©Operative Dentistry, 2016, **41-3,** 305-317

Effect of Storage Time on Bond Strength and Nanoleakage Expression of Universal Adhesives Bonded to Dentin and Etched Enamel

P Makishi • CB André • APA Ayres
AL Martins • M Giannini

Clinical Relevance

The bond strength of a universal adhesive to dentin or etched enamel can be similar to that of conventional restorative systems in the long term; however, the bonding efficacy of both bonding techniques may decrease with aging, leading to leakage formation at the adhesive/dentin interface.

SUMMARY

Purpose: To investigate bond strength and nanoleakage expression of universal adhesives (UA) bonded to dentin and etched enamel.

Methods: Extracted human third molars were sectioned and ground to obtain flat surfaces of dentin (n = 36) and enamel (n = 48). Dentin and etched enamel surfaces were bonded with one of two UAs, All-Bond Universal (ABU) or Scotchbond Universal (SBU); or a two-step self-etching adhesive, Clearfil SE Bond (CSEB). A hydrophobic bonding resin, Adper Scotchbond Multi-Purpose Bond (ASMP Bond) was applied only on etched enamel. Following each bonding procedure, resin composite blocks were built up incrementally. The specimens were sectioned and subjected to microtensile bond strength (MTBS) testing after 24 hours or one year water storage, or immersed into ammoniacal silver nitrate solution after aging with 10,000 thermocycles and observed using scanning electron microscopy. The per-

*Patricia Makishi, DDS, PhD, Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil

Carolina Bosso André, DDS, MS, Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil

Ana Paula Almeida Ayres, DDS, MS, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

Adriano Luis Martins, DDS, MS, Laboratory of Oral Pathology-Oral Diagnosis Department, Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil

Marcelo Giannini, DDS, MS, PhD, associate professor, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

*Corresponding author: Avenida Limeira, 901, Piracicaba, SP 13414-903, Brazil; e-mail: pmakishi@gmail.com

DOI: 10.2341/15-163-L

centage distribution of silver particles at the adhesive/tooth interface was calculated using digital image-analysis software.

Results: The MTBS (CSEB = SBU > ABU, for dentin; and CSEB > ABU = SBU = ASMP Bond, for etched enamel) differed significantly between the adhesives after 24 hours. After one year, MTBS values were reduced significantly within the same adhesive for both substrates (analysis of variance, Bonferroni *post hoc*, $p<0.05$), and no significant differences were found among the adhesives for etched enamel. Silver particles could be detected within the adhesive/dentin interface of all specimens tested. Kruskal-Wallis mean ranks for nanoleakage in ABU, SBU, and CSEB were 16.9, 18.5 and 11, respectively ($p>0.05$).

Conclusions: In the short term, MTBS values were material and dental-substrate dependent. After aging, a decrease in bonding effectiveness was observed in all materials, with nanoleakage at the adhesive/dentin interface. The bonding of the UAs was equal or inferior to that of the conventional restorative systems when applied to either substrate and after either storage period.

INTRODUCTION

The ultimate goal of adhesive dentistry is to provide simple and fast adhesive application with durable bonding to enamel and dentin.[1] Self-etching adhesives contain acid monomers to enable etching of dental structures, hydrophilic monomers to enhance wettability, and hydrophobic bond resin monomers that infiltrate into the demineralized rough enamel or porous dentin surface,[2] providing monomer conversion and strengthening of the tooth-resin interface. Self-etching primer systems combine the etching and the priming steps into one, whereas all-in-one systems combine a self-etching primer and a bonding agent into one application step.

Among the self-etching adhesives available in the market, there is a growing interest in multi-mode adhesives, or so-called universal adhesives (UA). They are designed with the same concept as all-in-one adhesives but incorporate the versatility of being adaptable to different clinical situations.[3] According to the manufacturers, this new category of simplified one-bottle adhesives is indicated for direct and indirect restorations without the need for a primer. This adhesive can be applied to dentin or enamel using a self-etching approach or after selectively etching the enamel. Some UAs contain 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer in their formulation. This functional monomer is considered one of the most effective monomers with regard to chemical interaction and durability.[4,5]

Although the initial bonding performance of UAs to dentin seems to be material and technique dependent,[6,7] recent studies have reported good long-term performance of this new category of adhesives.[3,8] In addition, it has been suggested that similar to other self-etching adhesives,[9] pre-etching the enamel increases the bond strength values for UAs, based on a short-term evaluation.[10-12] Despite promising results, there are only a few studies with regard to the bonding effectiveness of MDP-containing UAs compared with those investigating conventional restorative systems bonded to dentin and etched enamel structures.[13]

Nanoleakage is leakage at nanometer-sized channels, which can occur within the hybrid layer and/or in the adhesive layer, in the absence of marginal gaps.[14,15] This phenomenon has been widely implicated as an important factor that leads to the degradation of the bonding to dental tissue.[14-16] It may be caused by insufficient infiltration of resin into the demineralized collagen network or by incomplete polymerization of hydrophilic monomers in the submicron interfacial spaces. The unprotected collagen fibrils that result from this may be vulnerable to degradation by oral and bacterial enzymes.[17,18] Under *in vitro* evaluations, interfacial sealing, bond strength, and artificial aging appear to be related to one another,[19] and these can be considered potential clinical predictors of the success of a restoration.[20]

Therefore, the aim of this study was to compare the MTBS and nanoleakage expression of two UAs currently in use with those of a conventional two-step self-etching adhesive to dentin and etched enamel substrates. In addition, on etched enamel substrate, use of UAs was also compared with a conventional technique consisting of a hydrophobic bonding resin applied directly to etched enamel. The null hypotheses tested were 1) there is no significant difference in bond strength between the materials tested after 24 hours or one year of water storage when bonded to dentin or etched enamel; and 2) after 10,000 cycles of thermal stress, the use of UAs does not result in more nanoleakage along the adhesive/tooth substrate interface compared with the conventional adhesives tested.

Table 1: *Materials Used in This Study*

Adhesive System (Batch Number)	Composition	Dentin Self-etch Mode	Enamel Etch-and-rinse Mode
All-Bond Universal (1200003968); BISCO, Schaumburg, IL, USA	Adhesive: MDP, Bis-GMA, HEMA, ethanol, water, initiators	Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10-15 sec per coat. Evaporate excess solvent by thoroughly air-drying with an air syringe for 10 sec until there is no visible movement of the material. Light cure for 10 sec.	Apply etchant for 15 sec. Rinse for 10 sec. Remove excess water with absorbent pellet for 2 sec. Apply adhesive as for the self-etching mode.
Scotchbond Universal (472387); 3M ESPE, St Paul, MN, USA	Adhesive: MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Apply adhesive to the preparation with a microbrush and rub it in for 20 sec. Gently air blow-dry for 5 sec until there is no visible movement of the material. Light cure for 10 sec.	Apply etchant for 15 sec. Rinse for 10 sec. Air dry for 2 sec. Apply adhesive as for the self-etching mode.
Clearfil SE Bond (Primer: 01115A Bond: 01671A); Kuraray Noritake Dental Inc, Tokyo, Japan	Primer: MDP, HEMA, camphorquinone, hydrophilic dimethacrylate, water Bond: MDP, Bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, *N,N*-diethanol *p*-toluidine bond, colloidal silica	Apply primer to tooth surface and leave in place for 20 sec. Blow-dry. Apply bond to the tooth surface and then create a uniform film using a gentle air flow. Light cure for 10 sec	Apply etchant for 15 sec. Rinse for 10 sec. Air dry. Apply adhesive as for the self-etching mode.
Adper Scotchbond Multi-Purpose Bond (N205453); 3M ESPE, St Paul, MN, USA	Bond: Bis-GMA, HEMA, triphenylantimony	N/A	Apply etchant for 15 sec. Rinse for 10 sec. Air dry. Apply adhesive with slight agitation for 10 sec. Air dry. Apply adhesive. Light cure for 10 sec.

