Application of ethanol improves the resin-dentin bond strength of a two-bottle, self-etching primer-adhesive

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ABSTRACT: Purpose: To investigate whether the application of ethanol to a two-step self-etching adhesive can improve its resin-dentin bond performance. Methods: Four different ethanol concentrations were added to the adhesive of Clearfil SE Bond to create four ethanol primers (40, 60, 80 or 100% ethanol). 24 extracted third molars were divided into four groups. Each group corresponded to one of the four hydrophilic ethanol primer concentrations. The teeth in the control group were bonded with Clearfil SE Bond according to the manufacturer’s instructions. The primed teeth in the experimental groups were treated with the ethanol primer prior to application of the solvent-free adhesive. Microtensile bond strengths (mTBS) were tested 24 hours after specimen preparation. Another 14 teeth were bonded using the same methods to evaluate mTBS after 1 year. Nanoleakage was evaluated under field-emission scanning electron microscopy before and after aging. Results: In the 24-hour group, the mTBS in the 60% ethanol/40% adhesive primer group increased significantly (21.6%, P < 0.05) over the no ethanol control. After 12-month water storage, the bond strength of that experimental group was still higher than that of the control group (19.5%, P < 0.05). Before aging, the nanoleakage was clearly seen in the control group but hardly any was seen in the experimental group. After aging, the nanoleakage increase in the experimental group was much less than that in the control group. (Am J Dent 2015;28:224-228).

CLINICAL SIGNIFICANCE: The results showed that application of ethanol primers to primed teeth prior to application of the solvent-free adhesive can greatly improve the bond performance of Clearfil SE Bond.

Introduction

The Tjäderhane et al1 review of dentin bonding concluded that the retention of excess water in etch-and-rinse adhesive bonded with the wet bonding technique fuels the degradation of hybrid layers by endogenous dentin matrix proteases. This is the result of Kanca's water wet bonding technique where 50 vol% water replaces 50 vol% mineral in dentin after acid etching solubilized it to a depth of 8-10 μm. This residual water can induce phase changes in solvated comonomer blends.3,4 The residual water is responsible for silver nanoleakage.6,7 Because acid etching uncovers endogenous dentin matrix proteases and activates them, the residual water provides the water needed to hydrolyze collagen fibrils.8 Ethanol wet bonding was developed in an attempt to remove residual water from acid etched dentin by replacing that water with ethanol, a much better solvent for monomers, that prevents phase changes and greatly reduces not only nanoleakage but hybrid layer degradation over time.10-14

Self-etching adhesives are applied to dry dentin and are not rinsed. The maximum residual water is only 20-30%.15 This water is necessary to ionize the acidic monomers and to solubilize any apatite ions liberated by weak self-etching monomers. Residual water is less than half that seen in etch-and-rinse adhesives. In the two-bottle self-etching primer adhesives, the presence of water is limited to the primer and the adhesive contains no volatile solvents. Most such adhesives contain hydroxyethyl methacrylate (HEMA) that can absorb residual water from the primed dentin into the bottom of the adhesive before polymerization. HEMA-water mixtures form hydrogels that sequester the water and prevent it from interacting with dimethacrylates in the adhesive that might undergo phase changes in the absence of HEMA.

The HEMA-water hydrogels in self-primed dentin may provide activated dentin matrix proteases (MMPs 2, 8, 9 and cathepsins). With the water that is necessary for cleavage of collagen polymers, a slow hybrid layer degradation is seen, even in self-etching adhesives.16,17

If the residual water in primed dentin could be removed prior to application of the water-free adhesive, then these adhesives could be formulated without HEMA, allowing more dimethacrylates to be added, creating stronger cross-linked polymer networks.

The present study added increasing amounts of ethanol to solvent-free adhesives to determine if they can increase the bond strength of Clearfil SE Bond to dentin. If addition of ethanol to Clearfil SE Bond adhesive increased bond strength, then that adhesive could be reformulated without HEMA, allowing proportional increases in dimethacrylates.

