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In Vitro Longevity of Bonding Properties of Universal Adhesives to Dentin

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Clinical Relevance

Clinicians should opt to use methacryloyloxydecyl dihydrogen phosphate–containing universal adhesives to improve the bonding longevity of dentin interfaces.

SUMMARY

Purpose: To evaluate the immediate and 6-month resin-dentin bond strength (μTBS) and nanoleakage (NL) of universal adhesives that contain or do not contain methacryloyloxydecyl dihydrogen phosphate (MDP) and are used in the etch-and-rinse and self-etch strategies.

Methods and Materials: Forty caries-free extracted third molars were divided into eight groups for μTBS (n=5). The groups were bonded with the Clearfil SE Bond (CSE) and Adper Single Bond 2 (SB) as controls; Peak Universal, self-etch (PkSe) and etch-and rinse (PkEr); Scotchbond Universal Adhesive, self-etch (ScSe) and etch-and-rinse (ScEr); and All Bond Universal, self-etch (AlSe) and etch-and-rinse (AlEr). After composite restorations, specimens were longitudinally sectioned to obtain resin-dentin bonded sticks (0.8 mm^2). The μTBS of the specimens was tested immediately (IM) or after 6 months of water storage (6M) at 0.5 mm/min. Some sticks at each storage period were immersed in silver nitrate and photo developed, and the NL was evaluated with scanning electron microscopy. Data were analyzed with two-way repeated-

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measures analysis of variance and Tukey test (α=0.05).

Results: At the IM period, PkSe and PkEr showed μTBS similar to the control adhesives (p>0.05) but increased NL pattern and lower μTBS after 6M (p<0.05). ScSe and ScEr showed intermediary μTBS values at the IM period but remained stable after 6 months (p>0.05). AlSe showed the lowest μTBS (p<0.05), but μTBS and NL remained stable after 6M (p>0.05). AlEr showed higher IM μTBS but showed higher degradation after 6M (p<0.05).

Conclusions: Universal adhesives that contain MDP showed higher and more stable μTBS with reduced NL at the interfaces after 6 months of water storage.

INTRODUCTION

Current adhesive materials simplify bonding procedures by reducing the number of application steps and time required for application. This simpler protocol makes them less technique sensitive and allows for better application standardization.[1] All of these factors are responsible for the large increase in the use of self-etch adhesives among clinicians.[2]

Self-etch materials (Se; also known as nonrinsing adhesives or etch-and-dry adhesives) do not require a separate acid step, as demineralization and priming occur simultaneously.[3] The preliminary use of phosphoric acid increases the probability of clinical errors due to the need of rinsing and adequate management of dentin moisture.[4] Contrary to the etch-and-rinse approach (Er), Se adhesives do not remove but incorporate the smear layer in the hybridized complex. Although a complete and thorough resin infiltration is not observed for some acidic Se systems,[5,6] some studies report a lower incidence of postoperative sensitivity after placement of direct composite posterior restorations.[7]

On the other hand, some drawbacks may be listed for these Se materials. They do not produce an enamel conditioning pattern that is as retentive as that produced by phosphoric acid,[8,9] which is likely responsible for the higher rates of marginal discoloration in the enamel margins of cervical restorations.[10] Selective enamel etching on the enamel margins with phosphoric acid is the most recently accepted technique to solve this problem, showing good results in both *in vitro*[11,12] and *in vivo* studies.[13]

Keeping this concept in mind, a novel family of bonding systems, known as "universal" or "multi-mode" adhesives,[14,15] was recently launched in the market. They are one-step Se adhesives that can be associated with phosphoric acid etching, mainly for enamel etching,[16] which gives the dentist a more versatile adhesive system.[17]

Universal adhesive differs from the current Se systems by the incorporation of monomers that are capable of producing chemical adhesion to the dental substrates.[14,15] It is believed that this incorporation may increase the durability of the bonds produced with simplified Se adhesives, which was shown to be limited for the current Se under *in vitro* and *in vivo* studies.[10,18] To the extent of our knowledge, the literature is still scarce with regard to the longevity of bonds produced by universal adhesives.[19]

Thus, the aim of this study was to evaluate the immediate and six-month resin-dentin bond strength (μTBS) and nanoleakage (NL) of universal adhesive systems used in the Er and Se approaches. The following null hypotheses were tested: 1) the immediate and six-month resin-dentin μTBS of universal adhesives is not influenced by the adhesive strategy selected (Er or Se) and 2) the immediate and six-month NL of universal adhesives is not influenced by the adhesive strategy selected.

METHODS AND MATERIALS

Tooth Selection and Preparation

Forty extracted caries-free human third molars were used. The teeth were collected after obtaining the respective patients' informed consent under a protocol approved by the local Ethics Committee Review Board. The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within six months of extraction. A flat dentin surface was exposed after wet grinding the occlusal enamel on a No. 180-grit SiC paper. The exposed dentin surfaces were further polished on wet No. 600-grit silicon-carbide paper for 60 seconds to standardize the smear layer.

