Effect of Ethanol Wet Bonding Technique on the Durability of Resin-Dentin Bond with Contemporary Adhesive Systems

Nagpal R*/ Manuja N**/ Pandit IK***

Objective: *To evaluate the effect of ethanol wet bonding technique on the immediate and long term bond strength of simplified etch and rinse adhesive systems to dentin.* ***Study Design:*** *96 extracted human permanent molars were ground to expose the flat dentin surface. The teeth were divided into four groups (n=24) according to the adhesives used, either Tetric N Bond or Solobond M and bonding techniques i.e. water wet bonding or ethanol wet bonding (EWB). Composite cylinder was bonded to each specimen using the respective adhesive technique. Ten samples from each group were then tested immediately for shear bond strength evaluation and two samples for SEM analysis. The remaining samples (12) were tested after 6 month storage in distilled water.* ***Results:*** *Upon immediate testing, there was no significant difference in the mean shear bond strength of the groups regardless of the bonding technique or adhesive used. The bond strength fell dramatically in the water wet bonded specimens after 6 months water storage, while the bond strength of both the adhesives was maintained when EWB technique was used. SEM observation revealed good interfacial adaptation in EWB groups even after six months.* ***Conclusion:*** *For both simplified etch and rinse adhesives used, ethanol wet bonding technique led to significant preservation of the resin dentin bond over 6 months.*

Key Words: *Resin-dentin bond, durability, ethanol wet bonding, bond strength.*

INTRODUCTION

Despite significant improvement in the adhesive systems, the tooth-adhesive interface is the weakest area of composite resin restoration. Compared with the long term stability of resin-enamel bonds, the durability of resin-dentin bonds is relatively poor.[1,2] A 30% to 40% decrease in bond strength has often been observed after 3-6 months of *in vitro* aging.[3] This may be attributed to the heterogeneous character of dentin structure and/or intrinsic shortcomings in the design of the contemporary adhesives.[4]

*Rajni Nagpal, MDS, Associate Professor, Department of Conservative Dentistry, Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India .

**Naveen Manuja, MDS, Associate Professor, Department of Pediatric Dentistry Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India.

***I K Pandit, MDS, Principal, Professor and Head, Department of Pediatric Dentistry, DAV Dental College, Yamunanagar, Haryana, India.

From Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India

Send all correspodence to

Naveen Manuja, Associate Professor, Department of Paediatric Dentistry, Kothiwal Dental College and Research Centre, Moradabad-244001,Uttar Pradesh, India.
Phone: +91-9997048380
E-mail: naveenmanuja@gmail.com

In dentin, a high quality interface is achieved when adhesive monomers thoroughly infiltrate and encapsulate exposed collagen fibrils till the entire depth of demineralized dentin, creating a resin-dentin interdiffusion zone known as hybrid layer.[4] However, discrepancy between dentin demineralization and resin infiltration may result in denuded collagen fibrils at the base of the hybrid layer.[5] With the conventional water wet bonding technique, water fills the intrafibrillar and interfibrillar spaces between collagen fibrils of demineralized dentin, after acid-etching. Ideally, resin monomers should replace water in these spaces. However, it may be impossible for the resin monomers to completely displace water around collagen fibrils.[6] Nanoleakage studies have identified water-filled channels within hybrid layers, indicating that not all residual water is removed. [7]

Moreover, contemporary dentin adhesives cannot replace free and loosely bound water from the intrafibrillar spaces of water saturated collagen fibrils even when resin monomers are able to encapsulate the collagen fibrils. Residual water prevents intimate contact between resin and collagen fibrils. These denuded or poorly encapsulated collagen fibrils are susceptible to hydrolysis by host derived matrix metalloproteinases [MMPs] which are a class of zinc- and calcium-dependent endopeptidases (collagenases, gelatinases and co-elastinases) capable of degrading all extra-cellular matrix components. Human dentin contains at least MMP-2, MMP-8, MMP-9 and MMP-20.[8-10] As MMPs are hydrolases, the existence of water is necessary for them to hydrolyze peptide bonds in collagen resulting in the degradation of the resin-dentin interface.[11] Therefore,

durability of bonding to water-saturated dentin remains questionable. Thus, procedures that improve resin infiltration by coating each collagen fibril with resin may prevent collagen-bound MMPs from access to water and make resin-dentin bonds more durable.

Moreover, the comonomer blends used in contemporary adhesives produce very hydrophilic polymers that absorb 5% to 12% water, resulting in plasticization that lowers their mechanical properties.[12,13] Ito, Tay and Yiu *et al* emphasized that, to enhance the durability of resin-dentin bonds, future dentin adhesives should be rendered more hydrophobic.[12,14,15] Presumably, the more hydrophobic the resins, the lower the water sorption, the smaller the plasticization effect and the more durable the bonding to dentin.

Accordingly, the promising concept of Ethanol-Wet Bonding [EWB] has been proposed by Pashley *et al*, Tay *et al* and Sadek *et al*, in which ethanol instead of water is used to support the demineralised dentin collagen matrix.[16-18] Ethanol-wet bonding represents a new philosophical approach to dentin bonding with etch and rinse adhesives. Ethanol-wet bonding embraces the important concept of water replacement from interfibrillar and intrafibrillar spaces within the demineralized collagen matrix with ascending concentrations of ethanol. In this technique, ethanol, a polar solvent with less hydrogen bonding capacity than water is used for chemical dehydration of the demineralized collagen network, to create a comparatively hydrophobic, ethanol-suspended demineralized collagen matrix for infiltration by hydrophobic resin monomers.

Several other methods have been previously advocated to achieve superior infiltration of monomers, and to inhibit the breakdown of denuded collagen fibrils along the bottom of hybrid layer by host-derived MMPs. Protease inhibitors such as chlorhexidine as additional primers have been recommended to inhibit the intrinsic collagenolytic activity of human dentin, to reduce the aging of bonded interfaces and to increase the stability of the dentinal collagen fibrils within the hybrid layer.[19,20] However, parameters like duration of application, concentration and interfering chemicals may influence the effect of matrix metalloproteinase inhibitors (MMPI) applied.

Ethanol-wet-bonding differs conceptually from the use of chlorhexidine, a potent MMP inhibitor in preventing hybrid layer degradation. Since the use of chlorhexidine with a water wet-bonding technique does not provide a mechanism for progressive removal of intrafibrillar water from the collagen matrix, the chlorhexidine may eventually leach out of the hybrid layer because of its electrostatic binding characteristics, with water functioning as the desorption medium.[21] Thus, the long-term effectiveness of chlorhexidine as a MMP inhibitor may be compromised when water is incompletely removed during water wet-bonding with etch-and-rinse adhesives.

