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Do Matrix Metalloproteinase Inhibitors Improve the Bond Durability of Universal Dental Adhesives?

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Summary: The aim of this study was to evaluate the effects of matrix metalloproteinases (MMPs) inhibitors on the microtensile bond strength (μTBS) and the adhesive–dentin interface of two universal dentin bonding agents, Single Bond Universal and All Bond Universal, after 12 months of water storage. Seventy extracted, caries-free, human third molars were used in this study. Of these, 50 were used for μTBS testing and 20 were used for scanning electron microscopy. The two bonding agents were applied to flat dentin surfaces in five different ways: self-etch mode, etch-and-rinse mode with 37% phosphoric acid, etch-and-rinse mode with phosphoric acid containing 1% benzalkonium chloride, etch-and-rinse mode with phosphoric acid and 2% chlorhexidine, and etch-and-rinse mode with 0.5 M ethylenediaminetetraacetic acid (EDTA) ($n = 5$ for each bonding agent in each group; $N = 50$). Half the specimens were subjected to μTBS tests at 24 h, while half were subjected to the tests after 12 months of water storage. For each bonding agent, inhibition, storage, and their interaction effects were tested by two-way analysis of variance and Bonferroni tests. For Single Bond Universal, the benzalkonium chloride ($p = 0.024$) and chlorhexidine groups ($p = 0.033$) exhibited significantly higher μTBS values at 24 h compared with the self-etch group. For All Bond Universal, all groups displayed similar bond strengths at 24 h ($p > 0.05$). After 12 months of water storage, the μTBS values decreased significantly in the benzalkonium chloride group for Single Bond Universal ($p = 0.001$) and the self-etch ($p = 0.029$), chlorhexidine ($p = 0.046$), and EDTA ($p = 0.032$) groups for All Bond Universal. These results suggest that the immediate dentin bond strength increases when universal bonding systems are applied in the etch-and-rinse mode, although the durability decreases. The use of chlorhexidine and EDTA can increase the bond durability of mild adhesives such as Single Bond Universal. SCANNING 38:535–544, 2016.
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Key words: universal adhesives, benzalkonium chloride, chlorhexidine, EDTA, durability

Introduction

The ability of dentin bonding agents to provide a good seal and stable adhesion to tooth substrates is one of the primary requirements for the durability of tooth-colored restorations (Reis *et al*., 2008). Formation of a resin-infiltrated hybrid layer composed of collagen fibrils embedded by methacrylate-based resins contributes to adhesion (Breschi *et al*., 2010a). However, despite successful immediate bonding, longevity of the adhesive interface remains questionable because of physical and chemical factors challenging the adhesive interface (Breschi *et al*., 2010b). Universal adhesives represent the last generation of adhesives in the market. Although they are designed under the "all-in-one" concept of existing one-step self-etch adhesives, it is possible to use them in the etch-and-rinse mode.

In theory, degradation of the adhesive–dentin interface should not occur when water within the intra- and interfibrillar compartments of a collagen matrix is completely replaced by resin (Sadek *et al*., 2010). However, the hydrophilic nature of self-etch systems and the poor infiltration capacity of etch-and-rinse resins into collagen can impair the covering step. Collagen fibrils poorly encapsulated by resin can be slowly hydrolyzed by endogenous matrix metalloproteinases (MMPs) (Mazzoni *et al*., 2006, 2007; Tjaderhane *et al*., 2013). Procedures that improve resin infiltration or resin coating of each collagen fibril

Conflicts of interest: None.

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may prevent MMPs from coming in contact with water, thus increasing the durability of resin–dentin bonds.

MMPs belong to a group of zinc- and calcium-dependent enzymes that cleave native collagenous tissues. Once activated by an adhesive procedure, they can slowly degrade the collagen fibrils of resin-infiltrated hybrid layers (Sorsa *et al*., 2004; De Munck *et al*., 2010; Pashley *et al*., 2011). Enzymatic degradation of the collagen matrix by host-derived enzymes plays a significant role in the destruction of the bonded interface (Tjaderhane *et al*., 2013). This results in the loss of adhesion of resin composites to dentin, which can be measured as a significant decrease in the microtensile bond strength (μTBS) (Pashley *et al*., 2011). To block enzymatic degradation while preserving the hybrid layers, investigators have applied MMPs inhibitors such as benzalkonium chloride (Sabatini and Patel, 2013; Sabatini and Pashley, 2015), chlorhexidine (Hebling *et al*., 2005; Carrilho *et al*., 2007a,b; Breschi *et al*., 2010b; Kim *et al*., 2010; Lenzi *et al*., 2012), and ethylenediaminetetracetic acid (EDTA) during adhesive application (Osorio *et al*., 2005).

Although studies on enzyme inhibitors are available, their effects on the bond durability of universal adhesives remain unclear. Manufacturers often recommend the use of universal adhesives in the etch-and-rinse mode; however, this application mode has been shown to result in MMPs activation (Zhang and Kern, 2009). From the above perspectives, we conducted the present study to evaluate the effects of three different MMPs inhibitors on μTBS values and the adhesive–dentin interfaces of two different universal dentin bonding agents applied using different methods, including the etch-and-rinse method, at 24 h and 12 months after water storage. The null hypotheses tested were as follows: (i) MMPs inhibitors do not improve the immediate μTBS of universal adhesives and (ii) MMPs inhibitors do not prevent bond degradation after 12 months of water storage.

