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Ethanol-Wet Bonding May Enhance Infiltration of Nanofillers into Dentinal Hybrid Layers.

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ABSTRACT

The objective of the present study was to examine nanofillers from two adhesives, Single Bond 2 and Prime & Bond NT along dentinal hybrid layers using energy dispersive x-ray spectroscopy (EDS) under ethanol-wet bonding or water-wet bonding conditions. Prepared dentin samples from twelve bovine incisors were divided into four groups randomly according to bonding technique and dentin adhesive system as following; (I) Single Bond 2 + water wet bonding technique, (II) Single Bond 2 + ethanol wet bonding technique, (III) Prime & Bond NT + water wet bonding technique, (IV) Prime & Bond NT + ethanol wet bonding technique. Bonded teeth were sectioned into 1-mm thick resin-dentin slabs. One slab from center of each tooth was used, totaling three slabs for each test group. Silicon detection within dentinal hybrid layer was measured with EDS in three regions across the photographed interfaces. Ethanol-wet bonding technique increased silica uptake within dentin hybrid layers interfaces of Single Bond 2 and Prime & Bond NT. However, there was no significant difference between Prime & Bond NT groups. Ethanol-wet bonding may enhance nanofiller infiltration into demineralized dentin matrices, thus may improve mechanical properties of hybrid layers. However, benefit may depends on solvent/monomer composition of resin adhesive system.

Keywords: Ethanol-wet bonding, nanofiller, resin, hybrid layer

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INTRODUCTION

Bonding mechanism of current resin dentin bonding systems is generally believed to be micromechanical as a result of infiltration of resin monomers into the demineralized collagen matrices of acid-etched dentin and their in situ polymerization [1]. This composite of polymeric resin and collagen fibrils is referred to as the hybrid layer [2]. Optimal infiltration of the resin monomers into mineral-depleted interfibrillar spaces depends on the keeping the demineralized collagen matrices in a hydrated and uncollapsed status [3]. This may be achieved through the use of a water-wet bonding technique [4].

There is a reduction in the tensile properties of dentin as it is depleted of its apatite mineral phase [5]. This is particularly so when the demineralized dentin matrices is prevented from dehydration and saturated with water [3]. Resin infiltration into the demineralized collagen matrices helps to improve the mechanical properties of the mineral-depleted dentin. It was reported that there was a positive correlation between the ultimate tensile strength of dentin adhesive resin and that of the resin-infiltrated demineralized dentin, suggesting that stronger resins could results in the formation of stronger hybrid layers [5, 6].

Simplified etch-and-rinse adhesives consist of combination of primer and bonding resins. Initially, they all contain unfilled resin monomers. However, simplified etch-and-rinse adhesives has been marketed that incorporates the use of nanofillers as a means of strengthening the resin matrix of adhesive. Nanofillers within these adhesives with a primary particle size of 7 nm. Manufacturers claimed that the nanofillers are small enough to infiltrate the interfibrillar spaces of demineralized dentin, which have been reported to be about 20 nm in width [3,7]. Benefit of infiltration of nanofillers into interfibrillar spaces could be strengthening the hybrid layer [8].

The idea of using nanofillers included in dentin adhesive systems to replace apatite phase lost following acid-etching of dentin is theoretically interesting. A stronger adhesive may then produce a stronger layer hybrid layer to resist the forces of polymerization shrinkage if nanofillers can uniformly penetrate the interfibrillar spaces within the demineralized collagen matrices. However, it was reported that this idea could not be achieved under water-wet bonding conditions due to the existence of noncollagenous proteins and proteoglycans which constitute biophysical barriers within the demineralized collagen matrices [9].

On the another hand, it was reported that ethanol-wet bonding technique, which dehydrates water-wet demineralized dentin matrices prior to resin infiltration cause a shrinkage of interfibrillar proteoglycans, resulting wider interfibrillar spaces when compared with water-wet bonding technique [10,11]. It would be reasonable to claim that if ethanol-wet bonding provide wider interfibrillar spaces as a results of the shrinkage of proteoglycans, it also enhance infiltration of nanofillers into demineralized dentin matrices. Therefore, the objective of the present study was to examine nanofillers from two adhesives, Single Bond 2 and Prime & Bond NT along dentinal hybrid layers using energy dispersive X-ray spectroscopy (EDS) under ethanol-wet bonding or water-wet bonding conditions.

MATERIALS AND METHODS

Twelve bovine incisors, stored in 0.02% sodium azide, were used within 6 months after extraction [1]. The roots were severed along the cemento-enamel junction with a low-speed diamond saw with water-cooling. Crowns were embedded into self-curing acrylic resin by using silicone molds. Enamel surfaces were removed with 240-grit silicon carbide abrasive papers under water, exposing dentin surfaces. Dentin surfaces than polished with 600-grit silicon carbide abrasive paper under water.

