Dental Materials Journal 2015; 34(4): 441–448

Effect of simplified ethanol-wet bonding on dentin bonding durability of etch-and-rinse adhesives

Cemal YESILYURT, Muhammet Kerim AYAR, Tahsin YILDIRIM and Mustafa Sadık AKDAG

Department of Restorative Dentistry, Faculty of Dentistry, Karadeniz Technical University, Trabzon, Turkey
Corresponding author, Muhammet Kerim AYAR; E-mail: mkayar82@gmail.com

This *in vitro* study evaluated the effects of simplified ethanol-wet bonding technique on dentin bonding durability of two etch-and-rinse adhesives to bovine dentin. Sixteen freshly extracted bovine incisors were divided into four groups according to bonding technique (water-wet or ethanol-wet bonding) and adhesive (Single Bond 2 or Prime & Bond NT). After etching and rinsing, dentin surfaces were left either water-moist or immersed in ethanol. Following adhesive application and composite build-up, bonded teeth were sectioned into sticks for microtensile bond strength (μTBS) testing conducted after 24-h and 12-month water storage. There were no significant differences in bond strength among the groups at 24 h. At 12 months, the bond strengths of adhesives to dentin were significantly decreased ($p<0.05$). Simplified ethanol-wet bonding did not improve the resin-dentin bonding durability of tested etch-and-rinse adhesives.

Keywords: Dentin bonding, Ethanol, Bond durability

INTRODUCTION

Despite significant technological advances that have improved the bonding reliability at resin-dentin interface[1], *in vitro* and *in vivo* studies continued to reveal degradation of resin-dentin interfaces and reduction in bond strength over time[2,3]. Although recent long-term clinical trials of adhesive resin systems in non-carious cervical lesions demonstrated favorable clinical performance[4,5], degradation of resin-dentin interfaces remains a hindrance to more durable and clinically acceptable restorations[2]. Bond degradation at resin-dentin interfaces was speculated to be due to water sorption[6], hydrolysis of ester linkages of methacrylate resins[7], and activation of endogenous dentin matrix metalloproteinases (MMPs)[8].

To preserve the intact hybrid layers, strategies to counter the diverse mechanisms of degradation include ethanol-wet bonding[9-12] and the use of chlorhexidine[13,14]. The anti-degradation efficacy of ethanol-wet bonding technique hinges on two primary mechanisms. On the one hand, this technique reduces water sorption by coaxing more hydrophobic resins to be deployed for dentin bonding[12]. On the other hand, this technique inhibits MMP activity with improved encapsulation of demineralized collagen fibrils by adhesive resin, by virtue of water being replaced from interfibrillar and intrafibrillar spaces by ethanol[10,11]. Adhesive resin monomers, which are more soluble in ethanol than in water, can better penetrate demineralized dentin matrices that are saturated with ethanol than with water. Therefore, the quality of resultant hybrid layer is expected to improve with improved resin encapsulation of demineralized collagen fibrils[10].

By the process of ethanol dehydration in ethanol-wet bonding technique, a hydrophilic dentin matrix can be rendered more hydrophobic because water is replaced by ethanol. Therefore, this process is crucial to deploy more hydrophobic resins for dentin bonding so as to reduce water sorption over time. Nonetheless, hydrophilic adhesive resins can be used in conjunction with ethanol-wet bonding technique to enhance resin encapsulation of collagen fibrils. Most hydrophilic adhesive resins are ethanol-soluble monomers that can encapsulate and protect fibrils against MMPs[10].

Recent *in vitro* water storage aging studies showed that the ethanol-wet bonding technique successfully improved the durability of resin-dentin bonds with experimental, relatively hydrophobic adhesive resins[9,11,12]. To date, however, no commercial hydrophobic adhesive resin systems were specially developed for use with ethanol-wet bonding technique. It would be profitable, therefore, if ethanol-wet bonding could improve the bonding durability at resin-dentin interfaces when used with the current simplified etch-and-rinse adhesives, which are more hydrophilic in nature. In a recent review on state-of-the-art etch-and-rinse adhesives, it was shown that ethanol-wet bonding increased resin-dentin bonding durability[10]. Nonetheless, knowledge remained scarce on the effects of ethanol-wet bonding on the bonding durability of current etch-and-rinse adhesives.

