International Journal of Adhesion and Adhesives 88 (2019) 43–49

Contents lists available at ScienceDirect

International Journal of Adhesion and Adhesives

journal homepage: www.elsevier.com/locate/ijadhadh

The sonic application of universal adhesives in self-etch mode improves their performance on enamel

M.A. Muñoz[a], I. Luque-Martinez[a], V. Hass[b], M.F. Gutierrez[c], A. Reis[d], A.D. Loguercio[d,*]

[a] Centro de Investigación Interoperativo en Ciencias Odontológicas y Medicas, Faculty of Dentistry, Universidad de Valparaíso, Valparaíso, Chile
[b] Postgraduate Program in Dentistry, University of Northrn Paranara, Londrina, PR, Brazil
[c] Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Chile
[d] Department of Restorative Dentistry, State University of Ponta Grossa, Paraná, Brazil

ARTICLE INFO

Keywords:
Microshear bond strength
Degree of conversion
Enamel
Etch-and-rinse
Self-etch
Sonic application
Universal adhesive systems

ABSTRACT

The objective of this study was to evaluate the effect of sonic application of universal adhesives on the enamel microshear bond strengths (μSBS), *in situ* degree of conversion (DC) and etching pattern. Ninety-six extracted third molars were sectioned in four parts (buccal, lingual, proximal) and divided into 12 groups, according to the combination of 1) *adhesive system* (All-Bond Universal [ABU], G-Bond Plus [GBP], Prime&Bond Elect (PBE), and Scotchbond Universal Adhesive [SBU]), and 2) *adhesive application mode* (manual active etch-and-rinse [M-ER], manual active self-etch [M-SE], and sonic vibration self-etch [S-SE]). Specimens were stored in water at 37 °C during 24 h and tested at 1.0 mm/min (μSBS). DC was evaluated in the enamel-resin interfaces using micro-Raman spectroscopy. The enamel-etching pattern was evaluated under a field- emission scanning electron microscope. Data were analyzed with two-way ANOVA and Tukey's test ($\alpha = 0.05$). S-SE application increased μSBS and DC for all universal adhesives when compared to M-SE ($p < 0.05$). S-SE application resulted in mean bond strengths that were statistically similar to those obtained with the respective ER application mode ($p > 0.05$). A deeper enamel-etching pattern was observed for all universal adhesives in the etch-and-rinse strategy. An improvement in etching ability was observed in S-SE application compared to M-SE application. In light of the improved performance of universal adhesives when applied sonically in SE mode, selective enamel etching with phosphoric acid may not be crucial for their adhesion to enamel. The sonic application of universal adhesives in self-etch mode may be a practical alternative to enamel etching.

1. Introduction

The available adhesive systems presented in the dental market can be classified into two categories or bonding strategies: etch-and-rinse (ER) in versions of two or three application steps, and self-etch (SE), in versions of one or two application steps [1–3]. More versatile adhesive systems that can be used in both adhesive strategies in their simplest version (2-step ER or 1-step SE) were released by manufacturers so that clinicians can choose the adhesive strategy based on the dental substrate and their preference [4–7]. This new group of dental adhesives has been recently introduced in the market as “universal” or “multi-mode” adhesives and represents the latest generation of adhesives [6,8–12].

However recent studies observed a reduced enamel bonding effectiveness when the universal adhesives were applied on enamel as SE adhesives [5,6,8,10,13], in a similar way to what had been reported for the earlier 1-step SE adhesives [14–17]. In comparison with phosphoric acid, the self-etch and universal adhesives are less acidic, a characteristic that limits their demineralization ability in creating appropriate micro-retentive porosities. As a consequence, enamel bonding remains unsatisfactory [2,3,18].

This is not a new problem in a way that different and successful approaches were already tested in vitro to increase enamel bonding, such as prior phosphoric acid etching [4,5]. However, clinical studies that compared the application of universal adhesive as SE associated or not with selective enamel-etching did not shown any difference in the retention rates of composite resin restorations in cervical lesions [18–22], except reduced marginal discrepancies at the restoration interface [21,23]. Additionally, this adds an additional step to the adhesive procedure. Recently, some authors showed that the active application of universal adhesives in SE mode to enamel appears as a viable alternative to selective enamel etching in terms of enamel

* Correspondence to: Universidade Estadual de Ponta Grossa. Departamento de Odontologia, Avenida Carlos Cavalcanti, 4748 – Uvaranas, Ponta Grossa, Paraná CEP: 84030-900, Brazil.
E-mail address: alelog@uepg.br (A.D. Loguercio).

https://doi.org/10.1016/j.ijadhadh.2018.10.013
Accepted 17 October 2018
Available online 01 November 2018
0143-7496/

bonding efficacy [5], as shown for 1-step SE [24,25]. A clinical trial corroborates these findings by showing reduced marginal discoloration on the enamel margins and higher 2-year retention when of 1-step SE adhesives were applied actively in non-carious cervical lesions [26].

The active application is prone to force exerted by the operator, which may interfere with the efficacy of technique if not standardized. Different apparatus for adhesive application were studied [27–30] and they all share the ability to standardize the procedure. Some recent studies demonstrated that the use of a sonic device with an oscillating frequency of 170 Hz improved the resin-dentin bond strength of 1-step SE adhesives [31,32]. The high-speed vibration of the microbrush creates pressure waves and shear forces in the adhesive [32], which may lead to a higher penetration of the resin monomers into porosities created by the demineralization. To the best of our knowledge, no study has so far evaluated whether or not this technique improves the bond strength of commercially available universal adhesives to enamel.

Therefore, the aim of this study was to compare the resin-enamel microshear bond strength (μSBS), the *in situ* degree of conversion (DC), and the enamel-etching pattern of four universal adhesive systems when applied in the manual active ER mode, manual active SE mode and sonic SE mode.

