

Effectiveness of immediate bonding of etch-and-rinse adhesives to simplified ethanol-saturated dentin

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Abstract: This study examined the immediate bond strength of etch-and-rinse adhesives to demineralized dentin saturated with either water or absolute ethanol. The research hypothesis was that there would be no difference in bond strength to dentin between water or ethanol wet-bonding techniques. The medium dentin of 20 third molars was exposed ($n = 5$). The dentin surface was then acid-etched, left moist and randomly assigned to be saturated via either water wet-bonding (WBT) or absolute ethanol wet-bonding (EBT). The specimens were then treated with one of the following etch-and-rinse adhesive systems: a 3-step, water-based system (Adper Scotchbond Multipurpose, or SBMP) or a 2-step, ethanol/water-based system (Adper Single Bond 2, or SB). Resin composite build-ups were then incrementally constructed. After water storage for 24 h at 37°C, the tensile strength of the specimens was tested in a universal testing machine (0.5 mm/min). Data were analyzed by two-way ANOVA and Tukey's test ($\alpha = 5\%$). The failure modes were verified using a stereomicroscope (40 \times). For both adhesives, no significant difference in bond strength was observed between WBT and EBT ($p > 0.05$). The highest bond strength was observed for SB, regardless of the bonding technique ($p < 0.05$). No significant interaction between adhesives and bonding techniques was noticed ($p = 0.597$). There was a predominance of adhesive failures for all tested groups. The EBT and WBT displayed similar immediate bond strength means for both adhesives. The SB adhesive exhibited higher means for all conditions tested. Further investigations are needed to evaluate long-term bonding to dentin mediated by commercial etch-and-rinse adhesives using the EBT approach.

Descriptors: Dentin; Tensile Strength; Dentin-Bonding Agents; Dental Restoration, Permanent.

Introduction

The wet-bonding technique has been regarded as being the main method for bonding etch-and-rinse adhesives to dentin.¹ In this bonding approach, the organic solvents added to hydrophilic monomers, such as acetone and ethanol, displace the water molecules from the demineralized collagen matrix.² As a consequence, depending on the adhesive composition, the solvent evaporation will facilitate the diffusion of the monomers throughout the demineralized dentin. This favors the correct adhesive polymerization to form an interfacial hybrid layer and also im-

proves the bond strength to dentin.³ However, several studies have demonstrated that the monomer infiltration is considered mostly incomplete,⁴ mainly due to the hydrophilicity of the adhesive monomers. This favors the creation of a porous hybrid layer that is more prone to water sorption and solubility.⁵ These characteristics help the leachable monomers to reach the pulp tissue through intertubular and/or dentin tubules and, consequently, increasing the potential toxicity and compromise the restoration longevity.⁶ Studies have shown that adding hydrophobic monomers to the adhesives allows for higher stiffness and increased stability in an aqueous environment, thus improving the durability of the bonded interface compared to that observed when hydrophilic-rich adhesives are applied.⁷

Recently, a study proposed to replace the residual water in a demineralized dentin matrix prior to the adhesive application by applying absolute ethanol to coax hydrophobic monomers into the ethanol-saturated dentin.⁸ The ultimate goal of this technique is to displace the residual water with ethanol, leaving the unsupported, uncollapsed demineralized collagen network suspended in ethanol-moist collagen fibrils. The closer the solubility parameter for hydrogen bonding forces (δ_h) of the mixture monomer/solvent is to that of the dentin, the better the compatibility and ability of the solvents to wet the dentin substrate.⁹ The EBT allows the relatively hydrophobic monomers to permeate the dentin substrate.⁸ Presumably, the more hydrophobic the resins, the lower the water absorption, the smaller the plasticization effect, and the more durable the bonding to dentin.⁵ The EBT is completed in two different ways:

- a series of increased ethanol concentrations or
- by saturating the dentin with absolute ethanol.⁹

Both strategies have shown promising performance, but a simplified bonding technique is considered to be more acceptable to the practitioners. Despite the promising findings of the ethanol wet bonding technique in coronal¹⁰ and intraradicular dentin,¹¹ the association of a simplified ethanol-wet bonding technique with a commercial total-etch system deserves evaluation.