Experimental Design

A total of five adhesive systems were evaluated. As control materials, the two-step Er, Adper Single Bond 2 (SB; 3M ESPE, St Paul, MN, USA), and the two-step Se, Clearfil SE Bond (CSE; Kuraray, Okayama, Japan), were used. The following three universal adhesive systems were tested in both the Er and Se strategies: Peak Universal Adhesive System (Peak LC Bond and Peak SE Primer Ultradent Products Inc, South Jordan, UT, USA), applied as a two-step Er (PkEr) and two-step Se (PkSe);

Scotchbond Universal Adhesive (3M ESPE), applied as a two-step Er (ScEr) and one-step Se (ScSe); and All Bond Universal (Bisco Inc, Shaumburg, IL, USA), applied as a two-step Er (AlEr) and one-step Se (AlSe). A total of eight experimental conditions were tested in this study, and five teeth were randomly assigned for each group.

Restorative Procedure and Specimen Preparation

The adhesive systems were applied as per the manufacturer's instructions (Table 1). After the bonding procedures, all teeth received a microhybrid composite restoration (Opallis, FGM Produtos Odontológicos, Joinville, SC, Brazil) in two increments of 2 mm. Each increment was light polymerized for 40 seconds using an LED light-curing unit set at 1200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia).

After the restored teeth had been stored in distilled water at 37°C for 24 hours, the specimens were sectioned longitudinally in the mesiodistal and buccal-lingual directions across the bonded interface using a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to obtain 25-30 resin-dentin sticks with a cross-sectional area of approximately 0.8 mm^2 as measured with a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). All sticks from each tooth were divided for μTBS and NL evaluation. Usually, six sticks per tooth were used for NL, three in each storage time; the remaining sticks were used for μTBS, half in the immediate time and half after six months of water storage time (37°C).

Microtensile Bond Strength

Resin-dentin bonded sticks were attached to a Geraldeli jig[20] with cyanoacrylate adhesive and tested under tension (Kratos Dinamometros, Cotia, SP, Brazil) at 0.5 mm/min until failure. The μTBS values were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode of the specimens was classified as cohesive (C; failure exclusive within dentin or resin composite), adhesive (A; failure at the resin-dentin interface), or mixed (M; failure at the resin-dentin interface, which included cohesive failure of the neighboring substrates). The classification was performed under a stereomicroscope at 100× magnification (Olympus SZ40, Tokyo, Japan). Specimens with premature failures were included in the tooth mean.

Nanoleakage

Three resin-bonded sticks from each tooth at each period were not tested in tension and were prepared for NL evaluation. The sticks were placed in an ammoniacal silver nitrate solution[21] in darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in photo-developing solution for eight hours under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Specimens were polished down to 2500-grit SiC paper and 1 and 0.25 μm diamond paste (Buehler Ltd) using a polishing cloth. They were ultrasonically cleaned, air dried, mounted on stubs, and coated with carbon-gold (Shimadzu IC 50, Tóquio, Japão). Resin-dentin interfaces were analyzed in a field-emission scanning electron microscope (SEM) operated in the backscattered mode (SSX-550, Shimadzu).

Three images were captured of each resin-dentin stick. The relative percentage of NL in the adhesive and hybrid layers was measured by a blinded operator (UTHSCSA ImageTool 3.0 software, Department of Dental Diagnostic Science at The University of Texas Health Science Center, San Antonio, TX, USA). Values originating from the same specimen were averaged for statistical purposes. The mean NL of all sticks from the same tooth was taken for statistical purposes.

Statistical Analysis

The experimental unit in the current study was the hemi-tooth. The μTBS and NL of all sticks from the same hemi-tooth were averaged for statistical purposes. The μTBS (MPa) and NL (%) means for every testing group were expressed as the average of five hemi-teeth used per group. The premature failures during specimen preparation were not included in the tooth mean. The μTBS (MPa) and NL (%) data were subjected to a two-way repeated-measure analysis of variance (adhesive vs storage time) and a post hoc test (Tukey post hoc test at α=0.05) for pairwise comparisons.

RESULTS

The percentage of specimens with premature failure and the frequency of each fracture pattern mode are shown in Table 2. Few premature (5.7% on average) and cohesive failures (4.6% on average) were observed. Most of the specimens showed adhesive or adhesive/mixed failures.

Regarding μTBS, the cross-product interaction adhesive vs storage time was statistically significant

(p=0.001). PkSe and PkEr showed higher immediate μTBS, which was statistically similar to the control adhesives (CSE and SB; $p>0.05$; Table 3). However, a significant decrease in μTBS was observed for these materials after six months of water storage ($p<0.05$).

The adhesives ScSe, ScEr, AlSe, and AlEr showed lower immediate μTBS compared with the control adhesives (CSE and SB; $p<0.05$; Table 3). In the Se mode, only ScSe and AlSe showed no significant decrease of the μTBS after six months ($p>0.05$). In the Er mode, only Al showed significantly lower μTBS after six months ($p<0.05$).

The cross-product interaction adhesive vs storage time was statistically significant (p=0.001; Table 4). PkSe and PkEr in both bonding strategies showed the highest NL at the immediate time ($p<0.05$; Table 4), which significantly increased after six months ($p<0.05$; Table 4). Sb and Al, when applied in Se and Er mode, showed lower NL at both storage periods, which was statistically similar to the control adhesives (CSE and SB; $p>0.05$; Table 4).

Representative backscattered SEM images of the resin-dentin interfaces for all experimental conditions are depicted in Figure 1. Specimens of the Peak Universal Adhesive System applied as Er and Se (Figure 1B,J) showed a thicker area of silver nitrate deposition throughout the hybrid and adhesive layer at the immediate period. This deposition resembles the classic images of water trees[21,22] (Figure 1). A higher amount of NL was detected for this adhesive after six months (Fig. 1F,N).

