©Operative Dentistry, 2019, **44-5,** 510-520

Bonding Performance of Universal Adhesive Systems Applied in Etch-and-Rinse and Self-Etch Strategies on Natural Dentin Caries

V Hass • AFM Cardenas • FSF Siqueira • RR Pacheco
PMW Zago • DO Silva • MC Bandeca • AD Loguercio

Clinical Relevance

Universal adhesive systems containing phosphate/carboxylic functional methacrylate derivatives are an interesting alternative when bonding to caries-affected dentin.

SUMMARY

Objective: This study investigated the bonding performance of three universal adhesive systems applied using etch-and-rinse (ER) or self-etch (SE) strategies on natural dentin caries.

Materials and Methods: Sixty human third molars were selected for this study: 30 naturally carious (CAD) and 30 sound (SD) teeth. The dentin surfaces were exposed, and teeth were randomly assigned to each evaluated adhesive system: Scotchbond Universal (SBU), Futurabond U (FBU), and Prime&Bond Elect (PBE) and an adhesive strategy: ER or SE. The adhesive systems were applied following the manufacturer's instructions, and the teeth were restored using a resin composite (Filtek Supreme Ultra, 3M). After 24 hours (distilled water at 37°C), samples were sectioned and evaluated using microtensile bond strength analysis (μTBS), micro-Raman spectroscopy to evaluate the degree of conversion within the hybrid layer (DC), and scanning electronic microscopy (SEM) to describe the morphology of the hybrid layer. The μTBS and DC data were analyzed using three-way analysis of variance and Tukey's test for means

*Viviane Hass, University North Parana, Post-Graduate Program in Dentistry, Londrina, Paraná, Brazil

Andres Felipe Millan Cardenas, Department of Restorative Dentistry, School of Dentistry, CEUMA University, São Luís, Maranhão, Brazil

Fabiana Suelen Figuerêdo de Siqueira, Department of Restorative Dentistry, School of Dentistry, CEUMA University, São Luís, Maranhão, Brazil

Rafael R Pacheco, Department of Restorative Dentistry, University of Detroit Mercy, Detroit, MI, USA

Patricia Maria Wiziack Zago, School of Dentistry, CEUMA University, São Luís, Maranhão, Brazil

Daniella Oliveira Silva, Post-Graduate Program in Dentistry, University Ceuma, São Luís, Brazil

Matheus C Bandeca, Department of Dentistry, CEUMA University, São Luís, Maranhão, Brazil

Alessandro D Loguercio, Restorative Dentistry, Ponta Grossa State University, Uvaranas, Ponta Grossa, Brazil

*Corresponding author: Rua José Montello, 1, Bairro Jardim Renascença II, São Luís, Maranhão 65075-120, Brazil; e-mail: vivikl_hass@hotmail.com

DOI: https://doi.org/10.2341/17-252-L

comparison (α=0.05). The SEM images were analyzed qualitatively.

Results: Reduced μTBS values were observed when comparing CAD with SD, regardless of adhesive system or strategy ($p<0.0001$). SBU showed statistically higher μTBS for both dentin substrates and strategies ($p<0.0001$). Furthermore, SBU showed greater integrity of the hybrid layer and resin tag formation compared with FBU and PBE. Mean μTBS values for FBU were higher for SD in the SE mode, whereas higher mean μTBS values were observed for CAD in the ER mode, both compared with PBE ($p<0.001$).

Conclusion: Bonding performance is reduced on a caries-affected substrate. The ER strategy was not able to improve the bonding performance on natural CAD for universal adhesive systems. Improved bonding performance was obtained when using the Scotchbond Universal system.

INTRODUCTION

In contemporary dentistry, minimally invasive approaches have been consistently developed and reinforced for restorative procedures, with one technique advocating the removal of the outer layer of highly infected, denatured caries-infected dentin.[1,2] The remaining caries-affected dentin tissue above the pulpal floor is to be preserved because it is a substrate that is suitable for remineralization and could prevent disease progression, reducing the unnecessary exposure of dental pulp tissues.[1,2]

Several chemical, biological, and physical modifications can occur as a result of the caries process, leading to a clinically distinct situation, which can affect adhesion to caries-affected dentin (CAD).[3,4] As a result, lower bond strength is found, compared with bonding procedures to sound dentin (SD),[5-7] and a poorly formed hybrid layer[7] can also be observed. However, most of the adhesion studies have focused on investigating SD only, which differs completely from the carious-dentin substrate. Furthermore, some studies are performed in artificially induced lesions,[8-11] which is a less challenging substrate than the one resulting from clinical caries.

In an attempt to simulate the caries process, several artificially induced caries protocols have been described.[12,13] These methods have a very important advantage in that they provide a standardized substrate, which is difficult to achieve with the natural lesions. However, it is known that no artificial carious lesion process is able to completely simulate the cascade of events involved in the natural caries process.[14,15] Additionally, the chemical and histologic characteristics promoted by the natural carious process (water content, blocked dentin tubules due to the deposition of intratubular dentin, among other factors) cannot be simulated.[16-18] Therefore, controversial results are found in the literature regarding different caries induction, bonding, or adhesive strategies.[4,6,7,9,10,13] This creates difficulty for the clinician when deciding which adhesive system or strategy to use with this sorely modified substrate.

More recently, new "universal" or "multimode" adhesives have been launched in the market.[19] Among the main advantages of these systems is the ability to use either a one-step self-etch approach or phosphoric acid (like an etch-and-rinse system) can be used.[20-23] Therefore, the use of universal systems seems favorable due to the broad versatility and facility of use by the clinician.