Abbreviations: Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; HEMA, hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; N/A: Not applicable (because Adper Scotchbond Multi-Purpose Bond was applied only on etched enamel substrate).

METHODS AND MATERIALS

Specimen Preparation

A total of 84 extracted intact human third molars were used according to the guidelines of the local Ethics Committee, under protocol number 143/2014. For dentin specimens (n=36), the occlusal one-third and the root of each tooth were cut using a diamond saw (IsoMet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. The exposed coronal flat dentin surface was polished with 600-grit silicon carbide paper (Norton, Vinhedo, Brazil) under running water to ensure that enamel isles were completely removed.

For enamel specimens (n=48), the root of each tooth was removed and a mesiodistal cut perpendicular to the middle occlusal surface was performed using a diamond saw (IsoMet, Buehler Ltd) under water cooling. Flat buccal and lingual enamel surfaces were obtained from 48 teeth after wet-grinding with 600-grit silicon carbide paper (Norton). All the specimens for enamel substrate were pre-etched with 35% phosphoric acid for 15 seconds (Scotchbond Universal Etchant; 3M ESPE, St Paul, MN, USA), rinsed thoroughly, and air-dried before each adhesive application.

The obtained dentin or etched enamel flat surfaces were assigned randomly to three groups (n=12 per adhesive/substrate) according to the material used: two UAs, All-Bond Universal (ABU; Bisco, Schaumburg, IL, USA) and Scotchbond Universal (SBU; 3M ESPE); or a two-step self-etch adhesive, Clearfil SE Bond (CSEB; Kuraray Noritake Dental Inc, Tokyo, Japan). Only on etched enamel substrate, for an additional group (n=12), consisting of a hydrophobic bonding resin, Adper Scotchbond Multi-Purpose Bond (ASMP Bond; 3M ESPE) was applied directly to the substrate. After each bonding procedure, all the specimens were restored with a composite resin, Filtek Z350XT (3M ESPE) in two increments of 2 mm each. Each increment was cured for 40 seconds using a quartz-tungsten-halogen light-curing unit (Optilux 501, Kerr, CA, USA; 600 mW/cm^2 intensity). The specimens were prepared according to each of the manufacturer's instructions (Table 1).

MTBS Measurement and Fracture Analysis

After being stored for 24 hours in water at 37°C, 10 restored teeth from each adhesive and each substrate (dentin: n=30; etched enamel: n=40) were sectioned serially using a low-speed diamond saw

(IsoMet, Buehler Ltd) under water cooling to produce parallel piped sticks (0.9 mm wide × 0.9 mm thick × 6 mm length) with their long axis perpendicular to the bonded interface. Half the number of sticks from each tooth and each substrate were selected randomly for immediate testing, and the remainder were stored for one year in distilled water.

For the MTBS testing, the ends of the sticks were carefully fixed with cyanoacrylate glue (Model Repair II Blue, Sankin Industry Co, Tokyo, Japan) to a jig in a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) and subjected to a tensile force at a crosshead speed of 1 mm/min. After the bond strength testing, the two ends of the fractured surfaces were mounted on brass stubs, gold-coated, and observed using scanning electron microscopy (SEM; JSM5600, JEOL Ltd, Tokyo, Japan) at magnifications of 100× and 1000×.

For dentin specimens, the failure mode of each beam was determined by two experienced researchers and then classified, by consensus, into categories: cohesive failure in composite resin (C); failure between composite resin and adhesive (CA); failure between adhesive and dentin (AD); mixed failure of composite resin, adhesive, and dentin (CAD); cohesive failure in adhesive (A); cohesive failure in hybrid layer (HL); and cohesive failure in dentin (D).

In a similar way, for etched enamel specimens, the failure mode of each beam was determined and classified into cohesive failure in composite resin (C); failure between composite resin and adhesive (CA); failure between adhesive and etched enamel (AE); mixed failure of composite resin, adhesive, and etched enamel (CAE); cohesive failure in adhesive (A); failure between enamel and dentin (ED); and cohesive failure in enamel (E).

Thermocycling Procedure

In addition, two teeth from each adhesive and each substrate were selected to undergo thermocycling. The specimens were fatigued with 10,000 thermocycles between 5°C and 55°C at a dwell time of 30 seconds per temperature and a transfer time of 10 seconds between baths (MSCT 3, Marnucci ME, Sao Carlos, Brazil).

Nanoleakage Evaluation

After the thermocycling procedure, the two teeth from each material and each substrate were vertically sectioned with a diamond saw (IsoMet, Buehler Ltd) under water coolant, across the adhesive/tooth substrate interface, into approximately 1-mm-thick slabs. Two central slabs were chosen from each tooth, forming a total of four specimens per material and per substrate. All the specimens were coated with two layers of nail varnish applied 1 mm from the bonded interface, followed by immersion into 50% (wt/vol) ammoniacal silver nitrate solution for 24 hours. Thereafter, they were rinsed thoroughly under running tap water and exposed to photodeveloping solution for eight hours under fluorescent light to reduce the penetration of the ammoniacal silver nitrate into metallic silver grains. Each slab was then wet-polished with 1200-grit silicon carbide paper (Norton) and sonicated for five minutes to remove the superficial silver adsorption.

Following air-drying, the specimens were gold-coated and examined by SEM in back-scattered electron mode and energy dispersive x-ray spectroscopy (EDS; JSM5600, JEOL Ltd) at a magnification of 2000×. Interfacial images were obtained from each specimen (n=10). The percentage distribution of metallic silver particles at the adhesive/tooth substrate interface was calculated with a digital image-analysis software (NIH ImageJ 1.60, Scion, Frederick, MD, USA) in a selected area on each image at 500× (20.2 × 250 μm, height × width). Initial energy spectra analyses were performed to determine the elemental composition of the entire area. In addition, select surface areas were mapped for elements including silver, calcium, and silicon.

Statistical Analysis

The MTBS data were statistically analyzed using a three-way analysis of variance (ANOVA) with the significance level defined as $\alpha = 0.05$; bond strengths to dentin or etched enamel were dependent variables, and the adhesive, the storage period, and the substrate were factors. A Bonferroni *post hoc* test with UNIANOVA syntax was used for multiple comparisons with significant differences in bond strength means. The nanoleakage data were statistically analyzed with a Kruskal-Wallis test, with the statistical significance defined as $\alpha = 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS for Windows, Version 16.0, SPSS Inc, Chicago, IL, USA).

