

# Degradation of Multimode Adhesive System Bond Strength to Artificial Caries-Affected Dentin Due to Water Storage

AC Follak • LL Miotti • TL Lenzi • RO Rocha • FZM Soares

## Clinical Relevance

When using multimode adhesives on sound dentin, clinicians can choose to use either an etch-and-rinse or a self-etch strategy in terms of bond strength; however, on caries-affected dentin, bonding degradation will be greater irrespective of etching mode.

## SUMMARY

The purpose of this study was to evaluate the influence of water storage on bond strength of multimode adhesive systems to artificially induced caries-affected dentin. One hundred twelve sound bovine incisors were randomly assigned to 16 groups ( $n=7$ ) according to the dentin condition (sound; SND, artificially induced caries-affected dentin; CAD, cariogenic challenge by pH cycling for 14 days); the adhesive system (SU, Scotchbond Universal Adhesive; AB, All-Bond Universal; PB, Prime

& Bond Elect; SB, Adper Single Bond 2; and CS, Clearfil SE Bond), and the etching strategy (etch-and-rinse and self-etch). All adhesive systems were applied under manufacturer's instructions to flat dentin surfaces, and a composite block was built up on each dentin surface. After 24 hours of water storage, the specimens were sectioned into stick-shaped specimens ( $0.8 \text{ mm}^2$ ) and submitted to a micro-tensile test immediately (24 hours) or after six months of water storage. Bond strength data (MPa) were analyzed using three-way repeated-measures analysis of variance and *post hoc* Tukey test ( $\alpha=5\%$ ), considering each substrate separately (SND and CAD). The etching strategy did not influence the bond strength of multimode adhesives, irrespective of the den-

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**tin condition. Water storage only reduced significantly the bond strength to CAD. The degradation of bond strength due to water storage was more pronounced in CAD, regardless of the etching strategy.**

## INTRODUCTION

To simplify the adhesive technique and improve its clinical versatility, materials referred to as universal or multimode adhesives have been developed. They generally consist of one-bottle adhesives that can be used both in etch-and-rinse and self-etch strategies. Moreover, manufacturers and previous studies recommend an alternative protocol: the selective enamel etching technique, which combines the advantages of acid etching on enamel with the simplified self-etching technique in dentin.<sup>1,2</sup>

Moreover, several multimode adhesives present phosphate monomers (such as 10-MDP)<sup>3</sup> in the composition in an attempt to improve bond strength longevity by chemical adhesion to hydroxyapatite. As the hybrid layer is prone to both collagen and resin matrix hydrolytic degradation,<sup>4-6</sup> it is extremely important to evaluate the bond stability achieved with multimode adhesive systems on different substrates.

Recent studies have evaluated different multimode adhesive systems, comparing the performance of etch-and-rinse and self-etch strategies.<sup>7-16</sup> A systematic review with meta-analysis demonstrated that the etch-and-rinse strategy produces higher values for enamel; however, on dentin, for the majority of the multimode adhesive systems included, both strategies produced similar values.<sup>17</sup>

High immediate bond strength values have been found for multimode adhesives<sup>7-9,11,12,14,15</sup>; however, the question about the long-term bonding effectiveness to dentin remains controversial.<sup>7,10,11,13-16</sup> Moreover, the influence of a substrate condition such as caries-affected dentin on the bond strength of multimode adhesives is unclear. In caries-affected dentin of primary molars, the effect of etching strategy was substrate dependent,<sup>18,19</sup> whereas in permanent dentin, the effect of approach strategy was not significant.<sup>20</sup>

Caries-affected dentin left after selective removal of carious tissue is a clinically relevant substrate in everyday dental practice. The selective removal of carious tissue can significantly reduce the risk of pulp exposure and postoperative pulp symptoms, which is advantageous for deep caries treatment.<sup>21</sup> However, caries-affected dentin differs in composi-

tion and microstructure compared with sound dentin,<sup>22</sup> and lower bond strengths have been reported when bonding to this substrate, regardless of the adhesive system tested.<sup>22-28</sup> It is also known that hybrid layer degradation is more pronounced on caries-affected dentin due to substrate-intrinsic characteristics.<sup>27</sup> Based on this, laboratory assessments of bonding to caries-affected dentin is relevant,<sup>22</sup> including the performance of universal adhesive systems on this kind of substrate.