Unfortunately, there is a lack of dental literature regarding the bonding performance to natural dentin caries, especially when using universal or multimode adhesive systems.[19-23] It is very important to investigate the performance of universal systems when bonding to natural dentin caries, as this substrate represents one of the major reasons for restorative procedures.[24,25] Unfortunately, the performance of these universal systems has been tested using SD[22,23,26] or artificially induced caries-affected dentin.[9,10,13]

The aim of this study was to evaluate the bonding performance of three universal adhesive systems on natural CAD, using both etch-and-rinse and self-etch strategies, compared with sound dentin. The null hypotheses tested were as follows: 1) there is no difference in the bonding performance to natural CAD or SD; 2) the bonding performance is the same for all universal adhesive systems evaluated; and 3) there is no difference between the self-etch and etch-and-rinse strategies on the bonding performance to the tested substrates.

METHODS AND MATERIALS

Tooth Selection and Preparation

Sixty human third molars (30 natural CAD and 30 SD) were obtained with informed consent from donors (20-35 years old) under Local Ethics Committee number 1750969. Teeth were stored at 4°C in 0.5% chloramine T for up to 1 month before use. For carious teeth, the inclusion criteria required teeth diagnosed with active lesions using tactile, visual, and radiographic analysis, described as a 5 on the International Caries Detection and Assessment System (ICDAS) scale.[27,28] The samples of carious teeth were prepared by exposing a flat midcoronal carious dentin surface using a slow speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. One operator was calibrated to achieve appropriate dentin for the lesion walls[29] determined by the tactile dentin texture, ensuring that only carious tissue was removed until firm dentin (resistant to hand excavator) was left on the pulpal wall and that the periphery of the lesion contained clean to hard dentin (similar to sound dentin)[29] using a hand excavator. The exposed dentin was then finished using 600-grit silicon carbide paper under irrigation with distilled water to standardize the dentin roughness and smear layer thickness. All teeth were thoroughly rinsed with water, followed by the removal of the surrounding enamel using a diamond bur in a high-speed handpiece (#2135, KG Sorensen; São Paulo, SP, Brazil) under water irrigation. All exposed dentin surfaces were further finished using a 600-grit silicon carbide (SiC) abrasive paper for 60 seconds.

Experimental Design

All teeth were assigned to an experimental group based on the type of substrate (CAD or SD) and then three levels for adhesive system: Scotchbond Universal (3M Oral Care, St. Paul, MN, USA, also known as Single Bond Universal in some countries), Futurabond Universal (Voco, Cuxhaven, Germany), and Prime&Bond Elect (Dentsply Caulk, Konstanz, Germany); and two levels for adhesive strategy: etch-and-rinse or self-etch, totaling 12 experimental groups (n=5). Detailed information about the composition of adhesive systems, batch numbers, and application modes are described in Table 1.

Bonding and Restorative Procedure

One single calibrated operator performed all adhesive protocols according to each adhesive system and the respective manufacturer's instructions (Table 1). After bonding, the dentin surfaces were restored using a resin composite build-up (Filtek Supreme Ultra, 3M Oral Care). The build-ups were 4-5 mm in height, with three to four increments of resin composite, individually light-cured for 40 seconds using a monowave LED curing unit (Radii Cal, SDI, Bayswater, Victoria, Australia; 1200 mW/cm^2). All the restored teeth were stored in distilled water at 37°C for 24 hours prior to preparation.

Specimen Preparation

After 24 hours of storage, teeth were longitudinally sectioned in both the mesio-distal and bucco-lingual directions across the bonded interface in a cutting machine (Buehler, Lake Bluff, IL, USA), resulting in resin-dentin sticks with a 1-mm^2 cross section. The number of premature failures per tooth during specimen preparation was recorded. The resin-dentin sticks originating from areas immediately above the pulp chamber covered by CAD were selected for testing (selected by dentin color, under 40× magnification). Selected sticks were randomly allocated to three different tests: two sticks for degree of conversion analysis, two for hybrid layer morphology observation, and all other remaining sticks were used for microtensile bond strength testing.

Microtensile Bond Strength (μTBS)

The cross-sectional area of each stick was confirmed using a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) with a precision of 0.01 mm. Each bonded stick was attached to a jig for microtensile testing using cyanoacrylate resin (Super Bonder Gel, Loctite, São Paulo, Brazil) and subjected to a tensile force using a universal testing machine (Kratos, São Paulo, SP, Brazil) at a crosshead speed of 0.5 mm/min. The failure modes were evaluated under stereomicroscopy at 40× magnification and classified as cohesive, adhesive, or mixed.

In Situ Degree of Conversion Within the Hybrid Layer (DC)

The degree of conversion (on prepared resin-dentin sticks) was analyzed using micro-Raman spectroscopy[30] (Horiba Scientific Xplora, Villeneuve d'Ascq, France) with a 785-nm diode laser through a ×100/0.9 NA air objective. The Raman signal was acquired with 600 lines/mm grafting, centered between 800 and 1800 cm^{-1}, and the setting parameters were as follows: 100 mW, spatial resolution of 3 μm, spectral resolution of 5 cm^{-1}, accumulation time of 30 seconds, with five co-additions. Spectra were obtained at the dentin-adhesive interface at three random sites (per stick) within the intertubular-infiltrated dentin. Spectra of uncured adhesives were taken as references. The ratio of double-bonds of uncured and after curing (monomer to polymer) in the adhesive was calculated using the following formula: $DC (\%) = (1 - [R\ cured/R\ uncured]) \times 100$, where R is the ratio of aliphatic and aromatic peak intensities at 1639 cm^{-1} and 1609 cm^{-1} in cured and uncured adhesives, respectively.

