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Effect of Different Protocols in Preconditioning With EDTA in Sclerotic Dentin and Enamel Before Universal Adhesives Applied in Self-etch Mode

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Clinical Relevance

A shorter ethylene diamine tetra-acetic acid (EDTA) application time of 30 seconds with a sonic device produced a higher bond strength when compared with the conventional two-minute EDTA application in sclerotic dentin.

SUMMARY

Objectives: The aim of this study was to investigate the effect of different protocols of 17% ethylene diamine tetra-acetic acid (EDTA) conditioning on the etching pattern and immediate bond strength of universal adhesives to enamel and sclerotic dentin.

Methods and Materials: Forty bovine teeth with sclerotic dentin and 20 human third molars were randomly divided into eight groups resulting from the combination of the main factors surface treatment (none, two-minute EDTA conditioning manual application, 30-second EDTA manual application, 30-second EDTA sonic application) and adhesives systems (Scotchbond Universal Adhesive [SBU] and Prime & Bond Elect [PBE]). Resin-dentin and enamel-dentin bond specimens were prepared and tested under the micro-

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tensile bond strength (μTBS) and microshear bond strength (μSBS) tests, respectively. The etching pattern produced on the unground enamel and the sclerotic dentin surfaces under the different protocols and adhesive systems was evaluated under scanning electron microscopy.

Results: For enamel, only the main factor adhesive was significant ($p<0.0001$), with SBU showing the highest μSBS. In sclerotic dentin, the lowest mean was observed for the group without EDTA application and the highest mean in the group with EDTA application with the sonic device for 30 seconds. Regardless of the EDTA protocol, the highest means of μTBS were observed for SBU ($p<0.05$).

Conclusions: EDTA conditioning improves the bonding performance of universal adhesives in the self-etch mode on sclerotic dentin, mainly when applied for 30 seconds with the aid of a sonic device. EDTA pretreatment also improves the retentive etching pattern of enamel, but it does not result in higher enamel bond strength.

INTRODUCTION

The primary aim of dentin bonding systems is to provide retention of restorative materials to the dental structure as well as to seal the dentin substrate. Fortunately, the immediate bonding effectiveness of most current adhesive systems is quite favorable,[1] but their effectiveness is often evaluated based on their ability to bond to sound dentin.

While sound dentin is frequently encountered in dental practice, a variety of other pathologically altered dentin substrates to include caries-affected and sclerotic dentin are also encountered.[2-5] Irrespective of the bonding strategy used, bonding to sclerotic dentin presents a challenge, which often results in diminished bond strengths.[2-6] This is due to partial or total obliteration of dentinal tubules with mineral crystals and to the presence of an acid-resistant hypermineralized layer.[5,7]

In fact, previous studies suggested that bonding to human sclerotic dentin could be improved by changing the adhesive protocol that is normally employed for sound dentin. Several methods have been suggested to include phosphoric acid pretreatment when using self-etch systems,[8,9] roughening of the sclerotic dentin surface with diamond burs,[6,8,10,11] increased application times of adhesive systems,[9] and preconditioning dentin with weak acids such as ethylene diamine tetra-acetic acid (EDTA).[11-13]

Among these strategies, preconditioning with EDTA seems a very promising approach. EDTA acts as a chelator and produces a shallow demineralization of the dentin,[14,15] which is likely responsible for the high immediate bond strength of self-etch adhesives to sound and sclerotic dentin.[8,14] In addition, EDTA conditioning also has an inhibitory effect on the matrix-bound endogenous metalloproteinases (MMPs) of demineralized dentin,[16,17] producing more stable adhesive interfaces in sound[14] and sclerotic[13] dentin. Furthermore, previous EDTA application can increase the bond strength of self-etch adhesives to enamel,[18,19] which solves one of the main drawbacks of self-etch adhesives.[20,21]

While the use of EDTA pretreatment has shown promise, it does involve an extra step and up to two minutes extra procedural time; one of the touted benefits of the current simplified adhesive systems is time saving.[13,16,22] Shorter EDTA application times and/or "active" EDTA application may allow the benefits of EDTA use with reduced time requirements. Previous studies have reported that agitation of EDTA in root canal systems provides more effective removal of smear layer and debris.[23-25]