Materials and Methods

Seventy extracted, caries-free, human third molars were used in this study. Of these, 50 were used for μTBS tests and 20 were used for scanning electron microscopy (SEM). They were stored in 0.5% chloramine solution at 4˚C and were used within 2 weeks. All teeth were obtained from patients who underwent extraction as a routine part of their treatment after providing written informed consent. The occlusal enamel was horizontally sectioned, and the exposed dentin surfaces were further polished using wet 600-grit silicon carbide paper for 60 s to achieve a standardized smear layer. After the flat dentin surfaces were prepared, the following groups were constituted according to the application of Single Bond Universal (3M ESPE, St. Paul, MN) or All Bond Universal (Bisco, Schaumburg, IL) (n = 25 for each adhesive).

- Self-etch group: the universal dentin bonding agent was applied using the self-etch mode on flat dentin surfaces according to the manufacturer's instructions (n = 5).
- Etch-and-rinse group: 37% phosphoric acid (3M ESPE) was applied to the surfaces for 15 s and rinsed thoroughly, following which the universal dentin bonding agent was applied using the etch-and-rinse mode according to the manufacturer's instructions (n = 5).
- Benzalkonium chloride group: 37% phosphoric acid containing 1% benzalkonium chloride (Bisco) was applied to the surfaces for 15 s and rinsed thoroughly, following which the universal dentin bonding agent was applied using the etch-and-rinse mode according to the manufacturer's instructions (n = 5).
- Chlorhexidine group: 37% phosphoric acid was applied to the dentin surfaces for 15 s and rinsed thoroughly. The flat surfaces were treated with an aqueous solution of 2% chlorhexidine (Cerkamed, Stalowa Wola, Poland) for 60 s (which was not rinsed off), following which the universal dentin bonding agent was applied using the etch-and-rinse mode according to the manufacturer's instructions (n = 5).
- EDTA group: 0.5 M EDTA was applied to the dentin surfaces for 60 s and rinsed thoroughly, following which the universal bonding agent was applied according to the manufacturer's instructions (n = 5).

The study design is depicted as a flowchart in Figure 1.

The ingredients and manufacturer's instructions for the dentin bonding agents used in this study are listed in Table I. After their application to the flat dentin surfaces, core build-ups measuring 5 mm in height were created using Filtek Ultimate Universal composite resin (nanofill resin composite A2; 3M ESPE) applied in three layers with a maximum 2 mm thickness each. Each layer was cured for 40 s using a quartz–tungsten halogen curing unit (Bisco,VIP Dental Curing Light, Schaumburg, IL). The light intensity output was maintained at a minimum of 600 mW/cm^2 and was monitored using a dental radiometer (Hilux Ledmax Light Curing Meter; Benlioğlu Dental Inc., Ankara, Turkey). All specimens were prepared by a dental specialist (N.T.).

After storage in distilled water for 24 h at 37˚C, the restored teeth were divided into two equal parts. Each half was longitudinally sectioned across the bonded interfaces in the mesiodistal and buccolingual directions using a slow-speed diamond saw to obtain resin–dentin beams with a cross-sectional area of approximately 0.9 mm^2. Beams prepared from the same teeth were randomly divided and assigned for immediate μTBS testing or μTBS testing after 12 months of storage in distilled water at 37˚C.