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

Adhesive System (Manufacturer/Batch Number)	Composition	Application Mode[a] Self-Etch Strategy	Application Mode[a] Etch-and-Rinse Strategy	pH Value[b]
Scotchbond Universal Adhesive (3M Oral Care, St. Paul, MN, USA; D-82229)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: 10-methacryloyloxydecyl dihydrogenphosphate monomer, dimethacrylate resins, 2-hydroxyethyl methacrylate, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane.	1. Apply actively the adhesive to the entire surface with a microbrush for 20 s. 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 thoroughly. 3. Blot excess water 4. Apply adhesive as for the self-etching mode.	2.8
Futurabond U (Voco, Cuxhaven, Germany; 1346518)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: 2-hydroxyethyl methacrylate, bisphenolA diglycidyl methacrylate, (poly)methylene dimethacrylate, acidic adhesive monomer, urethane dymethacrylate, catalyst, silica nanoparticles, ethanol.	1. Apply the adhesive to the entire preparation with a microbrush and rub it for 20 s. 2. Directed a gentle stream of air over the liquid for about 5 sec until it no longer moved and the solvent was evaporated completely. 3. Light cured 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.	2.3
Prime&Bond Elect (Dentsply Caulk, Konstanz, Germany; 1102221)	1. Scotchbond Universal Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide. 2. Adhesive: Mono-, di-and trimethacrylate resins; dipentaerythritol petaacrylate monophosphate diketone; organic phosphine oxide; stabilizers; cetylamine hydrofluoride; acetone; water.	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 has evaporated completely. 3. Light-cure for 10 s at 1200 mW/cm^2.	1. Apply etchant for 15 s. 2. Rinse thoroughly with water spray. 3. Air dry. 4. Apply adhesive as for the self-etch mode.	2.5

[a] *All adhesive systems were applied according to slight modified manufacturers' instructions.*
[b] *References 56-58.*

Morphologic Analysis of the Hybrid Layer

Specimens were polished using wet SiC paper (grits #1500, 2000, and 2500). After ultrasonic cleaning, specimens were demineralized in HCl (6 N) for 30 seconds and deproteinized in 1% NaOCl for 10 minutes to reveal the hybrid layer. Specimens were dehydrated in ascending grades of ethanol: 25% (20 minutes), 50% (20 minutes), 75% (20 minutes), 95% (30 minutes), and 100% (60 minutes).[31] Following preparation, the specimens were mounted and sputter coated with gold-palladium in a vacuum evaporator (SCD 050, Balzers Union, Balzers, Liechtenstein), and the entire surface was examined using a scanning electron microscope (Vega, Tescan, Warrendale, PA, USA). Three photomicrographs of representative surface areas were taken at 5000× magnification.

Table 2: *Percentage (%) of Specimens According to Fracture Mode for the Experimental Groups*

Adhesive System	Application Mode	Sound Dentin				Caries Affected-Dentin			
		A/M	CR	CD	PF	A/M	CR	CD	PF
SBU	ER	54 (100)	0 (0)	0 (0)	0 (0)	50 (91)	0 (0)	5 (9)	0 (0)
	SE	50 (98)	0 (0)	0 (0)	1 (2)	48 (92)	0 (0)	4 (8)	0 (0)
FBU	ER	49 (94)	0 (0)	0 (0)	3 (6)	41 (85)	0 (0)	6 (13)	1 (2)
	SE	50 (96)	0 (0)	0 (0)	2 (4)	40 (85)	0 (0)	4 (9)	3 (6)
PBE	ER	52 (95)	0 (0)	0 (0)	3 (5)	38 (83)	0 (0)	5 (11)	3 (6)
	SE	50 (96)	0 (0)	0 (0)	2 (4)	35 (78)	0 (0)	8 (18)	2 (4)

Abbreviations: A/M, adhesive-mixed; CD, cohesive fracture mode in dentin; CR, cohesive fracture mode in resin; ER, etch-and-rinse; PT, premature failures; SE, self-etch.

Statistical Analysis

The values obtained for specimens from the same experimental unit were averaged for statistical purposes for μTBS (MPa) and DC (%). Sticks with premature and cohesive failures were not included in the calculation of mean value for the tooth due to their low frequency in the experiment. The Kolmogorov-Smirnov test was used to assess whether the data from these tests followed a normal distribution. Bartlett's test was performed to determine whether the assumption of equal variances was valid (data not shown). After observing normality and equality of the variances, the data from μTBS (MPa) and DC (%) were subjected to a three-way analysis of variance (type of dentin, adhesive system, and adhesive strategy). Tukey's test was used for pair-wise comparisons for all analyses (α=0.05).

RESULTS

Microtensile Bond Strength

Approximately 13-15 bonded sticks were obtained per tooth, including the pretest failures. The mean cross-sectional area was 0.94 ± 0.8 mm^2, and no differences were detected among the experimental groups (p>0.05). The failure modes are shown in Table 2. The number of dentin cohesive failures increased in CAD compared with SD.

Means and SDs obtained from μTBS for all experimental groups are shown in Table 3. The cross-product interaction was statistically significant (p<0.0001). The higher mean μTBS values were observed for sound dentin compared with caries-affected dentin, regardless of the adhesive system or strategy (p<0.0001).

When Scotchbond Universal (SBU) was applied in the self-etch or etch-and-rinse mode, the highest mean μTBS values were observed in CAD and SD, which were statistically higher than those obtained for Futurabond U (FBU), and Prime&Bond Elect (PBE) for both strategies in both substrates (p<0.001). When FBU was compared with PBE, the former presented higher mean μTBS values for SD in the self-etch mode and higher mean μTBS values for CAD in the etch-and-rinse and self-etch mode compared with PBE (p<0.001).