The aim of the present study was to compare the 24-h and 12-month microtensile bond strengths (μTBSs) of two current etch-and-rinse adhesives to dentin using ethanol-wet bonding. The null hypothesis was that simplified ethanol-wet bonding would preserve the bonding stability of two etch-and-rinse adhesives to bovine dentin after 1 year of water storage.

MATERIALS AND METHODS

Tooth sample preparation

Sixteen bovine incisors, which were collected from

Received Dec 3, 2014: Accepted Feb 26, 2015
doi:10.4012/dmj.2014-335 JOI JST.JSTAGE/dmj/2014-335

bovines that were at least 2 years old, were used in this study. Teeth were stored in 0.02% sodium azide solution at 4°C for a maximum of 6 months prior to usage. Soft tissue around teeth was removed using a scalpel, and roots were removed using a low-speed diamond disc under water. Crowns were embedded in self-cure acrylic blocks using double-sided adhesive band. Enamel surfaces were ground using 320-grit silicon carbide papers to obtain flattened surfaces. Box-shaped cavities were prepared on exposed enamel surfaces using a coarse diamond bur (Microdont, Sao Paulo, Brazil) with a high-speed turbine. Prepared samples were randomly divided into four groups (n=4) according to adhesive and bonding technique as follows:

Group 1: Single Bond 2 with water-wet bonding;
Group 2: Prime & Bond NT with water-wet bonding;
Group 3: Single Bond with ethanol-wet bonding; and
Group 4: Prime & Bond NT with ethanol-wet bonding

Bonding procedures

In water-wet bonding groups (Groups 1 and 2), each of the two adhesives was applied to water-saturated, visibly moist, acid-etched dentin according to manufacturers' instructions (Table 1). In Group 1, Single Bond 2 was applied using a cotton applicator with a rubbing action for 30 s. In Group 2, Prime & Bond NT was applied using a cotton applicator and left for 20 s on the surface without any rubbing action. Following air-thinning, adhesives were light-cured for 10 s using a LED curing unit that had a light intensity of 1,200 mW/cm^2 (Elipar S10, 3M ESPE, St. Paul, MN, USA).

In ethanol-wet bonding groups (Groups 3 and 4), simplified ethanol-wet bonding technique was used[9]. Using an needle, acid-etched dentin surfaces were saturated with absolute ethanol for 1 min. Surfaces were kept visibly wet with ethanol during this time and prior to adhesive resin application. The remaining steps of the bonding procedure were as per the water-wet bonding groups.

Following adhesive application, resin composite (Valux Plus, 3M ESPE) was incrementally built up on the surfaces in three layers up to a height of 4 mm. Each increment layer was cured for 20 s using a LED curing unit that had a light intensity of 1,200 mW/cm^2 (Elipar S10, 3M ESPE).

Microtensile bond strength (μTBS) test

All bonded specimens were stored in tap water at 37°C for 24 h before μTBS test. Resin-dentin specimens of approximately 0.7×0.7 mm^2 dimensions were obtained for μTBS test using a diamond saw (Micracut 125, Metkon, Bursa, Turkey) at 300 rpm under copious water. Thinner specimens were obtained for this study to accelerate the aging effect of water storage. It was assumed that the adverse effects of water on resin-dentin bond strength stemmed from the diffusion of water into resin-dentin interfaces[15].

μTBS sticks obtained from each tooth of all groups were randomly divided into two similarly sized subgroups. One subgroup was used for 24-h bond strength testing. The remaining sticks were stored in distilled water for a 12-month storage period before the second μTBS test. Storage medium did not include any antimicrobial agent, and neither was the storage medium changed to maintain the mechanical properties of dentin. Frequent changing of storage solutions would cause significant dentin mineral loss, thus decreasing the mechanical properties of dentin substrates[16].

After μTBS specimens were fixed to a jig using cyanoacrylate glue (Pattex, Henkel, Duesseldorf, Germany), they were stressed in tension until failure at a crosshead speed of 1 mm/min using Bisco microtensile testing machine. μTBS was derived by dividing the applied force at the time of fracture by the bonded area (mm^2). Modes of failure at resin-dentin bonded interfaces were determined using a stereo microscope at ×40 magnification (Meade Bresser Biolux, Meade Bresser, Rhede, Germany). They were recorded as: Adhesive failure (failure at resin-dentin interface), Cohesive failure (failure entirely within dentin substrate or resin composite), or Mixed failure (failure at resin-dentin interface including cohesive failure of one of the substrates).