This study evaluated the influence of the sim-

plified ethanol-wet bonding technique on the bond strength to mid-coronal dentin by using commercial ethanol and water-based etch-and-rinse adhesive systems. The purpose of this *in vitro* study was to evaluate the effectiveness of both a 3-step and a 2-step etch-and-rinse adhesive system, which were applied by using water or ethanol wet bonding. The research hypothesis tested was whether the ethanol bonding technique would produce similar bond strengths to dentin when compared to those observed by the water wet-bonding technique, regardless of the adhesive system.

Methodology

Twenty sound human third molars were selected and used in accordance with a protocol approved by the institutional Research Ethics Committee. Teeth were stored in a saline solution containing 0.1 % thymol at 4°C and used within 4 months of extraction. The cusps were abraded with a water-cooled rotating diamond wheel (Isomet 1000, Buehler; Lake Bluff, USA) to expose a flat surface free of enamel tissue in the mid-coronal dentin surface. Using the highest pulp horn as a reference, a remaining dentin thickness varying from 1.5 to 2.0 mm was used to standardize the mid-coronal dentin. Also, a magnifying lens was used to check whether remaining enamel areas were noted. The dentin surfaces were further polished with a wet #600-grit SiC paper for 60 s to standardize the smear layer. After that, each exposed surface was acid-etched for 15 s with a 37% phosphoric acid gel and water-rinsed for 15 s. The specimens were then randomly divided into 2 groups (n = 10) according to the bonding approach:

1. Water wet-bonding technique (WBT) - Excess water was removed from the surface with absorbing paper and the dentin remained moist.
2. Ethanol wet-bonding technique (EBT) - The ethanol wet-bonding substrate was achieved by applying absolute ethanol (100% ethanol) to the demineralized dentin surface. Absolute ethanol was applied using a microbrush for 30 s. The dentin surface was saturated with 100% ethanol to completely replace the saturated water in the demineralized dentin matrix. Special care was

Table 1 - Chemical composition of the adhesives applied.

Adhesive systems	Composition	Lot #
Adper Scotchbond Multipurpose Plus (SBMP)	Primer: aqueous solution of HEMA and a polyalkenoic acid copolymer Adhesive: BisGMA and HEMA resins	7KP
Adper Single Bond 2 (SB)	BisGMA, GDMA, UDMA, HEMA, nanofillers, water, ethanol, methacrylate functional copolymer of polyacrylic and polyitaconic acids	6BC

Abbreviations: BisGMA: bisphenol A-glycidyl methacrylate; HEMA: hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; GDMA: glycerol dimethacrylate. **Note:** The brand name of Adper Single Bond 2 is used in Latin America and Oceania, while Adper Scotchbond 1 XT is used in Europe, Adper Single Bond Plus in the USA and Adper Single Bond 1 XT in South Africa.

taken to ensure that the dentin surface was always visibly moist. Excess ethanol was removed using an absorbing paper.

The adhesive systems selected were a 3-step, etch-and-rinse, water-based adhesive system (Scotch-Bond Multipurpose Plus, 3M ESPE, St. Paul, USA) and a 2-step, etch-and-rinse, ethanol/water-based adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, USA). Table 1 describes the adhesives and their compositions.

Both adhesives were applied to the dentin surface after WBT or EBT according to the manufacturer's directions. After application, the adhesives were light cured using a halogen light curing unit (Demetron, Kerr, Danbury, USA) with a power density of 570 mW/cm² for 10 s. Four layers of 1 mm-thick resin composite (Filtek 350XT, 3M ESPE, St. Paul, USA) were applied to obtain specimens with the same dentin/resin composite proportion. Each layer was light-cured for 40 s using the same light-curing unit. The bonded teeth were stored in distilled water at 37°C for 24 h before testing.