When both strategies were compared, the only significant difference was observed for SBU in SD. Higher mean μTBS values were achieved when SBU was applied using the etch-and-rinse mode compared with the self-etch mode (p<0.001).

In Situ Degree of Conversion Within the Hybrid Layer (DC)

The means and SDs obtained from the degree of conversion within the hybrid layer are shown in Table 4. The cross-product interaction was statistically significant (p<0.001). Higher mean DC values were observed for SD compared with CAD for both SBU and FBU (p<0.001). However, no significant difference was observed for PBE regardless of the type of dentin or adhesive strategy (p>0.05).

Table 3: *Means and SDs (MPa) Obtained for Microtensile Bond Strength for All Experimental Groups*[a]

Dentin Substrate	SBU		FBU		PBE	
	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse
Sound	46.9 ± 2.9 b	53.6 ± 2.9 a	32.0 ± 2.9 c	32.9 ± 1.7 c	27.7 ± 1.4 d	31.2 ± 1.5 c, d
Caries affected	19.8 ± 1.6 e	19.2 ± 1.0 e	13.2 ± 1.3 f	13.3 ± 2.6 f	8.6 ± 1.4 g	9.3 ± 0.3 g

[a] *Different letters indicate mean values statistically different (three-way analysis of variance and Tukey's test;* p<*0.05).*

Table 4: *Means and SDs (%) Obtained for Degree of Conversion Within the Hybrid Layer for All Experimental Groups*[a]

Dentin Substrate	SBU		FBU		PBE	
	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse	Self-Etch	Etch-and-Rinse
Sound	67.3 ± 3.6 a	68.7 ± 6.9 a	58.7 ± 1.7 b	61.2 ± 6.6 a,b	57.1 ± 2.8 b,c	55.6 ± 2.8c
Caries affected	50.3 ± 4.4 c,d	52.7 ± 4.9 c	50.8 ± 4.3 c,d	44.5 ± 4.6 d	58.1 ± 1.4 b	56.9 ± 2.5 b,c

[a] *Different letters indicate mean values statistically different (three-way analysis of variance and Tukey's test;* $p<0.05$*).*

When SBU was applied in either the self-etch or etch-and-rinse modes, the highest mean DC values were observed for SD, which were statistically higher compared with those obtained from FBU (only in the self-etch strategy) and PBE for both strategies and both substrates ($p<0.001$). However, when comparing CAD results, the highest mean DC values were achieved with PBE, which were statistically higher than those obtained from FBU ($p<0.001$). The results from SBU in CAD showed intermediary values.

Morphologic Analysis of the Hybrid Layer

Photomicrographs (5000×) obtained from the morphologic analysis of all resin-dentin interfaces for both SD and CAD by scanning electronic microscopy (SEM) are shown in Figure 1. For SD, a hybrid layer with more integrity was observed with a greater number and longer resin tags compared with CAD. For all systems, these features were more likely to occur in the etch-and-rinse technique compared with the self-etch technique. CAD images presented hybrid layers with scarce and incomplete tag formation for all adhesive systems.

When comparing the adhesive systems, SBU demonstrated greater frequency of tag formation for both strategies and substrates of FBU and PBE. FBU demonstrated more regular resin tag formation compared with PBE. When evaluating the self-etch mode, resin tag formation was more evident for SD, whereas PBE showed more collapsed collagen fibrils with more porosity signals. FBU demonstrated few