RESULTS

The means and standard deviations of dentin and etched enamel MTBS values obtained in this study are presented in Table 2. The three-way ANOVA showed that the bond strength results for dentin and

Table 2: *Mean MTBS of Different Adhesives Applied in a Self-etch Mode on Dentin Substrate and in an Etch-and-rinse Mode in Enamel Substrate*

Material	Dentin Substrate		Etched Enamel Substrate	
	After 24 h Water Storage	After One y Water Storage	After 24 h Water Storage	After One y Water Storage
All-Bond Universal (ABU)	39.9 (11.9) A, a	25.1 (13.2) A, b	40.8 (5.9) A, c	30.7 (9.4) A, d
Scotchbond Universal (SBU)	63.1 (8.2) B, a*	45.9 (8.4) B, b**	40.7 (13.0) A, c*	28.4 (10.8) A, d**
Clearfil SE Bond (CSEB)	63.4 (9.2) B, a	44.1 (4.2) B, b**	59.7 (13.8) B, c	27.1 (5.7) A, d**
Adper Scotchbond Multi-Purpose Bond (ASMP Bond)	N/A	N/A	40.4 (7.7) A, a	24.4 (6.5) A, b

Abbreviation: N/A: Not applicable (because ASMP Bond was tested only on etched enamel substrate).
[a] *Data are presented as the mean (standard deviation) in megapascals (MPa; n = 10 teeth). Identical capital letters in a column indicate the absence of any statistically significant difference. Identical lowercased letters in a row within the same substrate between after 24 h and one y water storage indicate the absence of any statistically significant difference. Comparisons within the same material and storage period between different substrates, and marked with one asterisk for 24 h and two asterisks for one y water storage are statistically significant. (Analysis of variance and Bonferroni* post hoc *test; significance at* $p<0.05$*).*

etched enamel were influenced significantly by the adhesive used, by the storage period, and by the substrate. The interaction of these three factors was not significant ($p=0.055$). On the other hand, significant statistical interaction was observed between adhesive material and storage period ($p=0.011$) and between adhesive and substrate ($p<0.001$), but no significant statistical interaction was found between storage period and substrate ($p=0.710$).

A Bonferroni *post hoc* test revealed the presence of statistically significant differences between the MTBS results after 24 hours or one year of water storage for both substrates. For dentin, CSEB and SBU showed higher bond strength values than those of ABU after 24 hours or one year of storage. However, there was a significant decrease in MTBS values within all adhesives tested after one year of storage ($p<0.05$). For etched enamel, CSEB showed significantly higher bond strength values after 24 hours; no significant difference was observed among ABU, SBU, and ASMP Bond at this time point. After one year of water storage, a significant decrease in bond strength was observed within all adhesives compared with their baselines, and the bond strength values did not differ from each other among all adhesives ($p>0.05$). Using ABU, there were no significant MTBS differences between the dentin and etched enamel when the substrates were compared after the same storage period. For SBU, the bonding to dentin did in fact result in a significantly higher bond strength than etched enamel after 24 hours. The same was true when using both SBU and CSEB after one year of water storage.

The failure modes of the tested groups are summarized in Figure 1. Representative high magnification SEM micrographs of the fracture mode patterns are shown in Figure 2. For dentin, the C failure mode predominated for SBU and CSEB at both time points, whereas ABU showed an increased incidence of AD mode of failure after one year storage. For etched enamel, a slight increase of C and CAE mode of failure was observed in most of the materials in the long term.

Representative images of silver-challenged specimens for dentin and etched enamel substrates after 10,000 thermocycles are illustrated in Figures 3 and 4, respectively. High magnification of SEM micrographs after the silver challenge revealed the existence of nanoleakage formation only for dentin specimens after 10,000 thermocycles. Images in which the total percentage distribution of silver tracer within the interface was calculated are shown in Figure 3. For the nanoleakage test, no significant statistical difference was observed among all adhesives after 10,000 thermocycles (Kruskal-Wallis test, $p>0.05$). A distinctive silver-spotted pattern of nanoleakage formation could be recognized along the adhesive/dentin interface in all specimens, and its silver percentage distribution means and mean ranks can be visualized in Table 3. In addition, SEM/EDS images of the interface with silver particles are shown in Figure 5. Elemental silver was identified by EDS analysis, confirming the results obtained.

DISCUSSION

In this study, the bonding strength of UAs was tested on dentin and etched enamel and compared with a conventional two-step self-etching adhesive. The MTBS test was chosen owing to its advantages over other bond strength tests: Bonding measurement can be achieved for very small areas, and multiple beams can be obtained from a single tooth.[21] It also allowed us to divide the number of beams obtained from each specimen and to evaluate them at two storage times.

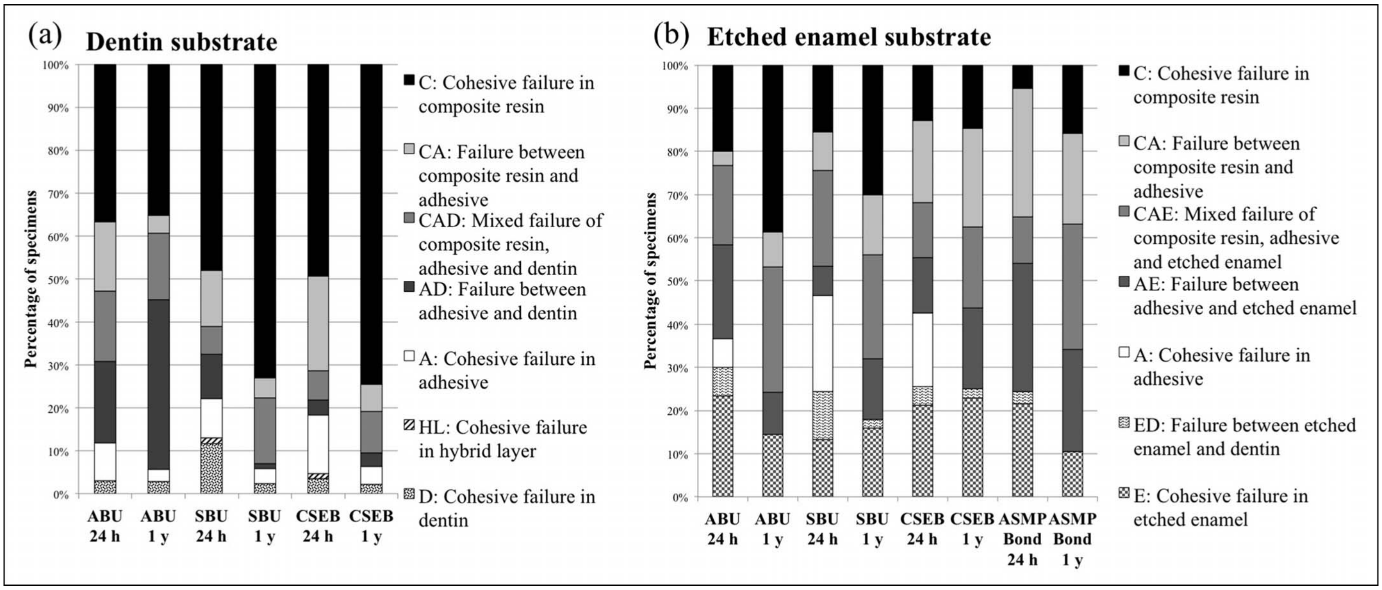

Figure 1. *Distribution (%) of failure modes of the adhesive materials tested after 24 hours (24 h) and one year (1 y) water storage in (a): dentin and (b): etched enamel substrates.*