Scanning electron microscopic observation

Three fractured specimens which presented adhesive

Table 1 Composition and manufacturer instructions of etch-and-rinse adhesives used in this study

Brands, LOT	Compositions	Instructions for use
Single Bond 2 (3M ESPE, St Paul, USA), N240989	Bis-GMA, HEMA, dimethacrylates, polyalkenoid acid copolymer, initiator, 34% water, ethanol	Etch for 15 s, rinse and dry gently. Scrub for 30 s, air-thin, light-cure for 10 s.
Prime&Bond NT (DENTSPLY De Trey, Konstanz, Germany), 0901000595	PENTA, UDMA, T-resin (cross-linking agent) , D-resin, (small hydrophilic molecule), butylated hydroxitoluene, 4-ethyl dimethyl aminobenzoate, cetilamine hydrofluoride, acetone, silica nanofiller	Etch for 15 s, rinse and dry gently. Apply primer/bond, leave for 20 s, gently air dry, light-cure for 10 s

Bis-GMA: Bisphenol-A-glycidyl methacrylate, DMA: Dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, PENTA: Dipentaerythritolpenta-acrylate phosphate, TEGDMA: Triethylene glycoldimethacrylate.

or mixed failure were randomly selected from each group. Each selected specimen was fixed in 10% neutral buffered formalin for 48 h. After drying at room temperature for 24 h[17], specimens were sputter-coated with gold. Dentin surfaces of fracture sites were observed under a scanning electron microscope (SEM; JEOL 6400, JEOL, Tokyo, Japan) with different magnifications (×85–2,500).

Statistical analysis

Two-way analysis of variance (ANOVA) with Tukey's HSD test were performed to determine the effects of independent variables (adhesive resin and storage period) on dependent variable (μTBS) per wet bonding technique. After performing two-way ANOVA, one-way ANOVA and/or pairwise comparisons were performed. Statistical significance was set in advance at p=0.05.

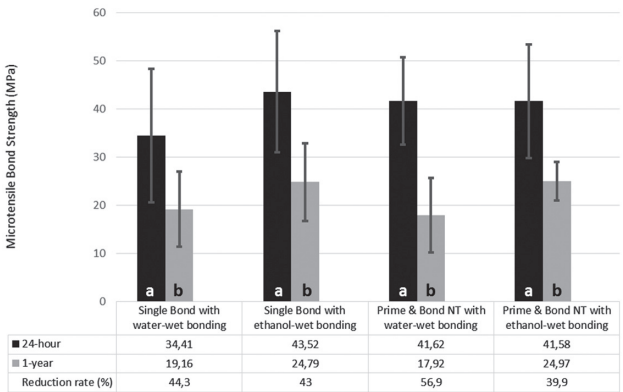


Fig. 1 Graphical presentation of microtensile bond strength results.
Same letters on the graph represent absence of statistical difference (p>0.05).

RESULTS

Microtensile bond strength

At the 24-h testing period, simplified ethanol-wet bonding technique did not affect the resin-dentin bond strength of all the adhesives tested (p>0.05) (Fig. 1). After 12-month water storage, the bond strengths of both simplified ethanol-wet bonding and control groups of all the adhesives tested were significantly reduced (p<0.05) (Fig. 1). Storage in distilled water reduced the bond strength of control groups by 44.32% and 56.94% for Single Bond and Prime & Bond NT respectively. For the simplified ethanol-wet bonding groups, reductions were 43.04% and 39.95% for Single Bond 2 and Prime & Bond NT respectively. At the 12-month testing period, simplified ethanol-wet bonding technique did not improve the stability of resin-dentin bonds of all the adhesives tested (p>0.05) (Fig. 1).

Failure mode distribution

Adhesive failure was higher in Group 1 (Single Bond 2 with water-wet bonding) than the other groups at 24-h testing period; at 12-month testing period, similar adhesive failure rates were seen among all the groups (Table 2). Reduction in mixed failure rate and increase in adhesive failure rate were due to storage time, not because of wet bonding technique.