There are two versions of the ethanol-wet bonding technique for use with experimental hydrophobic adhesives.[22] In the progressive ethanol replacement, ethanol saturation is achieved using a series of ascending ethanol concentrations, taking approximately 3-4 minutes, which defies the principle of user-friendliness.[23] It is time consuming and impractical for clinical application. To overcome this obstacle, simplified dehydration protocol with single-step 1 min application of 100% ethanol has been developed so that it can be accomplished within a clinically relevant time frame.[24] However, it is extremely technique-sensitive and does not completely replace water and therefore may not be successfully used with hydrophobic adhesives. On the other hand, it may be assumed that, contemporary hydrophilic adhesives will be more tolerant to the presence of residual water as compared to hydrophobic adhesives after ethanol-wet bonding in a simplified dehydration protocol.[25] Therefore, alternative version of the simplified ethanol-wet bonding technique is to apply hydrophilic adhesives to ethanol-saturated demineralized dentin.

Although various studies have investigated the role of EWB in dentin, still gaps remain in our knowledge regarding the effect of EWB on the durability of contemporary simplified adhesive systems when applied in a clinically relevant application time. Therefore, this study was designed to evaluate the effect of EWB on the immediate and long term bond strength of simplified etch and rinse adhesives to dentin.

MATERIALS AND METHOD

Study was performed in ninety-six freshly extracted permanent human molars. Teeth with any caries, cracks, abrasions, attrition and restorations were excluded from the study. The teeth were thoroughly cleaned and stored in 0.1% thymol in distilled water and used within six months of extraction.

Flat dentin surface was prepared by removing the occlusal third of the tooth crown with the Isomet low speed diamond saw (Buehler, Lake IL, USA) to expose mid-coronal dentin. This was followed by manual polishing of the dentinal surface with 600 grit silicon carbide paper to create a standardized smear layer. The samples were embedded in an auto-polymerizing acrylic resin (RR self- cure acrylic resin, Dentsply) placed perpendicular to the acrylic resin surface.

Clear tape with 3mm punch hole was used to define the pre-treatment and bonding area. All the samples were acid etched with respective acid etchants for 15 seconds followed by rinsing and blot drying and were randomly divided into four groups (n=24): according to the two bonding techniques (water-wet or ethanol-wet bonding) and two simplified etch and rinse adhesive systems (table 1).

Group 1 (WWB-TN) : After acid etching, rinsing & blot drying excess water, dentin surface was bonded with Tetric N Bond according to manufacturer's instructions.

Group 2 (EWB-TN) : After acid etching and rinsing with water, dentin surface was chemically dehydrated by application of 100% ethanol for 1 min & gently blot dried prior to bonding with Tetric N Bond.

Group 3 (WWB-SB) : After acid etching, rinsing & blot drying excess water, dentin surface was bonded with Solobond M according to manufacturer's instructions.

Group 4 (EWB-SB) : After acid etching and rinsing with water, dentin surface was chemically dehydrated by application of 100% ethanol for 1 min & gently blot dried prior to bonding with Solobond M.

For ethanol-wet bonding groups, the etched dentin surface was inverted into 100% ethanol for 1 minute never allowing the ethanol saturated dentin to evaporate to dryness.

Half samples i.e. 12 from each group were scheduled to undergo experimentation immediately and the other half were stored in distilled water at 37^0C for 6 months.

Table 1: Composition and manufacturer's instructions of the adhesives

Adhesive	Composition	Manufacturer's instructions
Tetric N-Bond (IVOCLAR VIVADENT)	15% 2- Hydroxyethyl methacrylate (HEMA), 11 % Phosphonic acid acrylate, 53 % Dimethacrylates, 20-30%Ethanol, Water, Finely dispersed bonded nano silica fillers, Initiators based on Camphoroquinone, Stabilizers.	1.Acid-etch for 15 to 30 seconds, rinse and dry leaving dentin visibly moist. 2. Apply a thick layer of Tetric N-Bond and brush the material gently into the dentin for at least 10 seconds. 3. Remove excess material and the solvent by a gentle stream of air so that the adhesive completely covers the surface without pooling. 4. Light-cure Tetric N-Bond for 10 seconds.
Solobond M (Voco)	5-10% 2- Hydroxyethyl methacrylate (HEMA), 10-25% Bis-GMA, 2.5 % Hydroxypropyl methacrylate, 5-10% Dimethacrylates, 50-100% acetone, Finely dispersed bonded silica filler, Water, Initiators based on Camphoro-quinone, Stabilizers.	1. After acid-etching and rinsing, air dry gently so that dentin is left visibly moist. 2. Apply Solobond M and allow to act for 30 seconds. Then disperse Solobond M with a faint air jet and light polymerize for 20 seconds.

Transparent plastic tubes of internal diameter 3mm and 2mm height with thickness 0.5mm were pre-cut and placed perpendicular to etched and bonded dentinal surface. A hybrid resin composite (Flitek Z350 XT) was loaded into the pre-cut tubes and bonded to the adhesive by light curing at 500mW/cm^2 for 40 seconds with the light tip in contact with plastic cylinder. The tubes were then removed. The bonded specimens were finally placed in distilled water at room temperature for 24 hrs.

Ten samples from each subgroup were subjected to immediate shear bond strength testing in a universal testing machine (Instron, ADMET, Enkay Enterprises, India). The specimens were placed and stabilized by the jig, while a straight knife-edge rod (2.0mm) was applied at the tooth restoration interface at a cross-head speed of 0.5mm/min. Load was applied until restoration failure. The remaining ten samples were subjected to shear bond strength evaluation after storage in distilled water at 37°C for 6 months. The mode of failure of the bond to tooth restoration interface was determined with a stereomicroscope (Nikon SMZ10, Tokyo, Japan) at 10x magnification, then classified into three categories: adhesive, cohesive and mixed type of failures. Adhesive: failure at the resin/dentin interface, cohesive: failure exclusive within dentin or composite, mixed: failure at the resin/dentin interface that included cohesive failure of the neighboring substrates.

Four samples per group were used for SEM analysis. Two samples from each group were prepared immediately and two samples were stored for 6 months prior to the SEM study. The samples were sectioned buccolingually through the composite build up and ground on wet 210 grit silicon carbide paper to a flat surface in order to observe the interface. The specimens were decalcified in 6 N HCl for 30 seconds, rinsed in distilled water and deproteinized by 10-minute immersion in 1% NaOCl, and then rinsed in distilled water. After acid base treatment, the specimens were subjected to dehydration in ascending grades of ethanol up to 100% (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes), then transferred to a critical point dryer for 30 minutes. The specimens were then gold sputter coated and the resin-dentin interfacial adaptation was observed under a SEM (LEO 430, England).