Therefore, the aim of this *in vitro* study was to evaluate the influence of water storage on bond strength of multimode adhesive systems to artificially induced caries-affected dentin. The null hypotheses tested were as follows: 1) water aging has no effect on bond strength of multimode adhesives systems to sound and artificially induced caries-affected dentin, irrespective of the etching strategies, and 2) the multimode adhesives present similar bonding, independent of the etching strategies on either sound or artificially induced caries-affected dentin.

## METHODS AND MATERIALS

### Tooth Selection and Preparation

One hundred twelve freshly extracted bovine incisors were stored in 0.5% aqueous chloramine T at 4°C for a maximum of 30 days and used in this study. The teeth were divided into dentin substrates (sound and artificially induced caries affected), and for each substrate, teeth were allocated into eight groups (n=7). The root portion was removed using a diamond disc in a low-speed hand piece. The buccal surfaces were ground under water cooling using a 100-grit SiC paper in a polishing machine (EcoMet 250, Buehler, Lake Bluff, IL, USA) to expose and obtain flat dentin surfaces. Further, for both substrates (sound dentin and artificially induced caries-affected dentin), buccal surfaces were ground manually using 600-grit SiC paper for 60 seconds to create a standardized smear layer.

### Artificial Caries Induction

The teeth were randomly assigned in two groups according to the substrate condition: sound (SND) and artificially induced caries-affected dentin (CAD). After procedures to obtain a standardized smear layer, teeth allocated to the sound dentin group were kept in distilled water and those to the artificially induced caries-affected dentin group were submitted to artificial caries induction by a pH-cycling model.<sup>29,30</sup> Teeth were individually submitted to immer-

sion for eight hours in 10 mL of demineralizing solution (2.2 mM  $\text{CaCl}_2$ , 2.2 mM  $\text{NaH}_2\text{PO}_4$ , and 50 mM acetic acid, adjusted pH of 4.8 with 1 M KOH) and for 16 hours in the same volume of remineralizing solution (1.5 mM  $\text{CaCl}_2$ , 0.9 mM  $\text{NaH}_2\text{PO}_4$ , and 0.15 mM KCl with adjusted pH of 7.0). Solutions were changed at every cycle for 14 days, and the solution pH was confirmed on each cycle using a digital pH meter (Digimed, DM22, ServMed Analítica, Guarulhos, SP, Brazil).

### Bonding and Restorative Procedures

Teeth from each dentin substrate (sound dentin and artificially induced caries-affected dentin) were randomly reallocated into eight groups according to the adhesive system and etching strategy ( $n=7$ ). The three multimode adhesives systems evaluated were as follows: Scotchbond Universal (SU; 3M ESPE, St Paul, MN, USA), All-Bond Universal (AB; Bisco, Schaumburg, IL, USA), and Prime & Bond Elect (PB; Dentsply Caulk, Milford, DE, USA). All materials were applied on dentin surfaces in either a self-etch (SE) or etch-and-rinse (ER) protocol. As control groups for each strategy, a two-step etch-and-rinse Adper Single Bond 2 (SB; 3M ESPE) and a two-step self-etch Clearfil SE Bond (CS; Kuraray Noritake Dental, Tokyo, Japan) were used. A single trained operator applied the adhesive systems on dentin surfaces strictly under the manufacturers' instructions (Table 1).

After hybridization, a block ( $\sim 10 \times 7 \times 5$  mm) of resin composite (Filtek Z250, shade A2, 3M ESPE) was incrementally built up on dentin surfaces, and each increment was light cured for 20 seconds using a light-emitting diode curing unit (Emitter B, Schuster, Santa Maria, RS, Brazil) delivering 800  $\text{mW/cm}^2$  and checked with a radiometer (Demetron Research Corp, Danbury, CT, USA) every three blocks. All specimens were stored in distilled water at 37°C for 24 hours.