It was hypothesized that ethanol could greatly improve the quality of the hybrid layer created by self-etching adhesives by removing residual water, and therefore decreasing nanoleakage in hybrid layers, increasing their bond strength and improving bond durability. The first test null hypothesis was that the “ethanol wet bonding” technique could not increase the immediate bond strength of self-etching adhesives. The second test null hypothesis was that the “ethanol wet bonding” technique could not decrease nanoleakage in dentin hybrid layers created by self-etching adhesives. The third test null hypothesis was that the “ethanol wet bonding” technique could not improve the bond durability of self-etching adhesives over time.

Materials and Methods

Bond strength evaluation - Preparation of ethanol solutions containing different concentrations of hydrophobic resin monomers - A two-step self-etching primer adhesive, Clearfil
SE Bond, was used for this study. Different amounts of water-free Clearfil SE Bond adhesive (hydrophobic resin monomers) were added into 100% ethanol to prepare ethanol primer solutions containing four different concentrations of ethanol: 40 wt%, 60 wt%, 80 wt% or 100 wt%.

**Tooth preparation** - Twenty-four unerupted, caries-free third molars were collected from patients with informed consent. The study protocol was reviewed and approved by the Ethics Committee for Human Studies, Peking University, Beijing, China (Approval number: PKUSSIRB-2012060). These molars were stored in 0.9% NaCl containing 0.02% sodium azide at 4°C for no more than 1 month. The 24 teeth were randomly divided into four groups. Each group corresponded to one of the following four concentrations of ethanol/adhesive primers: 40, 60, 80 or 100 wt% ethanol / 60, 40, 20, 0 wt% adhesive.

The occlusal enamel of each tooth was removed. Each tooth was hemisected facio-lingually into two halves using a slow-speed water-cooled saw equipped with a diamond-impregnated disk (Isomet 1000). Each half was randomly assigned to either control or experimental groups, respectively. The teeth were polished on wet 600-grit silicon carbide paper by hand for 30 seconds to create standard smear layer, a relatively smooth mid-coronal dentin surface with a thin smear layer between 0.8-1.2 μm.¹⁸

**Bonding procedures** - The control group teeth were bonded with Clearfil SE Bond according to the manufacturer’s instructions. That is, the SE primer was applied and left for 20 seconds; air dried for 10 seconds; then the SE adhesive was applied and distributed evenly with mild air flow, followed by light curing for 20 seconds using an LED light-curing unit (Elipar S10) with an output of 700 mW/cm². For the experimental group teeth, after applying the water-containing acidic primer for 20 seconds and air drying for 10 seconds, three consecutive layers of one of the four ethanol/adhesive primer solutions were applied for 10 seconds over a 30-second period. Then, the bonded surface was air-dried with mild air flow for 10 seconds. Finally, the undiluted, full strength SE adhesive was applied and light cured for 20 seconds. Five or six increments of resin composite (Clearfil AP-Xc) were added to the bonded surfaces and individually light-cured for 20 seconds. The teeth were then stored in distilled water at 37°C for 24 hours.

**Micromorphological analysis of the hybrid layers** - All silver nanoleakage specimens were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. The sticks were placed in 50% ammoniacal silver nitrate in darkness for 24 hours. The ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.¹⁸ Then, the sticks were rinsed thoroughly in distilled water for 5 minutes, and immersed in photo developing solution (RP X-OMAT Iv) for 8 hours under fluorescent light.