Sonic and ultrasonic application of adhesives may improve the longevity of bonds to tooth structure without greatly increasing time requirements for bonding. This technology has existed since 1950 and is used in periodontics and endodontics.[26] The technique can be used in the application of dental adhesive systems with potential benefit of improved bond strengths.[27] The vibration at high speed favors the fluid dynamics in the substrate as well as eliminates bubbles and solvent evaporation.[28]

To bring the benefits of prior application of EDTA to the most difficult-to-treat substrates—such as sclerotic dentin and enamel—the use of a prototype sonic device (SMART, FGM Dental Products, Joinville, SC, Brazil) may be beneficial in the application of EDTA to sclerotic dentin and enamel prior to the use of self-etch adhesives (compared with manual application).

The aim of this study was to investigate the effect of different preconditioning protocols with EDTA on the bond strength and etching pattern prior to application of universal adhesive systems to enamel and sclerotic dentin. This study tested two null hypotheses: 1) different preconditioning protocols with EDTA do not affect the bonding effectiveness of adhesives to sclerotic dentin and b) different precon-

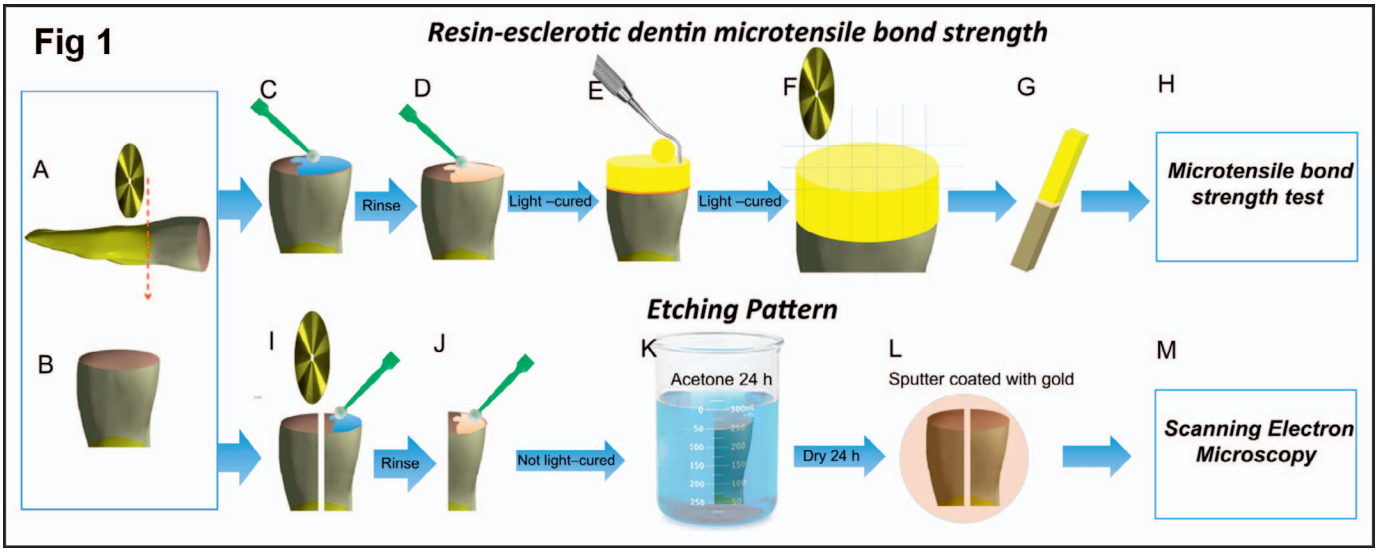

Figure 1. *Schematic drawing showing specimen preparation and microtensile bond strength testing. (A): The roots of all bovine teeth were removed by sectioning at the cementum-enamel junction. (B): Sclerotic tooth surface ready to receive the adhesive protocols. (C): Ethylene diamine tetra-acetic acid (EDTA) application according to each group. (D): Application of the universal adhesive systems (Scotchbond Universal Adhesive or Prime & Bond Elect) according to guidelines of the manufacturers. (E): Composite resin crowns were constructed. (F): Specimens were cut perpendicularly with a low-speed diamond saw to obtain resin-dentin specimens (G) for microtensile testing (H). (I): For the etching pattern, the teeth were cut in half; in one half, the adhesive was applied, and in the other half, the sclerotic dentin surface was left intact. (J): In the halves in which the adhesive protocol was performed, the groups received the application of EDTA; the adhesive application was per the manufacturer's instructions, except that the adhesive was not light cured. (K): Specimens were stored in an acetone bath for 24 hours (K), to remove all the resin monomers from the surface. (L): Samples were dehydrated for 24 hours, sputter coated with gold, and examined by scanning electron microscopy (M).*

ditioning protocols with EDTA do not affect the bonding effectiveness of adhesives to enamel.

METHODS AND MATERIALS

Resin-Dentin and Resin-Enamel Bond Strength

Sclerotic Dentin—Forty extracted bovine incisors with exposed sclerotic dentin in the incisal area due to abrasion/erosion and with an extremely vitreous appearance[12] were used in this part of the study. Bovine substrate was used instead of human teeth, as it is easy to obtain and has been considered a reliable substitute for human teeth.[29]