SEM micrographs

Figures 2–4 show the representative SEM micrographs of resin-dentin interfaces of both water-wet and ethanol-wet bonding groups. For all specimens which were fractured after 24-h water storage, intertubular dentin seemed to be completely covered by adhesive and neither funnel-shaped nor lateral dentinal tubules were observed. For specimens which were fractured after 12-month water storage, porosity increased within intertubular dentin and funnel-shaped or exposed lateral dentinal tubules existed independently of adhesive agent or wet bonding technique.

Table 2 Distribution of failure modes (%) at resin-dentin bonded interfaces

Duration	24-h			One year		
Groups	Adhesive	Mix	Cohesive	Adhesive	Mix	Cohesive
Group 1 (Single Bond with water-wet bonding)	80	20	—	70	—	30
Group 2 (Prime & Bond NT with water-wet bonding)	55	30	15	70	5	25
Group 3 (Single Bond with ethanol-wet bonding)	50	35	15	70	5	25
Group 4 (Prime & Bond NT with ethanol-wet bonding)	65	25	10	60	5	35

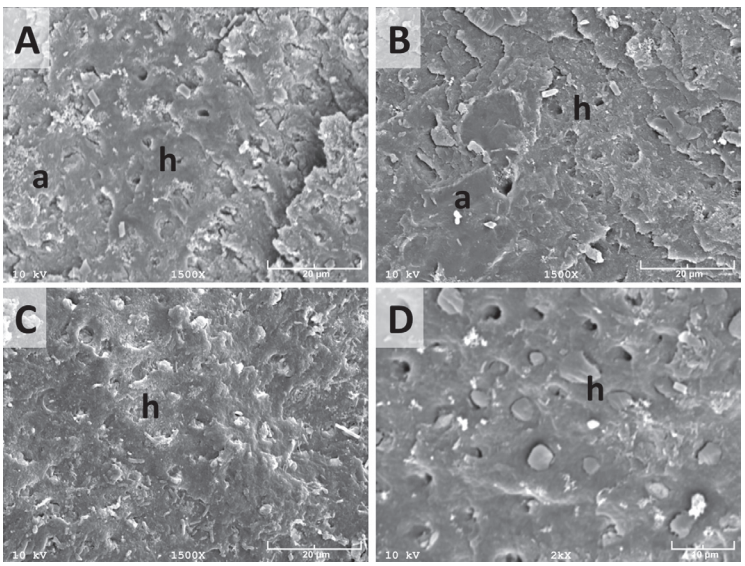


Fig. 2 Representative scanning electron micrographs (SEM) of the dentin side of fractured specimens of different groups at 24-h testing time, where a=adhesive resin, h=hybrid layer. Water-wet bonding with Single Bond 2 (A) and Prime & Bond NT (B); ethanol-wet bonding with Single Bond 2 (C) and Prime & Bond NT (D).
(A) High magnification (×1,500) demonstrates failure localized at the hybrid layer. Intertubular dentin seems to be completely covered by adhesive. No funnel-shaped dentinal tubules or lateral dentinal tubules are evident. (B) High magnification (×1,500) shows partial cohesive failure of adhesive (a) and hybrid layer (h). Intertubular dentin seems to be completely covered by adhesive. (C) High magnification shows cohesive failure of hydrid layer (h). Exposed dentinal tubules are filled by resin tags, while intertubular dentin seems to be completely covered by adhesive. (D) High magnification (×2,000) demonstrates cohesive failure of hydrid layer (h). Intertubuler dentin and most of exposed dentinal tubules are well infiltrated by adhesive resin.