2. Materials and methods

2.1. Tooth selection and preparation

A total of 96 extracted, caries-free, human third molars were used. An informed consent form was obtained from the participants that donate their teeth. Additionally, the study was approved by the local Ethics Committee Review Board. All teeth were disinfected in 0.5% chloramine, stored in distilled water at 4 °C and used within six months after extraction.

The roots of all teeth were removed. The dental crowns were then sectioned in a mesio-facial to disto-lingual direction and also in a disto-facial to mesio-lingual direction, to produce four enamel specimens (buccal, lingual, mesial and distal surfaces). Three hundred eighty-four enamel specimens, originated from 96 teeth, were ground wet with # 180 and 600-grit SiC paper for 60s. Sixty teeth were used for enamel microshear bond strength (μSBS), 24 teeth were used for measurement of the *in situ* degree of conversion (DC) in the enamel-resin interfaces and the remaining 12 teeth were used for evaluation of the enamel-etching pattern by field-emission scanning electron microscope (FESEM).

2.2. Experimental design

Twelve experimental conditions resulting from the combination of the two independent variables: 1) *adhesive system* (All-Bond Universal [ABU, Bisco Inc., Schaumburg, IL, USA], G-Bond Plus [GBP, GC Corporation Tokyo, Japan - also known as G-ænial Bond in some countries], Prime&Bond Elect [PBE, Dentsply Caulk, Milford, DE, USA], and Scotchbond Universal Adhesive [SBU, 3 M ESPE, St. Paul, MN, USA, also known as Single Bond Universal in some countries]; and 2) *adhesive application mode* (manual active etch-and-rinse mode [M-ER], manual active self-etch mode [M-SE], and sonic self-etch mode [S-SE]. The composition, application mode and batch number are described in Table 1.

Each enamel specimen originated from the same teeth was randomly assigned to one of the four adhesive systems by computer-generated tables. Then, within each adhesive, specimens were divided randomly using the same method into the different application modes. This process was applied for each method. In summary, we obtained 32 specimens per group. Twenty was used for μSBS test; eight was used for in situ DC measurement and four were used for evaluation of the enamel-etching pattern. A person not involved in the experimental tests did the randomization process.

2.3. Resin-enamel microshear bond strength (μSBS)

Enamel specimens were mounted individually on a polyvinyl chloride ring filled with acrylic resin (AutoClear, DentBras, Pirassununga, São Paulo, Brazil) displaying the enamel specimen surface on the top of the cylinder. The delimitation of the bonding area was performed according to Shimaoka et al. [33]. Using a Hygienic Ainsworth-style rubber-dam punch (Coltene, Alstätten, Switzerland) we performed six to eight perforations with an internal diameter of 0.8 mm in an acid-resistant, double-faced adhesive tape (Adelbras Ind. e Com. Adesivos Ltda, São Paulo, Brazil). This adhesive tape was then attached to the surface of the enamel specimens. The size of the enamel specimen determined the number of perforations.

The universal adhesive systems were then applied according to the respective manufacturer's instructions, except for the variation of the application in sonic vibration mode of the self-etch mode (Table 1). A single operator performed all bonding procedures according to the following description:

(1) Manual active etch-and-rinse mode (M-ER): the phosphoric acid gel was applied and left undisturbed for the time recommended for each manufacturer, following by water-rinsing with an air-water syringe for 10 s. Each adhesive was actively applied with a microbrush (Microbrush International, Grafton, WI, USA) on the enamel surface under manual pressure for the time recommended for each manufacturer (Table 1). We calibrated the operator to apply the adhesive in an analytical balance so that we could measure a mass value. In average, the mass measured was 35 g. This is equivalent of 0.345 N, which resulted in a pressure of 345 N/m^2 when applied with a 1-mm tip microbrush [34,35].
(2) Manual active self-etch mode (M-SE): each adhesive was actively applied on the enamel surface for the time recommended for each manufacturer (Table 1). The microbrush (Microbrush International, Grafton, WI, USA) was scrubbed on the enamel surface under manual pressure as reported above [34,35].
(3) Sonic self-etch mode (S-SE): The same microbrush was attached to the tip of a sonic applicator (released on the dental market as Smart by FGM Prod. Odontológicos, Joinville, SC, Brazil). The sonic device produces an oscillating vibration of 10,200 rpm or 170 Hz, measured by the Blackman-Harris sound method [36]. The sonic device has five different oscillating frequencies. The middle frequency (170 Hz) of the device was used [32,37]. It is important to report that the microbrush attached to the sonic device vibrates at the same oscillating frequency (170 Hz) of the device when in a non-contact condition. When the vibrating microbrush contacts the enamel surface, a reduction of this oscillating frequency may occur, which depends on the force exerted by the operator on the enamel surface. To avoid extreme variations, the operator only put the microbrush in contact with the enamel without much pressure [37].

After the application of the adhesive system, six to eight polyethylene transparent Tygon tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA), with an internal diameter of 0.8 mm and a height of 0.5 mm were positioned over the double-faced tape, with their lumen coinciding with the circular areas exposed by the perforations. Resin composite (Filtek Z350, 3M ESPE, St. Paul, MN, USA) was carefully packed inside each tube, and a clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place. The resin composite was light-cured for 20 s using a LED light-curing unit set at 1200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia). The operator checks the light intensity prior to each experiment, with a radiometer (Demetron L.E.D. Radiometer, Kerr Sybron Dental Specialties, Middleton, WI, USA). These procedures were carried out under magnifying loupes [38].