The teeth were extracted from the mandibles of three-year-old animals that had been slaughtered on a commercial scale for meat consumption. After harvesting, they were stored in distilled water at 4°C for no longer than one week before being used in this experiment. The roots of all teeth were sectioned, and the coronal pulp was removed (Figure 1).

The teeth were then embedded in chemically activated resin, with the exposed dentin surfaces parallel to the horizontal plane (Figure 1). The teeth were randomly assigned into eight experimental groups (n=5) according to the combination of the main factors "adhesive system" (two levels) and "surface treatment" (four levels), using a table of random numbers. A person not involved in the research protocol performed the randomization procedure using computer-generated tables.

In the control group, adhesives were applied as per the manufacturers' instructions (Table 1). In the group EDTA 30 seconds, a solution of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) was applied manually with a microbrush actively for 30 seconds. In the group EDTA two minutes, the same solution was applied manually on the sclerotic dentin surfaces with a microbrush actively for two minutes. In the group EDTA 30 seconds + sonic, the 17% EDTA solution was applied as in the earlier group, but the solution was agitated with a sonic device. For this purpose, the microbrush was first attached to the prototype of the Smart sonic device (FGM Dental Products, Joinville, SC, Brazil).

The prototype produced an oscillating vibration of 10,200 rpm or 170 Hz as measured by the Blackman-Harris sound method.[27] The Smart device (FGM) has five different oscillating frequencies (144.5, 150, 170, 223.5, and 267.5 Hz). This study employed the middle frequency of the device. After application of the EDTA in the experimental groups, the sclerotic dentin surfaces were copiously rinsed with water for

Table 1: *Adhesive Systems, Batch Number, Composition, and Mode of Application*

Adhesive (Batch Number)	Composition	Self-etch Strategy
Scotchbond Universal Adhesive (SBU) (130811)	1.Adhesive: MDP phosphate monomer, dimetacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the entire preparation with a microbrush and rub it actively for 20 s
		2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely[a]
		3. Light cure for 10 s at 1200 mW/cm^{2}[b]
Prime & Bond Elect (PBE) (523652)	1. Adhesive: Mono-, di-, and trimetacrylate resins; PENTA diketone; organic phosphine oxide; stabilizers; cetylaminehydrofluoride; acetone; water	1. Apply generous amount of adhesive to thoroughly wet all tooth surfaces
		2. Agitate for 20 s
		3. Gently dry with clean air for at least 5 s; surfaces should have a uniform, glossy appearance[a]
		4. Light cure for 10 s at 1200 mW/cm^{2}[b]

Abbreviations: MDP, methacryloyloxydecyldihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; PENTA, dipentaerythritolpenta acrylate monophosphate.
[a] *As per the manufacturers' instructions; as reported by manufacturers' material safety data issue or technical profile.*
[b] *The intensity of light curing was standardized for all materials.*

two minutes, and the surfaces were only slightly dried with an air stream without dehydration.