Statistical Analysis

The shear bond strength values were statistically analysed using Tukey's HSD test and three way ANOVA (Multivariate Assesment) at a significance level of p= 0.05.

RESULTS

The mean shear bond strength values obtained after immediate and delayed testing are depicted in table 2. No significant difference in immediate shear bond strength of the groups was observed regardless of the bonding technique or adhesive used. However, after 6 months of water storage the magnitude of shear bond strength fell significantly for both the adhesives with water wet bonding but no significant reduction in bond strength could be observed when the adhesives were applied following ethanol-wet bonding technique.

Table 2: Immediate and Delayed mean shear bond strength values (MPa) of all groups.

Subgroups (n=20)	Immediate (n=10)		Delayed (n=10)	
	Mean	SD	Mean	SD
Group 1	21.71[a]	5.58	12.23[b]	3.46
Group 2	24.57[a]	5.45	25.31[a]	6.30
Group 3	22.74[a]	3.53	13.15[b]	5.16
Group 4	23.77[a]	5.63	22.96[a]	3.88

Same superscript letters indicate no statistically significant difference between groups

Stereomicroscopic evaluation of the fractured surfaces revealed mainly mixed fractures after 24 hours testing. After 6 months of water storage main mode of fracture was adhesive in water wet

bonded groups while groups with EWB depicted mixed type of fractures as the main failure mode in both the etch and rinse adhesives (Table 3).

Table 3: Failure modes in different groups

Experimental Groups (n=10)	Adhesive	Cohesive	Mixed
Group 1 (Immediate)	1	0	9
Group 2 (Immediate)	2	1	7
Group 3 (Immediate)	2	0	8
Group 4 (Immediate)	1	0	9
Group 1 (Delayed)	5	0	5
Group 2 (Delayed)	1	1	8
Group 3 (Delayed)	6	0	4
Group 4 (Delayed)	2	0	8

Figures 1 to 4 depict the resin-dentin interfaces of etch and rinse adhesives [Tetric N bond or Solobond M] with different bonding techniques viewed 48 hours after bonding. Good interfacial adaptation with absence of gap was observed. Figures 5 to 8 show interface of dentin and resin with the same etch and rinse adhesives and viewed after 6 months of water storage. The interfacial gap was negligible in the samples with EWB for both the adhesives but the water wet bonded groups of both adhesives depicted presence of interfacial gap suggesting bond deterioration after six months.

DISCUSSION

Although incorporation of hydrophilic and acidic resin monomers has substantially improved the initial bonding of present-day etch and rinse and self-etch adhesives to intrinsically wet dental substrates, the problems with durability continue to plague adhesive dentistry.[1,26] To assess durability, the most commonly used artificial aging technique is long-term water storage as used in this study. The bonded specimens are stored in fluid at 37°C for a specific period. This period may vary from a few months up to 4-5 years or even longer. Most studies report significant decreases in bond strengths, even after relatively short storage periods.[27] Decrease in bonding effectiveness is, supposed to be caused by degradation of interface components by hydrolysis. To mimic the clinical situation more closely, artificial saliva solutions can also be used, but bond strength reductions obtained have been reported to be similar to those obtained with pure water degradation.[28]

In the present study, the difference between immediate shear bond strengths of all the groups was not significant regardless of the adhesive or bonding technique used. Accordingly, a good interfacial adaptation was also observed under scanning electron microscope for all the groups. However, after 6 months of water storage, a significant fall in bond strength was recorded for the water wet bonded groups of both the adhesives (Groups 1 and 3) resulting in predominantly adhesive mode of failure as observed under stereomicroscope. Scanning electron microscopic observation of the water wet bonded groups at six months also revealed presence of generalized gap along the rein dentin interface. Similarly, Erhardt et al reported significant reduction in the dentin bond strength of two etch and rinse adhesives after long term water storage.[29]

Figure 1: SEM photomicrograph of resin dentin interface after WWB with Tetric N-Bond obtained 24 hours after bonding. (D- dentin, C- composite, A- adhesive)

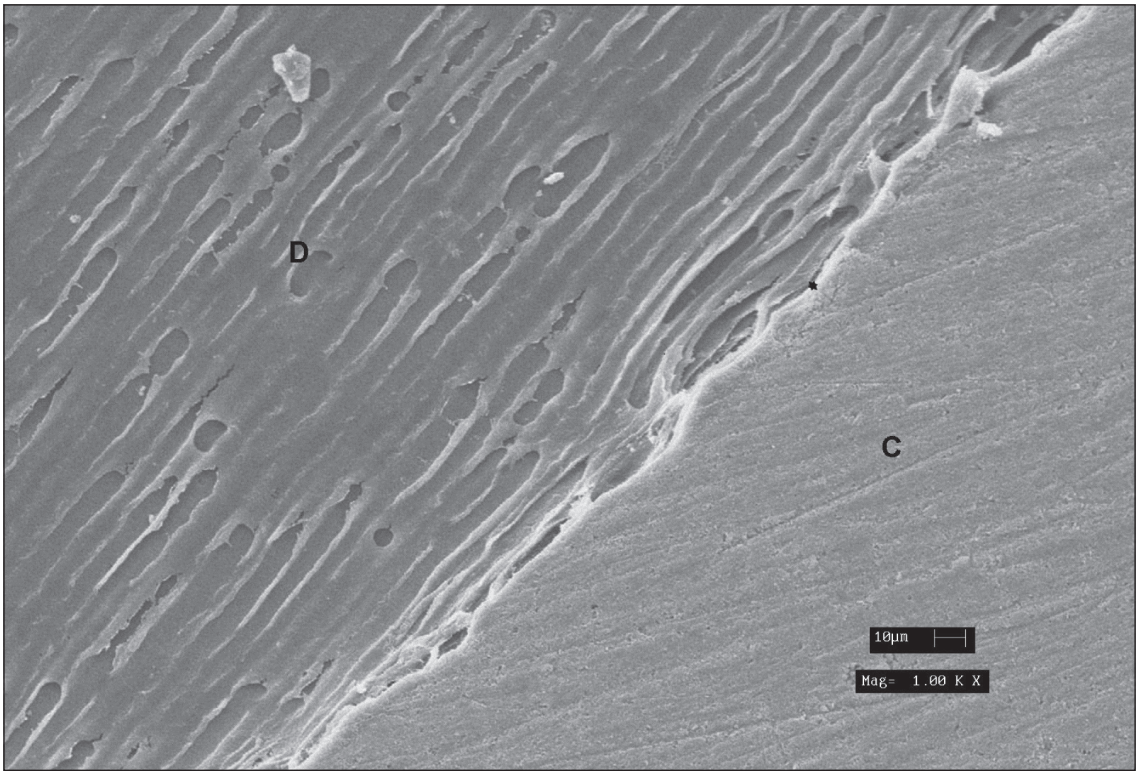


Figure 2: SEM photomicrograph of resin dentin interface after EWB with Tetric N-Bond obtained 24 hours after bonding. (D- dentin, C- composite, A- adhesive)