Prepared dentin samples were divided into four groups randomly according to bonding technique and dentin adhesive system as following; (I) Single Bond 2 + water wet bonding technique, (II) Single Bond 2 + ethanol wet bonding technique, (III) Prime & Bond NT + water wet bonding technique, (IV) Prime & Bond NT + ethanol wet bonding technique. The composition of the dentin adhesive systems is summarized in Table 1. All dentin surfaces were etched with 37% phosphoric acid gel for 15 sec, rinsed with water and kept water-moist. In water wet bonding groups, dentin adhesive systems were applied directly to water-moist acid-etched dentin surfaces according to manufacturer's instructions. In the ethanol-wet bonding groups, water-moist surfaces were converted ethanol-moist surfaces by applying absolute ethanol for 1 min with needle. Special care was given to prevent evaporation of ethanol from surfaces and dryness. Then, dentin adhesives were applied to ethanol-moist

acid-etched dentin surfaces according to manufacturer's instructions. Respective manufacturer's instructions for dentin adhesives are summarized in Table 1.

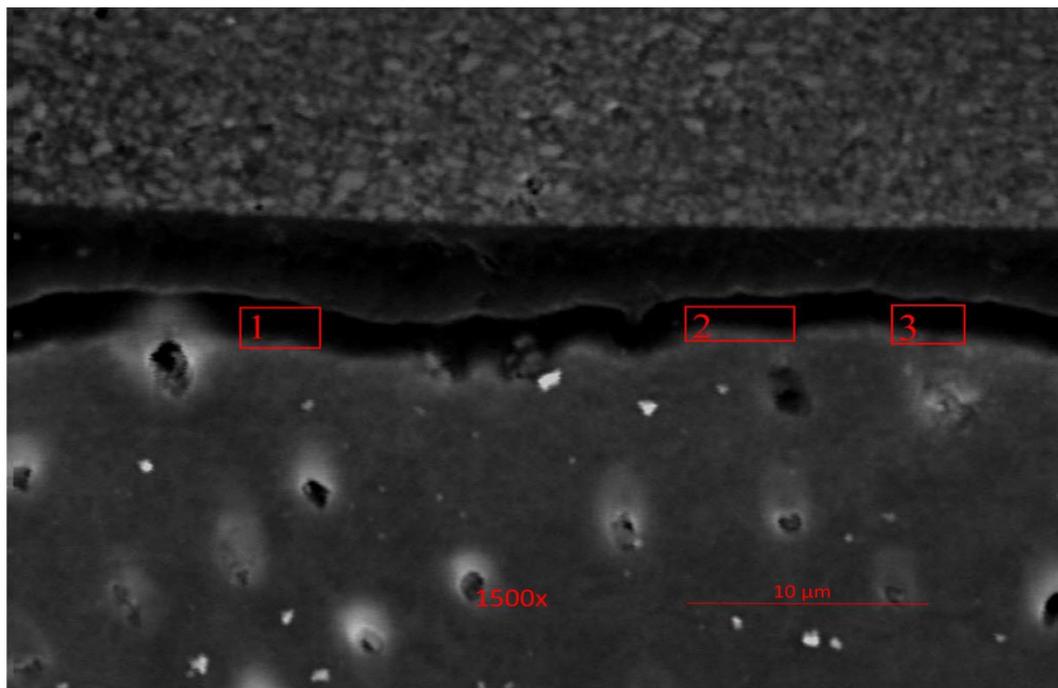
Table 1 Components, and manufacturer's instructions of the simplified-step etch-and-rinse adhesives used in the present study.

Adhesives	Components	Manufacturer's instructions
Single Bond 2 (3M ESPE, St Paul, USA)	Dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photoinitiator	Apply acid-etchant 15 s. Wash 10 s. Blow dry for 2 s. Apply adhesive 15 s with agitation. Air thin 5 s. Photocure 10 s. Apply composite.
Prime & Bond NT, (DENTSPLY, De Trey, Konstanz, Germany)	PENTA, TEGDMA, Bis-GMA, cetylamine hydrofluoride, acetone, nanofiller (amorphous silicon dioxide 8 nm), resin R5-62- 1, T-resin, D-resin, CQ	Apply acid-etchant 15 s. Wash 10 s. Blow dry for 2 s. Apply adhesive 20 s without agitation. Air thin 5 s. Photocure 10 s. Apply composite.
Bis-GMA: bisphenol A diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, PENTA: dipentaerythritol pentacrylate phosphoric acid ester, TEGDMA: triethylene glycol dimethacrylate		

After bonding, Valux Plus that is a composite resin material with 2-mm thickness was placed on the surface and light-cured for 40 seconds 600 mW/cm² of light energy. Following resin buildup placement, bonded teeth were sectioned into 1-mm thick resin-dentin slabs. One slab from center of each tooth was used, totaling three slabs for each test group. Then, specimens were embedded into self-cure acrylic resin and specimens were polished using wet 800-, 1000-, 1200- and 2000-grit SiC paper and finished with diamond polishing paste by means of polishing machine.

The specimens were air dried, mounted on aluminum stubs and placed in a desiccator for 24 h. The specimens were then coated with a thin layer of gold and resin-dentin interfaces were analyzed by scanning electron microscopy (SEM) operated in the secondary electron mode and using an Iridium Ultra energy-dispersive X-ray spectrometer⁵. Resin-dentin interfaces were photographed at 1.500x magnification. Silicon detection within dentinal hybrid layer was measured with EDS in three regions across the photographed interface (Figure 1). Calcium (Ca), phosphate (P), and Silica (Si) were selected for elemental analysis. Silica represents the nanofillers. Calcium and phosphate represent the element of dentin.