The specimens were stored in distilled water at 37 °C for 24 h and then, the Tygon tubes and the double-faced adhesive tape were

Table 1
Adhesive system (batch number), composition[a] and application mode of the adhesive systems according to the manufacturers' instructions[b].

Adhesive (batch number)	Composition[a]	Etch-and-rinse mode	Self-etch mode	Sonic vibration mode of self-etch
All-Bond Universal – ABU (1200006111)	1. Etchant: 32% phosphoric acid, benzalkonium chloride, xantham gum (Uni-Etch) 2. Adhesive: MDP, Bis-GMA, HEMA, ethanol, water, initiators	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Remove excess water with absorbent pellet or high volume suction for 1–2 s. 4. Apply adhesive as for the self-etch mode.	1. Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10–15 s per coat. Do not light cure between coats. 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance. 3. Light cure for 10 s.	1. Apply two separate coats of adhesive, with sonic vibration in the preparation with a microbrush attached to the sonic applicator, for 10–15 s per coat. Do not light cure between coats. 2. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance. 3. Light cure for 10 s.
G-Bond Plus - GBP (1102221)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide. (Scotchbond Universal Etchant) 2. Adhesive: Acetone, dimethacrylate, 4 methacryloxyethyltrimellitate anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator, distilled water.	1. Apply 34% phosphoric acid gel for 10 s. [c] 2. Rinse for 5 s and gently dry. 3. Apply adhesive as for the self-etch mode.	1. Apply using the disposable applicator. 2. Leave undisturbed for 10 s after the end of application. 3. Dry thoroughly for 5 s with oil free air under maximum air pressure. Use vacuum suction to prevent splatter of the adhesive. 4. Light-cure for 10 s at 1200 mW/cm^2.	1. Apply the adhesive with sonic vibration in the preparation, using the disposable applicator attached to the sonic applicator. 2. Leave undisturbed for 10 s after the end of application. 3. Dry thoroughly for 5 s with oil free air under maximum air pressure. Use vacuum suction to prevent splatter of the adhesive. 4. Light-cure for 10 s at 1200 mW/cm^2.
Prime&Bond Elect - PBE (1102221)	1. Etchant: 34% Tooth Conditioner Gel (34% phosphoric acid) 2. Adhesive: Mono-, di- and trimethacrylate resins; PENTA Diketone; Organic phosphine oxide; Stabilizers; Cetylamine hydrofluoride; Acetone; Water	1. Apply etchant for 15 s. 2. Rinse thoroughly for at least 15 s. 3. Dry by blowing gently with air syringe. 4. Apply adhesive as for the self-etch mode.	1. Apply generous amount of adhesive using microbrush. 2. Agitate for 20 s. 3. Gently dry with clean, dry air from a dental syringe forat least 5 s. Surface should have a uniform glossy appearance. 4. Light cure for 10 s.	1. Apply generous amount of adhesive with sonic vibration in the preparation, using microbrush attached to the sonic applicator 2. Agitate for 20 s. 3. Gently dry with clean, dry air from a dental syringe for at least 5 s. Surface should have a uniform glossy appearance. 4. Light cure for 10 s.
Scotchbond Universal Adhesive – SBU (D-82229)	1. Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide. (Scotchbond Universal Etchant) 2. Adhesive: MDP Phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply etchant for 15 s. 2. Rinse thoroughly with water. 3. Dry with water-free and oil-free air or with cotton pellets; do not overdry. 4. Apply adhesive as for the self-etch mode.	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 s.	1. Apply the adhesive to the entire preparation with a microbrush attached to the tip of a prototype sonic applicator, and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 s.

[a] MDP = methacryloyloxydecyl dihydrogen phosphate; Bis-GMA = bisphenol glycidyl methacrylate; HEMA = 2-hydroxyethyl methacrylate; PENTA = dipentaerythritol penta acrylate monophosphate.
[b] Except for the variation of the application in sonic vibration mode of self-etch
[c] In this case was used Scotchbond Universal Etchant.

carefully removed with a blade, exposing the composite cylinders. Each specimen was examined under a stereomicroscope at ×10 magnification. The bonded cylinder was discarded if there was evidence of porosities or gaps at the interface or pores in the composite resin.

The specimens were attached to a shear-testing fixture (Odeme Biotechnology, Joaçaba, SC, Brazil), and tested in a universal testing machine (Kratos IKCL 3-USB, Kratos Equipamentos Industriais Ltda, Cotia, São Paulo, Brazil). Then the set specimen-fixture was placed in the universal testing machine and a thin orthodontic wire (0.2 mm diameter) was looped around the base of each composite cylinder, contacting it in half of its circumference. The setup was kept aligned (resin-enamel interface, the wire loop and the center of the load cell) to ensure the correct orientation of the shear forces [39]. The crosshead speed was set at 1 mm/min until failure.

The μSBS values (MPa) were calculated by dividing the load at failure by the surface area (mm^2) to determine the shear bond strength. The failure mode was classified as cohesive ([C] failure exclusively within enamel or resin composite), adhesive ([A] failure at the enamel-resin interface), or mixed ([M] failure at the enamel-resin interface that included cohesive failure of the neighboring substrates). The failure mode analysis was performed under a scanning electron microscope (SEM) (SSX-550, Shimadzu, Tokyo, Japan).

2.4. In situ degree of conversion (DC)

The adhesives were applied on the enamel specimens and composite resin build-ups were constructed on the bonded enamel using the same materials and protocols described for the μSBS test. After specimen storage in distilled water for 24 h at 37 °C, the resin-enamel interfaces were longitudinally sectioned (1.5mm thick) across the bonded interface with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to obtain two slices of the enamel-resin interfaces.