### Microtensile Bond Strength ( $\mu\text{TBS}$ )

Specimens were sectioned in two perpendicular axes with an underwater cooled diamond saw in a cutting machine (Labcut 1010, Extex Co, Enfield, CT, USA) by a single blinded operator, obtaining stick-shaped specimens with a cross-sectional area of approximately 0.8  $\text{mm}^2$  measured individually with a digital caliper (Carbografite, Petrópolis, RJ, Brazil). Then, one half of the obtained specimens from each tooth were randomly assigned to be tested immediately (24 hours) and the other half after six months (6Mos) of water storage.

For microtensile testing, specimens were fixed to metallic devices (Odeme Medical and Dental, Joaçaba, SC, Brazil) with cyanoacrylate glue (Three Bond Super Gel, ThreeBond, Diadema, SP, Brazil) and submitted to the microtensile test in a universal testing machine (EMIC DL 1000, Equipment and Systems Ltda, São José dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min until fracture. Specimens that failed prior to the test or during cutting or fixing procedures were recorded as pretesting failures (PTFs) and included in the bond strength means. A single blinded operator performed the microtensile test. All fractured specimens were observed under 40 $\times$  magnifying stereoscope (Discovery.v20, Zeiss, Oberkochen, Germany) to identify and classify the type of failure as adhesive/mixed (failure at the resin–dentin interface or mixed with cohesive failure of the neighboring substrate) or cohesive (dentin or resin).

### Scanning Electron Microscopy

Representative specimens from each dentin substrate (sound and artificially induced caries-affected dentin) were gold sputtered and analyzed with scanning electron microscopy (SEM; Jeol-JSM-T330A, JEOL, Tokyo, Japan) operated in the secondary electron mode with 5 kV.

### Statistical Analysis

The experimental unit in the study was the tooth. Thus, the means of  $\mu\text{TBS}$  (MPa) values of specimens tested at 24 hours or 6Mos were averaged for statistical purposes. The sample size had been determined that, considering a mean difference of 20% among groups and expecting a variation coefficient of 20%, a minimum of seven teeth per group was required to achieve a power of 0.8 and an  $\alpha$  error probability of 5%.

Three factors were considered in statistical analysis: adhesive system (AB, PB, SU, SB, and CS), etching strategy (ER and SE), and evaluation time (24 hours and 6Mos). Analyses were performed for each substrate separately (SND and CAD).

A normal distribution of the data was confirmed by the Kolmogorov-Smirnov test. Data were analyzed by a three-way repeated-measures analysis of variance (ANOVA) and *post hoc* Tukey tests at a significance level of 0.05, using a statistical software package (Minitab, Minitab Inc, State College, PA, USA).

PTFs were included in the bond strength means with a value of 0.<sup>31,32</sup>

Table 1: Adhesive systems (manufacturers and batch number), composition, and application mode<sup>a</sup>

Adhesive System/Batch	Composition	Application Mode
All Bond Universal (Bisco Inc, Schaumburg, IL, USA) (1500000055)	Bis-GMA, 10-MDP, HEMA, ethanol, initiators, water	SE: 1. Dispense one to two drops into a clean well. 2. Apply two separate coats, scrubbing the preparation with a microbrush for 10-15 seconds per coat. Do not light cure between coats. 3. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 seconds, there should be no visible movement of the adhesive. The surface should have a uniform glossy appearance. 4. Light cure for 10 seconds. ER: 1. Etch enamel and dentin using an etchant for 15 seconds. Rinse thoroughly. Remove excess water by blotting the surface with an absorbent pellet or high volume evacuation for one to two seconds, leaving the preparation visibly moist. 2. Apply adhesive as self-etch technique.
Prime Bond Elect (Dentsply Caulk, Milford, DE, USA) (140304)	Mono-, di- and trimethacrylate resins, PENTA, diketone, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, acetone, water	SE: 1. Place two to three drops into a clean well. Immediately apply generous amounts of adhesive to thoroughly wet all the tooth surfaces. Agitate the applied adhesive for 20 seconds. Re-wetting of the microbrush may be required in order to coat the preparation for the full 20 seconds. 2. Remove excess solvent by gently drying with clean, dry air from a dental syringe for at least five seconds. Surface should have a uniform glossy appearance. 3. Light cure for 10 seconds. ER: 1. Apply Caulk 34% tooth conditioner gel. Condition enamel for at least 15 seconds and dentin for 15 seconds or less. Remove gel with aspirator and/or vigorous water spray and rinse conditioned areas thoroughly for at least 15 seconds. Remove rinsing water completely by blowing gently with an air syringe or by blot drying with a cotton pellet. 2. Apply adhesive as self-etch strategy.
Scotchbond Universal (3M-ESPE, St. Paul, MN, USA) (509806)	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Initiators, Silane	SE: 1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 seconds. 2. Direct a gentle stream of air over the liquid for about five seconds until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 seconds. ER: 1. Apply etchant for 15 seconds. Rinse thoroughly and air dry or cotton pellet. Do not overdry! 2. Apply adhesive as in the self-etch strategy.