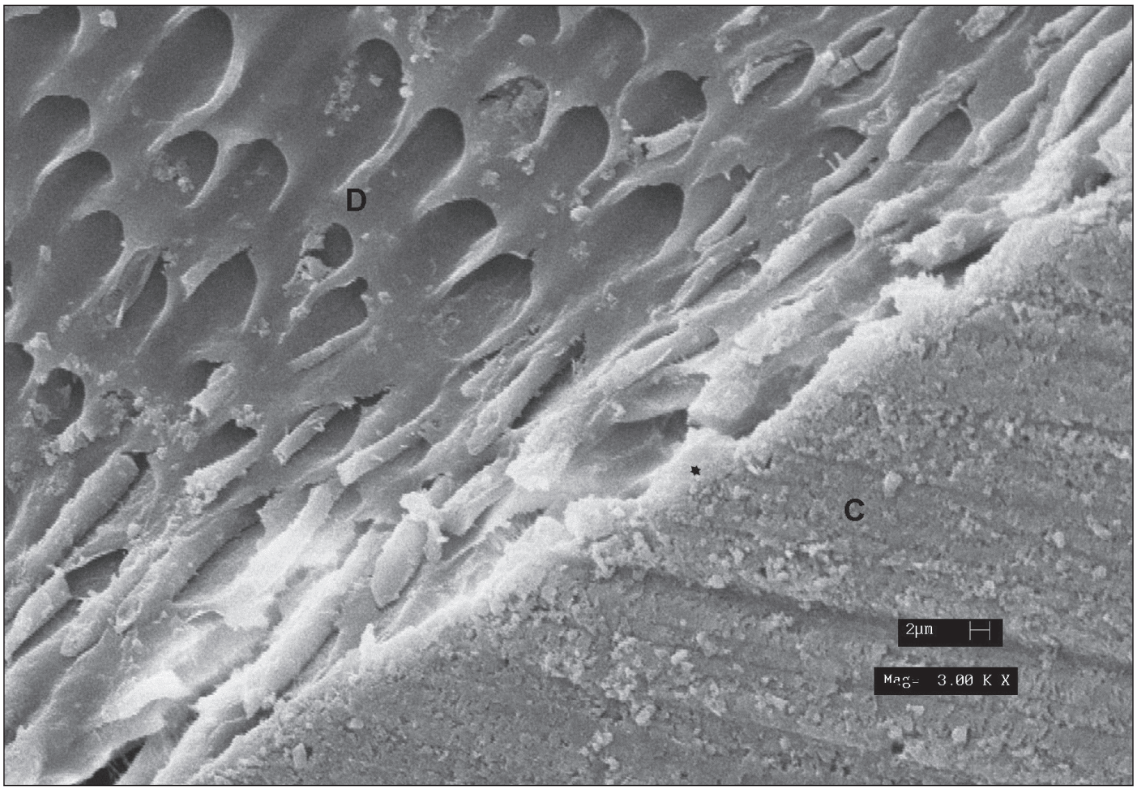


Figure 3: SEM photomicrograph of resin dentin interface after WWB with Solobond obtained 24 hours after bonding. (D- dentin, C- composite, A- adhesive)

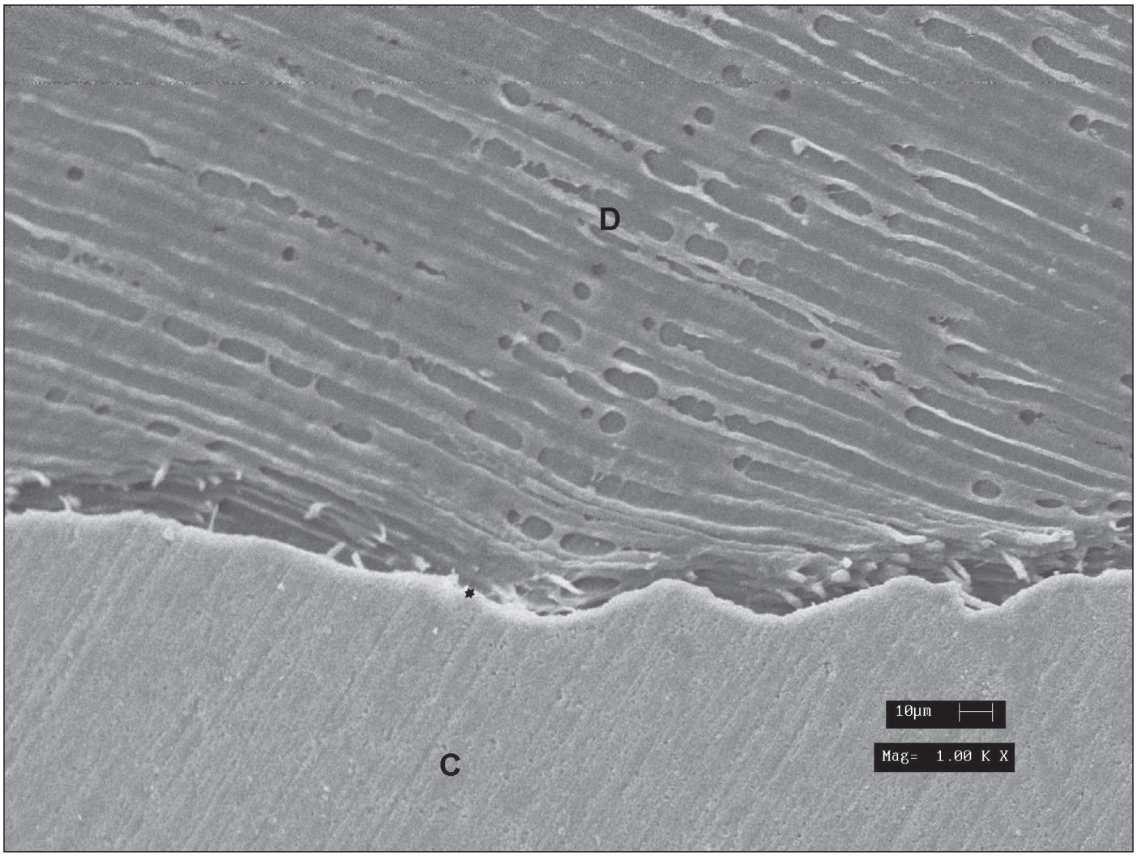


Figure 4: SEM photomicrograph of resin dentin interface after EWB with Solobond obtained 24 hours after bonding. (D- dentin, C- composite, A- adhesive)