The resin-enamel interfaces were wet polished with #1500; 2000 and 2500-grit SiC paper for 15 s each. Then, specimens were ultrasonically cleaned for 5 min in distilled water and then stored in water for 24 h at 37 °C prior to performing the DC readings. The DC measurements were performed in a micro-Raman spectrometer (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). The micro-Raman spectrometer was first calibrated for zero and then for coefficient values using a silicon specimen. Specimens were analyzed using the following Raman parameters: 20-mW Neon laser with 532-nm wavelength, spatial resolution of ≈ 3 μm, spectral resolution ≈ 5 cm^{-1}, accumulation time of 30 s with 6 co-additions, and magnification of x100 (Olympus UK, London, UK) to beam diameter of ≈ 1 μm [40–42].

The spectra were taken at the resin-enamel interface at three different sites for each specimen and also of the uncured adhesives for reference. Post-processing of spectra was performed using the dedicated Opus Spectroscopy Software version 6.5 (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). The ratio of double-bond content of monomer to polymer in the adhesive was calculated according to the following formula: DC (%) = (1 – R cured/R uncured) × 100, where R is the ratio of aliphatic and aromatic peak areas at 1639 cm^{-1} and 1609 cm^{-1} in cured and uncured adhesives.

2.5. Enamel-etching pattern

All adhesive in their specific application mode were applied to enamel according to the description done for the μSBS testing (Table 1) except for the fact that the adhesives were not light cured. The enamel surfaces were then immediately stirred in acetone for 24 h to remove the monomers from the enamel surface [43]. For the ER mode, before adhesive application, phosphoric acid gel was applied to enamel for 15 s, rinsed for 10 s and air-dried according to the description of Table 1.

All specimens were allowed to dry for 24 h under vacuum, then mounted on aluminum stubs with carbon tape, sputter-coated with gold-palladium and observed under FESEM (Hitachi S-4700, Hitachi High Technologies America, Inc., Pleasanton, CA, USA) at an accelerating voltage of 5.0 kV and a working distance of 12–13 mm.

2.6. Statistical analysis

The μSBS of all specimens with adhesive/mixed failure mode from the same enamel specimen were averaged for statistical purposes. Similarly, the same procedure was performed for the DC measurements, so that the experimental unit in this study was the enamel specimen. Specimens with cohesive and premature failures were not included in data analysis.

Data from μSBS and *in situ* DC were analyzed separately using two-way ANOVA (adhesive vs. adhesive strategy) and Tukey's post-hoc test at $\alpha = 0.05$. The enamel pattern etching was only evaluated qualitatively.

3. Results

3.1. Microshear bond strength

The majority of the specimens showed adhesive (51.8–73.2%) or adhesive/mixed (14.3–32.1%) failures (Table 2). For all adhesives tested, the S-SE application mode resulted in statistically significant higher mean μSBS when compared with the M-SE application ($p < 0.05$; Table 3). Each universal adhesive applied in the S-SE resulted in mean bond strengths that were statistically similar to those obtained with the respective M-ER application mode ($p > 0.05$; Table 3).

SBU showed higher bond strength values in the M-SE when compared with the other adhesives in the same strategy ($p < 0.05$; Table 3). No significant difference between adhesives were seen when they were applied in the M-ER approach or in the S-SE application mode ($p > 0.05$; Table 3).

3.2. Degree of conversion

For all adhesives tested, the change in the application mode from M-SE to S-SE application mode resulted in statistically higher DC ($p < 0.05$; Table 4). Each universal adhesive applied in the S-SE application mode resulted in means that were statistically similar to those obtained with the respective M-ER application mode ($p > 0.05$; Table 4). ABU showed higher DC results regardless the adhesive strategy ($p < 0.05$; Table 4). In general, GBP and PBE showed lower DC ($p < 0.05$; Table 4).

Table 2
Number of specimens and percentage of the total of specimens (%) according to fracture mode for all experimental groups[a].

Adhesive	Strategy	Fracture mode			
		A	C	A/M	PF
ABU	M-ER	35 (60.4%)	8 (13.8%)	13 (22.4%)	2 (3.4%)
	M-SE	41 (73.2%)	6 (10.7%)	8 (14.3%)	1 (1.8%)
	S-SE	39 (61.9%)	4 (6.3%)	17 (27.0%)	3 (4.8%)
GBP	M-ER	32 (58.2%)	7 (12.7%)	12 (21.8%)	4 (7.3%)
	M-SE	35 (67.3%)	5 (9.6%)	10 (19.3%)	2 (3.8%)
	S-SE	38 (66.7%)	3 (5.3%)	13 (22.8%)	3 (5.2%)
PBE	M-ER	29 (51.8%)	6 (10.7%)	18 (32.1%)	3 (5.4%)
	M-SE	33 (62.3%)	7 (13.1%)	11 (20.8%)	2 (3.8%)
	S-SE	37 (61.6%)	6 (10.0%)	16 (26.7%)	1 (1.7%)
SBU	M-ER	34 (58.6%)	8 (13.8%)	14 (24.1%)	2 (3.5%)
	M-SE	40 (72.7%)	4 (7.3%)	9 (16.4%)	2 (3.6%)
	S-SE	36 (61.0%)	5 (8.5%)	15 (25.4%)	3 (5.1%)

[a] A– adhesive fracture mode; C – cohesive fracture mode; A/M- adhesive/mixed fracture mode; PF – premature failure

Table 3
Means and standard deviations of the microshear bond strength (μSBS; MPa) obtained in each experimental group*.

Adhesive	Strategy		
	M-SE	S-SE	M-ER
ABU	13.6 ± 0.8 c	20.0 ± 1.1 a	19.7 ± 1.1 a
GBP	12.5 ± 1.3 c	18.8 ± 1.0 a	17.5 ± 1.5 a,b
PBE	13.6 ± 1.0 c	17.8 ± 1.4 a,b	21.4 ± 1.0 a
SBU	16.9 ± 1.3 b	20.3 ± 1.0 a	20.2 ± 1.0 a

* Means identified with the same letters are statistically similar (Tukey´s test, $p \geq 0.05$).