The roots were then sectioned approximately 2 mm below the cemento-enamel junction, perpendicular to the long axis of the tooth, using a diamond-impregnated disk (Extec, Enfield, USA) in a specific cutting machine (Isomet 1000, Buehler, Lake Bluff, USA), under water-cooling at 300 rpm. The teeth were then longitudinally sectioned in both "x" and "y" axes across the bonded interface using the same water-cooling cutting device. The resulting bonded stick-shaped specimens with a cross-sectional area of 0.8 (± 0.2) mm² were then cemented to the testing device using cyanoacrylate cement (Zapit,

Table 2 - Microtensile bond strength (MPa) of each adhesive as a function of bonding technique.

Adhesive systems	Water-wet bonding (WBT)	Ethanol-wet bonding (EBT)
SBMP	34.8 ± 6.5 Bb	35.2 ± 2.9 Bb
SB	44.9 ± 3.9 Aa	42.9 ± 4.5 Aa

Values are Mean ± SD (n = 5). Different upper case letters in each column: significant (p < 0.05). Same lower case letters for rows: not significant (p > 0.05).

DVA Inc., Corona, USA). The specimens were attached to a universal testing machine (Instron Model 3342, Instron Corp., Canton, USA) and stressed in tension at a cross-speed of 0.5 mm/min until failure. After testing, the fractured specimens were carefully removed from the apparatus and the cross-sectional area was measured with a digital caliper at the site of failure. The results were recorded, and the debonded stress values were converted into MPa. Pre-test failures were not noted. The distribution of the failure mode of remaining composite and dentin fragments was also evaluated at 40× magnification using a dissecting microscope (Stereozoom, Bausch & Lomb, Rochester, USA). Failure mode patterns were classified as follows:

- A = adhesive between dentin and adhesive system;
- M = mixed and
- C = cohesive in resin composite or dentin.

Data was statistically analyzed (2-way ANOVA, Tukey's test [$\alpha = 0.05$]).

Results

The bond strength means (in MPa) of the experi-

mental groups are summarized in Table 2.

Tukey's test showed no significant difference between the bond strength means for water and ethanol-wet bonding for both adhesive systems ($p > 0.05$). SB exhibited higher bond strength mean values regardless of the bonding approach ($p < 0.05$). ANOVA revealed that the interaction between adhesive and technique was not significant ($p = 0.597$). No premature failures were reported either during the cutting procedure or during the test. Failure modes of the tested interfaces demonstrated that the majority of the bonds failed in an adhesive mode.

Discussion

Adhesive systems are designed to provide dentin adhesion via the interaction of hydrophilic monomers in an organic solvent throughout a collagen-rich humid tissue.¹² Depending on the solvents, replacing the water contained in the dentin matrix may cause shrinkage.¹³ Water has not been shown to produce stiffening in the demineralized matrix.¹⁴ Humid, water-saturated dentin matrix, $\delta_h = 40.4$ (J/cm³)^{1/2}, favors the breaking of interpeptide H-bonds, allowing the dentin matrix to maintain itself expanded.¹⁵ As the collagen fibrils are intrinsically wet, a full impregnation of hydrophilic monomers such as HEMA would be expected. However, this may only occur under ideal conditions. The results of the present study warrant the acceptance of the research hypothesis that there is no difference in the bond strength means when water and ethanol bonding techniques were compared. A statistical equivalence was noticed when the EBT was used prior to the application of both adhesive systems ($p > 0.05$).

It has been claimed that, while the demineralized dentin matrix is dehydrated by the solvents, the interfibrillar spaces would be preserved if the δ_h values of these solvents were high.¹⁴ That study demonstrated the need to examine and optimize a short, clinically acceptable application time compared to that currently used in dentin bonding procedures. In addition, the optimal infiltration of collagen fibrils by the adhesive monomers would occur when the polar surface-free energy components are similar. According to Nishitani *et al.*,¹⁵ the solubility param-

eter for polar forces (δ_p) for ethanol-saturated dentin is 12.5 (J/cm³)^{1/2}, which is closer to that of the adhesives tested (data not shown). Adding ethanol as a solvent to adhesive systems has shown to favor the infiltration of collagen fibrils and makes the dentin matrix more hydrophobic and stable over time.¹⁶ For this reason, manufacturers have blended hydrophobic monomers in the adhesive formulations to promote bonding impregnation in a clinically acceptable time.¹⁷