Table 1: Continued.		
Adhesive System/Batch	Composition	Application Mode
Adper Single Bond 2 (3M-ESPE, St. Paul, MN, USA) (N520165)	Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Initiators	1. Apply etchant for 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. The surface should appear glistening without pooling of water. 2. Immediately after blotting, apply two to three consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator. Gently air thin for five seconds to evaporate solvents. 3. Light cure for 10 seconds.
Clearfil SE Bond (Kuraray Noritake Dental Inc., Tokyo, Japan) (Primer: 01233A Bond: 01865A)	PRIMER: 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, <i>N,N</i> -Diethanol-p-toluidine, Water BOND: 10-MDP, Bis-GMA, HEMA Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, <i>N,N</i> -Diethanol-p-toluidine, Colloidal silica	PRIMER: 1. Dispense the necessary amount of PRIMER into a well of the mixing dish immediately before application. 2. Apply PRIMER to the entire cavity wall with a sponge or a disposable brush tip. Leave it in place for 20 seconds. Use caution not to allow saliva or exudate to contact the treated surfaces for at least 20 seconds. 3. After conditioning the tooth surface for 20 seconds, evaporate the volatile ingredients with a mild oil-free air stream. BOND: 1. Dispense the necessary amount of BOND into a well of the mixing dish 2. Apply BOND to the entire surface of the cavity with a sponge or a disposable brush tip. 3. After application, make the bond film as uniform as possible using a gentle oil-free air stream. 4. Light-cure the BOND for 10 seconds with a dental curing light.
Abbreviations: bis-GMA, bisphenyl-glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl-dihydrogen-phosphate. <sup>a</sup> According to information provided by manufacturers.		

RESULTS

Statistical analyses were performed separately for each substrate (SND and CAD). Significant cross-interaction among the three factors (adhesive system × strategy × time) was found on both SDN ( $p=0.005$ ) and CAD ( $p=0.012$ ) analyses. Table 2 presents the  $\mu$ TBS values (means and standard deviation) and contrasts found in the interactions separately for each substrate.

On SND, the etching strategy did not influence the bond strength for any tested universal adhesive, regardless the water storage. After 6Mos of water storage, all materials showed a trend of reducing numerical values, but with no significant differences, except the AB with ER strategy, which showed a significant degradation of bond strength values.

Etching strategies also produced similar values when compared among each other on CAD. However, considering degradation over time on CAD, different behavior was observed because a significant reduction in bond strength values was found, except for AB on ER strategy and PB on SE strategy.

Bond strength values of multimode adhesive systems in the ER strategy were similar to SB, which was used as the control etch-and-rinse system. Only PB–ER after 6Mos of water storage presented lower values than the control. The same was found for multimode systems used in the self-etching strategy on sound dentin; no significant differences were found compared with CS, the self-etch system that was used as a control. On CAD, significant differences were only found for bond strength values of PB and SU using the SE strategy, with values that