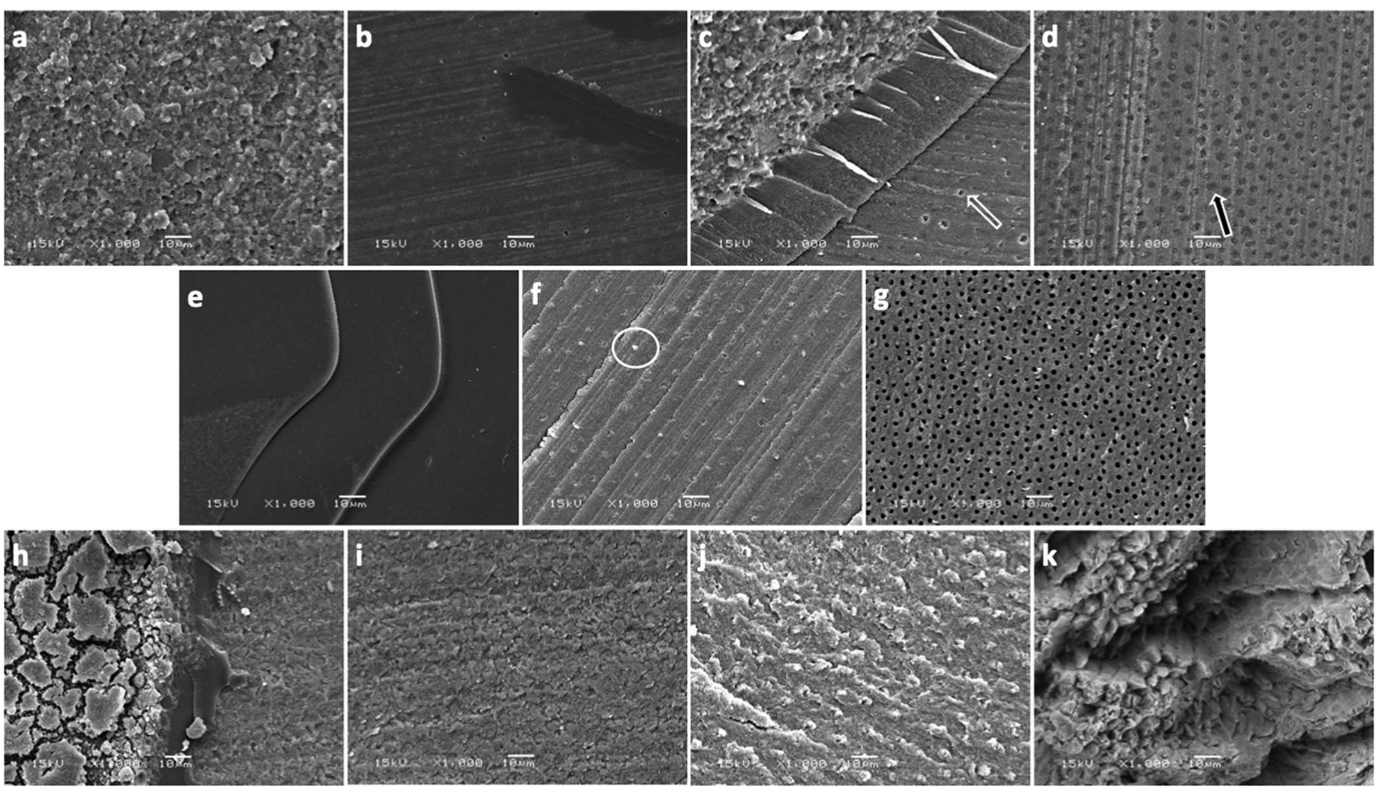

Figure 2. *SEM (1000×) showing representative fracture patterns on dentin (a-g) and etched enamel (h-k) substrates. Images are ordered as follows: (a): cohesive failure in composite resin, C; (b): failure between composite resin and adhesive, CA; (c): mixed failure of composite resin, adhesive, and dentin, CAD; (d): failure between adhesive and dentin, AD; (e): cohesive failure in adhesive, A; (f): cohesive in hybrid layer, HL; (g): cohesive failure in dentin, D; (h): mixed failure of composite resin, adhesive, and etched enamel, CAE; (i): failure between adhesive and etched enamel, AE; (j): failure between etched enamel and dentin, ED; and (k): cohesive failure in etched enamel, E. Demineralized dentinal tubules can be observed in (c) (arrow). Smear plugs in dentinal tubules can be visualized in (d) (black arrow). Circled area in (f) shows complete resin infiltration in the dentinal tubules.*

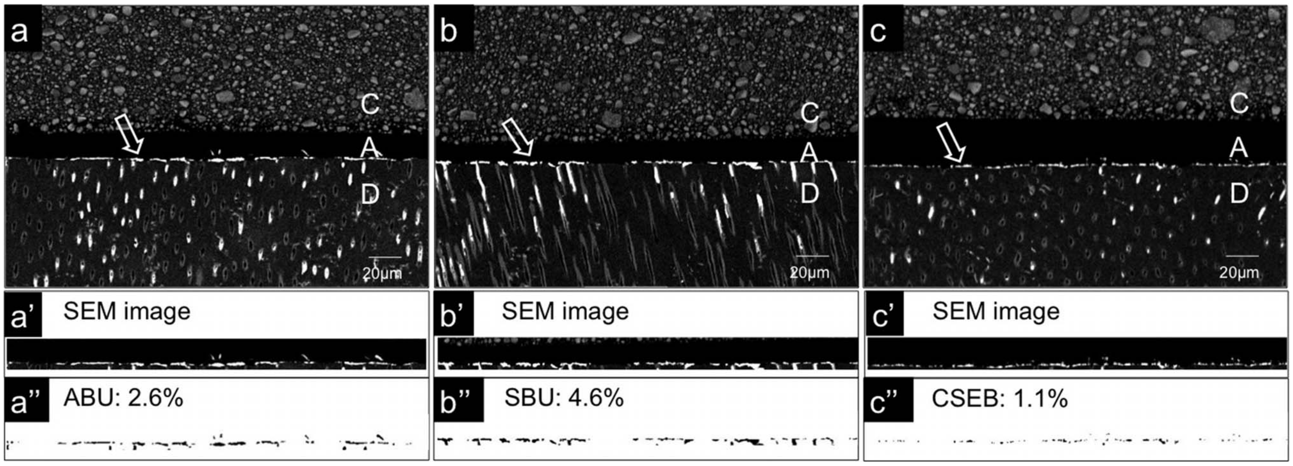

Figure 3. *Representative back-scattered scanning electron micrographs of nanoleakage at the adhesive/dentin interface in (a–c) after 10,000 thermocycles, the corresponding selected interfacial area (a′–c′), and the scored binary image obtained by the digital image analysis software (a″–c″). Images are ordered as follows: (a) ABU; (b) SBU; (c) CSEB. (a–c) Spotted silver patterns can be visualized in all the adhesives (arrow). (a′–c′) Standardized selected interfacial areas to be analyzed by the digital image analysis software. (a″–c″) The selected image was converted to a binary image to distinguish the silver area (black target pixels) from the resin and dentin (white background pixels). Percentage (%) distribution of metallic silver particles at the selected interface of the scanning electron micrograph image was calculated by the digital image analysis software; the results are indicated on each binary image: (a″) ABU; (b″) SBU; (c″) CSEB. Abbreviations: C, composite resin; A, adhesive; D, dentin.*