After that, the adhesive systems Scotchbond Universal Adhesive ([SBU] 3M ESPE, St Paul, MN, USA) and Prime & Bond Elect ([PBE] Dentsply, Milford, DE, USA) were applied by a single and calibrated operator according to the manufacturers' instructions (Table 1). After the bonding procedure, all teeth received a microhybrid composite restoration (Opallis, FGM Dental Products, Joinville, SC, Brazil) in three increments of 1 mm. Each increment was light cured for 40 seconds using a light-curing unit set at 1200 mW/cm^{2} (Radii Cal, SDI Limited, Bayswater, Victoria, Australia). The specimens were stored in water at 37°C for 24 hours.

To perform the microtensile bond strength test (μTBS), the specimens were cut perpendicularly with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) to obtain resin-dentin specimens (0.8 mm^{2} cross-sectional dimensions on average) from each tooth for microtensile testing (Figure 1). The bonded area was measured to the nearest 0.01 mm with a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). If premature debonding occurred during sectioning, the number of specimens was recorded. Specimens were attached to a Geraldelli jig[30] with cyanoacrylate adhesive and stressed under tension (Kratos Dinamometros, Cotia, SP, Brazil) at 0.5 mm/min until failure. Bond strengths were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode of the specimens was classified as adhesive/mixed if failure occurred at the resin-dentin bond interface with or without cohesive failure of the neighboring substrates and as cohesive if the failure occurred at the substrate (resin or dentin). The classification was done under a stereomicroscope at 40× magnification (Olympus SZ40, Tokyo, Japan).

Enamel Substrate—Twenty extracted and caries-free human third molars were used in this part of the study. The teeth were collected after obtaining the patients' informed consent under a protocol approved by the Ethics Committee Review Board of the local university. The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within six months after extraction.

The roots of all teeth were removed by sectioning at the cementum-enamel junction. The dental crowns were then sectioned in the diagonals across the long axis of teeth to produce four enamel specimens (buccal, lingual, and proximal; Figure 2).[31] Eighty enamel specimens, originating from 20 teeth, were ground wet with No. 180 and 600-grit SiC paper for 60 seconds, and each surface was mounted in a polyvinyl chloride ring filled with acrylic resin (AutoClear, Dent Bras, Pirassununga, SP, Brazil), showing the buccal, lingual, and proximal enamel surface on the top of the cylinder. Enamel surfaces were ground flat for development of uniform tensile forces during microshear loading.

For the microshear bond strength (μSBS), the delimitation of the bonding area was performed according to Shimaoka.[32] Four to six perforations with an internal diameter of 0.8 mm were made in an acid-resistant double-faced adhesive tape (Adel-