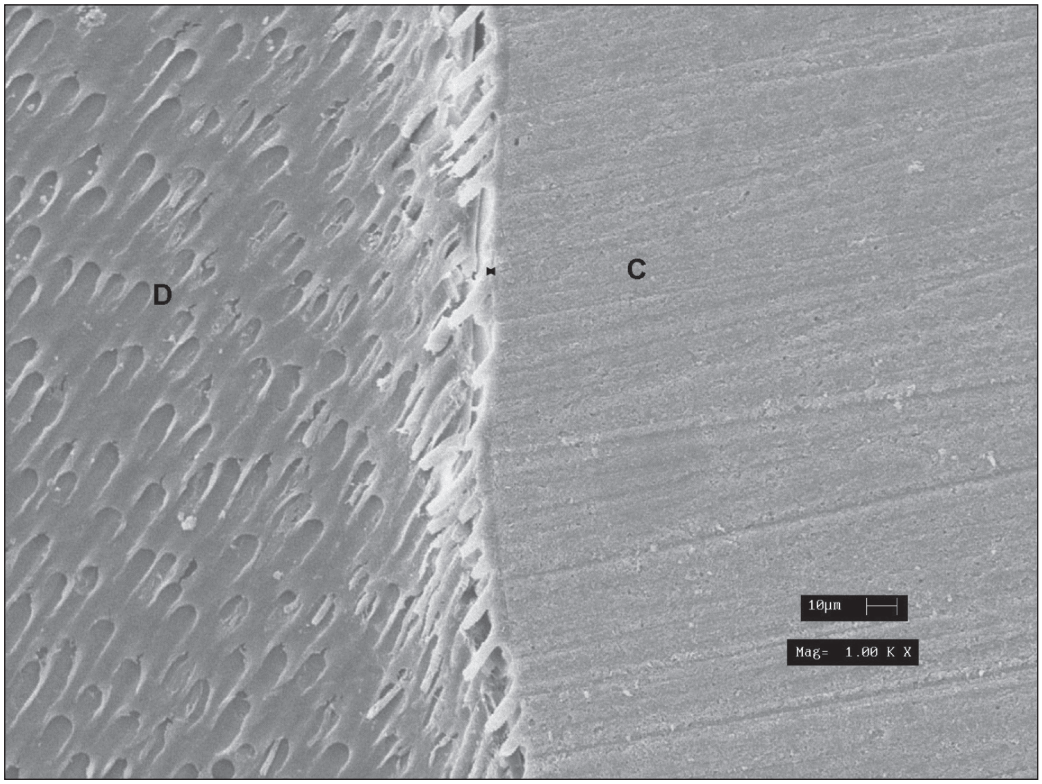


Figure 5: SEM photomicrograph of resin dentin interface after WWB with Tetric N-Bond and six months water storage. (D- dentin, C- composite, A- adhesive, arrow- interfacial gap)

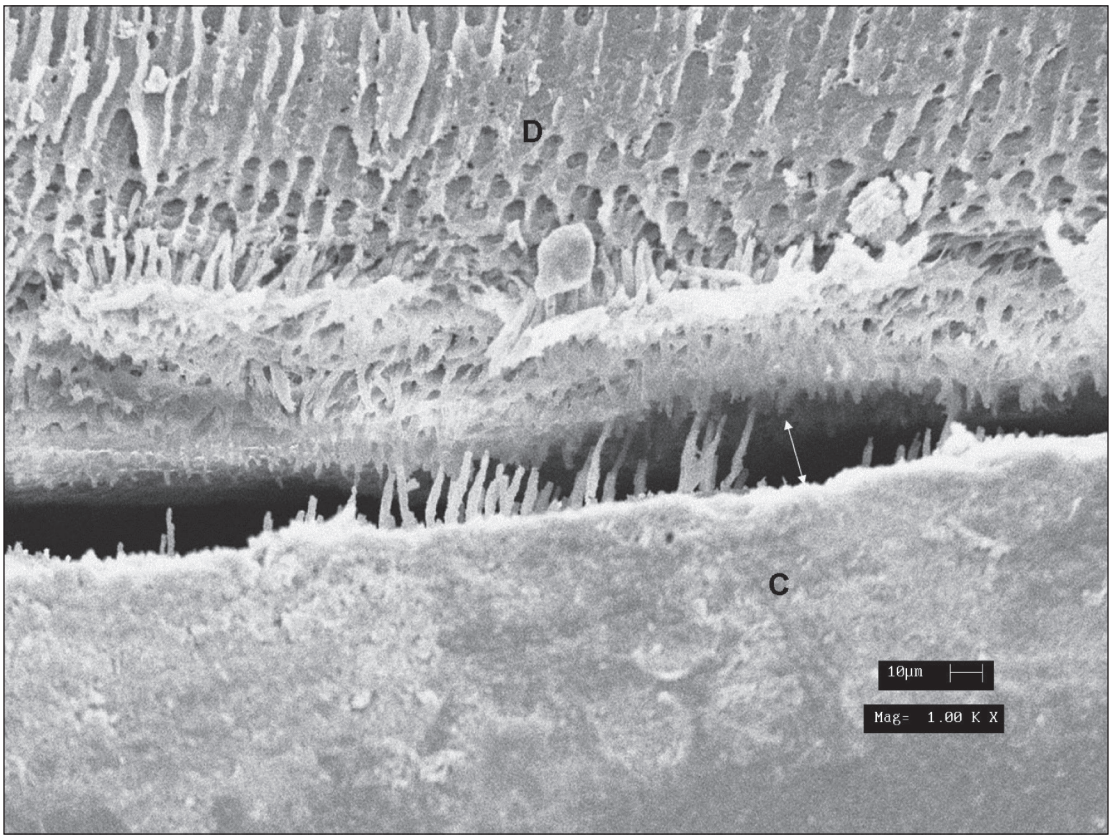


Figure 6: SEM photomicrograph of resin dentin interface after EWB with Tetric N-Bond and six months water storage. (D- dentin, C- composite, A- adhesive)