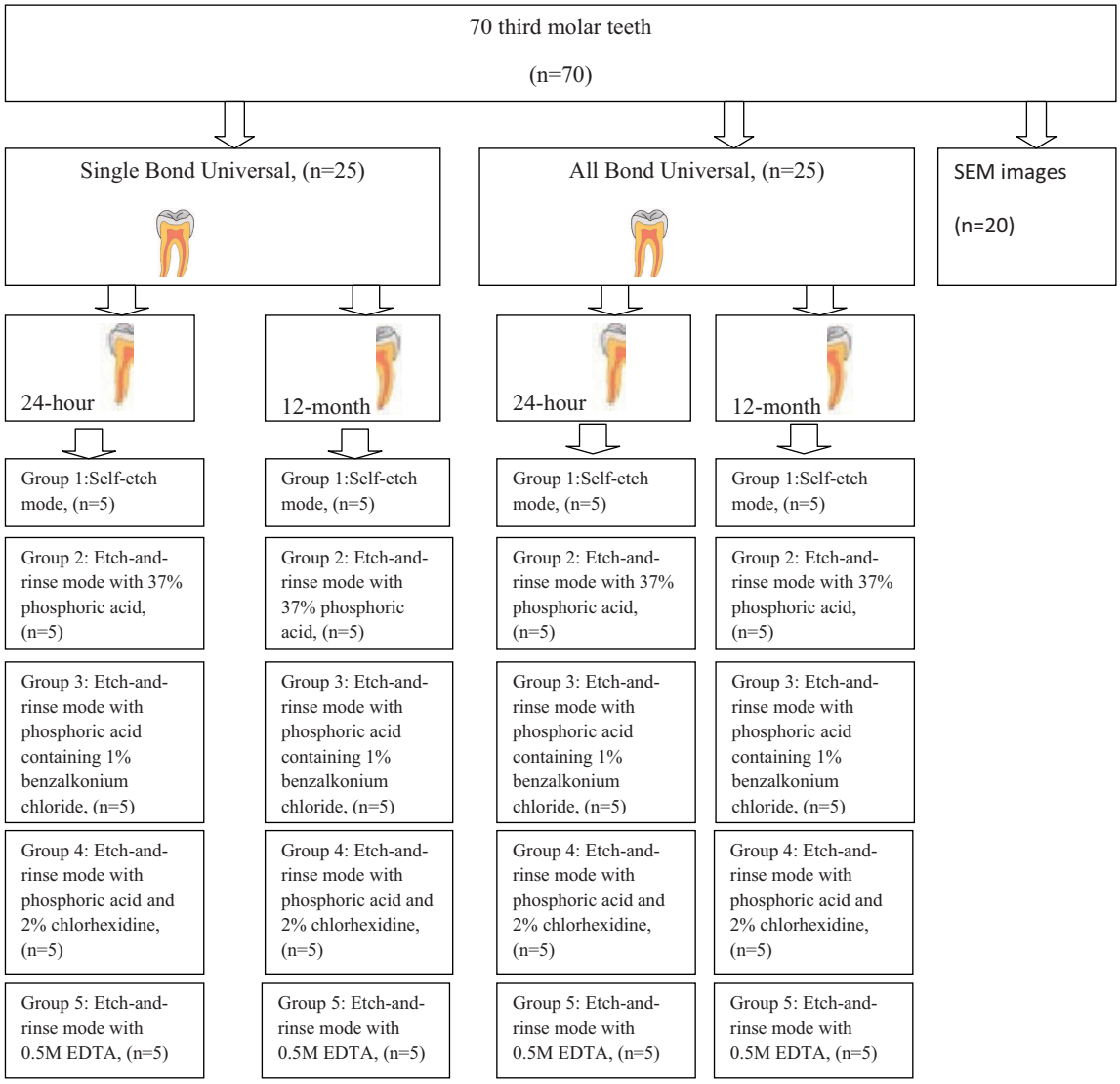


Fig 1. Overview of the study design.

The beams were attached to a modified device for μTBS testing using cyanoacrylate resin (Zapit Dental Ventures of North America, Corona, CA) and were subjected to a tensile force in a universal testing machine (Micro Tensile Tester; Bisco Inc., Schaumburg, IL) at a crosshead speed of 0.5 mm/min. The tensile load was applied until specimen failure. The failure load was recorded for each specimen, and the failure modes were evaluated at 30× magnification (Olympus SZ61, Munster, Germany). The failure modes were classified as follows: cohesive within dentin, cohesive within composite resin, and adhesive/mixed (failure at the resin–dentin interface or failure combined with cohesive failure of the neighboring substrates). The pretest failures were excluded from statistical analyses.

For each dentin bonding agent, two teeth per group (n = 20 in total) were used for SEM (JEOL JCM-5000 NeoScope™; JEOL, Tokyo, Japan) observation of the resin–dentin interface. The specimens were examined at various magnifications.

SEM Observations

For each dentin bonding agent, two teeth per group (n = 20 in total) were used for SEM observation of the resin–dentin interface. The specimens were vertically sectioned in a buccolingual plane through the center of the restoration and polished with 600-, 800-, and 1200-grit silicon carbide abrasive papers under running water. Subsequently, for fine polishing, they were treated with 1, 0.3, and 0.05 μm alumina powder slurry using polishing cloths. The specimens were immersed in 10% phosphoric acid solution for 15 s, following which they were rinsed with water for 15 s and dried for 10 s. Next, the specimens were treated with 10% sodium hypochlorite for 30 s, rinsed thoroughly with water, and fixed in glutaraldehyde solution (pH 7.4) for 2 h. Then, they were dehydrated through ascending series of ethanol (25–100%) and dried at room temperature for 24 h. Following dehydration, the samples were sputter-coated with gold (Emitech K-550X sputter

TABLE I Composition of the universal adhesives used in this study and their application procedures (information supplied in the safety data sheets and material instructions)

Universal adhesive	pH	Composition	Self-etch strategy	Etch-and-rinse strategy
Adper Single Bond Universal (3M ESPE, St. Paul, MN)	2.7	MDP phosphate monomer Dimethacrylate resins HEMA Vitrebond copolymer Filler Ethanol Water Initiator Silane	1. Rub the entire tooth structure for 20 s. 2. Direct a gentle stream of air over the liquid for approximately 5 s, until it no longer moves. 3. Light cure for 10 s.	1. Apply the etchant for 15 s. 2. Rinse thoroughly with water for 15 s. 3. Apply the adhesive as per the self-etch mode.
All-Bond Universal (Bisco, Schaumburg, IL)	3.1	MDP phosphate monomer Bis-GMA HEMA Ethanol Water Initiators	1. Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10–15 s per coat. Do not light cure between coats. 2. Evaporate any excess solvent by thorough air-drying with an air syringe for at least 10 s, until there is no visible movement of the material. The surface should have a uniform glossy appearance 3. Light cure for 10 s.	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Remove excess water with an absorbent pellet or high-volume suction for 1–2 s. 4. Apply the adhesive as per the self-etch mode.

coater; Emitech, Ashford, U.K.; operating at 20 kV) and examined under SEM (JEOL JCM-5000 NeoScope™; JEOL) at various magnifications.