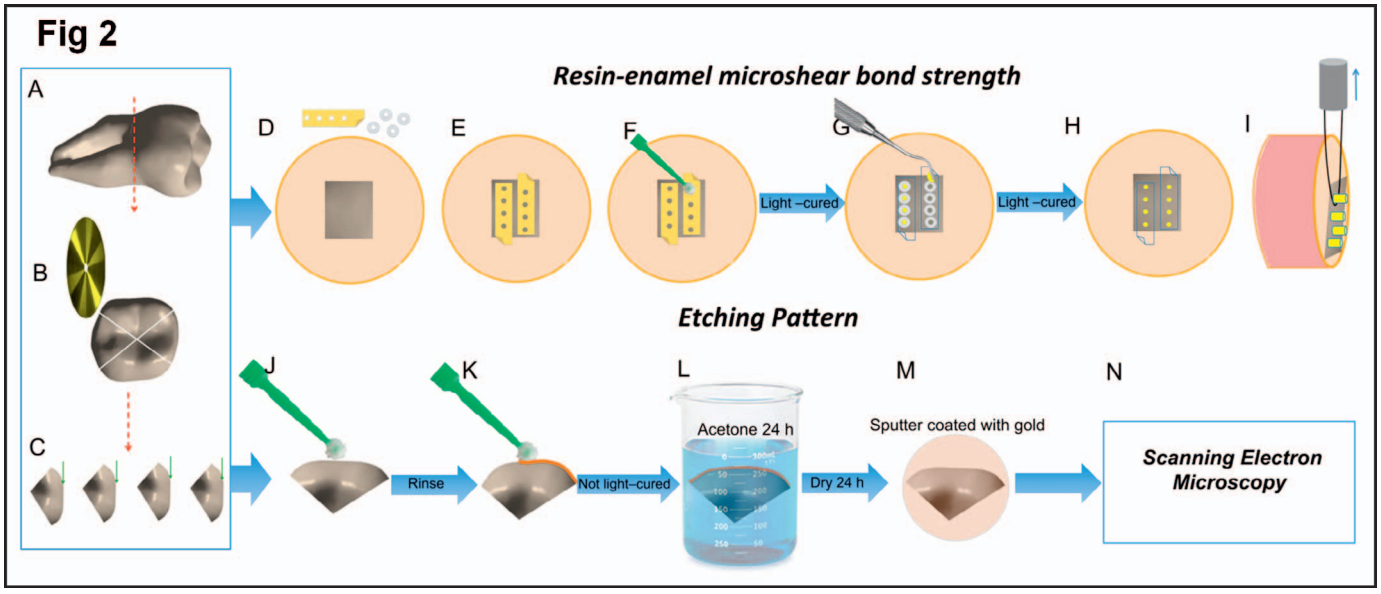

Figure 2. *Schematic drawing showing specimen preparation and microtensile bond strength testing. (A): The roots of all human teeth were removed by sectioning at the cementum-enamel junction. (B): Dental crowns were then sectioned in diagonals across the long axis of teeth. (C): Four enamel specimens (buccal, lingual, and proximals) were produced. (D, E): For resin-enamel microshear bond strength, each enamel specimen was mounted on a PVC ring filled with acrylic resin (displaying the enamel surface on the top of the cylinder); a double-faced adhesive tape was then attached to the enamel specimens to delimit the bonding area. (F): Adhesive application and light curing. (G): The Tygon tubes were placed on the enamel surface, and the lumen was filled with composite resin and light cured. (H): After storage, Tygon tubes and adhesive tapes were removed, leaving bonded resin composite cylinders only on the enamel surface. (I): Each tooth was placed in a jig and assembled in a universal testing machine for microshear bond strength testing with an orthodontic loop around the composite resin specimens. (J): For the etching pattern, the application of the EDTA protocols was done as performed in each group. (K): Adhesives systems (Scotchbond Universal Adhesive or Prime & Bond Elect) were applied, except that the adhesive layer was not light cured after application. (L): Specimens were stored in an acetone bath for 24 hours, to remove all the resin monomers from the surface. (M, N): Then, the samples were dehydrated for 24 hours, sputter coated with gold, and examined by scanning electron microscopy.*

bras Ind. e Com. Adesivos Ltda, SP, Brazil) with a hygienic Ainsworth-style rubber dam punch (Coltene, Alstätten, Switzerland). This adhesive tape was then attached to the enamel specimens (Figure 2).

The variation in the number of perforations was due to the different dimensions of the ground enamel specimens. Before adhesive application, all specimens were randomized into different groups as described for the sclerotic dentin (Figure 2). A single and calibrated operator applied the universal adhesive systems on enamel and sclerotic dentin.

After the application of the adhesive system, four to six polyethylene transparent Tygon tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA), with the same internal diameter of the perforations and a height of 0.5 mm, were positioned to face over the double-faced tape, ensuring that their lumen coincided with the circular areas exposed by the perforations (Figure 2).