For both control adhesives, as well as the universal adhesives Scotchbond Universal Adhesive tested in Er and Se strategies, a thinner deposition of silver nitrate was observed, mainly restricted to the base of the hybrid layer at the immediate time (Figure 1). This NL remained stable after six months of water storage (Figure 1).

DISCUSSION

The results of the present study demonstrated that the universal adhesive tested had a heterogeneous behavior, since some adhesives diminished the bonding performance over the course of time and some did not.

Although the adhesive Peak showed high immediate μTBS values, this material produced an adhesive interface with high deposition of NL at the immediate time. NL represents the location of defects at the resin-dentin interface that may serve as pathways for degradation of the resin-dentin bond over time.[22] Silver nitrate is capable of occupying nanometric-sized spaces present around the exposed collagen fibrils where the monomers were unable to infiltrate or where residual water was not displaced by the adhesive or even in areas with incomplete monomer conversion,[22] factors preponderant for the degradation of the bond interface.

PkSe can be categorized as an aggressive Se,[23,24] as this material has a very low pH (Peak SE Primer, pH=1.2) when compared with the other adhesives (Sb, pH=3.0; Al, pH=2.4).[25] This might be why this material showed the highest NL at the immediate period. It was already reported that acid and unpolymerized monomers are more present in acidic adhesive infiltrate than are polymerizable monomers.[5,6] Also, the hydrolysis of the ester bond of acidic monomer results in a strong phosphoric acid[5] that continues to demineralize the surrounding dentin.

Only the Peak material recommends the application of an extra layer of Peak LC Bond. Various *in vitro*[12,26,27,28] and *in vivo* studies[29,30] have shown that the application of an additional layer increases the performance of one-step Se adhesives, provided that this is a layer with a hydrophobic nature. This additional layer incorporates nonsolvated hydrophobic monomers at the bonding interface, which diminishes the relative concentration of solvents retained and nonreacted monomers in the adhesive layer,[31] making it less permeable[32,33] and less prone to the effects of degradation over the course of time.[34,35]

However, Peak LC Bond appears to be as hydrophilic as Peak SE Primer, since there are no hydrophobic monomers listed in the composition of Peak LC Bond (Table 1). In this way, the material does not take advantage of having a second adhesive layer; the high level of hydrophilicity must be responsible for the degradation of the adhesive interface.

Peak LC Bond is hydrophilic and is the recommended material to be used in the Er strategy. In the Er approach, the primer was not applied; the low pH of the Peak LC Bond (pH=2.0) might have caused an additional etching of the dentinal substrate. This probably resulted in an increase in the demineralization and collagen exposure,[24,36] thereby increasing the NL[37] even when used in the Er strategy, as can be seen in Figure 1.

Sc and Al are one-step Se adhesives and are therefore highly hydrophilic. In three of the four groups tested with these two adhesives, no degrada-

Table 1: *Adhesive System (Batch Number), Composition,[a] and Application Mode of the Adhesive Systems Used According to the Manufacturer's Instructions*

Adhesive (Batch Number)	Composition	Self-Etch Strategy (Se)	Etch-and-Rinse Strategy (Er)
Adper Single Bond 2 (BPBR)	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5-nm-diameter spherical silica particles	NA	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2-3 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air thin for 5 s 6. Light-cure for 10 s at 1200 mW/cm^2
Clearfil SE Bond (Primer: 00954A - Bond: 01416[a])	1. Primer: water, MDP, HEMA, camphorquinone, hydrophilic dimethacrylate 2. Bonding: MDP, Bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, N,N-diethanol p-toluidine bond, colloidal silica	1. Apply primer to tooth surface and leave in place for 20 s 2. Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4. Light-cure for 10 s at 1200 mW/cm^2	NA
Peak Universal Adhesive System (Peak SE Primer: 0N062–Peak LC Bond: Y062)	1. Etchant: 35% phosphoric acid (Ultraetch) 2. Primer: ethyl alcohol, methacrylic acid, 2-hydroxyethyl methacrylate (Peak SE Primer) 3. Adhesive: Ethyl alcohol, 2-hydroxyethyl methacrylate (Peak LC Bond)	1. Initial use of Peak SE requires activation of the two components separated in the syringe 2. Application of the Peak SE with microbrush for 20 s using continuous scrubbing on dentin; do not scrub enamel 3. Thin/dry for 3 s using air/water syringe or high-volume suction directly over preparation 4. Apply a puddle coat of Peak LC Bond with gently agitate for 10 s 5. Thin/dry 10 s using to air pressure 6. Light polymerize for 10 s at 1200 mW/cm^2	1. Apply etchant for 20 s 2. Rinse for 5 s 3. Air dry 2 s 4. Apply a puddle coat of Peak LC Bond with gently agitate for 10 s 5. Thin/dry 10 s using to air pressure 6. Light-cure for 10 s at 1200 mW/cm^2
Scotchbond Universal Adhesive (D-82229)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide. (Scotchbond Universal Etchant) 2. Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s; if necessary, rewet the disposable applicator during treatment 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely 3. Light-cure for 10 s at 1200 mW/cm^2	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry 2 s 4. Apply adhesive as for the self-etch mode