Statistical Analyses

All statistical analyses were performed using SPSS for Windows version 20.0 (IBM, Chicago, IL). Kolmogorov–Smirnov tests were used to test the normality of data distribution. Variables are expressed as means ± standard deviations. For each dentin bonding agent, inhibition, storage, and their interaction effects were tested by two-way analysis-of-variance (ANOVA) models. All pairwise comparisons for inhibition and storage were performed using Bonferroni adjustments. A two-sided p-value of <0.05 was considered statistically significant.

Results

μTBS Values

The mean μTBS values with standard deviations and the values obtained in failure analyses are summarized in Table II. Two-way ANOVA revealed a significant influence of the inhibitors and time ($p < 0.05$). The application of MMPs inhibitors using etch-and-rinse strategies did not affect the short-term μTBS values for All Bond Universal, although it significantly affected those for Single Bond Universal.

For the Single Bond Universal specimens, at 24 h, the highest μTBS value was obtained for the benzalkonium chloride (45.55 ± 2.09 MPa) and chlorhexidine (45.22 ± 6.32 MPa) groups. These values were significantly higher than those for the self-etch group ($p = 0.024$ and 0.033, respectively). The bond strengths of the Single Bond Universal specimens decreased over time in all groups, although the decrease was significant only in the benzalkonium chloride group ($p = 0.001$). After 12 months of water storage, the EDTA group exhibited significantly higher μTBS values compared with the self-etch and benzalkonium chloride groups. The decrease in bonding effectiveness was different for each group (interaction factor, $p = 0.1010$), with the highest values observed for the EDTA group.

For the All Bond Universal specimens, at 24 h, the highest μTBS was obtained for the benzalkonium chloride (46.59 ± 4.98) and EDTA groups (46.29 ± 5.92). The bond strengths of the All Bond Universal specimens showed a significant decrease over the 12 months storage period in the self-etch ($p = 0.029$), chlorhexidine ($p = 0.046$), and EDTA groups ($p = 0.032$). After 12 months of water storage, the bond strengths of the All Bond Universal specimens in the etch-and-rinse, benzalkonium chloride, chlorhexidine, and EDTA groups were still higher than those of the specimens in the control group, although the differences were not statistically significant

TABLE II Mean and standard deviation microtensile bond strength (MPa) values for the different groups and the failure rates (%)

Universal adhesive		Group	Storage	μTBS	Fracture pattern		
					A/M	C	D
Single Bond Universal							
Two-way ANOVA		Self-etch group	24 h	36.09 ± 4.46 Aa	60.6	36.4	3
			12 months	34.81 ± 7.09 Ax	78.3	21.7	0
		Etch-and-rinse group	24 h	43.33 ± 3.41 Aab	58.4	32.4	9.2
			12 months	37.67 ± 3.40 Axy	79	18.6	2.4
		Benzalkonium chloride group	24 h	45.55 ± 2.09 Ab	58	32.3	7.7
			12 months	35.07 ± 3.64 Bx	75.5	24.5	0
Inhibitor	$p = 0.002$	Chlorhexidine group	24 h	45.22 ± 6.32 Ab	70.4	22.8	6.8
Storage	$p = 0.002$		12 months	41.19 ± 3.98 Axy	76	20	4
Interaction	$p = 0.101$	EDTA group	24 h	43.60 ± 3.78 Aab	66.1	38.3	5.6
			12 months	43.97 ± 5.63 Ay	73.4	24.4	2.2
All Bond Universal							
Two-way ANOVA		Self-etch group	24 h	38.36 ± 8.43 Ia	78.6	21.4	0
			12 months	30.07 ± 6.10 IIx	90	10	0
		Etch-and-rinse group	24 h	43.81 ± 3.61 Ia	65.5	29	5.5
			12 months	38.54 ± 6.19 Ix	71	29	0
		Benzalkonium chloride group	24 h	46.59 ± 4.98 Ia	70.2	20.4	9.4
			12 months	39.51 ± 6.66 Ix	71	29	0
Inhibitor	$p = 0.002$	Chlorhexidine group	24 h	38.92 ± 4.01 Ia	60.5	35.4	4.1
Storage	$p < 0.001$		12 months	31.37 ± 5.97 IIx	73.5	23.5	3
Interaction	$p = 0.978$	EDTA group	24 h	46.29 ± 5.92 Ia	57	34.2	8.8
			12 months	38.13 ± 4.47 IIx	76	24	0

A/M = adhesive/mixed failure, C = cohesive failure in the composite, D = cohesive failure in the dentin. The p-values in the second column represent inhibitor–storage time interactions assessed using two-way ANOVA. Means followed by similar capital letters are not significantly different (24 h/12 months comparison). Means followed by similar lowercase letters (comparison between inhibitors) are not significantly different (a,b = 24 h; x,y = 12 months).

($p > 0.05$). Modification of the etching procedures using MMPs inhibitors had an effect on the μTBS values ($p = 0.002$).

Fracture Analyses

For both Single Bond Universal and All Bond Universal specimens, the most common failure mode was mixed failure, i.e. failure within the adhesive resin or resin composite at 24 h (Table II). After 12 months of water storage, the rate of adhesive or mixed failure increased in each group compared with that at 24 h. The second most common type of failure was cohesive failure within the composite, with an incidence of 10–38.3%. Cohesive failure within dentin was the least frequent pattern.