In addition, specimens were subjected to thermal aging prior to the nanoleakage test, and interfacial analysis for silver deposits was performed using SEM/EDS images. The use of 10,000 thermal cycles has been suggested to correspond to approximately one year of *in vivo* functioning.[22] EDS can produce quantitative and qualitative analysis of various elements' distribution and is considered to be a sensitive and accurate chemical component detection method.[23] Using EDS, the presence of elemental silver at specific locations could be confirmed. In this way, it was less likely that the investigators would misinterpret brightness caused merely by the electron microscope edge effect as indicative of silver particles.[24] Besides, this study used a digital image-analysis software to score the percentage of silver tracer particles within the adhesive/dentin interface. The percentage of silver particles within a selected area was calculated on the basis of the contrast and brightness of each pixel on the digital image.[25]

In the current study, ABU, SBU, and CSEB were bonded to dentin and etched enamel substrates, and their compositions include MDP as functional monomer. MDP has been reported to interact chemically with hydroxyapatite and to form a hydrolytically stable bond with calcium.[4,26] Indeed, no significant statistical difference of MTBS values was observed between SBU and CSEB for dentin substrate after 24 hours or one year of water storage; however, significantly lower bond strength was found for ABU. Conflicting results have been reported regarding the bond performance of ABU when applied in a self-etch mode.[3,6,7] Potentially, the ultramild acidity (pH=3.1)[13] of this material compared with that of SBU (pH=2.7)[13] and CSEB primer (pH=2.1)[6] may have limited the penetration of ABU's resin monomers into the dentinal tubules and intertubular dentin. In addition, ABU, in its formulation, contains bisphenol-A diglycidyl methacrylate (Bis-GMA), which is a highly viscous monomer.[27] It has been

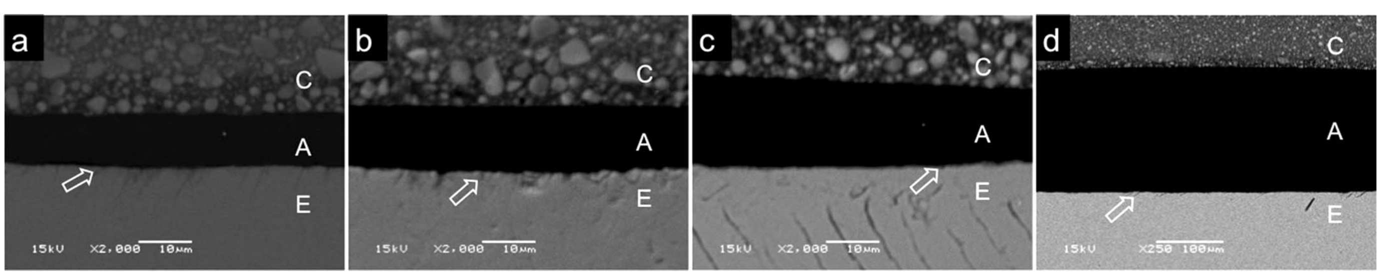

Figure 4. *Representative back-scattered SEM micrographs of nanoleakage at the adhesive/etched enamel interface in (a): ABU; (b): SBU; (c): CSEB; and (d): ASMP Bond after 10,000 thermocycles. Silver particles could not be visualized at the interface. Resin tags are indicated with the arrow. Abbreviations: C, composite resin; A, adhesive; D, dentin.*

Table 3: *Mean and Mean Ranks of Silver Penetration Values (%) at the Adhesive/Dentin Interface of Different Adhesive Systems*

Material	Dentin Substrate	
	After 10,000 Thermocycles	
	Mean	Mean Ranks
All-Bond Universal (ABU)	2.2 (1.1)	16.9 A
Scotchbond Universal (SBU)	4.4 (3.8)	18.5 A
Clearfil SE Bond (CSEB)	1.1 (1.0)	11.0 A

[a] *Data are presented as the mean (standard deviation) and the mean ranks. Identical small-cap letters in the column of mean ranks values indicate the absence of any statistically significant difference (Kruskal-Wallis test; significance at* $p<0.05$*).*

suggested that a more active application may be beneficial to increasing its bond strength to dentin.[7]

After one year of storage, a significant decrease in bond effectiveness to dentin substrate was observed for all the adhesives tested. Over time, storing the cut beams in water may have accelerated the degradation of the bonding resin or collagen fibrils,[1,28,29] especially for dentin specimens.[30] The presence of water also may have caused swelling and a reduction in the frictional forces between the polymer chains as well as hydrolysis of the filler-matrix interfaces, leading to a decrease in the mechanical properties of the resin.[31,32] Thus, after the long-term storage, mainly an increase of cohesive failure in composite resin and mixed failure of composite resin, adhesive, and dentin were observed for both SBU and CSEB. For ABU, a considerable number of failures between the adhesive and dentin were observed after 24 hours, and the numbers increased after one year of storage. It can be suggested that the growth of the initial defects at the adhesive/dentin interface resulted in the increase of this failure mode pattern in the long term. In addition, smear plugs could be observed in some MTBS-fractured surfaces of ABU. The limited penetration of monomers into the dentin may have weakened the bond performance of this adhesive.

Regardless of the adhesive used, some extent of silver deposits could be detected along the adhesive/dentin interface after thermal aging. In a recent study,[33] a similar leakage pattern was reported at the bottom of the hybrid layer for ABU and SBU after 10,000 thermocycles. Meanwhile, studies have shown that no or little silver particle was observed at the adhesive/dentin interface after 24 hours for ABU, SBU, and CSEB when applied in a self-etch approach to dentin.[6,8,24] The location of silver deposits may indicate areas of the hybrid layer where water remained after evaporating the solvents.[34] Besides MDP, all of the adhesives tested also contain hydroxyethyl methacrylate (HEMA) in their composition. Tay and others[35] have stated that when water is incompletely removed from the primed dentin, porous anionic hydrogels are formed through copolymerization with HEMA and acidic resin monomers. In addition, the presence of water may result in regions of incomplete polymerization in the resin matrix.[35] These regions may allow water permeation, accelerating water sorption, and extraction of unpolymerized or degraded monomers, affecting the durability of the bonding.[35] This might explain the continuous line of silver deposits on the hybrid layer observed in this study.

In contrast, no silver uptake was found for the adhesives tested on etched enamel substrate after 10,000 thermocycles. Resin tags could be observed at the adhesive/enamel interface for all the adhesives using SEM. This finding suggests that the penetration of resin monomers into the etched enamel surface may have encapsulated its crystallite components, providing an effective sealing ability and protecting the outermost enamel from dissolution.[36] There is still a lack of scientific data regarding the sealing performance of ABU, SBU, and ASMP Bond on etched enamel; however, the combined adhesive protocol of CSEB with prior etching of the enamel has been reported to produce scarce leakage in high C-factor cavities *in vitro*,[37] and this protocol has been applied clinically with success regarding marginal integrity and absence of discoloration.[38]

Phosphoric acid etching of enamel prior to adhesive application has been reported to increase its bond performance when compared with the self-etching approach alone.[9,39,40] It is assumed that the use of prior phosphoric acid etching promotes a deeper enamel demineralization, thus increasing the potential for chemical interaction and micro-mechanical interlocking.[41] In this study, CSEB showed significantly higher values in MTBS to etched enamel surfaces than did ABU and SBU after 24 hours, in agreement with findings from a recent study.[12] The CSEB primer penetration into the etched enamel, followed by the application of its bonding agent, may have contributed to the higher initial bond strength values when compared with ABU and SBU, which, as UAs, combine etchant, primer, and the bonding into one application.

