Effects of simplified ethanol-wet bonding technique on immediate bond strength with normal versus caries-affected dentin

Vivek Aggarwal, Mamta Singla, Ritu Sharma, Sanjay Miglani, Saranjit Singh Bhasin

Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Jamia Millia Islamia, New Delhi; Department of Conservative Dentistry and Endodontics, SGT Dental College, Gurgaon, Haryana, India

Abstract

Aim: The aim of the present study was to evaluate whether the use of simplified ethanol-wet bonding (EWB) technique improved the immediate microtensile bond strength (μTBS) between resin composite and caries-affected dentin (CAD).

Materials and Methods: Twenty-four extracted carious human permanent molars were sectioned to expose the carious lesion. The carious dentin was excavated until CAD was exposed. The samples were divided into two groups: Water-wet bonding with Adper Scotchbond Multi-Purpose and a simplified EWB (three 100% ethanol applications for 30 s each), followed by application of an experimental hydrophobic primer and restoration. The samples were vertically sectioned to produce 1 mm × 1 mm thick slabs. The normal dentin (ND) slabs and CAD slabs were identified and were subjected to μTBS evaluation. Slabs from four teeth (two from each group) were evaluated under microscope. Data were analyzed using two-way ANOVA and post hoc Holm–Sidak test at P < 0.05.

Results: EWB improved the μTBS in ND but not in CAD group. The dentinal tubules in CAD group showed sclerotic activity with minimal or no hybrid layer.

Conclusions: Simplified ethanol bonding does not improve the bond strength in CAD.

Keywords: Caries-affected dentin; ethanol-wet bonding; hybrid layer; microtensile bond strength; water-wet bonding

INTRODUCTION

Bonding of composite resins to dentin possesses a challenge to the clinician. The dentin is intrinsically wet and highly organic as compared to enamel, which makes the penetration of resin monomers difficult. The traditional bonding strategies involve etching the dentinal surface and application of low viscous primer/bonding agents, which infiltrates into the demineralized dentinal tubules and form of a “hybrid” layer. It has been shown that the resin monomers are not able to fully infiltrate the demineralized dentin, and a part of demineralized dentin remains susceptible to hydrolytic degradation. Moreover, the remaining water can get entrapped in the hybrid layer and deteriorate the bond strength. The use of hydrophilic monomers acts as a semipermeable membrane and allows movement of water, classically described as “water tree” phenomenon. This exposes both resin and exposed collagen fibers to degradation by hydrolysis. To allow better penetration of resin monomers into water-rich dentinal tubules, ethanol-wet bonding (EWB) has been recommended. It involves gradual replacement of water present in interfibrillar spaces with increasing concentrations of ethanol and subsequently replacing ethanol with hydrophobic primers and resins.

Since the bonding of resins depends upon an effective infiltration of resin monomers, any surface/morphological...
variation in the dentin will affect the resin-dentin bond.\textsuperscript{10,11} The clinically relevant changes in the dentinal substrate are commonly induced by dental caries. The carious dentin has two layers: The outer carious “infected dentin” and the inner carious or “caries-affected dentin” (CAD).\textsuperscript{11} The infected dentin is highly decalcified with irreversibly denatured collagen fiber.\textsuperscript{11} The infected dentin needs to be completely excavated whereas the caries affects dentin is relatively less decalcified and does not require its removal.\textsuperscript{10,11} Although the dentinal tubular lumen is filled with acid resistant whitelock mineral casts, the CAD has reduced mechanical properties as compared with normal dentin (ND).\textsuperscript{11} Many authors have shown that bonding with CAD is somewhat inferior than a normal dentinal substrate.\textsuperscript{12-15} A very few studies have evaluated the effect of EWB on the CAD. Huang et al.\textsuperscript{16} evaluated the effect of EWB with hydrophobic adhesive to sound dentin and to the CAD. The authors reported that simplified EWB improved the bond strength in carious dentin but reduced the bond strength in ND.

The aim of the present study was to evaluate the effect of EWB on the microtensile bond strength (TBS) of normal and CAD. This study tested the null hypothesis that different bonding strategies or substrates have no effect on bond strength.