SEM Observations

For Single Bond Universal, at 24 h, although adhesive tags could not be clearly observed, the self-etch group exhibited a thick adhesive layer with a hybrid layer (Fig. 2(a)). A thick, uniform, and continuous adhesive layer with a hybrid layer was evident in the etch-and-rinse, benzalkonium chloride, chlorhexidine, and EDTA groups at 24 h (Figs. 3(a), 4(a), 5(a), and 6(a)). In the etch-and-rinse and benzalkonium chloride groups, the resin tags penetrated deeper into the etched dentin (Figs. 3(a) and 4(a)). In the chlorhexidine group, some shorter resin tags protruding from the orifices of the dentinal tubules and within the dentinal tubules were observed (Fig. 5(a)). In EDTA group, the dentinal tubules were occluded with a thick adhesive and a hybrid layer (Fig. 6(a)). After 12 months, SEM findings for Single Bond Universal were similar to those at 24 h. A thick, uniform, and continuous adhesive layer with a hybrid layer was observed in the self-etch group (Fig. 2(b)). In addition, funnel-shaped formations and resin tags in the dentinal tubules, with a thick, uniform, and continuous adhesive layer with a hybrid layer, were observed in the other groups (Figs. 3(b), 4(b), 5(b), and 6(b)). However, funnel-shaped formations and resin tags in the dentinal tubules were more pronounced in the etch-and rinse and chlorhexidine groups (Figs. 3(b) and 5(b)).

For All Bond Universal, at 24 h, the self-etch group exhibited a thick, uniform, and continuous adhesive layer with a hybrid layer without resin tags (Fig. 7(a)). The other groups exhibited well-formed, funnel-shaped, and long resin tags in the dentinal tubules with a thick, uniform, and continuous adhesive layer with a hybrid layer (Figs. 8(a), 9(a), 10(a), and 11(a)). After 12 months, the adhesive layer in the self-etch group was no longer uniform (Fig.7(b)). Furthermore, there were gaps (debonding) at the resin–dentin interface, with no resin

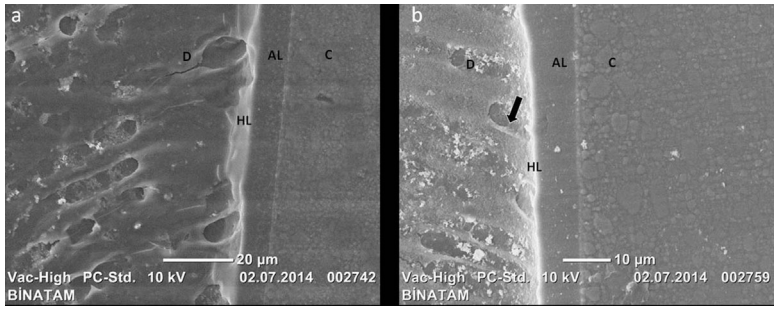


Fig 2. Standard electron microscopy (SEM) images for Single Bond Universal applied using the self-etch mode at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

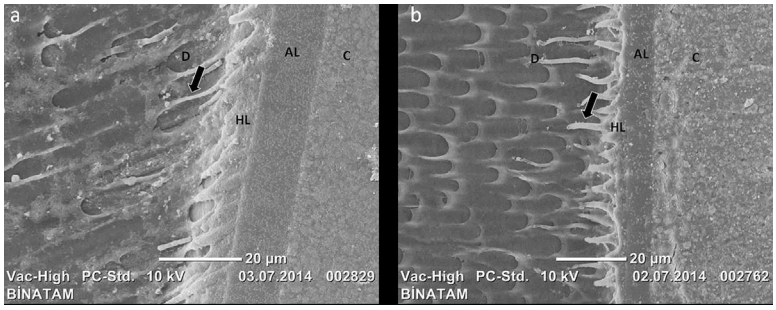


Fig 3. Standard electron microscopy (SEM) images for Single Bond Universal applied using the etch-and-rinse mode at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

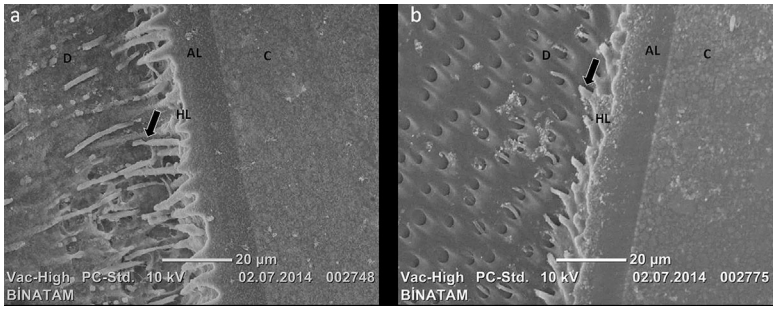


Fig 4. Standard electron microscopy (SEM) images for Single Bond Universal applied using the etch-and-rinse mode with phosphoric acid containing 1% benzalkonium chloride at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