Table 4
Means and standard deviations of the *in situ* degree of conversion (DC; %) obtained in each experimental group*.

Adhesive	Strategy		
	M-SE	S-SE	M-ER
ABU	70.3 ± 3.8 c	80.3 ± 3.1 a,b	84.7 ± 4.3 a
GBP	55.5 ± 3.2 e	62.1 ± 3.2 d	66.8 ± 4.3 d
PBE	58.7 ± 3.7 e	67.1 ± 3.4 c,d	69.5 ± 3.9 c,d
SBU	63.5 ± 4.2 d	70.5 ± 3.8 c	75.3 ± 4.8 b,c

* Means identified with the same letters are statistically similar (Tukey´s test, $p \geq 0.05$).

3.3. Enamel etching pattern

M-SE application of universal adhesives showed a slight exposure of the periphery of the enamel prisms for ABU and SBU, with no signs of hydroxyapatite dissolution. For the PBE and GBP adhesives, M-SE application resulted in islands of superficially dissolved enamel within areas without evidence of enamel inter-prismatic dissolution (Fig. 1, SE row). The enamel etching pattern was slightly improved with S-SE application in all adhesives, showing a similar pattern to the observed with the M-ER strategy in the following adhesives GB, PBE and SBU (Fig. 1, S-SE row). The M-ER strategy resulted in the deepest and more pronounced etching pattern (Fig. 1, ER row).

4. Discussion

The findings of the manual active application of SE mode was expected as an earlier study [5] demonstrated that this mode of could increase the μSBS and DC values of universal adhesives in the SE mode when compared to passive application. However, the most interesting finding of the present investigation is that the application of universal adhesives in SE mode with a sonic device produced bond strength values similar to the observed with the ER strategy. This is the first study that shows that the performance of universal adhesives in enamel can be similar in both bonding strategies when applied with a sonic device in the SE mode.

By using sonic application in the SE mode, we can overcome the disadvantages of the SE protocol, which is the lower bonding efficacy to enamel [15,16]. In recent studies, it was observed that the etching ability of universal adhesives applied in the SE mode does not produce a retentive pattern on the enamel surface [13,44]. It is likely that the superficial interaction of SE solutions with enamel prevents the acidic monomers to demineralize the substrate to the extent to produce an efficient etching pattern for micromechanical interlocking.

A completely different pattern was observed when the universal adhesives were applied in the SE mode with a sonic device. The sonic vibration applied to the microbrush imparts energy to the adhesive solution that is applied on the enamel. Then, these agitated monomers are able to achieve areas beyond those where the bristles touch. Fresh monomers are taken more effectively and progressively to the sub-superficial enamel, producing a deeper demineralization. This is the exact mechanism of action that underlies sonic vibration of liquids and solutions. The high-speed vibration applied to the microbrush creates pressure waves and shear forces due to the stimulation of the solution molecules. It also generates microscopy bubbles that are forcefully propelled against surfaces to which the solution is applied.

Although the comparison of different adhesive systems is not a primary objective of this study, in the M-SE application mode, the mean bond strengths of SBU was higher than the others universal adhesives. The differences observed among them could be explained by their different chemical composition. Most universal adhesives available on the market contain at least one monomer with potential for chemical bonding. In SBU there are two components in the adhesive solution that can exert such activity. According to the manufacturer, SBU contain methacryloxydecyl phosphate (MDP) [45] and a methacrylate-modified polyalkenoic acid copolymer (Vitrebond copolymer, VCP) [46,47]. MDP can demineralize dental structures and produce the chemical interaction with hydroxyapatite by the formation of an acid–base resistant zone [48,49] that is more stable and durable over time [4,50]. Additionally, the polyalkenoic acid also have the potential to adhere chemically to hydroxyapatite, due to the abundance of polar carboxyl groups for bonding with minerals [47].

However, when we compare the bond strength values of the adhesives applied in the S-SE mode, no significant differences are detected. This could mean that apart from the deeper and more retentive etching pattern produced by the sonic vibration mode, this application technique could have also improved the chemical bonding of the functional monomers with the minerals of enamel.

Additionally, a higher degree of conversion was observed in the S-SE than in the M-SE application mode. The fluid movement of monomers caused by sonic mode may have led to their approximation and more chemical interactions among their molecules must have occurred. Additionally, the same fluid movements are likely responsible for higher solvent evaporation as they are brought to the adhesive surface, thus facilitating the evaporation of solvents outward [31,32,37]. This is especially important for adhesives composed of solvents with low vapor pressure, such as water (23.8 mmHg at 25 °C) and ethanol (54.1 mmHg at 25 °C) [51]. Solvent evaporation may give room for changes in polymer topology by reducing in the intrinsic fraction of nanopores, allowing increased polymer cross-linking, degree of conversion and other mechanical properties of the polymer inside the enamel hybrid layer [52–55].

Earlier studies reported that higher degree of conversion values were correlated with improved mechanical properties in polymeric materials [40,56,57]. This is the reason why a positive and significant correlation between bond strengths and degree of conversion was detected for earlier SE adhesives on enamel [58], which is in agreement with the findings of the present study for universal adhesives used on S-SE application mode.

When we compare the DC of the adhesives, we can observe that GBP and PBE showed trend towards lower DC regardless of the adhesive strategies. Differently from the other universal adhesives, GBP and PBE are HEMA-free adhesives. While HEMA-containing adhesives undergo more pronounced water sorption [59,60], they are highly prone to phase separation at the interface [61,62], which may have been the limiting factor for higher DC. An earlier study has demonstrated the presence of multiple droplets under SEM micrographs for these two adhesives (GBP and PBE), which is a clear evidence of this phenomenon [5].