**MATERIALS AND METHODS**

Extracted nonrestored human maxillary and mandibular permanent molars, with occlusal dental caries, were selected. The samples were used within 1 week of their extraction. Twenty-four teeth with radiographic sign of dental caries extending up to middle-third of dentin were used in the study. Teeth were sectioned perpendicularly 5 mm below the cementoenamel junction to attach the samples to cold cure resin block. The teeth were sectioned through the deepest part of occlusal fissure, perpendicular to the long axis of crown, to expose the carious lesion and to remove enamel overhangs. The carious dentin was excavated by conventional excavators and round carbide burs in an air turbine handpiece. The extent of excavation was based on tactile sensation of hardness to an explorer. The caries was excavated until a relatively hard, CAD, surrounded by sound dentin, was exposed. The dentinal surface was ground with 600-grit silicon carbide (SiC), under copious water irrigation, till an even flat surface was obtained. A single operator prepared all samples to reduce interoperator variability.

The dentinal surface was etched with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15 s. The surface was thoroughly rinsed with water and left moist before bonding procedures. The samples were divided into two groups (n = 12) on the basis of water-wet bonding (WWB) or EWB. The samples in WWB group were restored using Adper Scotchbond Multi-Purpose (3M ESPE), a three-step etch-and-rinse adhesive. In EWB group, the wet dentin surface was treated with three 100% ethanol applications for 30 s each. An experimental hydrophobic primer was prepared by diluting the component 3 of Adper Scotchbond Multi-Purpose in 50 weight% with absolute ethanol. Two applications of experimental resin were done followed by nondiluted component 3 and light curing. The composition and mode of application of the bonding systems used in this study are presented in Table 1. After application of bonding agent, the samples were restored with a composite restorative material (Z350, 3M ESPE). The specimens were stored in distilled water at 37°C for 24 h. The samples were vertically sectioned to produce 1 mm thick flat slabs. The normal and CAD were identified in the flat slabs. The slabs were further sectioned into 1 mm wide and 1 mm thick slabs using a diamond saw, under water lubrication. The ND slabs and CAD slabs were identified and separated from each other. A total of four slabs (two ND slabs and two CAD slabs) were obtained from each tooth. The slabs from two teeth in each group were subjected to histological analysis. The remaining slabs (n = 10 teeth/group, 20 slabs each for normal and CAD) were mounted on a universal instron testing machine (Zwick GmbH and Co., Germany) with the help of a cyanoacrylate adhesive. The samples were stressed to failure at a crosshead speed of 0.5 mm/min. The TBS was calculated as the load at failure divided by the bonded area (1 mm²).

Slabs from remaining two teeth in each group were subjected to microscopic evaluation. The slabs were attached to a

---

**Table 1: Different adhesive systems used in the study with their composition and application mode**

| Adhesive system                                      | Composition                                                                 | Mode of application                      |
|------------------------------------------------------|----------------------------------------------------------------------------|------------------------------------------|
| Adper Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA) | Component 1 (etchant): 35% H₃PO₄, Component 2: (Scotchbond multi-purpose primer) HEMA, polyalkenoic acid polymer, water Component 3: (Scotchbond multi-purpose adhesive) Bis-GMA, HEMA, tertiary amines, photoinitiator | Apply etchant, wait 15 s, rinse 10 s Blot excess water Apply primer, dry gently for 5 s Application of adhesive Light-cure for 10 s |
| Experimental resin (50 weight % Adper Scotchbond Multi-Purpose with 50 weight% absolute ethanol) | Bis-GMA, HEMA, tertiary amines, photoinitiator | Apply primer Dry gently for 5 s Apply primer and dry gently for 5 s Apply nondiluted component 3 and light-cure for 10 s |

Bis-GMA: Bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate
Aggarwal, et al.: Ethanol-wet bonding with carious dentin

RESULTS

The μTBS of all groups is shown in Table 2. Overall, the ND (46.8 MPa) had significantly higher μTBS than CAD (24.5 MPa) (P < 0.05). EWB improved the μTBS in ND (41.4 MPa vs. 52 MPa); however, EWB did not improve the μTBS in CAD (25.5 MPa vs. 23.6 MPa) (P = 0.224) (two-way ANOVA) [Table 3].