Table 1: *Adhesive System (Batch Number), Composition,[a] and Application Mode of the Adhesive Systems Used According to the Manufacturer's Instructions (cont.)*

Adhesive (Batch Number)	Composition	Self-Etch Strategy (Se)	Etch-and-Rinse Strategy (Er)
All-Bond Universal (1200006111)	1. Etchant Uni-Etch: 32%phosphoric acid, benzalkonium chloride 2. Adhesive: MDP, 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; do not light polymerize between coats 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s—there should be no visible movement of the material; the surface should have a uniform glossy appearance 3. Light cure for 10 s at 1200 mW/cm^2	1. Apply etchant for 15 s 2. Rinse thoroughly 4. Apply adhesive as for the self-etch mode 3. Remove excess water with absorbent pellet or high volume suction for 1-2 s

[a] *bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, methacryloyloxydecyl dihydrogen phosphate.*

tion of the resin-dentin bonds was observed. This must be attributed to the presence of monomers capable of producing a chemical bond to the hard structures of teeth,[14,15] as opposed to the lack of this compound in Pk.

Sc and Al contain methacryloyloxydecyl dihydrogen phosphate (MDP) in their composition, as does CSE, which was the first Se adhesive to incorporate this component. Studies with CSE have demonstrated that MDP allows for a stable chemical bond to dentin over the course of time, both *in vitro*[30,38-40] and *in vivo*.[13,41,42] This monomer forms a stable nanolayer together with a deposition of stable MDP-Ca salts at the adhesive interface,[43] which increases its mechanical strength.[43,44] However, regardless of the MDP, the adhesives showed different behaviors.

The μTBS values of ScSe and AlSe at the immediate time were not equivalent to those of the control CSE. ScSe and AlSe are one-step adhesives, and this probably leads to the concentration of MDP being lower than it is in CSE, which has MDP incorporated into both the primer and the

Table 2: *Number of Specimens (%) According to Fracture Mode and the Premature Failure of All Experimental Groups*

Adhesive System	Application Mode	Time	Fracture Pattern			
			A	C	A/M	PF
Adper Single Bond 2	Er control	Immediate	51 (73.9)	10 (14.5)	6 (8.7)	2 (2.9)
		6 mo	49 (73.1)	4 (6.0)	9 (13.4)	5 (7.5)
Clearfil SE Bond	Se control	Immediate	50 (74.6)	3 (4.5)	10 (14.9)	4 (6.0)
		6 mo	53 (80.3)	2 (3.0)	8 (12.1)	3 (4.6)
Peak Universal	Er	Immediate	56 (80)	0 (0)	10 (14.3)	4 (5.7)
		6 mo	52 (75.4)	2 (2.9)	12 (17.4)	3 (4.3)
	Se	Immediate	58 (82.8)	3 (4.3)	7 (10)	2 (2.9)
		6 mo	48 (69.6)	1 (1.5)	13 (18.8)	7 (10.1)
Scotchbond Universal	Er	Immediate	53 (79.1)	2 (3.0)	10 (14.9)	2 (3.0)
		6 mo	50 (78.1)	0 (0)	13 (20.3)	1 (1.6)
	Se	Immediate	47 (71.2)	3 (4.6)	11 (16.7)	5 (7.5)
		6 mo	51 (73.9)	1 (1.4)	14 (20.3)	3 (4.4)
Allbond Universal	Er	Immediate	49 (71)	8 (11.6)	7 (10.1)	5 (7.3)
		6 mo	49 (73.1)	2 (3.0)	12 (17.9)	4 (6.0)
	Se	Immediate	51 (72.85)	4 (5.7)	9 (12.9)	6 (8.6)
		6 mo	47 (69.1)	5 (7.4)	10 (14.7)	6 (8.8)

Abbreviations: A, adhesive fracture mode; C, cohesive fracture mode; A/M, adhesive/mixed fracture mode; PF, premature failure.

Table 3: *Microtensile Bond Strength (μTBS) Values (Means ± Standard Deviations) of the Different Experimental Groups (*)*

Time	Adhesive System							
	Adper Single Bond 2	Clearfil SE Bond	Peak Universal Se	Peak Universal Er	Scotchbond Universal Se	Scotchbond Universal Er	Allbond Universal Se	Allbond Universal Er
Immediate	47.6 ± 5.5 a	42.9 ± 4.4 a, b	39.5 ± 5.1 b	44.3 ± 1.6 a, b	33.3 ± 3.2 c	34.7 ± 4.6 b, c	20.9 ± 4.1 e	38.5 ± 4 b
6 mo	38.8 ± 5.7 b	36.2 ± 2.7 b, c	27.9 ± 4.9 d	34.2 ± 4.2 c	33.6 ± 5.8 c	34.6 ± 6.2 c	20.4 ± 4.8 e	28.1 ± 4.3 c

(*) Means identified with identical lower case letters are statistically similar ($p > 0.05$)

bond.[43] Moreover, it has been demonstrated that the presence of 2-hydroxyethyl methacrylate, a component of Sc and Al, may compete with MDP by bonding to the calcium of hydroxyapatite, thereby harming the chemical bond of MDP to dentin.[43]

Many other variables in the composition of the ScSe and AlSe may account for the differences observed between these materials as, for instance, in the presence of the polyalkenoic acid copolymer (PAC) in Sc and the high concentration of solvent of Al. Sc contains specific PACs used in resin-modified glass ionomer Vitrebond (3M ESPE). PAC bonds chemically and spontaneously to hydroxyapatite in glass ionomer materials,[45] and a recent study demonstrated that the presence of PAC showed more bond strength than a PAC-free adhesive with the same composition.[41,45] Yoshida and others[43] hypothesized that PAC may compete with the MDP present in Sc. However, if we compare the longevity results of Sc (MDP+PAC) with SB (PAC), two materials with similar compositions, the only difference being the presence of MDP in the former, it seems that the association MDP-PAC enhanced the bonding ability, since ScSe and ScEr showed stable bonds even after six months of water storage.

