Effect of ethanol-wet-bonding technique on resin—enamel bonds

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KEYWORDS
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Abstract
Background/purpose: It was reported that more stable resin-dentin bonds were achieved by using ethanol-wet bonding. Because it would be difficult to saturate acid-etched dentin with ethanol without saturation enamel, the study was conducted to evaluate the microtensile bond strength (μTBS) of adhesive resins bonded to enamel using water- and ethanol-wet-bonding techniques.

Materials and methods: Flat enamel surfaces from extracted bovine incisor teeth were conditioned with 37% H₃PO₄ (for 15 seconds) before bonding. Two commercial adhesives (Single Bond 2 and All Bond 3) were applied to enamel using water- and ethanol-wet-bonding techniques (for 1 minute) as follows: group I, Single Bond 2 and water-wet bonding; group II, All Bond 3 and water-wet bonding; group III, Single Bond 2 and ethanol-wet-bonding; and group IV, All Bond 3 and ethanol-wet-bonding. Resin-bonded teeth were stored in distilled water (for 24 hours) and sectioned in beams for μTBS testing. μTBS results were analyzed by a two-way ANOVA and LSD test. The resin—enamel interfaces were examined by SEM.

Results: The following mean μTBS values were obtained: 17.4 MPa for group I, 26.4 MPa for group II, 28.7 MPa for group III, and 31 MPa for group IV. For both adhesives, the obtained adhesive-enamel μTBS values with the ethanol-wet-bonding technique were significantly higher than those of the water-wet-bonding technique (P < 0.05).

Conclusion: The ethanol-wet-bonding technique may increase the bond strength of commercial adhesives to enamel. The chemical composition of the adhesives can affect the bond strength of adhesives when bonding to acid-etched enamel, using the ethanol-wet-bonding technique. Some adhesive systems used in the present study may simultaneously be applied to enamel and...
Enamel bonding with an ethanol-wet-bonding technique

Introduction

In spite of all the achieved improvements in adhesive dentistry, there is a consensus that the stability of resin—dentin bonds is problematic. It is well known that resin bonding as a process is far more complex than most dentists realize, and a great number of factors, including water sorption and hydrolysis of the adhesive resin, incomplete resin infiltration, incomplete solvent evaporation, and imperfect monomer/polymer conversion, influence the bonding effectiveness of dental adhesives.

Dental bonding systems are resin blends that possess both hydrophilic and hydrophobic properties, and are thus called amphiphilic. In other words, adhesives are compounds containing both hydrophilic and hydrophobic monomers. Bisphenol A diglycidyl methacrylate (Bis-GMA) monomer is the main component of most dental adhesive systems. However, due to the presence of water in the current wet-bonding techniques, there is the potential for phase separation of the hydrophobic Bis-GMA monomer, which has limited water solubility. To avoid this problem, a hydrophobic Bis-GMA monomer is blended with the hydrophilic 2-hydroxyethyl methacrylate (HEMA) monomer. However, nanoleakage studies identified water-filled channels within hybrid layers, which indicated that not all of the residual water was removed. Recent studies revealed that the most important factor in the failure of resin—biomaterial interfaces, was to increase the hydrophilicity of current dental adhesives. With the aim of optimizing the penetration of hydrophobic monomers, like Bis-GMA, into the wet demineralized dentin, a new technique was developed called "ethanol-wet-bonding". This technique is used to chemically dehydrate the exposed collagen matrices without causing their collapse. The potential of the ethanol-wet-bonding technique to create durable resin—dentin bonds, by replacing most of the water in the demineralized collagen matrices, was demonstrated by in vitro studies using experimental and commercial adhesives. The use of ethanol to saturate demineralized dentin matrices, instead of water, facilitates infiltration of dimethacrylates through the interfibrillar spaces. ethanol-wet-bonding permits adhesives to achieve better sealing between the resin rags and tubule walls. Therefore, ethanol-wet-bonding can increase the durability of resin dentin bonds over longer time periods.