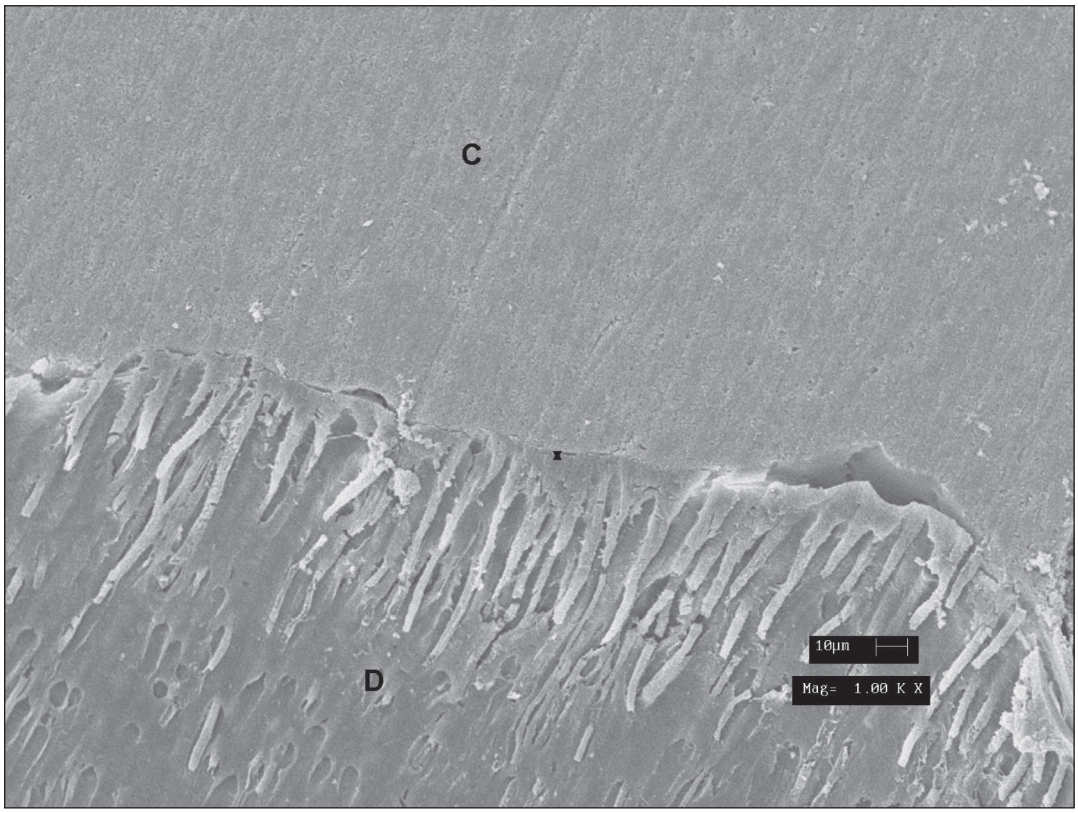


Figure 7: SEM photomicrograph of resin dentin interface of Solobond with WWB after six months water storage. (D- dentin, C- composite, A- adhesive, arrow- interfacial gap)

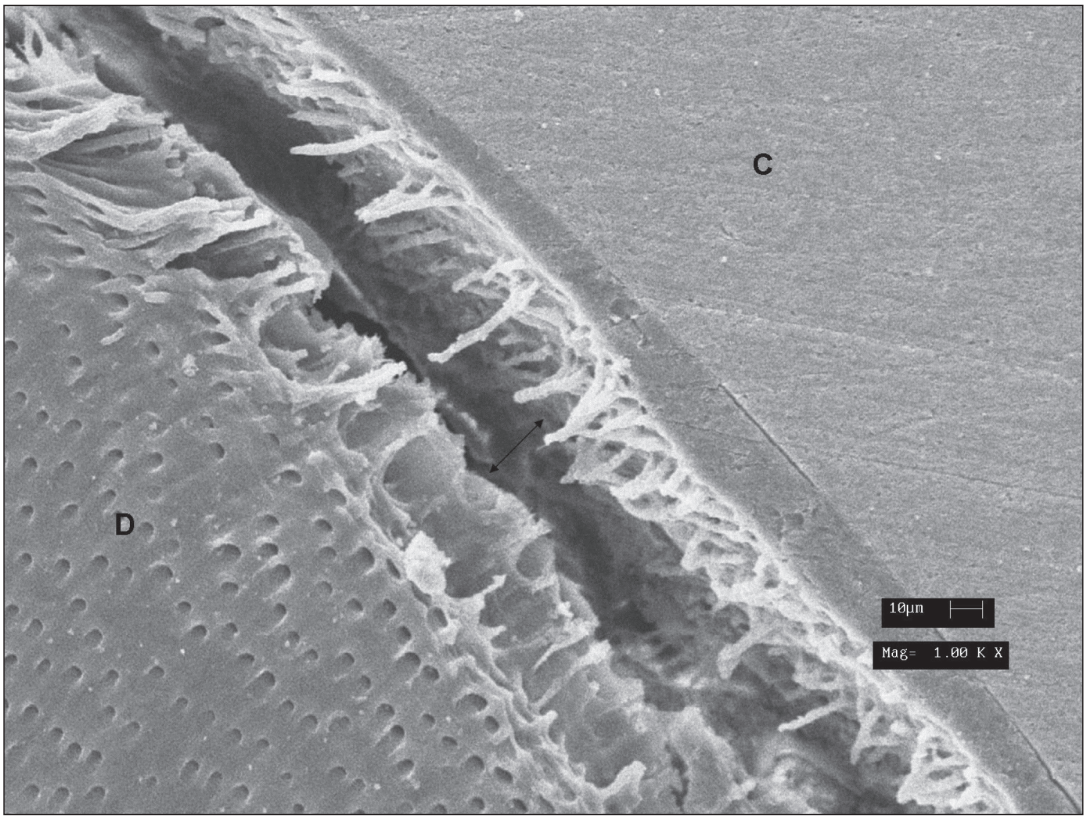
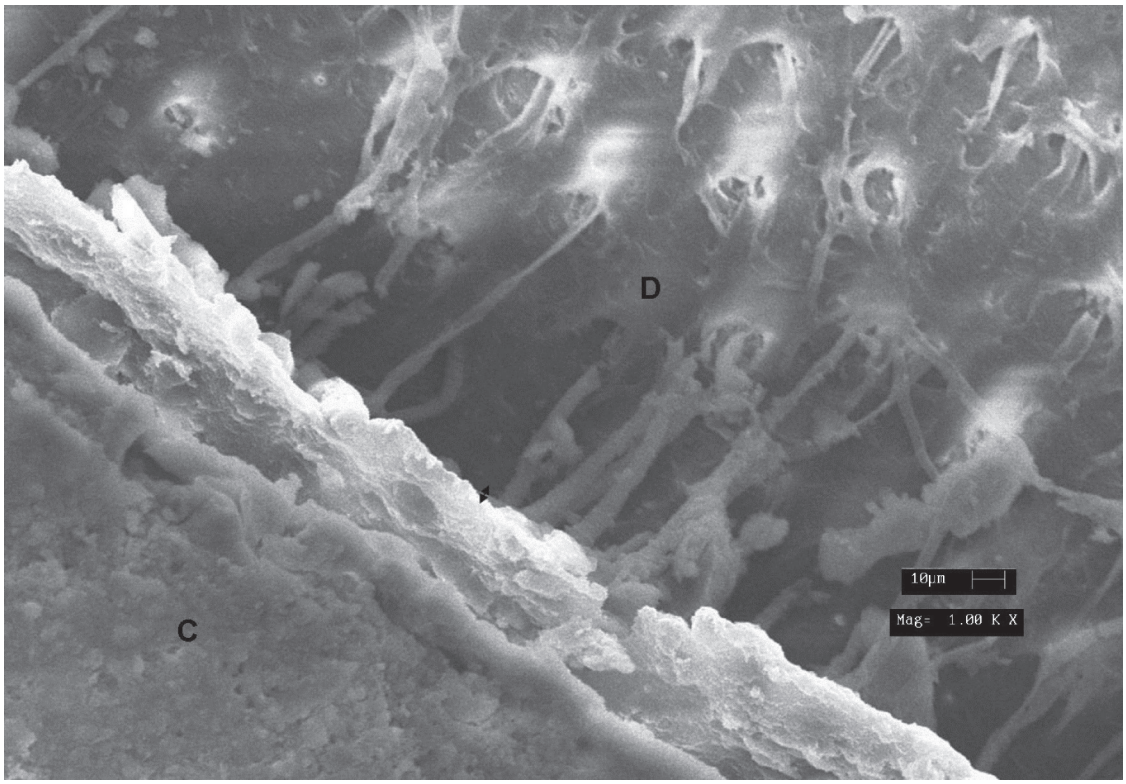