Al contains more solvent than Sc (30-60 wt% and 10-15 wt%, respectively).[46,47] This leads to more residual solvent retained in the hybrid layer and adhesive layer,[48] preventing the formation of a polymer with high reticulation.[49-51] As a consequence, a reduced degree of conversion[32] and μTBS values[52-55] is produced, making the adhesive interface more permeable after polymerization[56,57] and more prone to degradation over time.[3,58]

This may explain the lower results of AlSe in the immediate time interval in comparison with the other adhesives. This is in agreement with a recent study published by Munoz and others,[16] even when applied actively; whereas in the mentioned study, Al was applied passively.[16] Active/vigorous application improves the immediate and long-term results of the bond to dentin of the one-step SE adhesive systems,[59-62] because it increases the penetration of monomers into dentin and solvent evaporation. Agitation will also improve the efficacy of polymerization by improving the chemical interaction of the adhesive with the dental substrate, particularly for the acid Se adhesives.[37,63] In addition, unreacted acid monomers present in the superficial layer of the adhesive may be taken to a basal area of dentin, increasing demineralization of the substrate and diffusion of monomers and improving the interaction with the smear layer and subjacent dentin.[60-62]

As only AlEr demonstrated degradation of the μTBS values over the course of time after six months of evaluation, we could hypothesize that the presence of PAC is more important for Er adhesives than for Se. Some authors have indicated that the function of PAC is to improve the stability to humidity,[64,65] a crucial factor for Er adhesives, which, due to dentin demineralization, has a more sensitive technique when compared with that of the Se adhesives.[2]

We reject the first and second null hypotheses, given that the μTBS and NL values of universal

Table 4: *Nanoleakage (NL) Values (Means ± Standard Deviations) of the Different Experimental Groups (*)*

Time	Adhesive System							
	Adper Single Bond 2	Clearfil SE Bond	Peak Universal Bond Se	Peak Universal Bond Er	ScotchBond Universal Se	ScotchBond Universal Er	Allbond Universal Se	Allbond Universal Er
Immediate	13.1 ± 2.0 b	7.5 ± 2.9 a, b	31.6 ± 3.1 c	23.9 ± 4.2 c	5.1 ± 2.1 a	5.3 ± 1.1 a	6.0 ± 3.9 a	9.4 ± 1.8 b
6 mo	14.7 ± 4.1 b	8.6 ± 4.1 a, b	42.2 ± 2.6 d	34.4 ± 4.1 d	4.7 ± 2.8 a	5.4 ± 2.0 a	5.9 ± 1.4 a	8.9 ± 3.1 a, b

(*) Means identified with identical lower case letters are statistically similar ($p > 0.05$)