Although there are many advantages of ethanol-wet-bonding, it includes several shortcomings, and certain things must be considered. There are concerns about its biocompatibility and sensitivity to water contamination, and it also requires additional steps and time for the bonding procedure. However, when dentin is saturated with ethanol, it is difficult to keep the enamel wet with water or dry at the same time, like trying to keep enamel dry and the dentin wet. As to clinical considerations, if the enamel can be bonded effectively while it is wet with ethanol, the overall dentin-bonding procedure using ethanol-wet-bonding can be significantly simplified. Therefore, in this study, we assessed the effect of ethanol-wet bonding on resin—enamel bond strengths. The null hypothesis was that there was no significant influence of ethanol-wet-bonding on the adhesion of two commercial etch-and-rinse adhesives to enamel, compared to the conventional wet-bonding technique.

Materials and methods

Two commercially available etch-and-rinse adhesives were used in the present study. The adhesives used and their compositions are shown in Table 1.

Specimen preparation and bonding procedures

Eight freshly extracted bovine incisors were used in the present study. Teeth were stored in a 0.2% sodium azide solution at 4°C prior to use. Following separation of crowns from roots, the crowns were positioned on an acrylic block with the labial surface parallel to the floor. Labial surfaces of the teeth were flattened with a diamond bur (Microdont ISO 806 314.001.524.012, São Paulo, Brazil). Teeth were divided into four groups (n = 2) according to the

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**Table 1** Chemical formulations of the tested adhesives.

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Bond 2, 3M ESPE, St. Paul, MN, USA</td>
<td>Bis-GMA, HEMA, ethanol, water, polyalkenoic acid copolymer (Lot number: 20080912)</td>
</tr>
<tr>
<td>AllBond 3, Bisco Inc., Schaumburg, IL, USA</td>
<td>Part a: ethanol, MgNTG-GMA (Lot number: 0900000382)</td>
</tr>
<tr>
<td></td>
<td>Part b: Bis-GMA, BPDM, HEMA, photoinitiators, stabilizers (Lot number: 0900000018)</td>
</tr>
<tr>
<td></td>
<td>All Bond Resin: Bis-GMA, UDMA, TEGDMA, glass Filler (Lot number: 0900000225)</td>
</tr>
</tbody>
</table>

Bis-GMA = bisphenol A diglycidyl methacrylate; BPDM = biphenyl dimethacrylate or 4,4’-dimethacryloyloxyethoxyloxycarbonylbiphenyl-3,3’-dicarboxylic acid; HEMA = 2-hydroxyethyl methacrylate; MgNTG-GMA = magnesium nitric-triglycidyl glycicyld methacrylate.
bonding techniques (water-wet-bonding or ethanol-wet-bonding technique) and adhesive agents (Single Bond 2 or All Bond 3) as follows; group I, Single Bond 2 with water-wet bonding; group II, All Bond 3 with water-wet bonding; group III, Single Bond 2 with ethanol-wet-bonding; and group IV, All Bond 3 with ethanol-wet-bonding.

In all groups, prepared enamel surfaces were etched with a 37% phosphoric acid gel (Etch 37, Bisco, Schaumburg, IL, USA) for 15 seconds, rinsed, and left moist with water. The adhesive agents were applied to the moist surfaces in the water-wet-bonding technique groups (groups I and II) according to the manufacturer's instructions. For groups III and IV, the ethanol-wet-bonding technique was used. Briefly, ethanol-wet-bonded surfaces were achieved by covering the conditioned, water-rinsed enamel surfaces with absolute (99.5%) ethanol (Tekkim, Bursa, Turkey) for 1 minute. The procedure was performed by keeping the enamel surfaces visibly moist with ethanol prior to application of the resin blends. Then, the remaining bonding procedures were performed according to the respective-adhesive-manufacturer's instructions. Resin composite (Quadrant Universal LC, Cavex, Haarlem, The Netherlands), 4 mm thick, was built up using an incremental technique (four increments), and each increment from the occlusal surface was cured for 40 seconds using a curing unit with an intensity of 700 mW/cm² (Astralis 3, Ivoclar, Vivadent, Schaan, Liechtenstein). Prior to μTBS testing, samples were stored in water for 24 hours.

μTBS test

All composite-resin—enamel samples were sectioned into beams, with an approximate cross-sectional area of 1 mm², by a non-trimming method. We generated 60 ~ 70 sticks for each experimental subgroup. The μTBS of each stick was tested by attaching it to a movable jig, which was attached to a μTBS tester (Bisco) with cyanoacrylate adhesive (Zapit, Dental Venture of America, Corona, CA, USA). The sticks were stressed to failure in tension, using the μTBS tester at a cross-head speed of 1.5 mm/minute. Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV, Tokyo, Japan) at 40× magnification, to determine the mode of failure. Failure modes were classified as adhesive, cohesive (composite/dentin), or mixed. Only the mode of failure. Failure modes were classified as adhesive, cohesive (composite/dentin), or mixed. Only the mode of failure. Failure modes were classified as adhesive, cohesive (composite/dentin), or mixed. Only the mode of failure. Failure modes were classified as adhesive, cohesive (composite/dentin), or mixed.