Table 2: Bond strength mean values in MPa (standard deviation) for experimental groups <sup>a</sup> [tested sps/pretest failures]					
Material	Strategy	Sound Dentin		Caries-Affected Dentin	
		24 Hours	Six Months	24 Hours	Six Months
AB	ER	50.3 (12.4) A [52/0]	28.6 (11.3) B,C,D [57/0]	13.1 (2.7) b,c,d [46/14]	2.5 (0.3) d [49/2]
	SE	34.7 (7.5) A,B,C,D [53/2]	34.1 (10.8) A,B,C,D [57/0]	22.5 (4.4) a,b [55/5]	3.7 (2.4) d [58/0]
PB	ER	42.3 (8.3) A,B,C [62/2]	24.7 (8.1) C,D [71/0]	19.5 (6.1) a,b,c [49/10]	3.9 (1.6) d [58/0]
	SE	26.2 (17.1) C,D [48/14]	20.0 (5.7) D [53/0]	16.3 (10.9) b,c [41/22]	9.4 (7.9) c,d [55/0]
SU	ER	48.0 (14.2) A,B [53/4]	33.6 (15.4) A,B,C,D [58/0]	21.3 (6.1) a,b [56/5]	2.4 (0.2) d [63/0]
	SE	40.1 (8.9) A,B,C,D [55/2]	34.7 (8.8) A,B,C,D [59/0]	17.7 (4.4) b,c [55/5]	4.7 (3.2) d [58/0]
SB		52.6 (11.8) A [51/1]	47.3 (10.4) A,B [61/0]	22.4 (6.4) a,b [60/6]	3.4 (1.8) d [64/0]
CS		26.5 (9.6) C,D [52/18]	23.2 (7.7) C,D [54/0]	29.2 (7.3) a [58/4]	12.5 (11.3) b,c,d [60/0]

<sup>a</sup> Different letters indicate statistically significant differences ( $p<0.05$ ). Uppercase letters for sound dentin and lowercase for artificially induced caries-affected dentin.

were lower than the control at immediate evaluation.

Significant differences were found between control adhesives at immediate and 6Mos of water storage because the etch-and-rinse system (SB) presented higher values than the self-etch adhesive (CS).

Adhesive/mixed failure patterns were predominant for all experimental groups, except for SB ER on SND after 6Mos of water storage. Cohesive failures (resin or dentin) seemed to be more frequent in sound dentin and increased in the 6Mos groups (Figure 1). PTFs were numerically more evident in CAD compared with SND (Table 2).

DISCUSSION

Caries-affected dentin has been a common substrate through direct restoration procedures in a minimally invasive adhesive dentistry approach. Several studies tested different adhesive systems, with different strategies in an attempt to achieve better performance on this kind of substrate.<sup>18,22-26,28,33-35</sup> Intrin-

sic characteristics of caries-affected dentin usually lead to lower bond strength compared with sound dentin.<sup>22,26,27,33</sup> Thus, in our study, artificially induced caries-affected dentin was considered separately in the statistical analyses, enabling us to evaluate three universal adhesive systems while discriminating both etching strategy and storage time for each dentin condition. This is one of the first studies that assessed the long-term bond strength of different multimode adhesives to caries-affected dentin.

The bond strength values to CAD were clearly lower compared with SND. Carious dentin is more porous than sound dentin due to the demineralization process.<sup>22</sup> Lower mineral content in CAD can induce a decrease in bond strength.<sup>27</sup> A previous study reported that such a decrease could be the consequence of the collapse of collagen fibrils that prevents proper penetration of adhesive resin.<sup>36</sup> This behavior on substrates such as caries-affected dentin is well stated in the literature for self-etch and etch-and-rinse systems.<sup>22-26,28</sup>