All sticks were wet-polished with 600 SiC paper to remove the nail varnish. Then, the specimens were embedded in epoxy resin. After the epoxy resin had set, specimens were wet polished with 1,000, 1,200, 1,500 and 2,000 grit SiC paper. They were ultrasonically cleaned for 1 minute, air-dried, mounted on stubs and placed in a desiccator for 24 hours, and gold-coated (JEOL Fine Coat 1100). Resin-dentin interfaces were examined under a field-emission scanning electron micro-

**Microtensile bond testing** - The teeth were longitudinally sectioned across the bonded interface into 0.9 mm-thick slabs perpendicular to the bonded surfaces with a diamond saw (Isomet 1000). Each slab was then divided into 0.9 mm-wide sticks. Ten to 14 sticks (0.9 × 0.9 × 6 mm) from the center were obtained from each tooth and then were stored in 0.9% NaCl containing 0.02% sodium azide at 37°C for 24 hours before testing for the 1-day group or 1 year for the aged group. Each specimen was individually fixed to a custom-made testing jig with cyanoacrylate cement (RITE-Lok SF600). The specimens were then subjected to tensile load at a crosshead speed of 1.0 mm/minute until failure (EZ-L-1KN).¹⁸

**Debond pathway determination** - Both fractured surfaces of each specimen were observed under a stereomicroscope (SMZ 1500) with ×40 magnification to record the failure modes. The fracture modes were classified as follows: (1) cohesive failures in the resin composite or adhesive resin; (2) adhesive failures in the joint; (3) cohesive failures in dentin; and (4) mixed failures.

**Table 1. In vitro microtensile bond strengths (MPa).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration of hydrophobic resin monomers in ethanol</th>
<th>Sample size</th>
<th>μTBS (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (24 hrs)</td>
<td>100 0</td>
<td>48/6</td>
<td>66.2(8.9)</td>
</tr>
<tr>
<td>Experimental (24 hrs)</td>
<td>20 80</td>
<td>39/6</td>
<td>78.2(8.8)</td>
</tr>
<tr>
<td>Control (24 hrs)</td>
<td>100 0</td>
<td>38/6</td>
<td>69.3(10.5)</td>
</tr>
<tr>
<td>Experimental (24 hrs)</td>
<td>40 60</td>
<td>58/6</td>
<td>76.6(4.4)</td>
</tr>
<tr>
<td>Control (24 hrs)</td>
<td>100 0</td>
<td>48/6</td>
<td>63.5(8.4)</td>
</tr>
<tr>
<td>Experimental (24 hrs)</td>
<td>40 60</td>
<td>48/6</td>
<td>65.6(4.4)</td>
</tr>
<tr>
<td>Control (1 yr)</td>
<td>100 0</td>
<td>14/14</td>
<td>59.6(7.3)</td>
</tr>
<tr>
<td>Experimental (1 yr)</td>
<td>40 60</td>
<td>154/14</td>
<td>71.1(9.0)</td>
</tr>
</tbody>
</table>

Values are mean (SD) n= 6 or 14 per subgroup. Values identified by different superscript letters are significantly different (P< 0.05).

**Bond strength after 1-year aging** - Another 14 freshly extracted third molars were collected. Each tooth was hemisected; one half was assigned to the control group, while the other half was assigned to the experimental group. The tooth preparation, bonding procedures and specimen preparation were exactly the same as described above. As the 24-hour bond strengths of specimens bonded with the ethanol primer of 60% ethanol/40% adhesive were significantly higher than the control (P< 0.05, Table 1), that ethanol primer (60% ethanol/40% adhesive) was used for the 1-year aging group. After the sticks were prepared, two sticks were randomly selected from the control and experimental groups of each tooth. They were used for micromorphological analysis. The other sticks were stored in 0.9% NaCl containing 0.02% sodium azide for 1 year and then microtensile bond strengths were tested. The fracture modes were examined under a stereomicroscope.

**Statistical analysis** - One half of each tooth served as the control and the other half as experimental to minimize the influence from the teeth. In each group, the individual stick values were averaged to obtain a control or experimental mean bond strength per tooth and then all six halves in the 24-hour test or 14 halves in the 1-year test were averaged to obtain a mean for each group. Thus, the statistical unit was teeth, not sticks. Paired-sample t-test was used to compare the effects of the bonding methods (control vs. experimental) on bond strengths. Statistical significance was pre-set at α= 0.05.