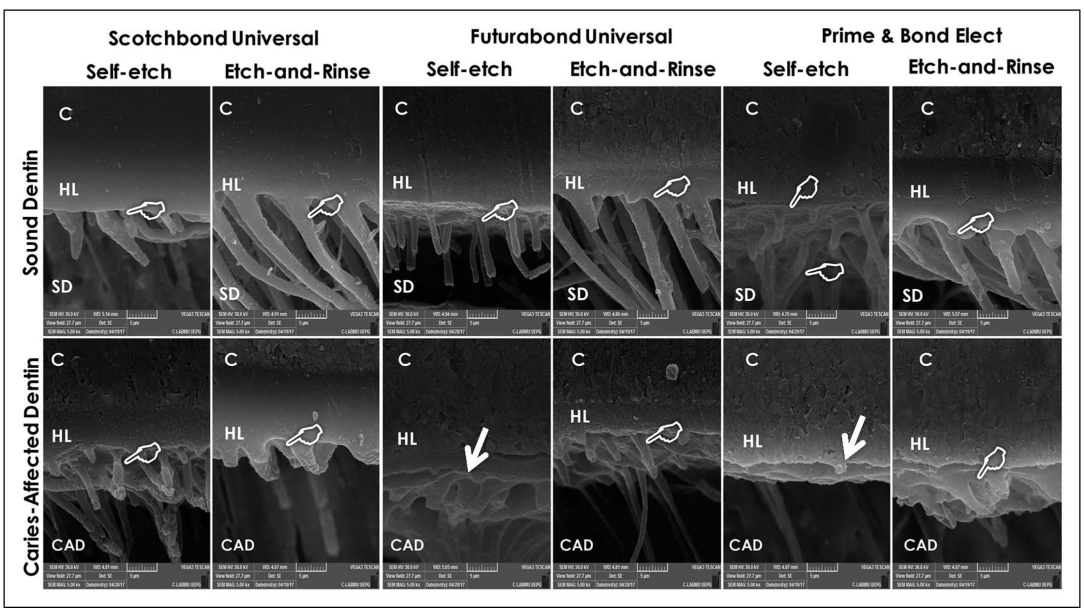

Figure 1. *Photomicrographs (magnification ×5000) obtained by scanning electron microscopy of all experimental groups. In the photomicrographs, it is possible to observe the variability of the hybrid layer formation (HL) between the SD and CAD. Note in SD more regularity and integrity of the hybrid layers, showing higher quantity and formation of longer resin tags (hands), mainly when the adhesives systems were applied in etch-and-rinse mode. The HLs promoted on CAD were evidenced by more porous zones, with incomplete resin tag formation. Observe in SBU, the HL showed better resin infiltration promoting HLs with more integrity than in FBU and PBE, regardless of substrate and adhesive strategy. Contrarily, PBE exhibited more disorganized HLs, for SD when applied in the self-etch mode. It is possible to identify confluence of tubules demonstrating poor adhesive penetration and collagen fibril encapsulation, in CAD note the very short and scarce resin tags present for the etch-and-rinse mode, and in the self-etch mode, there is almost total absence of hybridization signals (arrows). For FBU it is possible to observe intermediary hybridization performance, where in CAD, there was slight resin tag formation (arrows) in the self-etch mode and relatively longer tags in the etch-and-rinse mode. Abbreviations: C, composite resin; CAD, caries-affected dentin; HL, hybrid layer; SD, sound dentin.*

and short needlelike resin tags with the CAD substrate, although with a higher intensity than PBE. When using the etch-and-rinse mode for the CAD substrate, PBE demonstrated minimal resin tag formation, while resin tags were mostly absent when using the self-etch mode.

DISCUSSION

This is the first study that evaluated the bonding performance of universal adhesive systems on natural dentin caries. Although other studies have evaluated the bonding performance of universal systems on CAD, some in primary dentin,[9,13] and others in permanent dentin,[10,32] none of them evaluated natural caries lesions. However, studies involving natural caries lesions have an important disadvantage, which is the difficulty in standardizing the substrate for all evaluated teeth, and this, perhaps, is a potential limitation for studies using this experimental design. Several artificially induced caries protocols have been developed (using acid-gels,[33] pH cycling,[34] and microbiological agents[35]) with the intent of inducing changes to the dental substrate and allowing an easier understanding of the results.[14] However, the chemical caries induction methods have demonstrated controversial results due to the difference between the levels of demineralization and the duration of the demineralization and remineralization cycles not being standardized in pH cyclic protocols.[14] Additionally, the microbiological protocols provide softness, color alterations, and the presence of distinct zones,[14,35] but there are no standardized protocols for this method[36] and the few studies in the literature vary with the type of teeth evaluated, creating a situation where the studies are not comparable with each other.[14,33,37,38]

The events involved in the natural caries process promote a more dynamic environment. Natural caries is a diffusion-controlled process, involving not only the chemical dissolution of the inorganic material but also the exposure and the degradation of the organic matrix.[39,40] The different bacterial populations involved in the caries process drop the pH at different times,[35] creating a more challenging condition than the laboratory protocols. In addition to demineralization, the exposed organic matrix is degraded by the proteinases from microorganisms of the carious process and the host matrix metalloproteinase activated by bacterial acids, quantitatively and qualitatively affecting the structural substrate.[15,41] It is likely that these events combine to promote a distinct clinical condition that is, probably, more difficult to be completely simulated by artificial methods.

The current results showed that the bond strength was reduced when the bonding substrate was caries-affected dentin, regardless of the adhesive system (or technique) used. Thus, the first null hypothesis was rejected. These results were expected because the caries process is associated with the dynamic events of mineral loss and gradual denaturation of collagen fibrils.[7,42] These events can result in increased porosity in the intertubular dentin,[7] which can contribute to poor hybridization of the adhesive system for CAD,[18] as evidenced by SEM analysis for all CAD groups. Additionally, the reduced biomechanical properties for CAD compared with SD[7,42,43] can also be associated with reduced bond strength values for CAD. Unlike the intertubular dentin, the presence of an acid-resistant intratubular mineral deposit[18] can make resin tag formation difficult. According to SEM analysis, this tubule blockage in CAD could have interfered with acid-etching (ER strategy) and monomer penetration (ER and SE strategies) compared with SD.

These factors, such as a decrease in the biomechanical properties of dentin and lack of tag formation due to intratubular deposits, could support the lower bonding performance for CAD. Curiously, even when the current study was performed in natural CAD, the results agreed with other studies that used artificial caries protocols in permanent or primary teeth.[6,9,13,44,45] It is known that, although the artificial protocols are not able to completely simulate the cascade of events involved in the natural caries process, they do promote isolated alterations in the dentin substrate. According to Marquezan and others,[14] the pH-cycling protocol seems to resemble a natural affected caries dentin layer after caries removal. On the other hand, the microbiological method seems to more adequately simulate a dentin caries lesion with an evident infected layer, simulating a lesion prior to caries removal, although the artificial lesions are softer compared with natural lesions. The selection method of caries induction depends on what the researcher desires to study.[14] Isolated alterations would lead to better understanding the caries process and the potential effect on the bonding performance to a carious substrate. Thus, even when isolated, the alterations would impair the bonding performance compared with SD, consequently explaining the reason why different experimental designs obtained similar findings in bond strength tests.[6,9,13,44,45]

Our study also evaluated the degree of conversion within the hybrid layer and found reduced bond strength values for CAD, likely due to the lower degree of conversion inside the hybrid layer of two of three adhesive systems. The probable reason for this result is that the higher water content of CAD[43] could compromise the photopolymerization of the adhesive systems. Additionally, the phase separation phenomenon could be more prevalent for CAD compared with SD, because hydrophilic monomers tend to penetrate into the wet substrate, whereas hydrophobic monomers penetrate less,[18,46] which could affect the hybrid layer integrity. Furthermore, the phase separation phenomenon could affect the polymerization and, associated with the higher water content, influence the degree of conversion. However, the degree of conversion for PBE was not affected by the substrate.