On the other hand, after one year of storage, there was a significant decrease within the MTBS values of all materials for etched enamel compared with

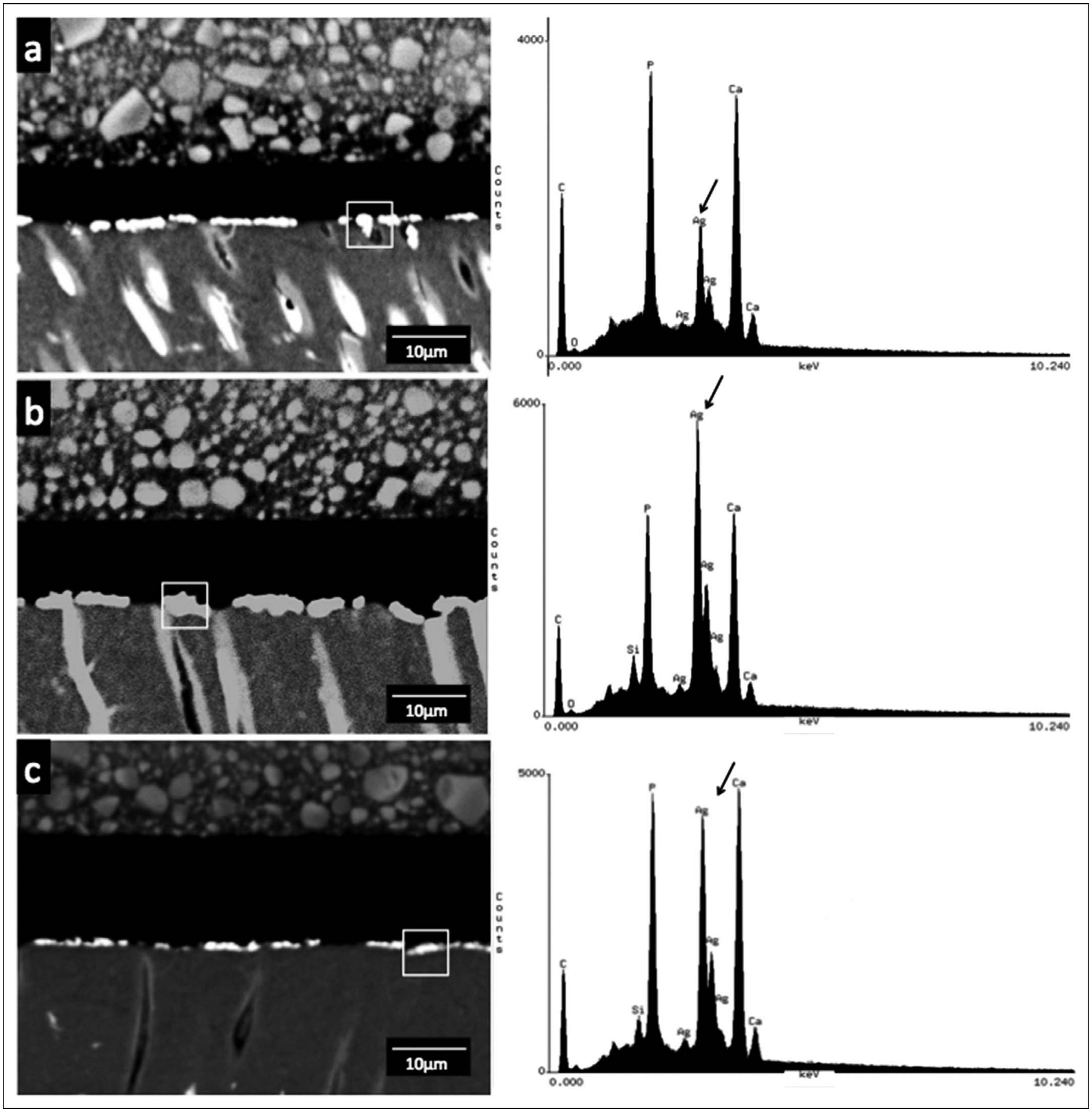

Figure 5. *Energy dispersive x-ray spectroscopy of the same specimens shown in Figure 3. (a): ABU; (b): SBU; and (c): CSEB. A distinct silver peak was observed on the elemental energy spectra (arrow). A map scan (white square) of the same specimen detected metallic silver particles.*

their baseline. It has been reported that the mixture of the hydrophilic monomers contained in the primer with the bonding agent may compromise the durability of the bond.[42-45] In addition, Miyazaki and others[45] observed a small, statistically insignificant decrease in the shear bond strength of self-priming adhesive systems to enamel after 30,000 thermocycles. Conversely, in the current study, storing the MTBS cut beams in water may be considered a form of accelerated aging.[1] Therefore, as with dentin, the collagen fibrils may have degraded and/or the mechanical properties of the resin may have decreased after one year when using etched enamel, regardless of the adhesive, because the incidence of cohesive failure in the composite resin increased slightly, as had the frequency of mixed failure in the composite resin, adhesive, and etched enamel.

ASMP Bond is a hydrophobic bonding resin that contains Bis-GMA and HEMA in its formulation. According to the manufacturer,[46] no primer is required when the preparation is in enamel substrate. Therefore, in the current study, the bonding agent was applied directly to a phosphoric acid-etched enamel surface, without the self-etching primer step. As Buonocore showed,[47] bonding to enamel only requires an acid-etch step followed by the application of an unfilled or low-filled hydrophobic resin on air-dried enamel, without the need for an intermediary primer step.[48-50] Because enamel contains very small amounts of water and organics, it has been suggested that the use of hydrophobic resin monomers alone may allow complete infiltration of bonding resin into the demineralized layer.[44,51] By omitting the self-etching primer step of the Adper Scotchbond Multi-Purpose Adhesive system, similar bond strength values were found for ASMP Bond, ABU, and SBU after 24 hours. Compared with the baseline, a significant decrease within MTBS values of ASMP Bond was observed after one year of storage. Although micromechanical interlocking might have contributed to the ASMP Bond results, the absence of MDP monomer as an additional chemical bonding may have accelerated the degradation process. In addition, the storage in water may have decreased the mechanical properties of the polymer matrix,[1] particularly in the thicker adhesive layer of ASMP Bond. It could be related to the increase in the mixed failure of composite resin, adhesive, and etched enamel observed for ASMP Bond after one year of storage.

It is interesting that no significant statistical difference in MTBS values was observed among ABU, SBU, CSEB, and ASMP Bond in etched enamel substrate after one year of storage. Indeed, it seems that the additional chemical bonding to etched enamel can be beneficial to provide bonding stability after long-term storage.[30,52] Takahashi[30] showed that the bond strength to enamel primed with experimental one-step MDP-containing adhesives remained unchanged after 30,000 thermocycles. Nevertheless, the micromechanical interlocking produced by phosphoric acid etching might be essential for effective bond to enamel.[36,53] This could explain the similar MTBS values and the good sealing ability obtained for the MDP-containing adhesives and ASMP Bond after aging.