**Micromorphological analysis of the hybrid layers** - All silver nanoleakage specimens were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. The sticks were placed in 50% ammoniacal silver nitrate in darkness for 24 hours. The ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.¹⁸ Then, the sticks were rinsed thoroughly in distilled water for 5 minutes, and immersed in photo developing solution (RP X-OMAT Iv) for 8 hours under fluorescent light.
Control (24 hrs) 100% 0% 27/61 15/61 12/61 7/61

Control (24 hrs) 100% 0% 57/141 40/141 41/141 3/141
Experimental (24 hrs) 40% 60% 20/58 10/58 9/58 19/58

xx/XX: xx=numbers of sticks tested reporting the indicated failure mode; XX= total sticks tested.

Control (24 hrs) 100% 0% 12/48 12/48 8/48 16/48
Experimental (24 hrs) 0% 100% 35/50 9/50 1/50 5/50

Results

Immediate bond strengths - None of a total of 685 specimens failed during the pre-testing phase. The means and standard deviations of the microtensile bond strengths (mTBS) are summarized in Table 1. When the concentration of hydrophobic resin monomers in the ethanol solution was 0% (i.e. when the primer was 100% ethanol), the bond strength of experimental group decreased to about one-half compared to controls (48.9%, P< 0.05). When the concentrations of hydrophobic resin monomers were 20% and 60% (i.e. 80% or 40% ethanol), the bond strength of the experimental groups increased to about one-half compared to controls (48.9%, P< 0.05). When the concentration of ethanol was 60% and 3.3%, respectively, however without statistical significance (P> 0.05). When the concentration of ethanol was 60% and adhesive concentration was 40%, the 24 hours bond strength of experimental groups increased significantly (21.6%, P< 0.05). After 12-month water storage, the bond strength of the experimental group was still higher than that of the control group (19.5%, P< 0.05) (Table 1).

Distribution of the failure modes - Table 2 summarizes the distribution of the failure modes. At the 24-hour testing period, the 100% ethanol/0% adhesive experimental group had many more adhesive joint failures compared with its corresponding control group. In the experimental groups containing 80%, 60% or 40% ethanol, the experimental groups had similar failure mode distribution with their control groups. In the aging experiment, after 1 year water storage, the experimental group bonded with 60% ethanol/40% adhesive still had similar failure mode distribution as its control group.

FE-SEM analysis - Backscattered electron images can reveal the size and distribution of silver grains that accumulate in water-filled interfacial voids after immersion in silver nitrate. At the 24-hour examination period, there was a significant difference of nanoleakage size and distribution between the groups. In the control group, silver deposits could be clearly seen; in the experimental group, few silver deposits could be seen. After 1-year water storage, the area of silver deposits increased significantly and assembled (white arrow) along the entire hybrid layer in the control group. The increase in nanoleakage was less in the experimental group than in the control group.

Table 2. Distribution of the failure modes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration of hydrophobic resin monomers in ethanol</th>
<th>Cohesive failures in the resin composite or adhesive resin</th>
<th>Failures in the adhesive joint</th>
<th>Cohesive failures in dentin</th>
<th>Mixed failures</th>
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<tr>
<td></td>
<td>Resin (%)  Ethanol (%)</td>
<td>12/48</td>
<td>12/48</td>
<td>8/48</td>
<td>16/48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35/50</td>
<td>9/50</td>
<td>1/50</td>
<td>5/50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/38</td>
<td>7/38</td>
<td>5/38</td>
<td>21/38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27/61</td>
<td>15/61</td>
<td>12/61</td>
<td>7/61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/58</td>
<td>10/58</td>
<td>9/58</td>
<td>19/58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14/48</td>
<td>10/48</td>
<td>10/48</td>
<td>14/48</td>
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<tr>
<td></td>
<td></td>
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<td>41/141</td>
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<td></td>
<td></td>
<td>54/154</td>
<td>52/154</td>
<td>44/154</td>
<td>4/154</td>
</tr>
</tbody>
</table>

Fig. 1. Representative SEM micrograph taken from stained, undemineralized sections of 24-hour composite-dentin sticks bonded with SE Bond adhesive (control group). A: adhesive; H: hybrid layer; D: dentin. The white arrow showed granular or dendritic silver deposits that represented sites of nanoleakage. These silver deposits were predominantly located along the bottom portion of the hybrid layer.