It is important to consider that applying absolute ethanol causes the dentin matrix to shrink 15%¹⁸ due to the formation of matrix interpeptide H-bonds. In this study, the demineralized dentin was treated with a copious amount of absolute ethanol for 30 s in order to create an ethanol-saturated dentin matrix. The saturation time varies extensively in the literature and has been previously reported as 15 s,¹⁹ 20 s²⁰ and 1 min,¹⁷ and a series of increased ethanol concentrations⁹ for 30 s has also been used. To test our hypothesis, we used 30 s to saturate the dentin matrix with ethanol in a clinically acceptable timeframe. The ideal clinical condition would be the application of adhesives in which solvent evaporation prevented further shrinkage during infiltration of the monomers.¹⁴ Thus, it can be speculated that applying absolute ethanol for 30 s lessens the stiffening effect on the dentin matrix because the bonding to dentin was unaffected.

It has been demonstrated that in ethanol-saturated dentin, the diameter of the collagen fibrils is smaller than those in water-saturated dentin, leaving larger interfibrillar spaces available for monomer impregnation.²⁰ As higher bond strength is correlated with wider interfibrillar spaces,²¹ the use of ethanol can increase bond strength.¹⁵ Saturating the dentin matrix with ethanol creates favorable circumstances for methacrylates (as BisGMA) to diffuse into interfibrillar spaces forming a hybrid layer and producing a higher mechanical property.¹⁸ A reasonable explanation for the equivalence of bond strength of both SB and SBMP to ethanol pretreated dentin could be related to both HEMA and BisGMA methacrylates present in the composition of both formulations. Despite their hydrophilicity (monomethacrylate HEMA) or hydrophobicity (di-

methacrylate BisGMA), both monomers are soluble in ethanol. The more resin (both mono- and dimethacrylates) that infiltrates acid-etched matrices, the higher are the resin-dentin bond strengths.¹⁵

Even though no improvement in the bond strength was noticed when using EBT, it is important to consider that recent studies have demonstrated that endogenous dentin matrix metalloproteinases (MMPs) are present within the dentin matrices and may be activated in the presence of water after the adhesive application.²² Consequently, the use of ethanol to replace water may stabilize the bonding to dentin over time.¹⁷ The results of the present study demonstrate that the bonding technique using absolute ethanol for 30 s had no influence on the immediate bond strength when associated with water and water/ethanol-based etch-and-rinse systems. Even though the EBT presents an additional clinical step, it may reduce technique sensitivity when both water/ethanol-based adhesives (SB and SBMP) are used. The EBT may successfully coax more hydrophobic monomers into the dentin matrix, thereby creating more hydrophobic and more stable hybrid layers that are less susceptible to hydrolysis over time. Recent studies have shown promising results

for EBT when using dehydration protocols with an increased series of ethanol concentrations associated with hydrophobic monomers. Future studies are necessary to evaluate the possible effects, both direct and indirect, of using adhesives that contain water and hydrophilic monomers (such as HEMA) on the long-term dentin bonding durability when associated with simplified ethanol saturated dentin.

Conclusion

Within the limitations of the present study it can be concluded that ethanol wet-bonding presents equivalent bonding to dentin when compared to the results observed with the water wet-bonding technique, regardless of the adhesive system tested (hypothesis accepted).