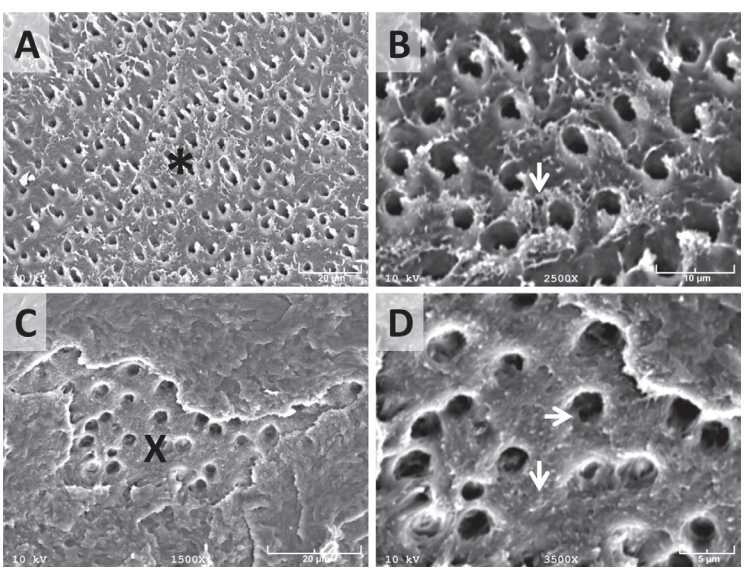


Fig. 3 Representative scanning electron micrographs (SEM) of the dentin side of fractured specimens of different groups at 12-month testing time.
Water-wet bonding with Single Bond 2 (A, B) and Prime & Bond NT (C, D).
(A) High magnification (×1,000) demonstrates failure localized at the hydrid layer. Porosity is observed for intertubuler dentin. (B) Higher magnification (×2,500) of the area indicated by asterik (*) in (A), which shows increased porosity and funnel-shaped dentinal tubules, indicating the degradation of exposed collagen fibrils over time (white arrow). (C) High magnification shows mixed failure of the adhesive resin and hydrid layer. (D) Higher magnification (×3,500) of the area indicated by 'X' in (C), which shows increased porosity in intertubuler dentin and exposed lateral dentinal tubules (white arrows).

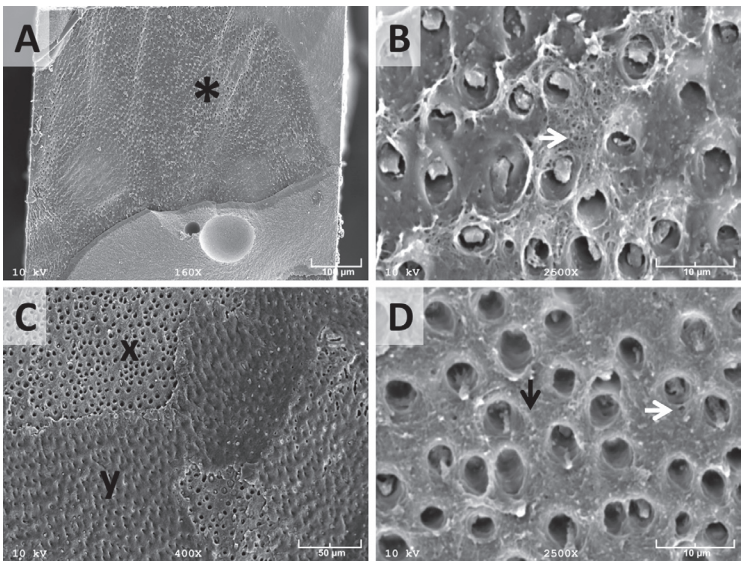


Fig. 4 Representative scanning electron micrographs (SEM) of the dentin side of fractured specimens of different groups at 12-month testing time.
Ethanol-wet bonding with Single Bond 2 (A, B) and Prime & Bond NT (C, D).
(A) Low magnification (×160) demonstrates mixed failure localized at the top of hybrid layer and composite resin. (B) Higher magnification (×2,500) of the area indicated by asterisk (*) in (A), which shows increased porosity in intertubular dentin at the top of hybrid layer, indicating the elution of resin monomers due to hydrolysis (white arrow). (C) Mid-high magnification (×400) shows mixed failure of the top (y) and bottom (x) layers of hybrid layer. (D) Higher magnification (×2,500) of the area indicated by 'X' in (C), which shows increased porosity in intertubular dentin (black arrow) and exposed lateral dentinal tubules (white arrow). Openings of funnel-shaped dentinal tubules are also evident.

DISCUSSION

Results of the present study did not support the null hypothesis, which stated that simplified ethanol-wet bonding would preserve the bonding stability of current etch-and-rinse adhesives to bovine dentin after 1 year of water storage. When Single Bond 2 and Prime & Bond NT were bonded to acid-etched dentin with simplified ethanol-wet bonding technique, significant reductions were seen in their mean 24-h and 1-year bond strengths. Therefore, the null hypothesis was rejected.

Unlike resin-enamel interfaces, degradation at resin-dentin interfaces occurs *via* the hydrolytic degradation of exposed collagen fibrils. In the presence of water, the latter occurs due to activation of endogenous MMPs and the hydrolysis of hydrophilic adhesive resins. Due to hydrolysis of the resin, elution of resin from hybrid layer over time increases the area of exposed collagen fibrils —which are susceptible to degradation by activated MMPs. Consequently, different degradation mechanisms may act synergistically to destroy the hybrid layer[18].