Fig. 2. Representative SEM micrographs taken from stained, undemineralized sections at 24 hours (a, c) and 1 year (b, d) composite-dentin sticks bonded with SE Bond adhesive using conventional bonding method (a, b) and ethanol wet bonding technique (c, d). A: adhesive; H: hybrid layer; D: dentin; R: resin composite. At the 24-hour examination period, there was a significant difference of nanoleakage size and distribution between the groups. In the control group, silver deposits could be clearly seen; in the experimental group, few silver deposits could be seen. After 1-year water storage, the area of silver deposits increased significantly and assembled (white arrow) along the entire hybrid layer in the control group. The increase in nanoleakage was less in the experimental group than in the control group.
Discussion

Although most adhesive monomers are solvated in ethanol, acetone or HEMA, they are near their solubility limit. When such mixtures are applied to water-saturated dentin matrices, the adhesive solvents immediately mix with the water-saturated matrix. This can induce phase changes that can progress to precipitation of monomers from solution. Both processes interfere with uniform resin infiltration of dentin and produce nanometer-size water-filled porosities within the adhesive.

The adhesive in Clearfil SE Bond contains no water or ethanol. It contains HEMA, bisGMA, and photoinitiators; no volatile solvents. When the adhesive is placed on primed dentin, it is theoretically possible for some residual water in the primed dentin to diffuse into the HEMA portion of the adhesive blend. That water might cause some nanoscopic phase changes in the SE adhesive that could interfere with copolymerization to HEMA with dimethacrylates.

By diluting the SE adhesive with 60 wt% ethanol and applying it three times in quick succession, relatively large amounts of ethanol are applied to the self-etched and primed dentin that may have removed far more residual water than is possible by air drying.

In the current experiment, in the 24-hour bond strength portion of the study, experimental teeth that had been primed with Clearfil SE primer and then treated with a 100% ethanol primer, prior to the application of SE adhesive provided microtensile bond strengths that were only 50% as great as the control group, indicating that repeated treatment with 100% ethanol may have extracted HEMA, photosensitizers and photoinitiators from the primed dentin. When the Clearfil SE adhesive was applied to the dry surface, it may not have wet that surface as well as occurred in the control.

This provides the rationale for incorporating adhesive components into the ethanol primer. As the adhesive contains HEMA, bisGMA and photoinitiators, ethanol/adsorptive primers may prevent ethanol-extraction of primer-deposited constituents by providing inward diffusion gradients of these compounds that prevent their extraction. Since these ethanol primers contain no water, they could selectively remove residual water from the primed dentin without removing much of the bonding reagents.

As the bond strength of the 60% ethanol/40% adhesive experimental group was significantly higher than that of its control group (P<0.05), the first null hypotheses should be rejected. Although the experimental bond strength of specimens bonded to 40% ethanol/60% adhesive or 80% ethanol/20% adhesive also increased, the increases were not statistical significant (P>0.05). Therefore, 60% ethanol/40% adhesive was the most ideal concentration ethanol to hydrophilic resin monomers. The second null hypotheses should also be rejected because the area of silver nanoleakage deposits was much greater in the control group than in the experimental group at the 24-hour examination period, as well as after 1-year water storage. After 1-year aging, the bond strength of the control group only fell from 76.6 to 71.2 MPa (P>0.05), while that of the experimental group only fell from 63.0 to 59.6 MPa (P>0.05). Since the long-term mTBS did not fall, the third null hypotheses was accepted.