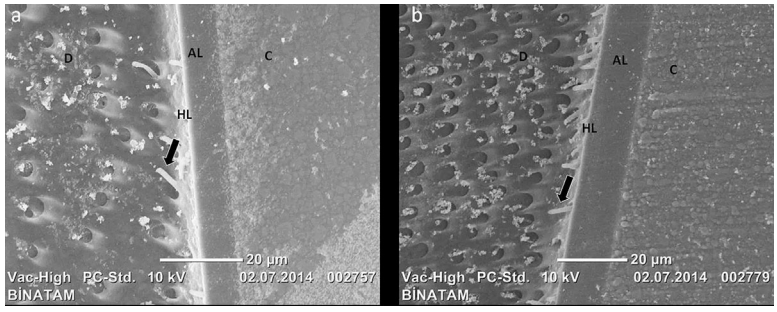


Fig 5. Standard electron microscopy (SEM) images for Single Bond Universal applied using the etch-and-rinse mode with chlorhexidine application at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

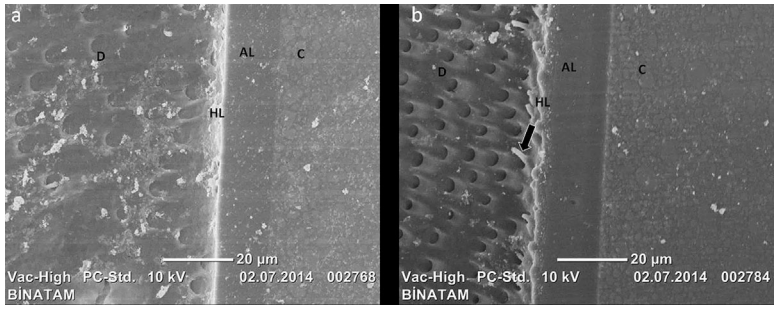


Fig 6. Standard electron microscopy (SEM) images for Single Bond Universal applied using the etch-and-rinse mode with EDTA at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

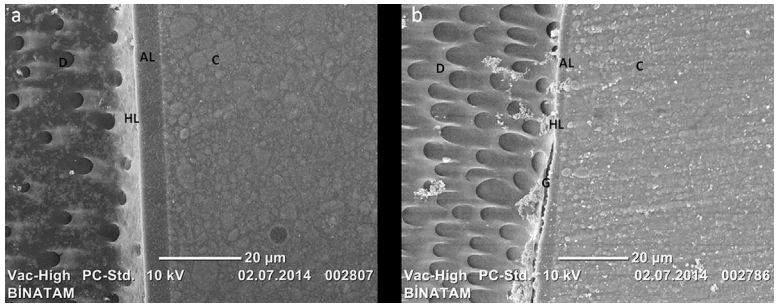


Fig 7. Standard electron microscopy (SEM) images for All Bond Universal applied using the self-etch mode at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag; G, microgap.

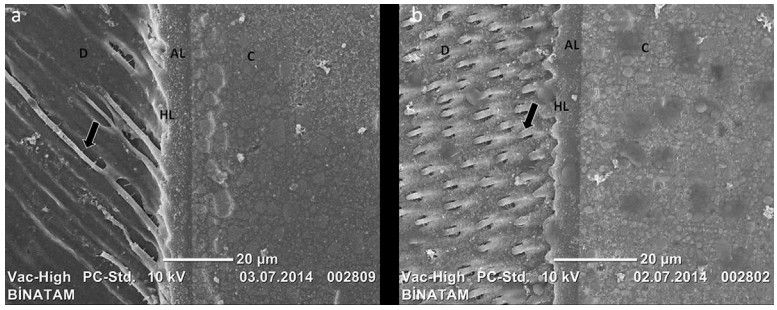


Fig 8. Standard electron microscopy (SEM) images for All Bond Universal applied using the etch-and-rinse mode at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

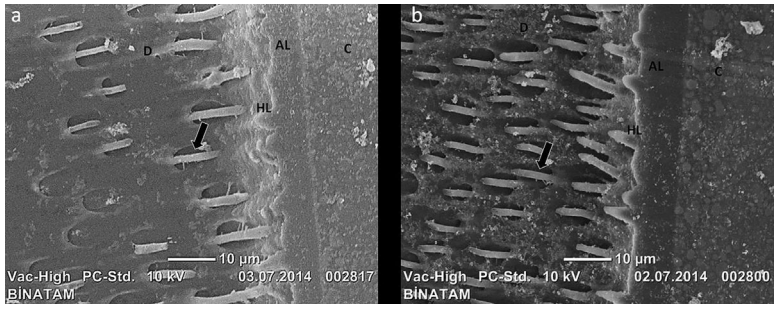


Fig 9. Standard electron microscopy (SEM) images for All Bond Universal applied using the etch-and-rinse mode with phosphoric acid containing 1% benzalkonium chloride at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

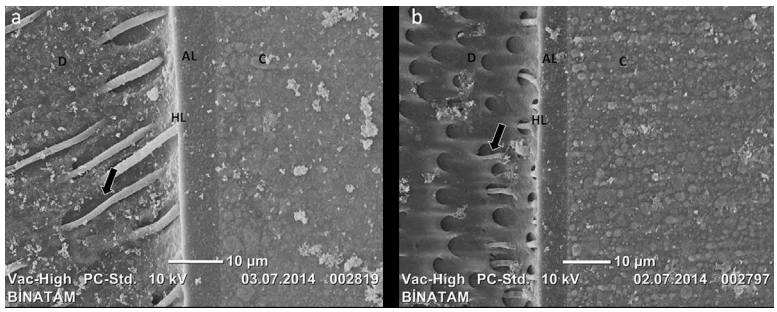


Fig 10. Standard electron microscopy (SEM) images for All Bond Universal applied using the etch-and-rinse mode with chlorhexidine application at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

tags (Fig. 7(b)). However, SEM findings for the other groups (Figs. 8(b), 9(b), 10(b), and 11(b)) were similar to those observed at 24 h. On the other hand, there were lesser funnel-shaped and long resin tag formations in the dentinal tubules after 12 months than at 24 h (Figs. 10(b) and 11(b)). In addition, extensive demineralization with no tubular appearance in the dentin was observed in the EDTA group after 12 months (Fig. 11(b)).