In previous studies on the durability of resin-dentin interfaces, Kato and Nakabayashi provided SEM findings in 1998 to show that the bond strength of etch-and-rinse adhesive reduced over time because of the degradation of non-resin-impregnated, demineralized dentin zone[19]. In 1999, Sano *et al.* revealed through an *in vivo* study that bond degradation of resin-dentin interfaces prepared with self-etch primers occurred because of the hydrolysis of resin within the hybrid layer rather than due to degradation of exposed collagen fibrils[17]. By SEM observation of the fracture surfaces of specimens aged *in vivo*, increased porosity was observed at the top of hybrid layer because of resin elution from between the collagen fibrils[17].

However, Hashimoto *et al.* confirmed the notion that collagen fibrils which were not well encapsulated with adhesive resin were susceptible to hydrolytic degradation *in vivo*[20]. Openings of lateral dentinal tubules were present on the fracture surfaces of 1-year-old specimens, which were not seen in the 24-h control group[20]. In another study, Pashley *et al.* revealed that in the absence of bacterial enzymes, host-derived MMPs within the dentin matrix could degrade exposed collagen fibrils within the hybrid layer[8].

In light of the μTBS results and SEM micrographs obtained in the present study, simplified version of the ethanol-wet bonding technique did not improve the bond durability of the tested adhesives' resin-dentin interfaces. One-year water storage resulted in significant bond strength reduction of both simplified ethanol-wet bonding and control groups irrespective of adhesive type. For the control groups, mean bond strengths were reduced by 44.32% and 56.94% for Single Bond and Prime & Bond NT respectively. For the simplified

ethanol-wet bonding groups, reductions were 43.04% and 39.95% for Single Bond 2 and Prime & Bond NT respectively. At 24 h, SEM micrographs of all the groups revealed that intertubular dentin within the hybrid layer was well impregnated with adhesive resin (Fig. 2). After 1-year water storage, porosity increased within the intertubular dentin for all groups and the presence of exposed lateral dentinal tubule openings indicated the degradation of hybrid layers (Figs. 3 and 4).

Several explanations could be offered for these findings. The cardinal step in ethanol-wet bonding is the chemical dehydration of water-saturated acid-etched dentin with a series of increasing ethanol concentrations (50, 70, 80, 95, and 100% three times at 30 s each), which culminates in an effect similar to epoxy resin tissue embedding used in transmission electron microscopy (TEM)[21]. TEM showed that interfibrillar spaces remained open and served as diffusion pathways for resin infiltration[22], thus improving resin encapsulation of exposed collagen fibrils and protecting them from degradation by MMPs and the plasticization effect of water[10]. While the simplified version of ethanol-wet bonding technique is certainly a more clinically relevant protocol, water-saturated acid-etched dentin surfaces were saturated with absolute ethanol for 60 s only —unlike the full chemical dehydration protocol which mimics epoxy resin tissue embedding[12,21].

In a study by Gregoire *et al.*, they claimed that treating acid-etched dentin with absolute ethanol twice for 10 s could leave water strongly bound to collagen molecules while excess water was replaced by ethanol prior to adhesive application[23]. However, pulpal pressure was not simulated in their study[23]. Osorio *et al.* showed that simplified ethanol application (100% ethanol for 60 s) caused collagen fibrils to collapse because of evaporation of water or absolute ethanol[24]. Therefore, simplified ethanol-wet bonding could not prevent collapse of collagen fibrils, and that residual water might cause exposure of collagen fibrils due to collapse of dentin matrices, phase separation of diffused resins[22], and reduced polymerization rates of infiltrated adhesive resins[25].

On the other hand, alongside trapped water, ethanol which is used as the solvent prior to adhesive resin/primer application may not completely evaporate after resin infiltration —within the clinically acceptable solvent evaporation periods. This phenomenon resembles the residual solvent problem which commonly plagues solvated adhesive resin systems[26]. Therefore, the limited dehydration ability of simplified ethanol-wet bonding technique, coupled with the presence of residual ethanol trapped within the hybrid layer prior to light-curing of adhesive resin, caused incomplete resin infiltration into exposed collagen matrices. Consequently, collagen fibrils were not encapsulated with resin, rendering them susceptible to MMP degradation at the bottom of the hybrid layer over time.