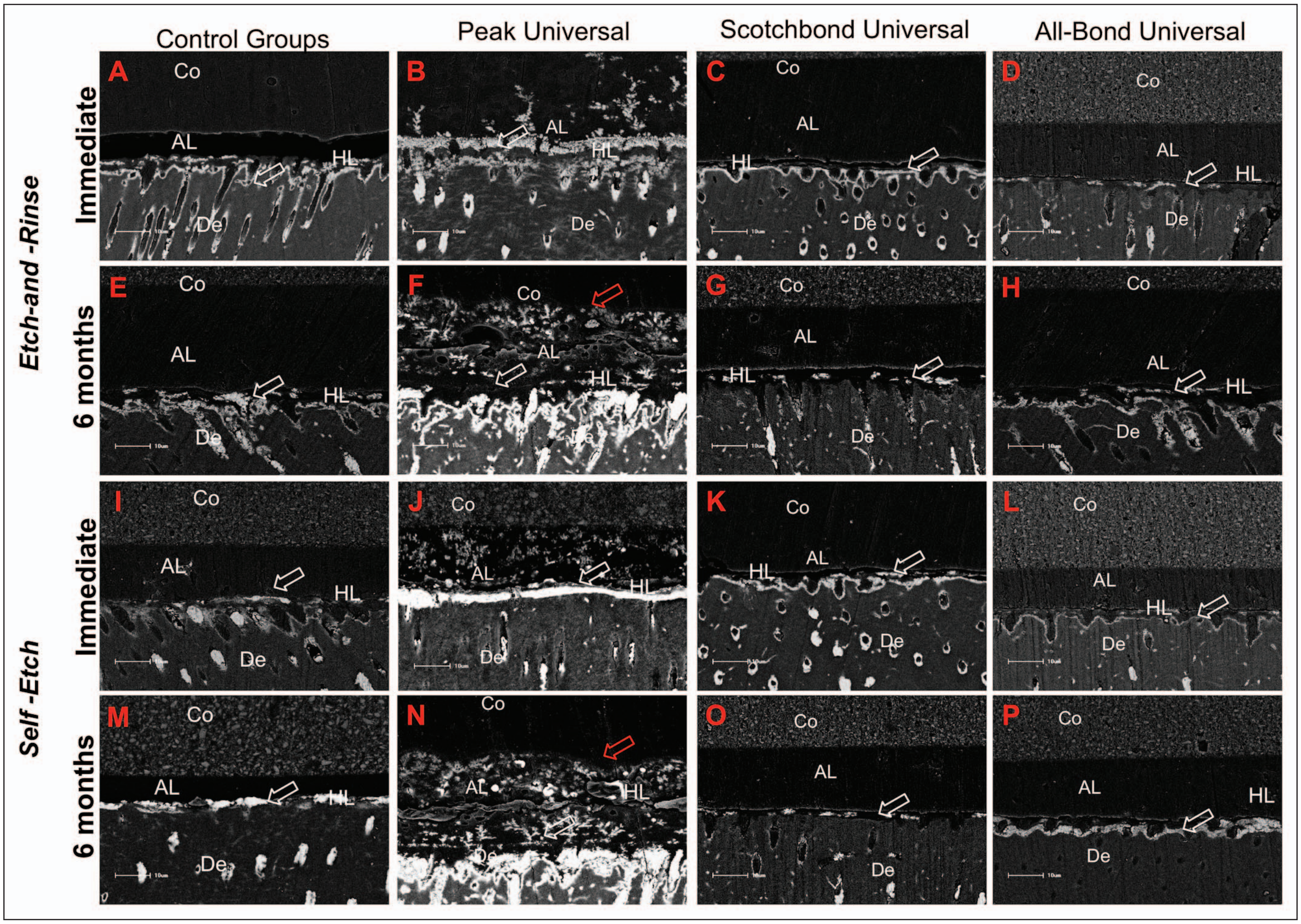

Figure 1. *Representative backscatter SEM images of the resin-dentin adhesive interfaces of each experimental group for immediate and six-month periods. Control etch-and-rinse group=Adper Single Bond 2 and control self-etch group=Clearfil SE Bond. For the Peak Universal Adhesive System, the amount of nanoleakage was higher (red and white arrows) than for the other materials and increased after six months for strategies Er (B, F) and Se (J, N). The amount of nanoleakage was lower and stable after six months within the hybrid layer for Scotchbond Universal (C, G, K, O), AllBond Universal (D, H, L, P), and controls (white arrows) (A, E. I, M). Co indicates composite; De, dentin; HL, hybrid layer; AL, adhesive layer; Se, self-etch; Er, etch-and-rinse.*

adhesives showed different results according to the Er or Se strategies employed.

CONCLUSIONS

Universal adhesives that contain MDP (Scochbond Universal Adhesive Er and Se and All Bond Universal Se) showed stable bond strengths and reduced NL, similar to the two-step SE adhesive tested (Clearfil SE Bond) after six months of water storage.

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Human Subjects Approval

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies. The approval code for this study is 17878/13. This study was conducted at State University of Ponta Grossa, Paraná, Brazil.

Conflict of Interest

The authors have no proprietary, financial, or other personal interests of any nature or kind in any product, service, and/or company that is presented in this article.