Resin composite (Opallis, FGM Dental Products, Joinville, SC, Brazil) was carefully packed into each tube, and a clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place. The composite was light cured for 40 seconds using a light-emitting diode light-curing unit set at 1200 mW/cm^2 (Radiical, SDI Limited, Bayswater, Victoria, Australia). These procedures were carried out under magnifying loupes.

After storage of the specimens in distilled water for 24 hours at 37°C, the Tygon tubes were carefully removed with a blade, exposing the composite cylinders (Figure 2). 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.

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, SP, Brazil). Each specimen was positioned into the universal testing machine, and a thin orthodontic wire (0.2-mm diameter) was looped around the base of each composite cylinder. The orthodontic wire contacted the composite resin cylinder at half of its circumference (Figure 2).

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.[27] The cross-head 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). The failure mode analysis was performed under a stereomicroscope at 100× magnification (Olympus SZ40) and classified as cohesive in enamel or resin composite, adhesive or mixed, which included adhesive and cohesive failure of the neighboring substrates.

Etching Pattern Examined by Scanning Electron Microscopy (SEM)

Sclerotic Dentin—Twenty-four additional bovine teeth were used for this part of the experiment. Teeth were randomly distributed using a table of random numbers into the same eight groups tested in the microtensile protocol. The teeth were cut perpendicular to their long axes using a slow-speed diamond saw (Isomet) to obtain two dentin halves. One half was used to evaluate the degree of dentin obliteration (control group), while the other half was treated according to one of the eight groups (n=3) described earlier in the μTBS test (Figure 1).

Initially, the specimens were then immersed in distilled water and left in an ultrasonic bath for five minutes to remove the debris from the surface.[33] Immediately after, the application was performed with EDTA for each experimental group of adhesives as described in Table 1, except that the adhesive layer was not light cured after application.

Each surface was rinsed with an acetone bath for 24 hours, to remove all resin monomers from the surface before being dehydrated for 24 hours in a desiccator containing colloidal silica. Finally, the samples were sputter coated with gold (Sputtering SCD050, BalTec, Balzers, Liechtenstein) and examined by SEM (SSX-550, Shimadzu, Tokyo, Japan) at 12 kV operated in secondary electron mode.

Enamel Substrate—Eight additional human teeth were used for this part of the experiment. The roots of all teeth were removed by sectioning at the enamel-cementum junction. The dental crowns were then sectioned in the diagonals across the long axis of teeth to produce four enamel slices (buccal, lingual, and proximal),[31] totaling 32 enamel specimens. In this part of the study, the enamel specimens were not ground as previously described and because we aim to evaluate the etching pattern in the most challenging condition (Figure 2).

First, the specimens were immersed in distilled water and left in an ultrasonic bath for five minutes to remove the debris from the surface.[33] Immediately after, the application was performed with EDTA for each experimental group of adhesives as described in Table 1, except that the adhesive layer was not light cured after application.

After that, the surfaces were immediately stirred in acetone for 24 hours to dissolve the monomers from the enamel surface.[34] All specimens were then allowed to dry for 24 hours in a desiccator, mounted on Al stubs, sputter coated with gold (Sputtering SCD050, Bal-Tec), and examined under the SEM (SSX-550, Shimadzu) at 12 kV operated in secondary electrons mode.

Statistical Analysis

All resin-dentin μTBS and resin-enamel μSBS values obtained from the same dentin and enamel surface, respectively, were averaged for statistical purposes.[35,36] The data from the μTBS and μSBS were then submitted to two-way analysis of variance (adhesive systems vs. surface treatment) and post hoc Tukey's test at a level of significance of 5%. The etching pattern produced on enamel and dentin substrates was evaluated only qualitatively.

RESULTS

Resin-Dentin Bond Strength

Approximately 12 to 20 resin-dentin bonded specimens were obtained from each tooth. The failure modes of all experimental groups are shown in Table 2. Most of the specimens (87%) presented adhesive/mixed failures. Dentin and resin cohesive failures were rarely observed. A small number of premature failures (1.6%) were observed. No significant difference was observed among groups (data not shown, chi-square test, $p>0.05$).