Statistical analyses

Distributions and equalities of variances of the μTBS data were respectively analyzed using one-sample Kolmogorov-Smirnov and Levene’s tests. Interactions between bonding techniques and adhesives were analyzed using a two-way analysis of variance (ANOVA). A least significant difference (LSD) test was applied for post-hoc comparisons. All statistical tests were performed using SPSS 13 (SPSS, Chicago, IL, USA), and statistical significance was set to P < 0.05.

Results

The μTBS, standard deviations (SD), number of the sticks, and percentages of failure modes of each group are summarized in Table 2.

<table>
<thead>
<tr>
<th>Bonding technique</th>
<th>Adhesives</th>
<th>Microtensile bond strength (MPa) as determined by number of beams (n)</th>
<th>PF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wet bonding</td>
<td>Single Bond 2 (Group I)</td>
<td>a 17.4 ± 4.6 (n = 24)</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>All Bond 3 (Group II)</td>
<td>b 26.4 ± 5.4 (n = 28)</td>
<td>46.6</td>
</tr>
<tr>
<td>Ethanol-wet-bonding</td>
<td>Single Bond 2 (Group III)</td>
<td>a,b 28.7 ± 7.6 (n = 41)</td>
<td>60.2</td>
</tr>
<tr>
<td></td>
<td>All Bond 3 (Group IV)</td>
<td>a 31 ± 7.3 (n = 23)</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Table 2 Results of the microtensile bonding test ranked according to group numbers and adhesive failure (PF) percentages. Number of sticks with adhesive (n) failure are also presented. Different superscripted letters indicate significant differences.

Specimen preparation for scanning electron microscopic (SEM) analysis

Four bovine teeth were used to analyze the enamel hybrid layers under SEM. First, all teeth were separated into two halves with a low-speed diamond saw under copious water. Tooth halves were divided into four groups, with two coupled halves each. One half of a tooth was subjected to water-wet bonding, while the other half of the same tooth was subjected to ethanol-wet-bonding. Labial surfaces of the obtained halves were ground as described above. Adhesive application protocols were applied to each half, according to the assigned groups. Composite was built up to a 2 mm height.

The enamel-bonding interface was assessed by SEM performed on two sections cut perpendicularly throughout the resin-bonded enamel surface of each tooth half, with a diamond saw under copious water. Cross-sections were polished with wet grinding using 2500-grit SiC paper and immersion for 20 seconds in 2 mol/L HCl, to delineate the resin-infiltrated enamel. After air-drying and sputter-coating with gold, each section was analyzed in a JEOL JSM-6400 SEM (Tokyo, Japan). Representative micrographs were obtained at a magnification of 2500×.
bond strengths, with significant differences among all groups. Group III yielded bond strengths with no significant difference from those of groups II and IV. There was no statistically significant difference between groups III and IV ($P = 0.179$). The mean $\mu$TBS value of group IV (All Bond 3 with ethanol-wet-bonding) was the highest among all groups (Table 2). The frequency of pre-test failures was higher in group I than in the other groups.

SEM micrographs of adhesive resins applied to etched-enamel, wetted with ethanol or water, revealed longer and smoother resin tag formation in group III, while there were slight differences between groups II and IV, in terms of the resin tag formation length and smoothness. However, the presence of a gray band covering the tips of resin tags may indicate that a more-acid-resistant layer was obvious in group IV (Fig. 1).

**Discussion**

The pivotal bonding mechanism of the current etch-and-rinse resin adhesives to dental substrates, is micro-mechanical interlocking, which is a diffusion-dependent phenomenon. Infiltration of resin monomers into the dentin and enamel depends on several factors related to both adhesive resins, including the molecular weights of the co-monomers and the necessity for good matching of solubility parameters of the monomer/solvent blends with the substrate being penetrated and related substrates. The inherent wetness of dentin makes it a difficult substrate on which to achieve durable bonds between adhesive resin and dentin. Solvents such as ethanol, deployed in primers, aim to help displace water from the dentin surface, which facilitates penetration of the resin monomers into microporosities of the exposed, acid-demineralized collagen network. Unlike dentin, enamel is not inherently wet and consists of a collagen-free, almost completely mineralized structure. Meanwhile, it was thought that considerably more water might be retained in micropores of etched enamel after air-drying, allowing non-uniform resin infiltration into acid-etched enamel. Previously, it was suggested that removal of retained water by drying using absolute ethanol, might increase the enamel resin adaptation.