Figure 8: SEM photomicrograph of resin dentin interface of Solobond with EWB obtained after six months water storage. (D- dentin, C- composite, A- adhesive)



In the current study, application of EWB technique to the acid-etched dentin resulted in practically no reduction in bond strength over 6 months of ageing. Scanning electron photomicrographs also corroborated the findings by depicting good interfacial seal in the ethanol wet bonded groups of both the adhesives (Groups 2 and 4) at six months.

Nishitani *et al* also reported that wet-bonding with ethanol achieved higher bond strengths even with hydrophilic resins than were possible with water-saturated matrices.[30] Sauro *et al* compared hybrid layers created with commercially available etch and rinse adhesive using water wet-bonding or ethanol-wet bonding, and reported significantly less micropermeability of the fluorescent tracer in hybrid layers created with ethanol wet-bonding.[31] They suggested that ethanol wet-bonding is capable of increasing resin uptake and producing better sealing of the collagen matrix, even with the use of hydrophilic adhesives. Hosaka *et al* also reported that ethanol wet bonding increased the durability of resin-dentin bonds.[32] Sauro et al evaluated the bond strength of resin dentin interfaces created with adhesives applied on root canal dentin using the water wet or ethanol wet bonding technique.[33] They reported that ethanol wet bonding technique gave higher bond strength values for all the adhesives tested.

Long term preservations of resin-dentin bonds made to acid-etched ethanol-saturated dentin, as observed here, may be attributed to several possible mechanisms. Firstly, the interfibrillar spaces of ethanol-saturated dentin are larger than those of water-saturated dentin. When dentin is acid-etched, the etchant dissolves apatite crystallites from within and between collagen fibrils. The interfibrillar spaces between collagen fibrils contain a hydrogel composed of proteoglycans. It has been speculated that the presence of this hydrogel may interfere with comonomer infiltration during bonding. However, ethanol removes the water from these spaces, causing the hydrogel to collapse.[34] Secondly, ethanol shrinks the diameter of collagen fibrils more than it shrinks the volume of the matrix.[17] With EWB, water is removed from the collagen intrafibrillar compartments resulting in higher intramolecular hydrogen bonding among the collagen molecules that causes shrinkage of the fibrils. As a consequence, the interfibrillar spaces of ethanol-saturated dentin are larger than those of water saturated dentin which allows for more resin infiltration. On the other hand, in water-wet bonding, water-saturated dentin matrix is too weak to resist evaporation stress during solvent evaporation leading to a dramatic shrinkage of dentin matrix, smaller interfibrillar spaces and deterioration of resin-dentin adhesion.

Thirdly, ethanol is a much better solvent for comonomers than water and prevents phase separation of hydrophobic resin monomers. Residual water on collagen and associated bound non-collagenous proteins such as MMPs is removed with the use of ethanol. This may permit adhesive monomers to actually dock with molecular cavities along the surfaces of collagen tripeptides that make up the microfibrils.[35] If adhesive monomers can truly coat collagen microfibrils and prevent access of MMPs to water, they may block the action of intrinsic collagenases known to be bound to collagen.[2,8]

The concept of enzyme immobilization by resin forms the basis of molecular imprinting of enzyme-template complexes by polymerized resinous materials.[36] In ethanol-wet-bonding, when comonomers dissolved in ethanol diffuse through interfibrillar spaces, they molecularly imprint those proteins after polymerization. The adhesive resin presumably infiltrates into and around these peptides and occupies their active (catalytic) sites.

Thus EWB technique may help in overcoming the deficiencies associated with contemporary etch-and rinse adhesives, mainly, their inability to replace free and loosely bound water from the intrafibrillar compartments of water-saturated collagen fibrils. Biomimetic remineralization provides an indirect evidence for this phenomenon, as intrafibrillar spaces created by simplified adhesives are amenable to remineralization by apatite crystallites.[37] However, when ethanol-wet-bonding was meticulously performed, neither nanoleakage nor intrafibrillar remineralization could be detected.[20] This indicates that resin can enter the intrafibrillar compartment if it contains ethanol, but not if it contains water. The ethanol saturated collagen matrix is rendered less hydrophilic and is more compatible with hydrophobic resin monomers and prevents phase separation of ethanol soluble hydrophobic resin monomers.[16]

However, ethanol replacement should be meticulously performed to prevent water-saturated collagen from exposure to air as the surface tension present along the air-collagen interface can easily result in collapse of the collagen matrix and prevent optimal infiltration of the adhesive monomers. During application of the adhesive, dentin matrix should be fully saturated with ethanol. Further research should aim to evaluate the effectiveness of ethanol wet bonding technique with other contemporary adhesive systems and to other dentinal substrates like pulp chamber and root canal dentin in clinical conditions.