Fig. 1. Presentative scanning electron image of resin-dentin interface, showing the regions were silver ion uptake was measured by SEM/EDS.



Descriptive statistics, mean, standard deviations were computed for silicon weight percent for each group. Normality of data was checked with Kolmogorov Smirnov test and homogeneity of variance across the groups was controlled with Levene test, primarily. As distribution of data normal and variance was homogeneity, parametric tests (one-way ANOVA and Tukey HSD test) were used to analyse data. A 5-percent significance level was used for all tests.

RESULTS

Means and standard deviation of silica uptake (%) of each group were as following; Single Bond 2 with water-wet bonding (0.29 ± 0.26), Single Bond 2 with ethanol-wet bonding (0.68 ± 0.34), Prime & Bond NT with water-wet bonding (0.21 ± 0.32) and Prime & Bond NT with ethanol-wet bonding (0.44 ± 0.27). One-way ANOVA revealed that there was a significant difference among groups ($p=0.000$). Ethanol-wet bonding technique increased silica uptake within dentin hybrid layers interfaces of Single Bond 2 and Prime & Bond NT. Post-hoc comparisons showed that silica uptake of hybrid layers made with Single Bond applied with ethanol-wet bonding technique was only significantly higher than those of all other groups ($p=0.000$). There is no significant differences between Prime & Bond NT groups ($p=0.784$).

DISCUSSION

The results obtained in the present study showed that nanofillers from the commercially available adhesives were detected within the dentinal hybrid layers when adhesives were applied by using ethanol-wet bonding technique. However, the same benefit was not shown when adhesives were applied by water-wet bonding technique. Furthermore, nanofillers from Prime & Bond NT adhesive were detected lesser within dentinal hybrid layers when either one of two wet bonding techniques was employed.

Some issues must be addressed before the aim of nanofiller infiltration into acid-etched dentin matrices may be realized. First, interfibrillar spaces within the demineralized collagen matrices may contain a hydrogel of noncollagenous proteins and proteoglycans that may physically impede the infiltration of the nanofillers [12]. Secondly, as the nanofillers and resin monomers differ markedly in molecular weight, the differences in diffusion rate may preclude complete infiltration of the nanofillers into the demineralized dentin matrices zone, even if the interfibrillar spaces are maintained extended [13].

Demineralized dentin matrices as a bonding substrate has always considered as a bed of collagen fibrils floating in water. However, it was demonstrated that the interfibrillar spaces were not simply filled with water after acid-etching [12]. Instead, interfibrillar spaces are filled with noncollagenous proteins and proteoglycans. Proteoglycans, being highly charged polyanions, have a tendency to bind large volumes of water, exerting swelling pressures on the hydrated collagen fibrils and maintaining the integrity of the interfibrillar spaces [14]. The presences of remnant proteoglycans within 20 nm wide interfibrillar spaces would impose severe physical restrictions to the infiltration of the nanofillers. However, it was speculated that dehydration of water-saturated demineralized dentin matrices with absolute ethanol for 1 min, namely deploying the ethanol-wet bonding technique might maintain interfibrillar spaces open as a results of shrinkage of proteoglycans [11]. This enhance resin hybridization quality of the resultant hybrid layers [15], and also may improve nanofiller infiltration into hybrid layers. Finding of the present study showed that ethanol-wet bonding increase amount of silica within hybrid layers of both of tested adhesives. However, benefit of ethanol-wet bonding for Single Bond 2 is significantly better than that of Prime & Bond NT.

Ethanol-wet bonding insignificantly increased silica amounts within hybrid layers of Prime & Bond NT adhesive. Differences in chemical compositions and size of nanofillers of the tested adhesives may play an important role on benefits of ethanol-wet bonding for nanofiller infiltration into acid-etched dentin. Size of nanofillers within the Single Bond 2 (5 nm) is smaller than that of Prime & Bond NT (8 nm). However, Prime & Bond NT uses acetone as a solvent whereas Single Bond 2 uses ethanol/water blend as a solvent to dissolves resin monomers within adhesive solution. Acetone with low hydrogen bonding capacity when compared to ethanol and water, may not able to keep interfibrillar spaces open during resin monomer and nanofiller infiltration. This may results that matrices may collapse again during adhesive blend infiltration, and then, squeeze out infiltrated resin monomer and/or nanofillers from matrices. Yet, solvent of Single Bond 2 is ethanol/water blend might maintain interfibrillar spaces enough open to infiltration of nanofillers along with resin monomers.

In conclusion, the idea of replacing apatite filler crystallites with nanofillers as a strengthening element for demineralized dentin can be achieved with ethanol-wet bonding. Shrinkage of proteoglycans as a results of absolute ethanol application may provide enough access for nanofiller infiltration into acid-etched ethanol-saturated dentin matrices. However, this benefit of ethanol-wet bonding may depend on chemical composition of adhesives.

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