Microscopical analyses

Figure 1 shows the hybrid layer in ND. The WWB group had a minimal hybrid layer with somewhat collapsed scaffold. The EWB group had a distinct wide hybrid layer with signs of resin infiltration into demineralized dentinal tubules. Figure 2 shows the CAD and resin interface. There is a lack of distinct hybrid layer. The dentinal tubules show some sclerotic activity, and there are signs of a tubular hard tissue formation.

DISCUSSION

The bond strength and microscopic analysis indicated that the use of an EWB approach improved the resin-dentin bonds with ND but not with CAD. Thus, the null hypothesis – different bonding strategies or substrates have no effect on bond strength – should be partially rejected.

The current dental resin adhesive is made hydrophilic to improve their penetration into water-rich dentinal substrate. These hydrophilic monomers have adhesion promoting property. However, due to increased hydrophilicity, these monomers can absorb water, both in cured and in uncurd states. This allows for water movement across the hybrid layer and can cause hydrolysis of resin and collagen fibers.

The dental adhesive requires a wet dentinal substrate for infiltration of resins. Drying the dentinal surface leads to formation of hydrogen bonds between the collagen fibers. This shrinks the collagen scaffold and reduces the interfibrillar space, thus limiting the penetration of resin monomers. To overcome this problem, the dentinal substrate is kept moist. Water, because of its polar activity, can easily break the H-bonds between the collagen fibers and re-expand the shrunk collagen fibers. This water surrounds the collagen fibers. During bonding procedures, the resin monomers should displace this water. However, it is very difficult to completely remove the water during priming of dentin. This problem gets worsen if hydrophilic monomers are present in dental adhesives. As stated above, the trapped water can lead to deterioration of resin-dentin bonds by hydrolytic degeneration. A viable solution to this problem is to replace the water with ethanol.

Ethanol can also expand the collagen fibers and has a property to stiffen the collagen fibers before solvent evaporation. The resin monomers can easily replace ethanol than water from interfibrillar spaces. The EWB involves two steps: First, the water in the interfibrillar spaces is gradually replaced with increasing concentrations of ethanol, and then the ethanol is gradually replaced by monomers mixed with ethanol and finally with neat hydrophobic monomers. The aim of EWB is to maintain the collagen fibers in an expanded condition and simultaneously allowing the penetration of hydrophobic monomers, with similar solubility parameters. Li et al. evaluated the EWB with contemporary etch-and-rinse adhesives and reported that...
Adhesion to enamel and dentin: Strategies to prevent hydrolytic degradation of the resin-dentin interface in normal dentin-water-wet bonding group. There is minimal hybrid layer with somewhat collapsed scaffold. The normal dentin-ethanol-wet bonding group shows a distinct wide hybrid layer.

Figure 1: (a) resin-dentin interface in normal dentin-water-wet bonding group, r denotes resin composite, and d shows dentin. There is minimal hybrid layer with somewhat collapsed scaffold. (b) The normal dentin-ethanol-wet bonding group shows a distinct wide hybrid layer.

Figure 2: Caries-affected dentin and resin interface lacking a distinct hybrid layer. Sclerotic dentin along with hard tissue formation can be appreciated.

EWB could improve the bonding efficacy, probably due to the good wettability of ethanol-saturated dentin and the structure of the hybrid layer. Similar results were reported by a study evaluating the bond strength of resin-dentin interfaces created with adhesives applied on root dentin using the WWB or EWB technique. The µTBS values were lower in CAD as compared to ND, even after EWB protocols. The adhesion of resin composites depends upon the structural and morphological properties of dentinal substrate. The microscopic evaluation showed better resin diffusion and improved hybrid layer in EWB group. The microscopic analysis results were similar to confocal microscopy results reported by Sauro et al. However, with CAD, the EWB did not improve the µTBS values. The µTBS values were lower in CAD as compared to ND, even after EWB protocols. The adhesion of resin composites depends upon the structural and morphological properties of dentinal substrate. In response to the carious process, there is deposition of minerals in the dentinal tubules, which may affect the infiltration of resin monomers. Various studies have reported that CAD substrate presents with lower bond strength than ND. The microscopic analysis in the present study showed signs of sclerotic activity and atubular hard tissue formation. This could have prevented the penetration of resin monomers, thus hampering hybrid layer formation. The intertubular dentin in CAD is partially demineralized with high intrinsic water content. The simplified approach of EWB might not be able to fully replace the water with ethanol and hydrophobic monomers.