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REFERENCES

1. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, Yoshida Y, Inoue S, & Suzuki K (2005) Technique-sensitivity of contemporary adhesives *Dental Materials Journal* **24(1)** 1-13.
2. Perdigao J (2007) New developments in dental adhesion *Dental Clinics of North America* **51(2)** 333-357.
3. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: methods and results *Journal of Dental Research* **84(2)** 118-132.
4. Reis A, Loguercio AD, Azevedo CL, de Carvalho RM, da Julio Singer M, & Grande RH (2003) Moisture spectrum of demineralized dentin for adhesive systems with different solvent bases *Journal of Adhesive Dentistry* **5(3)** 183-192.
5. Wang Y, & Spencer P (2005) Continuing etching of an all-in-one adhesive in wet dentin tubules *Journal of Dental Research* **84(4)** 350-354.
6. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26(9)** 1035-1042.
7. Unemori M, Matsuya Y, Akashi A, Goto Y, & Akamine A (2004) Self-etching adhesives and postoperative sensitivity *American Journal of Dentistry* **17(3)** 191-195.
8. Pashley DH, & Tay FR (2001) Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel *Dental Materials* **17(5)** 430-444.
9. Kanemura N, Sano H, & Tagami J (1999) Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces *Journal of Dentistry* **27(7)** 523-530.
10. Hafer M, Schneider H, Rupf S, Busch I, Fuchss A, Merte I, Jentsch H, Haak R, & Merte K (2013) Experimental and clinical evaluation of a self-etching and an etch-and-rinse adhesive system *Journal of Adhesive Dentistry* **15(3)** 275-286.
11. Rotta M, Bresciani P, Moura SK, Grande RH, Hilgert LA, Baratieri LN, Loguercio AD, & Reis A (2007) Effects of phosphoric acid pretreatment and substitution of bonding resin on bonding effectiveness of self-etching systems to enamel *Journal of Adhesive Dentistry* **9(6)** 537-545.
12. Frankenberger R, Perdigao J, Rosa BT, & Lopes M (2001) “No-bottle” vs “multi-bottle” dentin adhesives—a microtensile bond strength and morphological study *Dental Materials* **17(5)** 373-380.
13. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, & Van Meerbeek B (2010) Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching *Dental Materials* **26(12)** 1176-1184.
14. Perdigao J, Sezinando A, & Monteiro PC (2012) Laboratory bonding ability of a multi-purpose dentin adhesive *American Journal of Dentistry* **25(3)** 153-158.
15. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, & De Munck J (2012) Bonding effectiveness of a new “multi-mode” adhesive to enamel and dentine *Journal of Dentistry* **40(6)** 475-484.
16. Perdigao J, & Loguercio AD (2014). Universal or multi-mode adhesives: why and how? *Journal of Adhesive Dentistry* **16(2)** 193-193.
17. Perdigao J, Munoz M, Sezinando A, Luque-Martinez I, Staichak R, Reis A, & Loguercio A (2014) Immediate adhesive properties to dentin and enamel of a universal adhesive associated with a hydrophobic resin coat *Operative Dentistry* **39(5)** 489-499.
18. Heintze SD, Ruffieux C, & Rousson V (2010) Clinical performance of cervical restorations—a meta-analysis *Dental Materials* **26(10)** 993-1000.
19. Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosa M, Cadenaro M, Pashley DH, Tay F, & Breschi L (2014) Adhesive performance of a multi-mode adhesive system: 1-year *in vitro* study *Journal of Dentistry* **42(5)** 603-612.
20. Perdigao J, Geraldeli S, Carmo AR, & Dutra HR (2002) *In vivo* influence of residual moisture on microtensile bond strengths of one-bottle adhesives *Journal of Esthetic and Restorative Dentistry* **14(1)** 31-38.
21. Tay FR, Pashley DH, Suh BI, Carvalho RM, & Itthagarun A (2002) Single-step adhesives are permeable membranes *Journal of Dentistry* **30(7-8)** 371-382.
22. Sano H (2006) Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds *Journal of Dental Research* **85(1)** 11-14.
23. Tay FR, & Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I: depth of penetration beyond dentin smear layers *Dental Materials* **17(4)** 296-308.
24. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, & Vanherle G (2003) Buonocore memorial lecture: adhesion to enamel and dentin: current status and future challenges *Operative Dentistry* **28(3)** 215-235.
25. Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, & Bombarda NH (2013) Immediate bonding properties of universal adhesives to dentine *Journal of Dentistry* **41(5)** 404-411.
26. Feitosa VP, Leme AA, Sauro S, Correr-Sobrinho L, Watson TF, Sinhoreti MA, & Correr AB (2012) Hydrolytic degradation of the resin-dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing *Journal of Dentistry* **40(12)** 1134-1143.
27. Reis A, Albuquerque M, Pegoraro M, Mattei G, Bauer JR, Grande RH, Klein-Junior CA, Baumhardt-Neto R, & Loguercio AD (2008) Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? *Journal of Dentistry* **36(5)** 309-315.
28. Albuquerque M, Pegoraro M, Mattei G, Reis A, & Loguercio AD (2008) Effect of double-application or the application of a hydrophobic layer for improved efficacy of one-step self-etch systems in enamel and dentin *Operative Dentistry* **33(5)** 564-570.

29. Loguercio AD, & Reis A (2008) Application of a dental adhesive using the self-etch and etch-and-rinse approaches: an 18-month clinical evaluation *Journal of the American Dental Association* **139(1)** 53-61.

30. Reis A, Leite TM, Matte K, Michels R, Amaral RC, Geraldeli S, & Loguercio AD (2009) Improving clinical retention of one-step self-etching adhesive systems with an additional hydrophobic adhesive layer *Journal of the American Dental Association* **140(7)** 877-885.

31. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101.

32. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, Biasotto M, Contardo L, & Breschi L (2005) Degree of conversion and permeability of dental adhesives *European Journal of Oral Sciences* **113(6)** 525-530.

33. Breschi L, Cadenaro M, Antoniolli F, Sauro S, Biasotto M, Prati C, Tay FR, & Di Lenarda R (2007) Polymerization kinetics of dental adhesives cured with LED: correlation between extent of conversion and permeability *Dental Materials* **23(9)** 1066-1072.

34. Reis A, Grande RH, Oliveira GM, Lopes GC, & Loguercio AD (2007) A 2-year evaluation of moisture on microtensile bond strength and nanoleakage *Dental Materials* **23(7)** 862-870.

35. Reis A, de Carvalho Cardoso P, Vieira LC, Baratieri LN, Grande RH, & Loguercio AD (2008) Effect of prolonged application times on the durability of resin-dentin bonds *Dental Materials* **24(5)** 639-644.

36. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation *Journal of Dental Research* **90(8)** 953-968.

37. Wang Y, & Spencer P (2003) Hybridization efficiency of the adhesive/dentin interface with wet bonding *Journal of Dental Research* **82(2)** 141-145.

38. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH, & Tay F (2007) Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media *Dental Materials* **23(7)** 885-892.

39. Waidyasekera K, Nikaido T, Weerasinghe DS, Ichinose S, & Tagami J (2009) Reinforcement of dentin in self-etch adhesive technology: a new concept *Journal of Dentistry* **37(8)** 604-609.

40. Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, Sano H, & Van Meerbeek B (2005) Hydrolytic stability of self-etch adhesives bonded to dentin *Journal of Dental Research* **84(12)** 1160-1164.