In the case of ethanol-wet-bonding, the etched enamel surfaces were kept moist with pure ethanol prior to resin application. Results of the present study suggest that the ethanol layer on the etched surface did not hamper the adhesive abilities of the tested adhesives. The present study revealed that wetting etched and visibly moist enamel with absolute ethanol for 1 minute improved the initial bond strengths of all of the tested adhesives. In terms of failure modes, it was determined that adhesive failure was dominant in all Single Bond groups (groups I and II).

![Figure 1](image-url)  
**Figure 1**  SEM micrographs of adhesive resins applied to etched-enamel-wet with ethanol or water after 2 mol/L HCl treatment for 20 seconds. (A) SEM micrograph of Single Bond 2 applied to etched-enamel-wet with water; (B) SEM micrograph of Single Bond 2 applied to etched-enamel-wet with ethanol showing longer and smoother resin tag formation (black arrow). It seems that resin had penetrated into deeper interprismatic areas; (C) SEM micrograph of All Bond 3 applied to etched-enamel-wet with water; (D) SEM micrograph of All Bond 3 applied to etched-enamel-wet with ethanol showing slightly better resin tag formation than those of the former group. Gray band over tips of resin tags, possibly consists of residual apatite after 2 mol/L HCl treatment for 20 seconds is remarkable.
III) regardless of the bonding technique. However, it seems that ethanol-wet-bonding affected the failure mode pattern of All Bond 3. Although adhesive failure was dominant in the All Bond 3 group with water-wet-bonding (group II), cohesive failure in the enamel was more frequent in the All Bond 3 group with ethanol-wet-bonding (group IV). In short, high bond strength demonstrated more-cohesive failure in the enamel (Table 2).

The main principles of the ethanol-wet-bonding concept were explained using solubility parameter theories. First, according to solubility parameter theories, ethanol, with a lower hydrogen bond capacity than water, maintains the stability of the demineralized dentin matrix against surface tension forces, and keeps interfibrillar spaces open when demineralized collagen matrices are saturated with ethanol, thus allowing better resin infiltration. Second, ethanol is a more-suitable solvent than water for most resin monomers, and it consequently allows resins to infiltrate ethanol-saturated collagen matrices without phase separation.

In the case of etched enamel consisting of hydroxyapatite and keratin-like organic matrices, with higher stiffness than demineralized dentin matrix, enamel, even after etching, was not as delicate against surface tension forces that occur during solvent evaporation. For that reason, the first principle of the ethanol-wet-bonding concept did not contribute to the results of the present study.

Meanwhile, the latter principle was quite explanatory in several ways, including: (1) increasing resin infiltration as a result of increased solubility of monomers in ethanol-saturated etched enamel; (2) preventing phase separation of monomer blends during infiltration; and (3) enhancing mechanical properties of the resulting polymer, because of enhanced resin infiltration due to the absence of water and the polymerization of monomers due to phase separation.

It is clear that increasing the solubility of a resin in a medium improves resin infiltration and prevents phase separation. Substitution of water in nanoscale spaces around and within enamel crystallites (inter- and intra-crystallite spaces) and enamel rods affected by acid etching with ethanol, prior to resin infiltration, facilitates resin infiltration into these nanoscale spaces, due to their good solubility in ethanol.