EWB involves progressive dehydration with increasing ethanol concentrations. Traditionally, the dentin is saturated with 50% ethanol followed by 70%, 80%, 95%, and three applications of 100% ethanol for 30 s. This method is less technique sensitive and allows for better water removal from interfibrillar spaces. However, this progressive protocol takes a long time and is clinically difficult to perform. A simplified EWB has been introduced which involves three applications of 100% ethanol for 30 s. This method is less time-consuming and clinically acceptable. There are some possible limitations of this study. A major limitation was the lack of simulated pulpal pressure and aging. As described before, the water movement through the hybrid layer leads to hydrolytic degradation and subsequent deterioration of resin-dentin bonds. Another unavoidable limitation was the lack of standardization of CAD substrate. In natural carious lesions, the extent and amount of demineralization cannot be standardized, and there is always an inherited variability in the dentinal substrate.

CONCLUSIONS

Within the limitations of the present study, it can be concluded that the use of simplified EWB improves the µTBS in ND whereas it has no effect in CAD.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P et al. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003;28:215-35.
2. 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;24:90-101.
3. Manso AP, Marquezini L Jr., Silva SM, Pasheley DH, Tay FR, Carvalho RM. Stability of wet versus dry bonding with different solvent-based adhesives. Dent Mater 2008;24:476-82.
4. Carvalho RM, Chersoni S, Frankenberger R, Pasheley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 2005;26:1035-42.
5. Pereira GD, Paulillo LA, De Goes MF, Dias CT. How wet should dentin be? Comparison of methods to remove excess water during moist bonding. J Adhes Dent 2001;3:257-64.
6. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17-28.
7. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: A systematic review of current clinical trials. Dent Mater 2005;21:864-81.
8. Pasheley DH, Tay FR, Carvalho RM, Ruegggeberge FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007;20:7-20.
9. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersario IL, Geraldelli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer. A review. Dent Mater 2013;29:999-1011.
10. Perdigão J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater 2010;26:e24-37.
11. Ogushii K, Fusayama T. Electron microscopic structure of the two layers.
of carious dentin. J Dent Res 1975;54:1019-26.
12. Wang Y, Spencer P, Walker MP. Chemical profile of adhesive/carious-affected dentin interfaces using Raman microspectroscopy. J Biomed Mater Res A 2007;81:279-86.
13. Wei S, Sadr A, Shimada Y, Tagami J. Effect of caries-affected dentin hardness on the shear bond strength of current adhesives. J Adhes Dent 2008;10:431-40.
14. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, et al. Bonding of self-etch and total-etch adhesives to carious dentin. J Dent Res 2002;81:556-60.
15. Ceballos L, Camejo DG, Victoria Fuentes M, Osorio R, Toledano M, Carvalho RM, et al. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. J Dent 2003;31:469-77.
16. Huang X, Li L, Huang C, Du X. Effect of ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. Eur J Oral Sci 2011;119:310-5.
17. Ekambaram M, Yiu OK, Matlininna JP, King, NM, Tay FR. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. J Dent 2014;42:709-19.
18. de Barros L, Apolonio FM, Loguerco AD, de Saboia V. Resin-dentin bonds of etch-and-rinse adhesives to alcohol-saturated acid-etched dentin. J Adhes Dent 2013;15:333-40.
19. Li F, Liu XY, Zhang L, Kang JJ, Chen JH. Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. J Adhes Dent 2012;14:113-20.
20. Sauro S, Di Renzo S, Castagnola R, Grande NM, Plotino G, Foschi F, et al. Comparison between water and ethanol wet bonding of resin composite to root canal dentin. Am J Dent 2011;24:25-30.
21. Araújo JF, Barros TA, Braga EM, Loretto SC, Silva e Souza Pde A, Silva e Souza MH. One-year evaluation of a simplified ethanol-wet bonding technique: A randomized clinical trial. Braz Dent J 2013;24:267-72.
22. Sadek FT, Mazzoni A, Breschi L, Tay FR, Braga RR. Six-month evaluation of adhesives interface created by a hydrophobic adhesive to acid-etched ethanol-wet bonded dentine with simplified dehydration protocols. J Dent 2010;38:276-83.