PBE was the only evaluated adhesive system with acetone in its composition as a solvent. It is known that acetone demonstrates a higher vapor pressure compared with ethanol and/or water (as used for SBU and FBU), which may facilitate evaporation.[47] Solvent volatilization can also facilitate the polymerization reaction, because it reduces the distance among monomers, increasing the degree of conversion.[48] Although adhesive systems containing acetone as a solvent may demonstrate these advantages, a rapid acetone evaporation might not allow enough time for the monomers to infiltrate into dentin. It has been previously reported in the literature that adhesive systems with similar compositions to PBE did not promote uniform adhesive layer thickness across the interface (confocal microscopy), which requires twice the number of applications than recommended by the manufacturer, to obtain an acceptable resin-dentin bond strength.[26,49] Certainly, this could explain the reduced bond strength values observed for PBE and poor hybrid layer formation, mainly for CAD compared with FBU and SBU. The second null hypothesis was rejected, because different adhesive systems resulted in varied bonding performances.

Among the evaluated universal adhesive systems, SBU exhibited the highest bond strength results, regardless of the dentin substrate and technique. Universal adhesive systems have a composition similar to those of one-step self-etch adhesive systems, and most universal systems also contain specific carboxylate and/or phosphate monomers that can bond ionically to the Ca^{2+} in hydroxyapatite.[19] SBU contains the phosphate acid monomer, 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), which chemically bonds to hydroxyapatite, forming hydrolytically stable calcium salts in the form of "nano-layering" on hydroxyapatite.[50,51] Additionally, SBU contains a polyalkenoic acid copolymer in its composition, which interacts with apatite substrates following the same adhesion-decalcification reaction.[52,53] Thus, for SBU, both bonding mechanisms promoted micromechanical retention by diffusion of resin monomers and chemical adhesion. In the CAD substrate, even though it is partially demineralized, there is a reduced content of insoluble minerals in the dentinal tubules, which could be speculated to interact with the mineral content, explaining the morphology of the hybrid layer (SEM) obtained by SBU. FBU and PBE do not contain any of these compounds in their formulations; perhaps, the absence of these functional carboxylic or phosphate derivatives of methacrylate could explain the lower bond strength values compared with SBU. However, further studies are needed to determine whether the presence of carboxylate and/or phosphate monomers are able to maintain the bond strength values when subjected to storage and cycling methods, mainly for CAD.

The third null hypothesis was partially rejected, because the results showed that there is not a significant difference between etch-and-rinse and self-etch approaches for CAD compared with SD, indicating that the application of phosphoric acid is an unnecessary step during the bonding procedures for this substrate. This could be explained by the fact that CAD contains more residual mineral "β-tricalcium phosphate[18] (*whitlockite*) in the dentinal tubules, which is less soluble than hydroxyapatite at a pH lower than 5.5[54] compared with SD. Even though the adhesive strategy for universal systems is still controversial in the literature,[9,21,22] the bond strength should not be compromised by the strategy used,[55] because the ionization process resulting from self-etching acidic monomers should promote similar values compared with the etch-and-rinse mode. However, future studies need to be done evaluating which adhesive strategy is better in CAD after long-term exposure to water.

CONCLUSION

The use of the Scotchbond Universal system seems to be an interesting alternative when bonding to CAD, because it can lead to greater bond strengths and adequate hybrid layer formation compared with other adhesive systems tested. The application of phosphoric acid seems to be an unnecessary step during the bonding procedure to CAD when using universal adhesive systems.

Acknowledgements

The authors thank the support by Foundation for Research and Scientific Development of Maranhão (FAPEMA), the Research Funding supported by University Ceuma, and the interdisciplinary laboratory CLABMU of State University of Ponta Grossa for technique support. This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grant 305588/2014-1. This study was development during the Visiting Professor Scholarship of Prof Dr Alessandro D. Loguercio in the Ceuma University (São Luiz, MA, Brazil, 2014/2015).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Ethics Committee of University Ceuma. The approval code for this study is 1.750.969.

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.

(Accepted 12 March 2018)