Discussion

Despite the several studies conducted to determine the initial bond strength, data regarding the long-term durability of newly introduced universal adhesive systems have been limited to date. As a deviation from previous studies, the 1-year bond durability of two different universal adhesives applied with and without MMPs enzyme inhibitors was examined in the present study. In addition, the dentin–adhesive interface was examined using SEM at 24 h and 12 months after water storage. One of the most commonly used MMPs enzyme inhibitor is chlorhexidine, because it prevents the destruction of the adhesive interface over time. We also used benzalkonium chloride and EDTA for the same reasons, with the aim of determining the most effective enzyme inhibitor with regard to protection of the adhesive–dentin interface.

In the present study, the effects of MMPs on the bond durability were indirectly assessed through evaluation of resin–dentin bond degradation. This was also investigated by Sabatini and Patel (2013) and De Munck *et al*. (2010). The results of μTBS tests showed that the etch-and-rinse mode with phosphoric acid, phosphoric acid containing 1% benzalkonium chloride, and EDTA resulted in higher bond strengths compared with the self-etch mode for both universal adhesives tested at two different time points. Thus, our first hypothesis that MMPs inhibitors do not improve the immediate μTBS of universal adhesives was rejected. After 12 months of water storage, μTBS decreased for all groups. However, chlorhexidine and EDTA appeared to contribute to improved bond stability of Single Bond Universal after 12 months of water storage. Therefore, our second hypothesis that MMPs inhibitors do not prevent bond degradation after 12 months of water storage was partially accepted.

The pH of All Bond Universal is 3.1, making it an ultramild self-etch adhesive, whereas that of Single Bond Universal is 2.7, making it a mild self-etch adhesive. The interaction depth of the ultramild adhesives in dentin, known as the nano-interaction or nano-interdiffusion zone, differed by a few hundred nanometers (Koshiro *et al*., 2006). However, the key factor in this ultramild adhesive is 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), because of its composition and consequent ability to form a chemical bond with the mineral content of partially demineralized dentin (Yoshida *et al*., 2004). Both universal bonding agents used in this study displayed very similar μTBS values at

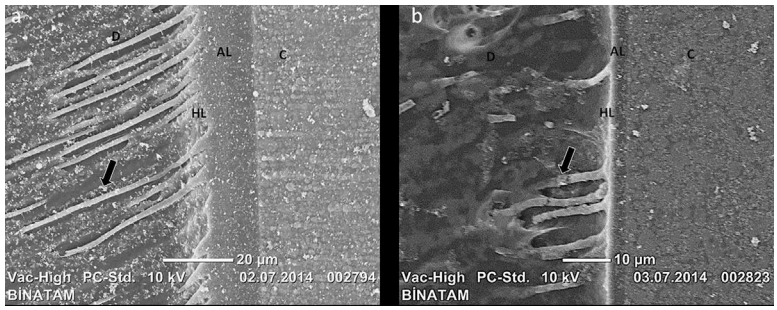


Fig 11. Standard electron microscopy (SEM) images for All Bond Universal applied using the etch-and-rinse mode with EDTA at (a) 24 h and (b) 12 months after water storage. C, composite resin; D, dentin; AL, adhesive layer; HL, hybrid layer; black arrow, resin tag.

24 h. This could be related with similar solvent and monomer contents in both adhesives (ethanol and water and 10-MDP, respectively).

In the present study, the etch-and-rinse, benzalkonium chloride, and chlorhexidine groups exhibited significantly higher μTBS values compared with the self-etch group for Single Bond Universal. On the other hand, all the All Bond Universal specimens showed similar values at 24 h. However, all four groups employing etch-and-rinse strategies showed higher μTBS values compared with the self-etch group for both universal adhesives. Phosphoric acid, which is commonly used, completely removes the smear layer and is also responsible for dentin demineralization up to depths of 5–8 μm (Pashley *et al*., 2011), depending on the concentration, pH, application duration, and viscosity (Nakabayashi *et al*., '82). After acid etching, a mineral-depleted collagen network is exposed, allowing resin infiltration through the nanometer spaces. This permits a strong mechanical interlocking between the adhesive and dentin, leading to high bond strength values. In agreement with Muñoz *et al*. (2013), we found higher μTBS values when the etch-and-rinse modes were used than when the self-etch mode was used for the application of both universal dentin bonding agents.