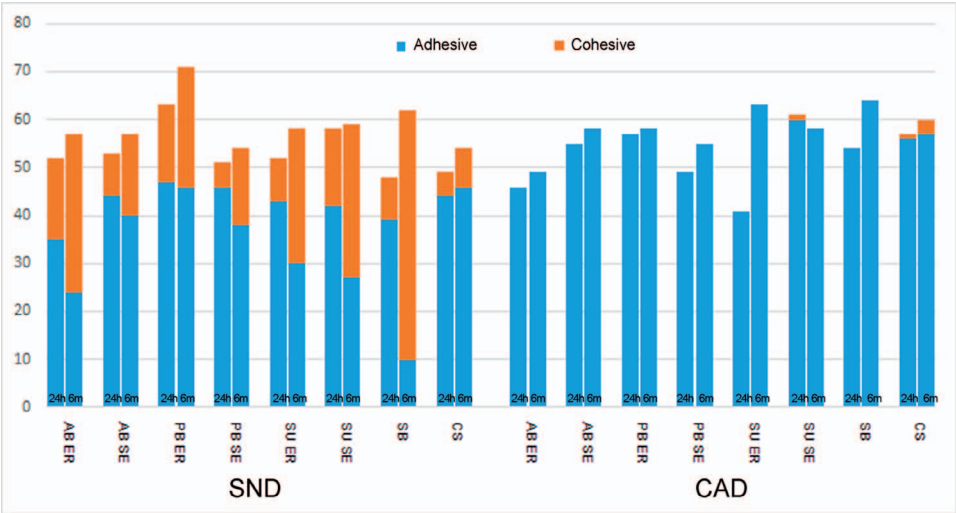


Figure 1. Fracture type distribution per experimental group.

The bond strength values remained stable in sound dentin after 6Mos of water storage, irrespective of the etching strategy, except for All Bond Universal using the etch-and-rinse strategy, which presented significant bond strength reduction after storage.

On the other hand, bonding to CAD presented a significant reduction for most experimental groups. There was an evident numerical drop in bond strength values for all the adhesive systems tested on this substrate. Only All Bond Universal using the ER strategy and Prime & Bond Elect using the SE strategy presented similar values after water aging on CAD. It is worth mentioning that these two adhesives, using their respective strategies, already produced the lowest immediate bond strength to CAD. Less mineral content, wettability, and other morphologic and chemical characteristics apparently had a strong influence on resin–CAD bond longevity due to higher permeability and a poor-quality hybrid layer.<sup>22,26,37</sup> In addition, the action of matrix metalloproteinase (MMPs) is more intense in caries-affected dentin.<sup>27</sup> The susceptibility to degradation of the interfaces created on CAD was reported in other studies evaluating self-etch and etch-and-rinse systems<sup>33,34</sup> and confirmed in our study, including the universal adhesive systems. Despite using a relatively short-term water storage, our study was able to demonstrate a more pronounced decrease in bond strength in caries-affected dentin, probably because of the substrate characteristics.

The etching strategy did not influence the immediate bond strength values for the multimode adhesives, regardless of the dentin condition and storage time, which is in accordance with previous studies that also evaluated both sound<sup>8,11,12,15</sup> and caries-affected dentin.<sup>18</sup>

The three multimode adhesives evaluated in this study present different compositions. Two of them (All Bond Universal and Scotchbond Universal) contain a monomer able to chemically bond to the dentin substrate (10-MDP). In addition, Scotchbond Universal incorporates a polyalkenoic acid copolymer (PAC) that also chemically interacts with the calcium of hydroxyapatite. Several studies showed that MDP-mediated chemical bonds maintained stable adhesion and prevented bond degradation when using the two-step self-etch adhesive Clearfil SE Bond, which was used as control in our study for SE strategy.<sup>38-40</sup>

Considering the presence of MPD in the universal adhesives evaluated in this study, Scotchbond

Universal (on both strategies) and All Bond Universal (on SE strategy) maintained bond strength after storage. Yoshida and others<sup>3</sup> showed that MDP-containing adhesives were able to form a nano-layer at the adhesive interface in different degrees, depending on the adhesive composition. They speculated that compositional differences and possibly different MDP concentrations could explain the distinct behavior of the MDP-containing adhesives. Moreover, they hypothesized that the presence of other components such as PAC or 2-hydroxyethyl methacrylate (HEMA) may compete with MDP on bonding sites to the calcium of hydroxyapatite.<sup>3</sup> These characteristics could explain the behavior of the MDP-containing adhesives tested in our study.

Similar performance was observed by Muñoz and others,<sup>13</sup> which supports the speculation that the presence of PAC is more important for etch-and-rinse adhesives as only All Bond Universal on ER strategy showed bond strength reduction after 6Mos. They indicated that PAC improves stability on a moist substrate, an important factor for etch-and-rinse adhesives. We hypothesized that the decreased performance of All Bond Universal on ER after water storage could be due to the etching strategy. The removal of available calcium by acid etching might have prevented any potential chemical bonding mediated by MDP since All Bond Universal with the SE strategy maintained bond strength values after 6Mos.