Indeed, there are always nanoscale voids and channels filled with water around and within enamel crystallites and rods. Water constitutes about 5–10% by volume of enamel, making the enamel porous. It is well known that these nanovoids and nanochannels play a critical role in the caries process. Recently, it was shown that a novel caries-prevention method, which uses a similar ethanol-dehydration protocol prior to application of a resin infiltrant to fill and block these nanovoids and nanochannels, was efficient in clinically arresting the dental caries process. Indirect evidence from clinical and in vitro studies associated with resin infiltration techniques, showed that they arrested and prevented dental caries, suggesting that nanovoids within the subsurface enamel are filled with resin monomers via chemical dehydration of etched enamel by ethanol. Therefore, it is meaningful to speculate that due to substitution of water within nanovoids and nanochannels around enamel crystals with ethanol, adhesive resin monomers can efficiently infiltrate these nanovoids, facilitating an extension of microscale retention of etch-and-rinse adhesives to nanoscale retention, which may explain results of the present study. Indeed, as noted by Tay et al. nano-retention means encapsulation of individual enamel crystallites by resin, which occurs when deploying hydrophilic total etching adhesives. With the results of the present study in mind, ethanol-wet-bonding may improve current nano-retention abilities of tested hydrophilic adhesives, due to the aforementioned aspects.

Furthermore, the success of ethanol-wet-bonding on enamel bonding can partly be attributed to the higher degree of conversion of resin monomers, and prevention of nano- and microphase separation of resin monomer blends, due to the nearly complete removal of water from the demineralized enamel by chemical dehydration of the demineralized enamel zone, which contains a higher extent of water than sound enamel due to the ethanol. It is known that the presence of water during polymerization of monomers results in a porous permeable polymer, and it was recently shown that 10% residual ethanol even significantly enhanced the degree of conversion of monomers. Therefore, mechanical properties of the resulting hybrid zone are greater.

Although ethanol-wet-bonding was developed to allow hydrophobic resin adhesives to bond to dentin, with the aim of reducing water sorption over time, it appears that a number of studies on ethanol-wet-bonding with commercial and more-hydrophilic adhesives are increasing over time, and also in vivo studies on ethanol-wet-bonding are ongoing. As the effects of ethanol-wet-bonding on the durability of hydrophilic-resin dentin were promising according to previous studies, this interest indicates that ethanol-wet-bonding is not so far from clinical practice. Therefore, commercially available dental adhesives, Single Bond 2, a simplified etch-and-rinse adhesive, and All-Bond 3, a conventional etch-and-rinse adhesive, were selected as resin adhesives in the present study.

According to the results of the present study, ethanol-wet-bonding increased the bond strengths of all tested adhesives to enamel. Results of the two-way ANOVA indicated that ethanol-wet-bonding resulted in significant improvement of the bond strength of Single Bond 2 compared to that of All Bond 3. More recently, Pashley et al found that ethanol-wet-bonding improved the durability of resin–dentin bonds using Single Bond 2. Therefore, it was speculated that Single Bond 2 can simultaneously be used with ethanol-wet-bonding for dentin and enamel bonding, despite there being limited information on the effects of ethanol-wet-bonding on the adhesiveness of commercial adhesives to dentin and enamel.

However, results of the present study indicate some implications of ethanol-wet-bonding on enamel for clinical practice, since enhancing the quality of resin–enamel bonding may improve the clinical performance of tooth-colored restorations that involve enamel borders, at least in reducing the incidence of marginal discoloration. Improved, more-durable resin–enamel bonds may be achieved by ethanol-wet-bonding in the future. Moreover, improved resin–enamel bonds may increase the durability of resin–dentin bonds obtained via ethanol-wet-bonding. It is well known that evidence from water storage-aging
studies suggested that resin–dentin bonds were protected by resin–enamel interfaces. Here, it was speculated that if the durability of resin–enamel bonds with ethanol-wet-bonding are greater than the conventional method, resin–dentin bonds should also be more durable at the same time.

Although the number of teeth employed in each group was rather small and intertooth differences existed which may have affected the results of the present study, the higher means and standard deviations obtained from specimens of a smaller diameter, make the μTBS test discriminative enough for detecting differences arising from treatment variables with the use of a smaller number of tooth specimens. Moreover, a previous study showed that intratooth variations (i.e., the number of beams from each tooth) were either similar or even larger than intertooth variations.

In conclusion, according to the results of the present study, ethanol-wet-bonding has the potential to improve resin–enamel hybridization, because of increasing infiltration of monomer blends into the etched enamel, preventing phase separation of the monomers, and enhancing their polymerization. The ethanol-wet-bonding technique on dentin and enamel may be used simultaneously and may provide the potential to create more-durable resin–dentin bonds, thanks to a more-qualified resin–enamel hybrid zone. In the future, there is a need to assess the durability of resin–enamel bonds and resin–dentin bonds with enamel borders, using ethanol-wet-bonding with different dehydration protocols.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References