From a clinical point of view, the use of a sonic device can be considered superior to manual active application, as it reduces the finger pressure variations of different operators and can guarantee homogeneous vibration of the adhesive. A notable advantage of sonic application over manual active application is that it does not require any calibration procedure, and it is probably less sensitive to operator

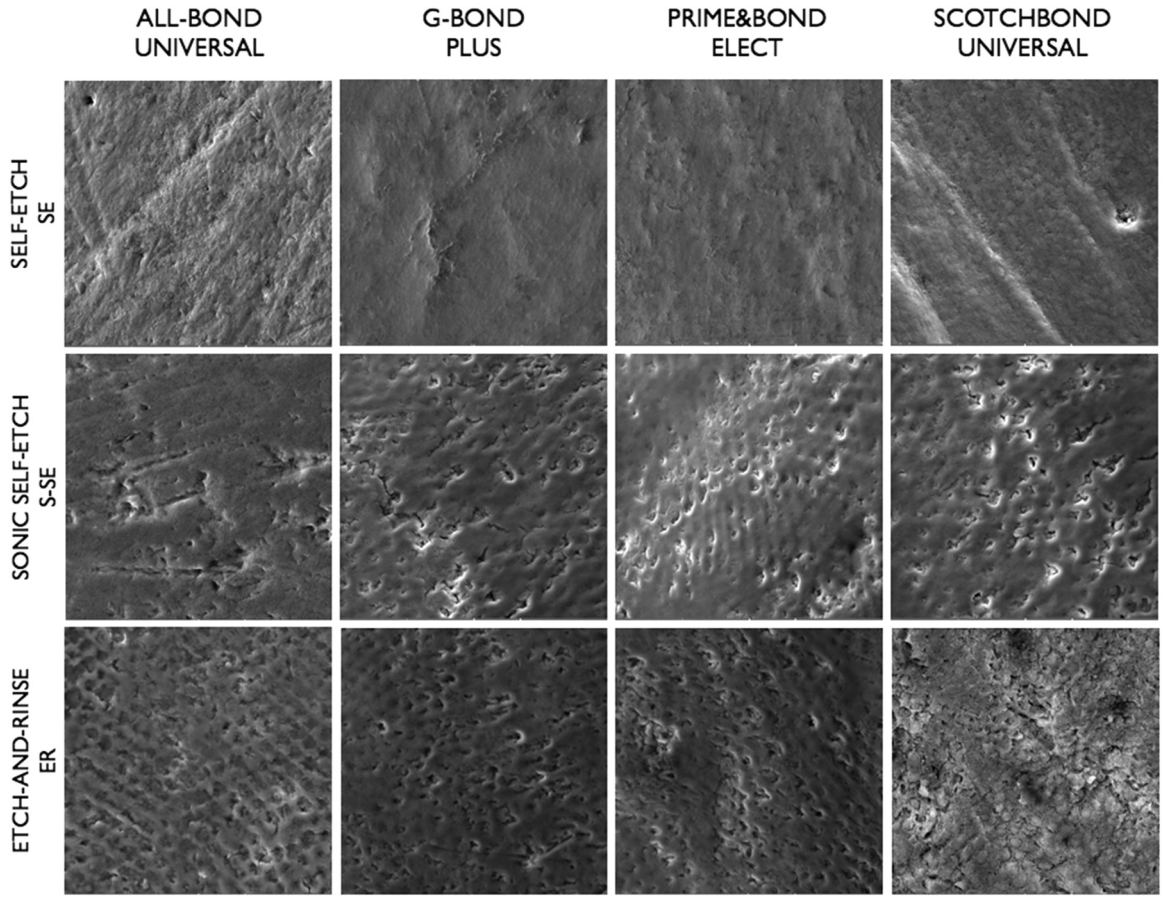

Fig. 1. Micrographs of enamel treated with the universal adhesives used in this study. Original magnification = 5000×. M-SE = manual active self-etch mode; S-SE = sonic self-etch mode; M- ER = manual active etch-and rinse mode.

experience. This clinical alternative is easy to implement and does not add more clinical steps to the bonding protocol [32]. In addition, the critical phosphoric acid etching is omitted, which makes the application procedure not only shorter but also easier [63]. Further clinical trials should be conducted to validate the results obtained with the sonic vibration self-etch application of universal adhesives in this laboratory study.

5. Conclusion

The sonic application of universal adhesives in self-etch mode to enamel increases the resin–enamel bond strength as well as the degree of conversion of the adhesive at the interface of all universal adhesives tested when compared to manual active self-etch application mode. The sonic application in universal adhesives in the self-etch mode is a viable alternative to selective enamel etching to improve enamel bonding.

Acknowledgements

This study was partially supported by in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and National Council for Scientific and Technological Development (CNPq) under grants 304104/2013-9 and 305588/2014-1.