The other universal adhesive evaluated (Prime & Bond Elect) is HEMA free and acetone solvated. Zhang and others<sup>16</sup> explained that the absence of degradation observed for this system might be related to the higher vapor pressure of acetone (compared with ethanol) that improves solvent evaporation and may lead to less retention of residual water within the adhesive. A recent study concluded that entrapment of residual water in resin–dentin bonds could compromise the performance of universal adhesives.<sup>9</sup> Furthermore, Prime & Bond Elect does not contain HEMA in composition, making it less hydrophilic. To avoid or reduce hybrid layer hydrolytic degradation, researchers have been developing less hydrophilic adhesives such as HEMA-free adhesives.<sup>5,41</sup> Therefore, we hypothesized that these characteristics may explain Prime & Bond Elect performance on dentin after 6Mos of aging.

The adhesive systems used as controls performed differently on SND. The etch-and-rinse system used as a control (Adper Single Bond 2) showed higher bond strength values than the self-etch system

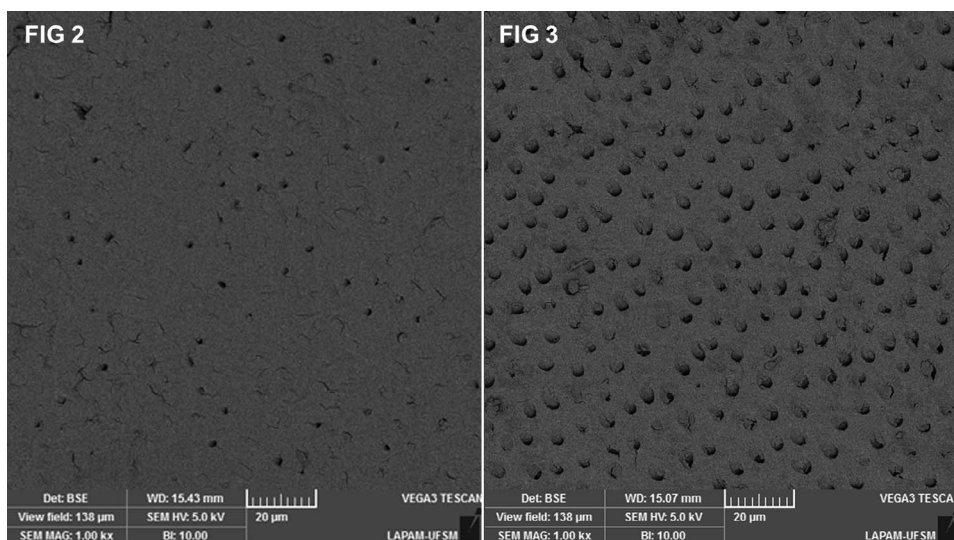


Figure 2. SEM image of representative specimen from sound dentin.

Figure 3. SEM image of representative specimen from artificially induced caries-affected dentin.

(Clearfil SE Bond); results have been previously reported.<sup>42-44</sup> We speculated that the results obtained with the self-etch system used as a control compared with the ER adhesive might be related to the fact that Clearfil SE Bond is the only tested adhesive that manufacturers do not recommend for active application. Several studies demonstrated that the active application enhanced immediate and long-term bond strength of self-etch adhesives.<sup>45-48</sup> The fact that Clearfil SE Bond has been available on the market for a long time could explain the manufacturer's instructions without using active application.<sup>39</sup>

This study used bovine dentin, which presents similarity compared with human dentin in several characteristics,<sup>49,50</sup> enabling its use for this purpose. A recent systematic review with meta-analysis supported the use of bovine tooth as a substitute to the human tooth in bond strength studies.<sup>51</sup> To confirm the differences for dentin substrates (SND and CAD), representative specimens for sound and artificially induced caries-affected dentin were analyzed by SEM (Figures 2 and 3).

Considering the obtained results, we failed to reject the first null hypothesis because the universal adhesives tested showed similar performance for both strategies on SND and CAD. Moreover, we partially rejected the second null hypothesis as water aging had no effect on SND for both strategies, and degradation over time was observed on CAD.

## CONCLUSIONS

Premature bond strength degradation occurred only to artificially induced caries-affected dentin. Etching

strategy did not influence the bond longevity of universal adhesives tested to artificial caries-affected dentin.

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## Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Santa Maria, Brazil.

## Conflict of Interest

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

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