Another possible explanation for the reduced bond strength in this study was the high hydrophilicity of commercial etch-and-rinse adhesives, which were developed to be used with water-wet bonding technique[27]. As manufacturers did not disclose the exact composition of their adhesive systems, the exact concentrations of hydrophilic monomers —such as HEMA in Single Bond 2, PENTA and D-resin in Prime & Bond NT[28]— were not known. Available information was that Single Bond 2 contained 15-5 wt% HEMA and 5 wt% water, and Prime & Bond NT contained 10% PENTA and an unknown amount of D-resin according to the material safety data sheets of the respective manufacturers.

In general, resin hydrophilicity is inversely related to the mechanical properties of resultant polymers due to hydrolysis and plasticization over time. For this reason, this property is not one expected to preserve the dentin bonding durability of commercial hydrophilic etch-and-rinse adhesives and experimental hydrophilic resin blends[27]. Hosaka *et al.* tested the dentin bond durability of different increasingly hydrophilic experimental resin blends which were bonded to acid-etched dentin saturated with water or ethanol. Although most resin-dentin bonds of water-saturated dentin did not change over time, those of ethanol-saturated dentin presented higher bond strengths and none showed significant reductions over time[9]. In another study, Sadek *et al.*[12] assessed the bond durability of a commercial etch-and-rinse adhesive which was deemed relatively hydrophilic when compared with solvated Bis-GMA-TEDGMA resin blend: the former adhesive's primer contained HEMA, polyalkenoic acid copolymer, and water. The resin-dentin bond strength of commercial hydrophilic adhesive with water-wet dentin was significantly reduced after 1-year water storage, whereas that of experimental hydrophobic adhesive with ethanol-wet bonding was preserved after 1-year storage[12].

Although information is scarce about the effects of simplified ethanol-wet bonding on the resin-dentin bonding durability of relatively hydrophilic experimental and/or commercial adhesive resins, results of the present study seemed to contradict some of the published findings[29]. These differences probably arose from differences in the study designs and artificial aging strategies used. Li *et al.* assessed the effects of simplified ethanol-wet bonding technique on the bond durability of commercial etch-and-rinse adhesives, including Single Bond 2 and Prime & Bond NT, after immersion in 10% NaOCl[29]. Although NaOCl challenge significantly reduced the bond strength of tested adhesives irrespective of wet bonding technique, significant differences were found between water-wet and ethanol-wet bonding groups of each adhesive after NaOCl immersion. Thus, they concluded that simplified ethanol-wet bonding technique improved the bonding efficacy of tested etch-and-rinse adhesives.

NaOCl immersion is responsible for only one aspect of the degradation process —namely, the chemical degradation of exposed collagen fibrils. It has no significant effect on the degradation process of resin

components, which is more critically affected by water uptake[30]. Therefore, these factors and considerations were probably not taken into account in the study by Li *et al.*[29]: effects of water uptake and dissolution of infiltrated resin into the hydrid layer, due to increased hydrophilicity of commercial adhesives, on bond durability of hybrid layer created by simplified ethanol-wet bonding.

When comparing the reduction rates in bond strength between the ethanol-wet bonding groups and control groups after 1-year water storage (Fig. 1), it was speculated that the different chemical compositions of the tested adhesives accounted for the different reduction rates. Single Bond 2 contained water, which then reduced the anti-degradation effects of ethanol-wet bonding. Prime & Bond NT did not contain any water and used only acetone as the solvent. This explained its diminished loss in bond strength with simplified ethanol-wet bonding, although the reduction in bond strength was statistically significant (Fig. 1).

CONCLUSIONS

Within the limitations of the present study, the following conclusions were drawn:

1. Simplified ethanol-wet bonding, which might be deemed the most clinically relevant ethanol-wet bonding technique, did not preserve the bond strength of commercial hydrophilic etch-and-rinse adhesives after 1 year of water storage.
2. Prime & Bond NT, which did not contain water and used only acetone as the solvent, demonstrated diminished loss in bond strength with ethanol-wet bonding when compared with Single Bond 2 which contained water in its composition.
3. Enzymatic degradation of exposed collagen fibrils between sound dentin and the hybrid layer caused the reduction in bond strength with ethanol-wet bonding. Degradation was caused by the synergistic effects of several factors: limited water removal capability of 60-s ethanol dehydration protocol, presence of residual ethanol after adhesive resin application, and the hydrophilic nature of tested adhesives.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that no financial interests exist for this study.