Enamel is a highly mineralized substrate composed of more than 90 wt% of hydroxyapatite, whereas dentin is a more complex, humid, and porous substrate containing a significant amount of mineral within an organic matrix.[54] This heterogeneous structure and surface morphology likely make dentin less inclined to bond with dental adhesives. Conversely, studies have found that MTBS values of pre-etched enamel surfaces can be similar or even lower than those of dentin surfaces.[55-57] In addition, Sadek and others[55] observed structural defects more frequently on enamel than on dentin specimens before loading, even when lower cutting speeds were used during specimen preparation. It was suggested that cracks propagate more quickly through enamel due to the substrate's brittle and isotropic nature; consequently, the MTBS at the enamel/adhesive interface was lower. With regard to the results of the present study, significantly higher MTBS values were observed using SBU on dentin than on etched enamel substrate; this was true after both 24 hours and one year of storage. In addition, CSEB showed similar initial bond strength regardless of the substrate used, but significantly higher MTBS values were found using dentin after one year. However, using ABU, no significant difference occurred between the two substrates within the same storage period.

Using x-ray diffraction, Yoshihara and others[58] found that MDP can chemically interact with the calcium of hydroxyapatite from dentin and enamel; however, significantly greater chemical reactivity was observed for dentin than enamel. In that same study, the authors suggested that the crystal structure and/or size of hydroxyapatite causes it to be less receptive to chemical interaction within enamel than within dentin. This may partially explain why the bonding effectiveness is lower when using enamel than when using dentin. These findings corroborate the results of the current study when using SBU and CSEB. Although ABU also contains MDP, there was no significant statistical difference between the two substrates, after either storage period. It may be that a more active application of ABU in dentin and enamel substrates increases solvent evaporation, changing the polymer topology by reducing the intrinsic fraction of nanopores and consequently allowing an increase of polymer cross-linking and a degree of conversion inside the dental tissue.[7,59]

In clinical use, UAs can be considered a good alternative with regard to user friendliness and their applicability to different substrates: enamel, dentin, alloys, ceramics, and composites. In a 36-month follow-up clinical report, a UA seemed to maintain good performance when used with non-carious cervical lesions by applying either the etch-and-rinse (wet and dry) strategy to dentin or the self-etch strategy (with or without selective etching) to enamel. However, signs of bonding degradation such as marginal staining were reported when the UA was applied in a self-etch mode without selective enamel etching.[60] That said, applying UAs to dentin using the etch-and-rinse strategy is controversial.[8] Indeed, on the basis of our results, it may be that UAs applied to dentin using a self-etch approach with selective enamel etching is more favorable. Further investigations are required regarding the interaction of UAs with different structures, such as caries-affected dentin and sclerotic dentin because alterations in the mineral content and structure of dentin may compromise the bond,[61] as well as the clinical stability of this promising new category of adhesives.

Within the limitations of this study, the MTBS values revealed statistically significant differences among adhesives and/or storage time for dentin and etched enamel substrates. Therefore, the first null hypothesis has to be rejected. On the other hand, all adhesives showed similar nanoleakage formation along the adhesive/dentin interface, and no silver presence was detected in etched enamel substrate groups after the artificial aging. Thus, the second null hypothesis has to be accepted.

CONCLUSIONS

In the short term, the MTBS values were adhesive and substrate dependent. After aging, the bonding effectiveness of both UAs and the conventional adhesive systems were equal on etched enamel substrate, without leakage; however, UAs may have a bonding performance that is equal or inferior to that of the two-step self-etching adhesive when using dentin as a substrate, with similar amounts of silver deposits at their interfaces.

Acknowledgements

This research was supported by the Brazilian National Council for Scientific and Technological Development (CNPq No. 307217-2014-0). The funding body had no role in study design; collection, analysis, and interpretation of data; writing of the report; or in the decision to submit the article for publication.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Piracicaba Dental School, State University of Campinas. The approval code for this study is 143/2014.

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 10 September 2015)

References

1. 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.
2. Moszner N, Salz U, & Zimmermann J (2005) Chemical aspects of self-etching enamel-dentin adhesives: A systematic review *Dental Materials* **21(10)** 895-910.
3. 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.
4. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, & Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **83(6)** 454-458.
5. Peumans M, Munck J, Van Landuyt K, Lambrechts P, & Van Meerbeek B (2005) Three-year clinical effectiveness of a two-step self-etch adhesive in cervical lesions *European Journal of Oral Sciences* **113(6)** 512-518.
6. 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.
7. Munoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, Bombarda NH, & Perdigao J (2014) Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives *Journal of Dentistry* **42(5)** 595-602.
8. Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosa M, Cadenaro M, Di Lenarda R, 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.
9. Erickson RL, Barkmeier WW, & Kimmes NS (2009) Bond strength of self-etch adhesives to pre-etched enamel *Dental Materials* **25(10)** 1187-1194.
10. 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.