REFERENCES

1. Franzon R, Guimaraes LF, Magalhães CE, Haas AN, & Araujo FB (2014) Outcomes of one-step incomplete and complete excavation in primary teeth: a 24-month randomized controlled trial *Caries Research* **48(5)** 376-383.
2. Maltz M, de Oliveira EF, Fontanella V, & Bianchi R (2002) A clinical, microbiologic, and radiographic study of deep caries lesions after incomplete caries removal *Quintessence International* **33(2)** 151-159.
3. Osorio R, Aguilera FS, Otero PR, Romero M, Osorio E, Garcia-Godoy F, & Toledano M (2010) Primary dentin etching time, bond strength and ultra-structure characterization of dentin surfaces *Journal of Dentistry* **38(3)** 222-231.
4. Sardella TN, de Castro FL, Sanabe ME, & Hebling J (2005) Shortening of primary dentin etching time and its implication on bond strength *Journal of Dentistry* **33(5)** 355-362.
5. Arrais CA, Giannini M, Nakajima M, & Tagami J (2004) Effects of additional and extended acid etching on bonding to caries-affected dentine *European Journal of Oral Sciences* **112(5)** 458-464.
6. Scheffel DL, Ricci HA, de Souza Costa CA, Pashley DH, & Hebling J (2013) Effect of reducing acid etching time on bond strength to noncarious and caries-affected primary and permanent dentin *Pediatric Dentistry* **35(7)** 199-204.
7. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, Carvalho RM, Nakajima M, & Pashley DH (2002) Bonding of self-etch and total-etch adhesives to carious dentin *Journal of Dental Research* **81(8)** 556-560.
8. de Almeida Neves A, Coutinho E, Cardoso MV, Lambrechts P, & Van Meerbeek B (2011) Current concepts and techniques for caries excavation and adhesion to residual dentin *Journal of Adhesive Dentistry* **13(1)** 7-22.
9. Lenzi TL, Raggio DP, Soares FZ, & Rocha Rde O (2015) Bonding performance of a multimode adhesive to artificially-induced caries-affected primary dentin *Journal of Adhesive Dentistry* **17(2)** 125-131.
10. Nicoloso GF, Antoniazzi BF, Lenzi TL, Soares FZ, & Rocha RO (2016) Is there a best protocol to optimize bond strength of a universal adhesive to artificially induced caries-affected primary or permanent dentin? *Journal of Adhesive Dentistry* **18(5)** 441-446.
11. Pinna R, Maioli M, Eramo S, Mura I, & Milia E (2015) Carious affected dentine: its behaviour in adhesive bonding *Australian Dental Journal* **60(3)** 276-293.
12. Schwendicke F, Eggers K, Meyer-Lueckel H, Dorfer C, Kovalev A, Gorb S, & Paris S (2015) In vitro induction of residual caries lesions in dentin: comparative mineral loss and nano-hardness analysis *Caries Research* **49(3)** 259-265.
13. Lenzi TL, Soares FZ, Raggio DP, Pereira GK, & Rocha RO (2016) Dry-bonding etch-and-rinse strategy improves bond longevity of a universal adhesive to sound and artificially-induced caries-affected primary dentin *Journal of Adhesive Dentistry* **18(6)** 475-482.
14. Marquezan M, Correa FN, Sanabe ME, Rodrigues Filho LE, Hebling J, Guedes-Pinto AC, & Mendes FM (2009) Artificial methods of dentine caries induction: a hardness and morphological comparative study *Archives of Oral Biology* **54(12)** 1111-1117.
15. Mazzoni A, Tjaderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, Pashley DH, & Breschi L (2015) Role of dentin MMPs in caries progression and bond stability *Journal of Dental Research* **94(2)** 241-251.
16. Nakajima M, Sano H, Zheng L, Tagami J, & Pashley DH (1999) Effect of moist vs. dry bonding to normal vs. caries-affected dentin with Scotchbond Multi-Purpose Plus *Journal of Dental Research* **78(7)** 1298-1303.
17. Tay FR, Pashley DH, Hiraishi N, Imazato S, Rueggeberg FA, Salz U, Zimmermann J, & King NM (2005) Tubular occlusion prevents water-treeing and through-and-through fluid movement in a single-bottle, one-step self-etch adhesive model *Journal of Dental Research* **84(10)** 891-896.
18. Wang Y, Spencer P, & Walker MP (2007) Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy *Journal of Biomedical Materials Research Part A* **81(2)** 279-286.
19. Perdigao J, & Swift EJ, Jr. (2015) Universal adhesives *Journal of Esthetic and Restorative Dentistry* **27(6)** 331-334.
20. Loguercio AD, de Paula EA, Hass V, Luque-Martinez I, Reis A, & Perdigao J (2015) A new universal simplified adhesive: 36-month randomized double-blind clinical trial *Journal of Dentistry* **43(9)** 1083-1092.

21. 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.

22. Munoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha NH, & Loguercio AD (2015) In vitro longevity of bonding properties of universal adhesives to dentin *Operative Dentistry* **40(3)** 282-292.

23. Sezinando A, Luque-Martinez I, Munoz MA, Reis A, Loguercio AD, & Perdigao J (2015) Influence of a hydrophobic resin coating on the immediate and 6-month dentin bonding of three universal adhesives *Dental Materials* **31(10)** e236-e246.

24. Heintze SD, & Rousson V (2012) Clinical effectiveness of direct class II restorations - a meta-analysis *Journal of Adhesive Dentistry* **14(5)** 407-431.

25. Opdam NJ, van de Sande FH, Bronkhorst E, Cenci MS, Bottenberg P, Pallesen U, Gaengler P, Lindberg A, Huysmans MC, & van Dijken JW (2014) Longevity of posterior composite restorations: a systematic review and meta-analysis *Journal of Dental Research* **93(10)** 943-949.

26. Luque-Martinez IV, Perdigao J, Munoz MA, Sezinando A, Reis A, & Loguercio AD (2014) Effects of solvent evaporation time on immediate adhesive properties of universal adhesives to dentin *Dental Materials* **30(10)** 1126-1135.

27. Gugnani N, Pandit IK, Srivastava N, Gupta M, & Sharma M (2011) International Caries Detection and Assessment System (ICDAS): a new concept *International Journal of Clinical Pediatric Dentistry* **4(2)** 93-100.

28. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, & Pitts NB (2007) The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries *Community Dentistry Oral Epidemiology* **35(3)** 170-178.

29. Innes NP, Frencken JE, Bjorndal L, Maltz M, Manton DJ, Ricketts D, Van Landuyt K, Banerjee A, Campus G, Domejean S, Fontana M, Leal S, Lo E, Machiulskiene V, Schulte A, Splieth C, Zandona A, & Schwendicke F (2016) Managing carious lesions: consensus recommendations on terminology *Advances in Dental Research* **28(2)** 49-57.