Only the main factors adhesive system ($p=0.01$) and surface treatment ($p<0.001$) were statistically significant. For both adhesives, the lowest mean μTBS values were observed when the adhesives were applied according to the manufacturer's directions (Table 3). Compared with the control group (without EDTA application), manual EDTA application either for 30 seconds or two minutes resulted in higher resin-dentin bond strength (Table 3; $p<0.05$). However, for both adhesives, the highest μTBS values were observed when the EDTA was applied for 30 seconds with the sonic device (Table 3).

Table 2: *Number of Specimens and Percentage (%) of Failure Types for All Experimental Groups in Sclerotic Dentin*

Adhesive System	Failure Type	Surface Treatment			
		Control	EDTA 30 s	EDTA 2 min	EDTA 30 s + Sonic Device
SBU	A	58 (93.6)	41 (82.3)	50 (80.4)	42 (76.3)
	D	0 (0)	3 (5.9)	2 (3.2)	2 (3.6)
	R	2 (3.2)	3 (5.9)	6 (9.5)	8 (14.5)
	M	2 (3.2)	3 (5.9)	5 (6.9)	2 (3.6)
	PF	0 (0)	0 (0)	0 (0)	1 (2)
PBE	A	42 (85.7)	43 (76.9)	42 (77.9)	49 (80.3)
	D	3 (6.1)	2 (3.5)	6 (11.1)	2 (3.3)
	R	0 (0)	4 (7.1)	5 (9.3)	4 (6.6)
	M	4 (8.2)	4 (7.1)	0 (0)	5 (8.2)
	PF	0 (0)	3 (5.4)	2 (1.7)	1 (1.6)

Abbreviations: A, adhesive failure; D, dentin cohesive failure; EDTA, ethylene diamine tetra-acetic acid; M, mixed failure; PBE, Prime & Bond Elect; PF, premature failure; R, resin cohesive failure; SBU, Scotchbond Universal Adhesive.

Resin-Enamel Bond Strength

Approximately four to six resin-enamel bonded specimens were obtained from each tooth. Table 4 shows the percentage of fracture patterns found in each group. Most of the specimens (92%) presented adhesive/mixed failures. No cohesive failure in enamel or cohesive failure in resin was observed for either adhesive. A small number of premature failures (8.3%) were observed. No significant difference was observed among groups (data not shown, chi-square test, $p>0.05$). Table 5 shows the mean values and standard deviations of the μSBS according to the experimental groups to be addressed. The cross-product interaction adhesive system versus surface treatment ($p=0.754$) as well as the surface treatment ($p=0.11$) were not statistically significant. Only the main factor adhesive was significant ($p<0.0001$), with SBU showing the highest μSBS when compared with PBE.

Etching Pattern on Sclerotic Dentin

The etching pattern produced by the different protocols on sclerotic dentin can be seen in Figures 3 and 4. The presence of a hypermineralized dentin substrate, with obliterated dentin tubules in the sclerotic dentin, can be confirmed by Figures 3A and 4A.

Without any additional treatment, the application of the universal adhesives in the self-etch mode produced dentin substrates with dentin tubules obliterated by mineralized deposits (Figures 3B and 4B). This is more evident for the PBE adhesive (Figure 4B).

The use of EDTA for 30 seconds under manual application (Figures 3C and 4C) improved the etching pattern, but mineralized deposits can still be seen as in the control groups. On the other hand, EDTA pretreatment for two minutes, followed by adhesive application, produced a dentin substrate with more open dentin tubules, with only some of them obliterated by mineralized deposits (Figures 3D and 4D). The application of EDTA for 30 seconds with the sonic device (Figures 3E and 4E) produced a dentin substrate that resembles that of the EDTA two minutes. Although mineralized deposits can still be seen in the EDTA 30 seconds + sonic group, they are located deeper in the dentin tubules.