41. Perdigao J, Kose C, Mena-Serrano A, De Paula E, Tay L, Reis A, & Loguercio A (2014) A new universal simplified adhesive: 18-month clinical evaluation *Operative Dentistry* **39(2)** 113-127.

42. Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, & Perdigao J (2013) A new universal simplified adhesive: 6-month clinical evaluation *Journal of Esthetic and Restorative Dentistry* **25(1)** 55-69.

43. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, & Meerbeek BV (2012) Self-assembled nano-layering at the adhesive interface *Journal of Dental Research* **91(4)** 376-381.

44. Yoshihara K, Yoshida Y, Nagaoka N, Fukegawa D, Hayakawa S, Mine A, Nakamura M, Minagi S, Osaka A, Suzuki K, & Van Meerbeek B (2010) Nano-controlled molecular interaction at adhesive interfaces for hard tissue reconstruction *Acta Biomaterialia* **6(9)** 3573-3582.

45. Mitra SB, Lee CY, Bui HT, Tantbirojn D, & Rusin RP (2009) Long-term adhesion and mechanism of bonding of a paste-liquid resin-modified glass-ionomer *Dental Materials* **25(4)** 459-466.

46. Chen L, & Suh BI (2013) Effect of hydrophilicity on the compatibility between a dual-curing resin cement and one-bottle simplified adhesives *Journal of Adhesive Dentistry* **15(4)** 325-331.

47. 3M ESPE (2013) Material Safety Data Sheet; Retrieved online December 6, 2013 from: http://multimedia.3m.com/mws/mediawebserver?mwsId=SSSSSuUn_zu8l00xMY_eMYt1Nv70k17zHvu9lxtD7SSSSSS--

48. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH, & Tay FR (2005) Solvent and water retention in dental adhesive blends after evaporation *Biomaterials* **26(34)** 6863-6872.

49. Ye Q, Spencer P, Wang Y, & Misra A (2007) Relationship of solvent to the photopolymerization process, properties, and structure in model dentin adhesives *Journal of Biomedical Materials Research Part A* **80(2)** 342-350.

50. Loguercio AD, Loeblein F, Cherobin T, Ogliari F, Piva E, & Reis A (2009) Effect of solvent removal on adhesive properties of simplified etch-and-rinse systems and on bond strengths to dry and wet dentin *Journal of Adhesive Dentistry* **11(3)** 213-219.

51. Paul SJ, Leach M, Rueggeberg FA, & Pashley DH (1999) Effect of water content on the physical properties of model dentine primer and bonding resins *Journal of Dentistry* **27(3)** 209-214.

52. Hass V, Folkuenig MS, Reis A, & Loguercio AD (2011) Influence of adhesive properties on resin-dentin bond strength of one-step self-etching adhesives *Journal of Adhesive Dentistry* **13(5)** 417-424.

53. Reis A, Ferreira SQ, Costa TR, Klein-Junior CA, Meier MM, & Loguercio AD (2010) Effects of increased exposure times of simplified etch-and-rinse adhesives on the degradation of resin-dentin bonds and quality of the polymer network *European Journal of Oral Sciences* **118(5)** 502-509.

54. Takahashi H, Sato H, Uno S, Pereira PN, & Sano H (2002) Effects of mechanical properties of adhesive resins on bond strength to dentin *Dental Materials* **18(3)** 263-268.

55. Cho BH, & Dickens SH (2004) Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength *Dental Materials* **20(2)** 107-115.

56. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, & Carrilho MR (2006)

Water sorption/solubility of dental adhesive resins *Dental Materials* **22(10)** 973-980.

57. 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 (2005) Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity *Biomaterials* **26(33)** 6449-6459.

58. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101.

59. do Amaral RC, Stanislawczuk R, Zander-Grande C, Michel MD, Reis A, & Loguercio AD (2009) Active application improves the bonding performance of self-etch adhesives to dentin *Journal of Dentistry* **37(1)** 82-90.

60. Miyazaki M, Platt JA, Onose H, & Moore BK (1996) Influence of dentin primer application methods on dentin bond strength *Operative Dentistry* **21(4)** 167-172.

61. Pleffken PR, de Almeida Lourenco AP, Torres CR, & Buhler Borges A (2011) Influence of application methods of self-etching adhesive systems on adhesive bond strength to dentin *Journal of Adhesive Dentistry* **13(6)** 517-525.

62. Loguercio AD, Stanislawczuk R, Mena-Serrano A, & Reis A (2011) Effect of 3-year water storage on the performance of one-step self-etch adhesives applied actively on dentine *Journal of Dentistry* **39(8)** 578-587.

63. Zhang Y, & Wang Y (2013) Effect of application mode on interfacial morphology and chemistry between dentine and self-etch adhesives *Journal of Dentistry* **41(3)** 231-240.

64. Van Meerbeek B, Conn LJ Jr, Duke ES, Eick JD, Robinson SJ, & Guerrero D (1996) Correlative transmission electron microscopy examination of nondemineralized and demineralized resin-dentin interfaces formed by two dentin adhesive systems *Journal of Dental Research* **75(3)** 879-888.

65. Spencer P, Wang Y, Walker MP, Wieliczka DM& Swafford JR (2000) Interfacial chemistry of the dentin/adhesive bond *Journal of Dental Research* **79(7)** 1458-1463.