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References

- 1 Perdigao J, Lopes M. Dentin bonding--questions for the new millennium. *J Adhes Dent.* 1999 Autumn;1(3):191-209.
- 2 Van Meerbeek B, Yoshida Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, *et al.* Hybridization effectiveness of a two-step *versus* a three-step smear layer removing adhesive system examined correlatively by TEM and AFM. *J Adhes Dent.* 1999 Spring;1(1):7-23.
- 3 Garcia FC, Wang L, Pereira LC, de Andrade e Silva SM, Junior LM, Carrilho MR. Influences of surface and solvent on retention of HEMA/mixture components after evaporation. *J Dent.* 2010 Jan;38(1):44-9.
- 4 De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K, Lambrechts P. Four-year water degradation of a resin-modified glass-ionomer adhesive bonded to dentin. *Eur J Oral Sci.* 2004 Feb;112(1):73-83.
- 5 Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, *et al.* Water sorption/solubility of dental adhesive resins. *Dent Mater.* 2006 Oct; 22(10): 973-80.
- 6 Ma S, Nakajima KF, Nishiyama N. Effects of storage temperature on the shelf life of one-step and two-step self-etch adhesives. *Oper Dent.* 2009 Jul-Aug;34(4):472-80.
- 7 Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater.* 2008 Jan;24(1):90-101.
- 8 Cadenaro M, Breschi L, Rueggeberg FA, Agee K, Di Lenarda R, Carrilho M, *et al.* Effect of adhesive hydrophilicity and curing time on the permeability of resins bonded to water vs. ethanol-saturated acid-etched dentin. *Dent Mater.* 2009 Jan;25(1):39-47.
- 9 Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, *et al.* Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. *J Biomed Mater Res A.* 2008 Jan;84(1):19-29.
- 10 Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, *et al.* Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. *J Dent Res.* 2009 Feb;88(2):146-51.
- 11 Carvalho CA, Monticelli F, Cantoro A, Breschi L, Ferrari M. Push-out bond strength of fiber posts luted with unfilled resin cement. *J Adhes Dent.* 2009 Feb;11(1):65-70.

- 12 Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. *J Dent.* 1997 Sep;25(5):355-72.
- 13 Pashley DH, Agee KA, Nakajima M, Tay FR, Carvalho RM, Terada RS, *et al.* Solvent-induced dimensional changes in EDTA-demineralized dentin matrix. *J Biomed Mater Res.* 2001 Aug;56(2):273-81.
- 14 Garcia FC, Otsuki M, Pashley DH, Tay FR, Carvalho RM. Effects of solvents on the early stage stiffening rate of demineralized dentin matrix. *J Dent.* 2005 May;33(5):371-7.
- 15 Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, *et al.* Effects of resin hydrophilicity on dentin bond strength. *J Dent Res.* 2006 Nov;85(11):1016-21.
- 16 Sadek FT, Pashley DH, Ferrari M, Tay FR. Tubular occlusion optimizes bonding of hydrophobic resins to dentin. *J Dent Res.* 2007 Jun;86(6):524-8.
- 17 Carvalho CA, Cantoro A, Mazzoni A, Goracci C, Breschi L, Ferrari M. Effect of ethanol application on post-luting to intraradicular dentine. *Int Endod J.* 2009 Feb;42(2):129-35.
- 18 Becker TD, Agee KA, Joyce AP, Rueggeberg FA, Borke JL, Waller JL, *et al.* Infiltration/evaporation-induced shrinkage of demineralized dentin by solvated model adhesives. *J Biomed Mater Res B Appl Biomater.* 2007 Jan;80(1):156-65.
- 19 Raina R, Loushine RJ, Weller RN, Tay FR, Pashley DH. Evaluation of the quality of the apical seal in Resilon/Epiphany and Gutta-Percha/AH Plus-filled root canals by using a fluid filtration approach. *J Endod.* 2007 Aug;33(8):944-7.
- 20 Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, *et al.* Bonding BisGMA to dentin--a proof of concept for hydrophobic dentin bonding. *J Dent Res.* 2007 Nov;86(11):1034-9.
- 21 Carvalho RM, Mendonca JS, Santiago SL, Silveira RR, Garcia FC, Tay FR, *et al.* Effects of HEMA/solvent combinations on bond strength to dentin. *J Dent Res.* 2003 Aug;82(8):597-601.
- 22 Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjaderhane L, *et al.* Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomaterials.* 2006 Sep;27(25):4470-6.