References

[1] Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater 2005;21:864–81.
[2] Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1–16.
[3] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.
[4] McLean Meyers E, Guillory V, Vandewalle K. Enamel bond strength of new universal adhesive bonding agents. Oper Dent 2015;40:410–7.
[5] Loguercio AD, Munoz MA, Luque-Martinez I, Hass V, Reis A, Perdigao J. Does active application of universal adhesives to enamel in self-etch mode improve their performance? J Dent 2015;43:1060–70.
[6] Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, De Munck J. Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. J Dent 2012;40:475–84.
[7] Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH, Tay FR. Bonding of universal adhesives to dentine–Old wine in new bottles? J Dent 2015;43:525–36.
[8] Perdigão J, Loguercio A. Universal or multi-mode adhesives: why and how? J Adhes Dent 2014;16:193–4.
[9] Wagner A, Wendler M, Petschelt A, Belli R, Lohbauer U. Bonding performance of universal adhesives in different etching modes. J Dent 2014;42:800–7.
[10] de Goes MF, Shinohara MS, Freitas MS. Performance of a new one-step multi-mode adhesive on etched vs non-etched enamel on bond strength and interfacial morphology. J Adhes Dent 2014;16:243–50.
[11] Munoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, Bombarda NH, Perdigao J. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. J Dent 2014;42:595–602.
[12] Perdigao J, Sezinando A, Monteiro PC. Laboratory bonding ability of a multi-purpose dentin adhesive. Am J Dent 2012;25:153–8.
[13] Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: a systematic review and meta-analysis. J Dent 2015;43:765–76.
[14] Knobloch LA, Gailey D, Azer S, Johnston WM, Clelland N, Kerby RE. Bond strengths of one- and two-step self-etch adhesive systems. J Prosthet Dent 2007;97:216–22.
[15] Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives - Part II: etching effects on unground enamel. Dent Mater 2001;17:430–44.
[16] Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent 1999;27:523–30.
[17] Beloica M, Goracci C, Carvalho CAR, Radovic I, Margvelashvili M, Vulicevic ZR, Ferrari M. Microtensile vs microshear bond strength of all-in-one adhesives to unground enamel. J Adhes Dent 2010;12:427–33.
[18] Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, Perdigao J. A new universal simplified adhesive: 6-month clinical evaluation. J Esthet Restor Dent