11. De Goes MF, Shinohara MS, & Freitas MS (2014) Performance of a new one-step multi-mode adhesive on etched vs non-etched enamel on bond strength and interfacial morphology *Journal of Adhesive Dentistry* **16(3)** 243-250.
12. McLean DE, Meyers E, Guillory V, & Vandewalle K (2015) Enamel bond strength of new universal adhesive bonding agents *Operative Dentistry* **40(4)** 410-417.
13. Rosa WL, Piva E, & Silva AF (2015) Bond strength of universal adhesives: A systematic review and meta-analysis *Journal of Dentistry* **43(7)** 765-776.
14. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, & Pashley DH (1995) Nanoleakage: Leakage within the hybrid layer *Operative Dentistry* **20(1)** 18-25.
15. Li H, Burrow MF, & Tyas MJ (2000) Nanoleakage patterns of four dentin bonding systems *Dental Materials* **16(1)** 48-56.
16. Hariri I, Shimada Y, Sadr A, Ichinose S, & Tagami J (2012) The effects of aging on shear bond strength and nanoleakage expression of an etch-and-rinse adhesive on human enamel and dentin *Journal of Adhesive Dentistry* **14(3)** 235-243.
17. Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, Carvalho R, & Pashley DH (1995) Comparative SEM and TEM observations of nanoleakage within hybrid layer *Operative Dentistry* **20(4)** 160-167.
18. Pioch T, Staehle HJ, Duschner H, & García-Godoy F (2001) Nanoleakage at the composite-dentin interface: A review *American Journal of Dentistry* **14(4)** 252-258.
19. Makishi P, Thitthaweerat S, Sadr A, Shimada Y, Martins AL, Tagami J, & Giannini M (2015) Assessment of current adhesives in class I cavity: Nondestructive imaging using optical coherence tomography and microtensile bond strength *Dental Materials* **31(9)** e190-e200.
20. Heintze SD, Rousson V, & Mahn E (2015) Bond strength tests of dental adhesive systems and their correlation with the clinical results—A meta-analysis *Dental Materials* **31(4)** 423-434.
21. Pashley DH, Sano H, Ciucchi B, Yoshiyama M, & Carvalho RM (1995) Adhesion testing of dentin bonding agents: A review *Dental Materials* **11(2)** 117-125.
22. Gale MS, & Darvell BW (1999) Thermal cycling procedures for laboratory testing of dental restorations *Journal of Dentistry* **27(2)** 89-99.
23. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, Van Meerbeek B, & Pashley DH (2004) *In vitro* effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM *Biomaterials* **25(25)** 5565-5574.
24. Yuan Y, Shimada Y, Ichinose S, & Tagami J (2007) Qualitative analysis of adhesive interface nanoleakage using FE-SEM/EDS *Dental Materials* **23(5)** 561-569.
25. Makishi P, Shimada Y, Sadr A, Wei S, Ichinose S, & Tagami J (2010) Nanoleakage expression and microshear bond strength in the resin cement/dentin interface *Journal of Adhesive Dentistry* **12(5)** 393-401.
26. 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.
27. Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, & Tay KC (2007) Bonding BisGMA to dentin—A proof of concept for hydrophobic dentin bonding *Journal of Dental Research* **86(11)** 1034-1039.
28. 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.
29. Reis AF, Giannini M, & Pereira PN (2008) Effects of a peripheral enamel bond on the long-term effectiveness of dentin bonding agents exposed to water *in vitro Journal of Biomedical Materials Research Part B: Applied Biomaterials* **85(1)** 10-17.
30. Takahashi H (2014) Effect of calcium salt of 10-methacryloyloxydecyl dihydrogen phosphate produced on the bond durability of one-step self-etch adhesive *Dental Materials Journal* **33(3)** 394-401.
31. Ferracane JL, Berge HX, & Condon JR (1998) *In vitro* aging of dental composites in water—Effect of degree of conversion, filler volume, and filler/matrix coupling *Journal of Biomedical Materials Research* **42(3)** 465-472.
32. Takeshige F, Kawakami Y, Hayashi M, & Ebisu S (2007) Fatigue behavior of resin composites in aqueous environments *Dental Materials* **23(7)** 893-899.
33. 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.
34. 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.
35. Tay FR, King NM, Chan KM, & Pashley DH (2002) How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *Journal of Adhesive Dentistry* **4(4)** 255-269.
36. Gwinnett AJ, & Matsui A (1967) A study of enamel adhesives. The physical relationship between enamel and adhesive *Archives of Oral Biology* **12(12)** 1615-1620.
37. Perdigao J, Monteiro P, & Gomes G (2009) *In vitro* enamel sealing of self-etch adhesives *Quintessence International* **40(3)** 225-233.
38. Peumans M, De Munck J, Van Landuyt K, & Van Meerbeek B (2015) Thirteen-year randomized controlled clinical trial of a two-step self-etch adhesive in non-carious cervical lesions *Dental Materials* **31(3)** 308-314.
39. Van Landuyt KL, Kanumilli P, De Munck J, Peumans M, Lambrechts P, & Van Meerbeek B (2006) Bond strength of a mild self-etch adhesive with and without prior acid-etching *Journal of Dentistry* **34(1)** 77-85.
40. Giannini M, Makishi P, Ayres AP, Vermelho PM, Fronza BM, Nikaido T, & Tagami J (2015) Self-etch adhesive systems: A literature review *Brazilian Dental Journal* **26(1)** 3-10.

41. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, & Van Landuyt KL (2011) State of the art of self-etch adhesives *Dental Materials* **27(1)** 17-28.

42. Sadr A, Shimada Y, & Tagami J (2007) Effects of solvent drying time on micro-shear bond strength and mechanical properties of two self-etching adhesive systems *Dental Materials* **23(9)** 1114-1119.

43. Reis AF, Carrilho MR, Ghaname E, Pereira PN, Giannini M, Nikaido T, & Tagami J (2010) Effects of water-storage on the physical and ultramorphological features of adhesives and primer/adhesive mixtures *Dental Materials Journal* **29(6)** 697-705.

44. Rathke A, Ostermeier V, Muche R, & Haller B (2013) Reconsidering the double etching of enamel: Do self-etching primers contaminate phosphoric acid-etched enamel? *Journal of Adhesive Dentistry* **15(2)** 107-114.

45. Miyazaki M, Sato M, & Onose H (2000) Durability of enamel bond strength of simplified bonding systems *Operative Dentistry* **25(2)** 75-80.

46. 3M ESPE Adper Scotchbond Multi-purpose Adhesive. Instruction sheet. Adper Scotchbond Multi-purpose Adhesive Instructions for Use. Retrieved online September 2, 2015 from: http://www.3m.com/3M/en_US/Dental/Products/Catalog/~/Adper-Scotchbond-Multi-Purpose-Adhesive?N=5144788+3294768970+3294857497&rt=rud

47. Buonocore MG (1955) A simple method of increasing the adhesion of acryl filling materials to enamel surfaces *Journal Dental Research* **34(6)** 849-853.

48. Buonocore MG, Matsui A, & Gwinnett AJ (1968) Penetration of resin dental materials into enamel surfaces with reference to bonding *Archives of Oral Biology* **13(1)** 61-70.

49. 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.

50. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P, & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **32(Supplement 6)** 119-144.

51. Li N, Nikaido T, Alireza S, Takagaki T, Chen JH, & Tagami J (2013) Phosphoric acid-etching promotes bond strength and formation of acid-base resistant zone on enamel *Operative Dentistry* **38(1)** 82-90.

52. Zhang Z, Wang X, Zhang L, Liang B, Tang T, Fu B, & Hannig M (2013) The contribution of chemical bonding to the short- and long-term enamel bond strengths *Dental Materials* **29(7)** e103-e112.

53. Erickson RL, Barkmeier WW, & Latta MA (2009) The role of etching in bonding to enamel: A comparison of self-etching and etch-and-rinse adhesive systems *Dental Materials* **25(11)** 1459-1467.

54. Teruel J de D, Alcolea A, Hernandez A, & Ruiz AJ (2015) Comparison of chemical composition of enamel and dentine in human, bovine, porcine, and ovine teeth *Archives of Oral Biology* **60(5)** 768-775.

55. Sadek FT, Cury AH, Monticelli F, Ferrari M, & Cardoso PE (2005) The influence of the cutting speed on bond strength and integrity of microtensile specimens *Dental Materials* **21(12)** 1144-1149.

56. Goracci C, Sadek FT, Monticelli F, Cardoso PE, & Ferrari M (2004) Influence of substrate, shape, and thickness on microtensile specimens' structural integrity and their measured bond strengths *Dental Materials* **20(7)** 643-654.

57. Cardoso PE, Sadek FT, Goracci C, & Ferrari M (2002) Adhesion testing with the microtensile method: Effects of dental substrate and adhesive system on bond strength measurements *Journal of Adhesive Dentistry* **4(4)** 291-297.

58. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, Van Landuyt KL, Osaka A, Suzuki K, Minagi S, & Van Meerbeek B (2011) Nanolayering of phosphoric acid ester monomer on enamel and dentin *Acta Biomaterialia* **7(8)** 3187-3195.

59. Loguercio AD, Munoz MA, Luque-Martinez I, Hass V, Reis A, & Perdigao J (2015) Does active application of universal adhesives to enamel in self-etch mode improve their performance? *Journal of Dentistry* **43(9)** 1060-1070.

60. 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.

61. Cardoso MV, de Almeida Neves A, Mine A, Coutinho E, Van Landuyt K, De Munck J, & Van Meerbeek B (2011) Current aspects on bonding effectiveness and stability in adhesive dentistry *Australian Dental Journal* **56(Supplement 1)** 31-44.