones (Fig. 1C and 1D respectively). These findings are not in agreement with those of Sadek et al. (2010), who affirm that 100vol% ethanol applied for 30 s induced low bond strength. On the other hand, Hosaka et al. (2009) showed acceptable performance of 100vol% ethanol applied for 1 min.

Authors have reported that the interfibrillar spaces between collagen fibrils contain a hydrogel composed of proteoglycans^{32,33}. It has been speculated that the presence of this hydrogel may interfere with comonomer infiltration during bonding^{5,11}. However, ethanol removes the water from these spaces, causing the hydrogel to collapse, and shrinks the dehydrated collagen fibrils in a greater extent than the overall shrinkage of the collagen mesh¹⁵, thereby enlarging the interfibrillar spaces and allowing more resin infiltration resulting in a resin-dentin interface with less denuded collagen fibrils^{28,37}. By LM, the dentin pre-treatment using 70vol% and 100vol% ethanol (Fig. 1C and 1D, respectively) presented less incidence of red zones than the control and 50vol% ethanol (Fig. 1A and 1B, respectively). It may be speculated that the former groups achieved improved resin infiltration and reduced resin-sparse collagen which could likely decrease the degradation of the resin-dentin interface.

Although ethanol-saturated dentin might be a better substrate for adhesive infiltration, it may be taking into account that ethanol evaporates faster than water, due to its higher vapor pressure²². Thus, the high volatility of 100vol% ethanol and its rapid evaporation may lead collagen fibrils to collapse unless a continuous and time-consuming application is performed²²⁻²⁵ which sometimes reduces the clinical feasibility of the technique. Although 70vol% ethanol also has a relatively high volatility, the presence of water in its composition decreases its evaporation thereby facilitating the exchange of water and maintenance of the interfibrillar spaces. In fact, this procedure allows better infiltration of the resin comonomer blend²⁹. However, in order to accomplish the optimal resin infiltration, the operator needs to avoid over-wet and over-dry situations which is easier to be

controlled using 70vol% ethanol^{22-25,29}. Furthermore, the water present in 70vol% ethanol facilitates the passage of ethanol through the bacteria cell wall which attains its well-known antibacterial properties^{34,38}.

The hydrophobic monomers are more soluble in ethanol than in water³⁵, and most of the dental adhesives are currently ethanol/water solvated mixtures of different sorts of monomers. Nevertheless, the degradation of the resin-dentin interface is remarkably reduced using more hydrophobic monomers which have higher affinity to ethanol-saturated dentin³⁶. Therefore, it is possible that most of current adhesives may exhibit enhanced penetration in ethanol-wet partially demineralized dentin matrix³⁶.

In an etched and rinsed dentin, the surface remains water-saturated avoiding the collagen shrinkage, and after the experimental treatments the ethanol concentration would certainly diminish. In other words, the 50vol% ethanol when in contact with this water-saturated dentin would be diluted (<50% ethanol), leaving the partially demineralized dentin predominantly water-wet. The same dilution could occur with 70vol% and 100vol% ethanol creating respectively <70% and <100% ethanol concentrations in the substrate. However, even after the dilution, these concentrations (70% and 100%) would likely saturate the partially demineralized dentin into a predominantly ethanol-wet surface due to the higher amount of ethanol. As previously demonstrated²², the overall solubility parameter of dental adhesive resins is higher in ethanol than in water. Therefore, the solubility and infiltration of the comonomer blend in a predominantly ethanol-wet substrate would be improved. This may explain the outcomes observed in the present investigation for 70vol% and 100vol% ethanol pre-treatment (Table 2 and Fig. 1). Indeed, in the 50vol% ethanol pre-treated dentin, this solvent was not able to significantly improve the BS and resin infiltration due to the increasing in the overall water content.

Despite the limitations of this in vitro study, the simplified application of 70vol% and 100vol% ethanol prior to the application of a two-step etch-and-rinse adhesive demonstrated to be effective in terms of initial dentin bonding performance. However, more investigations should be undertaken to confirm the efficacy of such dentin pre-treatments.

CONCLUSION

To conclude, the dentin pre-treatment using ethanol increased the bond strength of the resin-dentin interface when used in 70vol% or 100vol% concentrations.

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