30. Hass V, Luque-Martinez I, Sabino NB, Loguercio AD, & Reis A (2012) Prolonged exposure times of one-step self-etch adhesives on adhesive properties and durability of dentine bonds *Journal of Dentistry* **40(12)** 1090-1102.

31. Kenshima S, Francci C, Reis A, Loguercio AD, & Filho LE (2006) Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer *Journal of Dentistry* **34(10)** 775-783.

32. Giacomini MC, Scaffa P, Chaves LP, Vidal C, Machado TN, Honorio HM, Tjaderhane L, & Wang L (2017) Role of proteolytic enzyme inhibitors on carious and eroded dentin associated with a universal bonding system *Operative Dentistry* **42(6)** E188-e196.

33. Silverstone LM (1968) The surface zone in caries and in caries-like lesions produced in vitro *Braziliam Dental Journal* **125(4)** 145-157.

34. ten Cate JM, & Duijsters PP (1982) Alternating demineralization and remineralization of artificial enamel lesions *Caries Research* **16(3)** 201-210.

35. Clarkson BH, Wefel JS, & Miller I (1984) A model for producing caries-like lesions in enamel and dentin using oral bacteria in vitro *Journal of Dental Research* **63(10)** 1186-1189.

36. Ogawa K, Yamashita Y, Ichijo T, & Fusayama T (1983) The ultrastructure and hardness of the transparent layer of human carious dentin *Journal of Dental Research* **62(1)** 7-10.

37. Moron BM, Comar LP, Wiegand A, Buchalla W, Yu H, Buzalaf MA, & Magalhaes AC (2013) Different protocols to produce artificial dentine carious lesions in vitro and in situ: hardness and mineral content correlation *Caries Research* **47(2)** 162-170.

38. Silverstone LM (1966) The primary translucent zone of enamel caries and of artificial caries-like lesions *Braziliam Dental Journal* **120(10)** 461-471.

39. Chaussain-Miller C, Fioretti F, Goldberg M, & Menashi S (2006) The role of matrix metalloproteinases (MMPs) in human caries *Journal of Dental Research* **85(1)** 22-32.

40. van Strijp AJ, Jansen DC, DeGroot J, ten Cate JM, & Everts V (2003) Host-derived proteinases and degradation of dentine collagen in situ *Caries Research* **37(1)** 58-65, 68223.

41. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, & Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions *Journal of Dental Research* **77(8)** 1622-1629.

42. Bjorndal L (2002) Buonocore Memorial Lecture. Dentin caries: progression and clinical management *Operative Dentistry* **27(3)** 211-217.

43. Marshall GW, Habelitz S, Gallagher R, Balooch M, Balooch G, & Marshall SJ (2001) Nanomechanical properties of hydrated carious human dentin *Journal of Dental Research* **80(8)** 1768-1771.

44. Erhardt MC, Rodrigues JA, Valentino TA, Ritter AV, & Pimenta LA (2008) In vitro microTBS of one-bottle adhesive systems: sound versus artificially-created caries-affected dentin *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **86(1)** 181-187.

45. Erhardt MC, Toledano M, Osorio R, & Pimenta LA (2008) Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure *Dental Materials* **24(6)** 786-798.

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

47. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, & Pashley DH (1998) Effects of HEMA on water evaporation from water-HEMA mixtures *Dental Materials* **14(1)** 6-10.

48. Nunes TG, Garcia FC, Osorio R, Carvalho R, & Toledano M (2006) Polymerization efficacy of simplified adhesive systems studied by NMR and MRI techniques *Dental Materials* **22(10)** 963-972.

49. Platt JA, Almeida J, Gonzalez-Cabezas C, Rhodes B, & Moore BK (2001) The effect of double adhesive application on the shear bond strength to dentin of compomers using three one-bottle adhesive systems *Operative Dentistry* **26(3)** 313-317.

50. 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.

51. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Torii Y, Osaka A, Suzuki K, Minagi S, Van Meerbeek B, & Van Landuyt KL (2011) Self-etch monomer-calcium salt deposition on dentin *Journal of Dental Research* **90(5)** 602-606.

52. Fukuda R, Yoshida Y, Nakayama Y, Okazaki M, Inoue S, Sano H, Suzuki K, Shintani H, & Van Meerbeek B (2003) Bonding efficacy of polyalkenoic acids to hydroxyapatite, enamel and dentin *Biomaterials* **24(11)** 1861-1867.

53. Sezinando A, Serrano ML, Perez VM, Munoz RA, Ceballos L, & Perdigão J (2016) Chemical adhesion of polyalkenoate-based adhesives to hydroxyapatite *Journal of Adhesive Dentistry* **18(3)** 257-265.

54. Frank RM, & Voegel JC (1980) Ultrastructure of the human odontoblast process and its mineralisation during dental caries *Caries Research* **14(6)** 367-380.

55. Wagner A, Wendler M, Petschelt A, Belli R, & Lohbauer U (2014) Bonding performance of universal adhesives in different etching modes *Journal of Dentistry* **42(7)** 800-807.

56. Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH, & Tay FR (2015) Bonding of universal adhesives to dentine–Old wine in new bottles? *Journal of Dentistry* **43(5)** 525-536.

57. 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.

58. Gutierrez MF, Sutil E, Malaquias P, de Paris Matos T, de Souza LM, Reis A, Perdigao J, & Loguercio AD (2017) Effect of self-curing activators and curing protocols on adhesive properties of universal adhesives bonded to dual-cured composites *Dental Materials* **33(7)** 775-787.