2013;25:55–69.
[19] Perdigao J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, Loguercio AD. A new universal simplified adhesive: 18-month clinical evaluation. Oper Dent 2014;39:113–27.
[20] Lopes LS, Calazans FS, Hidalgo R, Buitrago LL, Gutierrez F, Reis A, Loguercio AD, Barceleiro MO. Six-month follow-up of cervical composite restorations placed with a new universal adhesive system: a randomized clinical trial. Oper Dent 2016;41:465–80.
[21] Loguercio AD, de Paula EA, Hass V, Luque-Martinez I, Reis A, Perdigao J. A new universal simplified adhesive: 36-month randomized double-blind clinical trial. J Dent 2015;43:1083–92.
[22] Lawson NC, Robles A, Fu CC, Lin CP, Sawlani K, Burgess JO. Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions. J Dent 2015;43:1229–34.
[23] Perdigao J, Swift Jr. EJ. Universal adhesives. J Esthet Restor Dent 2015;27:331–4.
[24] Ando S, Watanabe T, Tsubota K, Yoshida T, Irokawa A, Takamizawa T, Kurokawa H, Miyazaki M. Effect of adhesive application methods on bond strength to bovine enamel. J Oral Sci 2008;50:181–6.
[25] Torres CRG, Barcellos DC, Pucci CR, Lima GDG, Rodrigues CM, Siviero M. Influence of methods of application of self-etching adhesive systems on adhesive bond strength to enamel. J Adhes Dent 2009;11:279–86.
[26] Zander-Grande C, Amaral RC, Loguercio AD, Barroso LP, Reis A. Clinical performance of one-step self-etch adhesives applied actively in cervical lesions: 24-month clinical trial. Oper Dent 2014;39:228–38.
[27] Bagis B, Turkarslan S, Tezvergil-Mutluay A, Uctasli S, Vallittu PK, Lassila LVJ. Effect of ultrasonic agitation on bond strength of self-etching adhesives to dentin. J Adhes Dent 2008;10:441–5.
[28] Bagis B, Turkaslan S, Vallittu PK, Lassila LVJ. Effect of high frequency ultrasonic agitation on the bond strength of self-etching adhesives. J Adhes Dent 2009;11:369–74.
[29] Breschi L, Mazzoni A, Pashley DH, Pasquantonio G, Ruggeri A, Suppa P, Mazzotti G, Di Lenarda R, Tay FR. Electric-current-assisted application of self-etch adhesives to dentin. J Dent Res 2006;85:1092–6.
[30] Visintini E, Mazzoni A, Vita F, Pasquantonio G, Cadenaro M, Di Lenarda R, Breschi L. Effects of thermocycling and use of ElectroBond on microtensile strength and nanoleakage using commercial one-step self-etch adhesives. Eur J Oral Sci 2008;116:564–70.
[31] Mena-Serrano A, da Costa TRF, Patzlaff RT, Loguercio AD, Reis A. Effect of sonic application mode on the resin-dentin bond strength and dentin permeability of self-etching systems. J Adhes Dent 2014;16:435–40.
[32] Mena-Serrano A, Garcia EJ, Loguercio AD, Reis A. Effect of sonic application mode on the resin-dentin bond strength and nanoleakage of simplified self-etch adhesive. Clin Oral Investig 2014;18:729–36.
[33] Shimaoka AM, de Andrade AP, Cardoso MV, de Carvalho RC. The importance of adhesive area delimitation in a microshear bond strength experimental design. J Adhes Dent 2011;13:307–14.
[34] Loguercio AD, Stanislawczuk R, Mena-Serrano A, Reis A. Effect of 3-year water storage on the performance of one-step self-etch adhesives applied actively on dentine. J Dent 2011;39:578–87.
[35] Reis A, Pellizzaro A, Dal-Bianco K, Gones OM, Patzlaff R, Loguercio AD. Impact of adhesive application to wet and dry dentin on long-term resin-dentin bond strengths. Oper Dent 2007;32:380–7.
[36] Harris F. On the use of Windows for harmonic analysis with the discrete Fourier transform. Proc IEEE 1978;66:51–83.
[37] Cuadros-Sanchez J, Szesz A, Hass V, Patzlaff RT, Reis A, Loguercio AD. Effects of sonic application of adhesive systems on bonding fiber posts to root canals. J Endod 2014;40:1201–5.
[38] Munoz MA, Baggio R, Emilio Mendes YB, Gomes GM, Luque-Martinez I, Loguercio AD, Reis A. The effect of the loading method and cross-head speed on resin-dentin microshear bond strength. Int J Adhes Adhes 2014;50:136–41.
[39] Shimada Y, Yamaguchi S, Tagami J. Micro-shear bond strength of dual-cured resin cement to glass ceramics. Dent Mater 2002;18:380–8.
[40] Hass V, Dobrovolski M, Zander-Grande C, Martins GC, Gordillo LA, Rodrigues Accorinte Mde L, Gomes OM, Loguercio AD, Reis A. Correlation between degree of conversion, resin- dentin bond strength and nanoleakage of simplified etch-and-rinse adhesives. Dent Mater 2013;29:921–8.
[41] Perdigão J, Munoz MA, Sezinando A, Luque-Martinez IV, Staichak R, Reis A, Loguercio AD. Immediate adhesive properties to dentin and enamel of a universal adhesive associated with a hydrophobic resin coat. Oper Dent 2014;39:489–99.
[42] Hass V, Luque-Martinez I, Sabino NB, Loguercio AD, Reis A. Prolonged exposure times of one-step self-etch adhesives on adhesive properties and durability of dentine bonds. J Dent 2012;40:1090–102.
[43] Perdigão J, Lopes MM, Gomes G. In vitro bonding performance of self-etch adhesives: ii– ultramorphological evaluation. Oper Dent 2008;33:534–49.
[44] Tsujimoto A, Barkmeier WW, Takamizawa T, Watanabe H, Johnson WW, Latta MA, Miyazaki M. Influence of duration of phosphoric acid pre-etching on bond durability of universal adhesives and surface free-energy characteristics of enamel. Eur J Oral Sci 2016;124:377–86.
[45] Fukegawa D, Hayakawa S, Yoshida Y, Suzuki K, Osaka A, Van Meerbeek B. Chemical interaction of phosphoric acid ester with hydroxyapatite. J Dent Res 2006;85:941–4.
[46] Perdigao J, Sezinando A, Monteiro PC. Effect of substrate age and adhesive composition on dentin bonding. Oper Dent 2013;38:267–74.
[47] Sezinando A, Serrano ML, Perez VM, Munoz RA, Ceballos L, Perdigao J. Chemical adhesion of polyalkenoate-based adhesives to hydroxyapatite. J Adhes Dent 2016;18:257–65.
[48] Li N, Nikaido T, Takagaki T, Sadr A, Makishi P, Chen J, Tagami J. The role of functional monomers in bonding to enamel: acid-base resistant zone and bonding performance. J Dent 2010;38:722–30.
[49] Nikaido T, Ichikawa C, Li N, Takagaki T, Sadr A, Yoshida Y, Suzuki K, Tagami J. Effect of functional monomers in all-in-one adhesive systems on formation of enamel/dentin acid-base resistant zone. Dent Mater J 2011;30:576–82.
[50] Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, Meerbeek BV. Self-assembled nano-layering at the adhesive interface. J Dent Res 2012;91:376–81.
[51] Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757–85.
[52] Ikeda T, De Munck J, Shirai K, Hikita K, Inoue S, Sano H, Lambrechts P, Van Meerbeek B. Effect of evaporation of primer components on ultimate tensile strengths of primer-adhesive mixture. Dent Mater 2005;21:1051–8.
[53] Paul SJ, Leach M, Rueggeberg FA, Pashley DH. Effect of water content on the physical properties of model dentine primer and bonding resins. J Dent 1999;27:209–14.
[54] Cadenaro M, Breschi L, Rueggeberg FA, Suchko M, Grodin E, Agee K, Di Lenarda R, Tay FR, Pashley DH. Effects of residual ethanol on the rate and degree of conversion of five experimental resins. Dent Mater 2009;25:621–8.
[55] Reis A, Ferreira SQ, Costa TR, Klein-Junior CA, Meier MM, Loguercio AD. Effects of increased exposure times of simplified etch-and-rinse adhesives on the degradation of resin- dentin bonds and quality of the polymer network. Eur J Oral Sci 2010;118:502–9.
[56] Ferracane JL, Greener EH. The effect of resin formulation on the degree of conversion and mechanical properties of dental restorative resins. J Biomed Mater Res 1986;20:121–31.
[57] Hinoura K, Miyazaki M, Onose H. Effect of irradiation time to light-cured resin composite on dentin bond strength. Am J Dent 1991;4:273–6.
[58] Kanehira M, Finger WJ, Hoffmann M, Endo T, Komatsu M. Relationship between degree of polymerization and enamel bonding strength with self-etching adhesives. J Adhes Dent 2006;8:211–6.
[59] Hosaka K, Nakajima M, Takahashi M, Itoh S, Ikeda M, Tagami J, Pashley DH. Relationship between mechanical properties of one-step self-etch adhesives and water sorption. Dent Mater 2010;26:360–7.
[60] Takahashi M, Nakajima M, Hosaka K, Ikeda M, Foxton RM, Tagami J. Long-term evaluation of water sorption and ultimate tensile strength of HEMA-containing/-free one-step self-etch adhesives. J Dent 2011;39:506–12.
[61] Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005;84:183–8.
[62] Van Landuyt KL, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA in one-step self-etch adhesives. Dent Mater 2008;24:1412–9.
[63] Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P, Van Meerbeek B. Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives. J Adhes Dent 2009;11:175–90.