In the present study, the μTBS values for the etch-and-rinse and benzalkonium chloride groups at 24 h were very close to each other; this applied for both adhesive systems. Sabatini and Pashley (2015) examined adhesive interfaces treated with benzalkonium chloride for over 1 year. The researchers reported that treatment with both 0.5% and 1.0% benzalkonium chloride inhibited the total MMPs activity and prevented bond degradation when applied to acid-etched dentin for 60 s. As opposed to these findings, we found that μTBS values decreased significantly at 1 year for Single Bond Universal, not for All Bond Universal. Moreover, Sabatini and Pashley (2015) applied benzalkonium chloride in an adhesive blend, whereas we applied benzalkonium chloride in 37% phosphoric acid and washed the surfaces after application. Thus, the differences in results between our study and theirs can be attributed to differences in the methodology.

Sezinando *et al*. (2015) reported that the μTBS values of Scotchbond Universal adhesive regressed from 33.1 MPa to 26.8 MPa within 6 months when the etch-and-rinse mode was used, while the values decreased from 35.1 MPa to 30.5 MPa when the self-etch mode was used. For All Bond Universal, μTBS values decreased from 41.1 MPa to 32.2 MPa when the etch-and-rinse mode was used and from 24.1 MPa to 23.9 MPa when the self-etch mode was used. In agreement with Sezinando *et al*. (2015), in the present study, the bond strength of both universal adhesives after 12 months of water storage showed a greater decrease in the etch-and-rinse groups than in the self-etch group, with the exception of the chlorhexidine and EDTA groups for Single Bond Universal. The most critical process in the etch-and-rinse method is the priming step, which decreases technical sensitivity and facilitates the diffusion of hydrophobic resins through the water-filled collagen network (Meerbeek et al., 2003; Brackett et al., 2005). Sezinando *et al*. (2015) concluded that an extra hydrophobic layer improved the immediate and long-term *in vitro* performance (μTBS and nanoleakage) of universal adhesive systems. Universal bonding agents do not require the priming step, which can explain the decrease in μTBS values over time in the present study. One possible explanation for the degradation observed with the etch-and-rinse mode is the removal of almost all hydroxyapatite between collagens after acid etching and the lack of ability of 10-MDP to interact with the mineral content of dentin, which decreases the bond strength over time. A chemical interaction between residual hydroxyapatite and the functional monomers (10-MDP) in adhesives is expected to improve bonding (Landuyt *et al*., 2006), and this chemical interaction has been hypothesized to improve the resistance against degradation (Yoshida *et al*., 2004).

Osorio *et al*. (2005) reported that when EDTA was used to decalcify dentin, the bond strength values for Adper Scotchbond 1 were similar to those obtained when phosphoric acid was used. In agreement with Osorio *et al*. (2005), we found similar μTBS values for phosphoric acid-etched dentin and EDTA-treated dentin for both universal adhesives. Similarly, Sauro *et al*. (2009) reported that when EDTA or phosphoric acid was used to condition the dentin surface before bonding, the mean μTBS value did not change for Scotchbond 1XT or Optibond Solo Plus. Osorio *et al*. (2005) also reported that specimens pretreated with EDTA instead of phosphoric acid showed no significant decrease in bond strength after immersion in NaOCI. The researchers attributed the success of EDTA to improved resin infiltration into the EDTA-demineralized collagen matrix (Osorio *et al*., 2005).

The effectiveness of chlorhexidine in increasing the bond strength of universal adhesives has been examined in several studies (Hebling *et al*., 2005; Carrilho *et al*., 2007a,b; Breschi *et al*., 2010b; Kim *et al*., 2010; Lenzi *et al*., 2012). Lenzi *et al*. (2012) reported that dentin treatment with chlorhexidine (43.2 MPa) did not result in a significant change in μTBS values for the etch-and-rinse system Adper Single Bond 2 (41.7 MPa) at 24 h. In agreement with the findings of Lenzi *et al*. (2012), the specimens in the etch-and rinse and chlorhexidine groups in the present study exhibited μTBS values of 43.3 MPa and 45.2 MPa, respectively. Chlorhexidine inhibits dentin MMPs activity and consequently has a larger effect on the long-term bond strength (Carrilho *et al*., 2007b). In agreement with Hebling *et al*. (2005) and Carrilho *et al*. (2007b), the μTBS values in the chlorhexidine group for Single Bond Universal were similar at 24 h and 12 months in the present study. However, the μTBS values in the chlorhexidine group

for All Bond Universal showed a significant decrease after 12 months of water storage. As opposed to Single Bond Universal, All Bond Universal does not include polyalkenoic acid copolymer (so-called Vitrebond Copolymer). This copolymer binds to the calcium of the hydroxyapatite, in addition to MDP binding (Yoshida *et al*., 2012). More than 50% of the carboxyl groups in the polyalkenoic acid copolymer are capable of bonding with hydroxyapatite. Carboxylic groups replace phosphate ions on the substrate and form ionic bonds with calcium (Lin *et al*., '92). This additional bonding mechanism exhibited by the polyalkenoic acid copolymer present in Single Bond Universal may have contributed to the stable μTBS values over time, in addition to the use of chlorhexidine. Variations in hydrophilicity resulting from different amounts of 2-Hydroxylethyl methacrylate (HEMA) may also have contributed to the different degrees of hydrolytic stability for All Bond Universal and Single Bond Universal.

In conclusion, our results suggest that the performance of universal dentin adhesives depends on the adhesive strategy and the use of materials that promote adhesion. Mild self-etch adhesives seem to be more resistant to water degradation compared with ultra-mild adhesives. Chlorhexidine and EDTA can increase the bond durability of mild universal adhesives.

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