Self-etching adhesives produce much thinner hybrid layers (about 0.5–1.5 μm), compared to etch-and-rinse adhesives (about 5 μm). When self-etching adhesives were first introduced, clinicians argued that nanoleakage should not exist as etch and infiltrate simultaneously. This hypothesis was soon proved wrong by later studies that nanoleakage also exists in the hybrid layers created by self-etching adhesives. It is not caused by disparities between the depths of demineralization and resin infiltration, but by incomplete polymerization and/or hydrogel formation. Contemporary self-etching adhesives are also based on the “water wet bonding” theory since they contain 20–35% water to ionize the acidic monomers. Self-etching adhesive formulations usually include acidic hydrophilic monomers, water, HEMA, and bifunctional dimethacrylates. Water is indispensable for the self-etching adhesives to ionize the acidic monomers, which can release H+ to etch through the smear layer into the underlying dentin. Resin infiltration is also completed in the presence of water. As most acidic monomers and bifunctional monomers are not readily water-soluble, HEMA is introduced as a transitional polymerizable solvent. After monomer infiltration, the volatile solvents and water are partially removed by air-drying. However, HEMA can decrease the vapor pressure of water and interfere with water evaporation, resulting in the formation of homogenous hydrogels and water treeing. Therefore, in the hybrid layers created by self-etching adhesives, one can also observe the presence of silver nanoleakage, although the form of the nanoleakage created by self-etching adhesives have some differences from those created by etch-and-rinse adhesives. After polymerization, HEMA, residual water and unpolymerized acidic monomers can absorb water, resulting in the plasticization of the polymers that decreases their physical properties. The presence of silver nanoleakage in self-etch hybrid layers indicates the presence of uninfilled, naked collagen fibrils in those hybrid layers. Both etch-and-rinse adhesives and self-etching adhesives can expose and activate endogenous dentin matrix MMPs by etching adhesives. The authors did not offer any explanation for that result. They speculated that remnants of acetone or ethanol within the dentin tubules changed the action of the primer by diluting the primer ingredients, affecting the acidifying action of the primer on dentin. In the present study, ethanol was applied after the application of the primer, and therefore, it should not affect the etching ability of the primer. The application of ethanol after priming may have two principal effects. The first effect is to extract residual water from the hybridized matrix and replace it with ethanol. The second effect is to further facilitate dimethacrylate monomer infiltration into interfibrillar spaces, as well as into intrafibrillar spaces. As the resin monomers can infiltrate more completely into the ethanol-filled spaces that could not be infiltrated when water-filled, increasing the concentration of dimethacrylates in the hybrid layer should make the hybrid...
layers more hydrophobic.\(^{30}\) In addition, supplementing resin, such hybrid layers should adsorb less water over time.\(^{30}\) Therefore, the application of ethanol primers containing hydrophobic monomers can greatly improve the quality of hybrid layers. The increase of immediate bond strength and the almost absence of nanoleakage support that speculation.

In this study, the 1-year bond strengths of the control and experimental groups did not decrease significantly over 1-year water storage. This may be due to the excellent bond performance of mild two-step self-etching adhesives, such as Clearfil SE Bond. The 1-year aging period might not be enough to observe the bond strength decrease of Clearfil SE Bond. Longer aging periods should be tested in future studies. In this study, three layers of ethanol solution were applied in the experimental groups, which is more time consuming than the manufacturer’s instructions. Whether one layer or two layers of ethanol solution have the same effect on improving the bond quality of self-etching adhesives should also be evaluated in future studies.

The application of ethanol solution containing hydrophobic resin monomers can improve the quality of the hybrid layers of two-step self-etching adhesives when applied after use of the primer, to decrease the nanoleakage in the hybrid layer, and increase its bond strength and may improve its bond durability.

a. Kuraray, Osaka, Japan.

b. Buehler Ltd., Lake Bluff, IL, USA.

c. 3M ESPE, St. Paul, MN, USA.

d. Shimadzu, Tokyo, Japan.

e. Nikon, Tokyo, Japan.

f. Kodak, Wuxi, China.

g. JEOL, Tokyo, Japan.

h. FEI, Hillsboro, OR, USA.

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