CONCLUSION

The ethanol-wet-bonding technique significantly slowed down the rate of degradation of the bond strengths of both the simplified etch and rinse adhesives over 6 months of water storage. Biomimetic water replacement from the internal compartments of collagen fibrils may be the ultimate goal in improving the durability of resin-dentin bonds created by contemporary etch-and-rinse adhesives.

REFERENCES

1. Carrilho MRO, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent, 18: 315-319, 2005.
2. Pashley DH, Tay FR, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res, 83: 216-221, 2004.
3. Armstrong SR, Vargas MA, Chung I, Pashley DH, Campbell JA, Laffoon JE, *et al*. Resin-dentin interfacial ultrastructure and microtensile dentin bond strength after five-year water storage. Oper Dent, 29: 705-712, 2004.
4. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res, 16(3): 265-73, 1982.
5. Hashimoto M, Ohno H, Kaga M, Sano H, Endo K, Oguchi H. The extent to which resin can infiltrate dentin by acetone-based adhesives. J Dent Res, 81(1): 74–78, 2002.
6. Guo X, Spencer P, Wang Y, Ye Q, Yao X, Williams K. Effects of a solubility enhancer on penetration of hydrophobic component in model adhesives into wet demineralized dentin. Dent Mater, 23: 1473-1483, 2007.
7. Tay FR, Pashley DH. Water-treeing-a potential mechanism for degradation of dentin adhesives. Am J Dent, 16: 6-12, 2003.
8. Mazzoni A, Mannello F, Tay FR, Tonti GA, Papa S, Mazzotti G, et al. Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. J Dent Res, 86: 436–40, 2007.
9. Sulkala M, Larmas M, Sorsa T, Salo T, Tjaderhane L. The localization of matrix metalloproteinase-20 (MMP-20, anamelysin) in mature human teeth. J Dent Res, 81: 603–608, 2002.
10. Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, Tjaderhane L. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in dentin. Arch Oral Biol, 52: 121–127, 2007.
11. Zhang S, Kern M. The Role of Dentinal Host-derived Matrix Metalloproteinases in Reducing Dentin Bonding of Resin Adhesives. Int J Oral Sci, 1(4): 163–176, 2009.
12. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, *et al*. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials, 26: 6449-6459, 2005.
13. Malacarne J, Carvalho RM, deGoes MF, Svizero N, Pashley DH, Tay FR, *et al.* Water sorption/solubility of dental adhesive resins. Dent Mater, 22: 973-980, 2006.
14. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Asso, 69: 726-731, 2003.
15. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. Biomater, 25(26): 5789-5796, 2004.
16. Pashley DH, Tay FR, Carvalho RM, Rueggeberg 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 20: 7-20, 2007.
17. Tay FR, Pashley DH, Kapur RR, Carrilho MRO, Hur YB, Garrett LV, *et al.* Bonding BisGMA to dentin—a proof of concept for hydrophobic dentin bonding. J Dent Res 86: 1034-1039, 2007.
18. Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, *et al.* Application of the hydrophobic resin adhesive to acid etched dentine with an alternative wet bonding technique. J Biomed Mater Res A 84:19-29, 2008.
19. Carrilho MR, Geraldeli S , Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res, 86(6): 529-533, 2007.
20. 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 24(1): 90-101, 2008.
21. Blackburn RS, Harvey A, Kettle LL, Manian AP, Payne JD, Russell SJ. Sorption of chlorhexidine on cellulose: mechanism of binding and molecular recognition. J Phys Chem B, 111: 8775-8784, 2007.
22. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J et al. Limitations in Bonding to Dentin and Experimental Strategies to Prevent Bond Degradation. J Dent Res, 90(8): 953-968, 2011.

23. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. Ethanol wet bonding challenges current anti-degradation strategy. J Dent Res, 89: 1499-1504, 2010.
24. Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR*, et al*. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Dent Mater, 36:368-379, 2010.
25. Li F, Liu X, Zhang L, Kang J, Chen J. Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. J Adhes Dent, 14: 113-120, 2012.
26. Manuja N, Nagpal R, Pandit IK. Dental adhesion: mechanism, techniques and durability. J Clin Pediatr Dent, 36(3): 223-234, 2012.
27. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: Methods and results. J Dent Res, 84 (2): 118-132, 2005.
28. Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on dentin bond durability of resin cement. Dent Mater, 16:1-6, 2000.
29. Erhardt MC, Shinohara MS, Bedran-Russo AK, Amaral CM, Pimenta LA. Effect of long-term water storage on etch-and-rinse and self-etching resin-dentin bond strengths. Gen Dent, 56(4):372-7, 2008.
30. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, MazzoniA*, et al*. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 114: 160-166, 2006.
31. Sauro S, Watson TF, Mannocci F, Miyake K, Huffman BP, Tay FR*, et al*. Two-photon laser confocal microscopy of micropermeability of resin-dentin bonds made with water or ethanol wet bonding. J Biomed Mater Res B Appl Biomater, 90: 327-337, 2009.
32. 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, 88:146-151, 2009.
33. Sauro S ; Di Renzo S; Castagnola R; Grande NM.; Plotino G; Foschi F; Mannocci F Comparison between water and ethanol wet bonding of resin composite to root canal dentin. Am J Dent, 24(1): 25-30, 2011.
34. Scott JE, Thomlinson AM. The structure of interfibrillar proteoglycan bridges ('shape modules') in extracellular matrices of fibrous connective tissues and their stability in various chemical environments. J Anat 192(Pt 3): 391-405, 1998.
35. Vaidyanathan J, Vaidyanathan TK, Ramasubbu N, Ravichandran S. A computational and experimental analysis of ligand binding to type I collagen. Curr Comput-Aided Drug Des, 1: 397-422, 2005.
36. Jiang X, Jiang N, Zhang H, Liu M. Small organic molecular imprinted materials: their preparation and application. Anal Bioanal Chem, 389: 355-368, 2007.
37. Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. J Dent Res, 88:719-724, 2009.