REFERENCES

1) Sarr M, Kane AW, Vreven J, Mine A, Van Landuyt KL, Peumans M, Lambrechts P, Van Meerbeek B, De Munck J. Microtensile bond strength and interfacial characterization of 11 contemporary adhesives bonded to bur-cut dentin. Oper Dent 2010; 35: 94-104.
2) Hashimoto M, Nagano F, Endo K, Ohno H. A review: biodegradation of resin-dentin bonds. Jpn Dent Sci Rev 2011; 47: 5-12.
3) Sano H. Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds. J Dent Res 2006; 85: 11-14.
4) Peumans M, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Five-year clinical effectiveness of a two-step self-etching adhesive. J Adhes Dent 2007; 9: 7-10.
5) Van Landuyt KL, Peumans M, Fieuws S, De Munck J, Cardoso MV, Ermis RB, Lambrechts P, Van Meerbeek B. A randomized controlled clinical trial of a HEMA-free all-in-one adhesive in non-carious cervical lesions at 1 year. J Dent 2008; 36: 847-855.
6) Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005; 26: 6449-6459.
7) Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. Dent Mater 2005; 21: 895-910.
8) Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004; 83: 216-221.
9) Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, Pashley DH. Durability of resin-dentin bonds to water- *vs.* ethanol-saturated dentin. J Dent Res 2009; 88: 146-151.
10) Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater 2011; 27: 1-16.
11) Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. Ethanol wet-bonding challenges current anti-degradation strategy. J Dent Res 2010; 89: 1499-1504.
12) Sadek FT, Castellan CS, Braga RR, Mai S, Tjaderhane L, Pashley DH, Tay FR. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent Mater 2010; 26: 380-386.
13) Brackett WW, Tay FR, Brackett MG, Dib A, Sword RJ, Pashley DH. The effect of chlorhexidine on dentin hybrid layers *in vivo*. Oper Dent 2007; 32: 107-111.
14) Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjaderhane L, Ruggeri A Jr, Tay FR, Dorigo Ede S, Pashley DH. Chlorhexidine stabilizes the adhesive interface: a 2-year *in vitro* study. Dent Mater 2010; 26: 320-325.
15) Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, Oguchi H, Araki Y, Kubota M. Micromorphological changes in resin-dentin bonds after 1 year of water storage. J Biomed Mater Res 2002; 63: 306-311.
16) Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on dentin bond durability of resin cement. Dent Mater 2000; 16: 1-6.
17) Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, *in vivo*. J Dent Res 1999; 78: 906-911.
18) 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.
19) Kato G, Nakabayashi N. The durability of adhesion to phosphoric acid etched, wet dentin substrates. Dent Mater 1998; 14: 347-352.
20) Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000; 79: 1385-1391.
21) Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, Tay FR. Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. J Biomed Mater Res A 2008; 84: 19-29.
22) Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, Donnelly A, García-Godoy F. 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.
23) Grégoire G, Sharrock P, Delannée M, Delisle MB. Depletion of water molecules during ethanol wet-bonding with etch and rinse dental adhesives. Mater Sci Eng C Mater Biol Appl 2013; 33: 21-27.
24) Osorio E, Toledano M, Aguilera F, Tay FS, Osorio R. Ethanol wet-bonding technique sensitivity assessed by AFM. J Dent Res 2010; 89: 1264-1269.
25) Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res B Appl Biomater 2003; 66: 324-330.
26) Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH, Tay FR. Solvent and water retention in dental adhesive blends after evaporation. Biomaterials 2005; 26: 6863-6872.
27) Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Assoc 2003; 69: 726-732.
28) Perdigao J, Geraldeli S, Carmo AR, Dutra HR. *In vivo* influence of residual moisture on microtensile bond strengths of one-bottle adhesives. J Esthet Restor Dent 2002; 14: 31-38.
29) 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: 113-120.
30) Amaral FL, Colucci V, Palma-Dibb RG, Corona SA. Assessment of *in vitro* methods used to promote adhesive interface degradation: a critical review. J Esthet Restor Dent 2007; 19: 340-353.