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

Adhesive System	Surface Treatment				Main Factor Adhesive System[a]
	Control	EDTA 30 s	EDTA 2 min	EDTA 30 s + Sonic Device	
SBU	32.8 ± 2.8	40.3 ± 4.2	49.3 ± 4.7	54.4 ± 1.8	44.2 ± 9.0 A
PBE	28.4 ± 0.9	40.6 ± 1.7	40.9 ± 9.4	49.9 ± 3.7	40.0 ± 9.2 B
Main factor surface treatment*	30.6 ± 3.0 c	40.5 ± 3.2 b	45.1 ± 8.6 b	52.2 ± 3.7 a	—

Abbreviations: EDTA, ethylene diamine tetra-acetic acid; PBE, Prime & Bond Elect; SBU, Scotchbond Universal Adhesive.
[a] *Uppercase letters indicate comparison of the main factor adhesive, and lowercase letters indicate comparison between the main factor surface treatments. Different uppercase or lowercase letters indicate groups statistically different (analysis of variance, Tukey's test, $p<0.05$).*

Table 4: *Number of Specimens and Percentage (%) of Failure Types for All Experimental Groups in Enamel*

Adhesive System	Failure Type	Surface Treatment			
		Control	EDTA 30 s	EDTA 2 min	EDTA 30 s + Sonic
SBU	A	60 (78.5)	59 (94.2)	67 (87.6)	72 (98.3)
	D	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)
	M	16 (19.0)	7 (3.0)	9 (12.4)	0 (0)
	PF	4 (2.5)	5 (2.8)	0 (0)	3 (1.7)
PBE	A	49 (65)	62 (90.1)	45 (75.0)	58 (71.5)
	D	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)
	M	4 (6.5)	3 (1.9)	3 (5.0)	16 (25.5)
	PF	26 (28.5)	9 (8.0)	12 (20.0)	5 (3.0)

Abbreviations: A, adhesive failure; D, dentin cohesive failure; EDTA, ethylenediaminetetraacetic acid; M, mixed failure; PBE, Prime & Bond Elect; PF, premature failure; R, resin cohesive failure; SBU, Scotchbond Universal Adhesive.

Etching Pattern on Enamel

The etching pattern produced by the different protocols on enamel can be seen in Figures 5 and 6. In enamel, the application of SBU produced a shallow selective enamel demineralization (type I pattern) with some areas of unetched enamel (Figure 5A). However, this was not observed in the PBE group (Figure 6A). The use of EDTA in all groups produced a deeper demineralization of the enamel prism cores (Figures 5B,D and 6B,D), even for the PBE group (Figure 6), with fewer areas of no selective enamel etching. Differences between the different EDTA protocols are not very evident for both adhesives. The etching pattern produced by the SBU was visually more retentive than that of the PBE for both enamel and dentin (Figures 3 through 6).

DISCUSSION

Sclerotic dentin is a challenging substrate for dentin bonding. It is much more resistant to dissolution by acidic monomers because of the presence of a hypermineralized surface layer and partial/total obliteration of the dentinal tubules.[5] The presence of mineralized deposits inside the dentinal tubules hinders the formation of resin tags and reduces the thickness of the hybrid layer in the intertubular dentin.[8,37,38] These are possible reasons why lower bond strength values are observed in this substrate as compared with sound dentin to self-etch adhesives.[39,40]

Our study showed that pretreatment of sclerotic dentin with EDTA produced a significant increase in the immediate μTBS values for both universal adhesives, which led us to reject the first null hypothesis. This is in agreement with other published studies that reported higher μTBS values for self-etch adhesives when applied in EDTA-treated dentin substrates.[6,14,15]

Unlike previous studies, our study evaluated several different EDTA application protocols, to include a novel sonic application. We observed that the two-minute application time, as already tested in the literature,[13,16] produced μTBS values similar to a shorter manual application time of 30 seconds, which requires reduced clinical time. This means that clinicians can shorten the application time of the

Table 5: *Microshear Bond Strength (μSBS in MPa) Values (Means ± Standard Deviations) for the Different Experimental Groups*[a]

Adhesive System	Surface Treatment				Main Factor Adhesive System
	Control	EDTA 30 s	EDTA 2 min	EDTA 30 s + Sonic Device	
SBU	15.4 ± 1.2	15.2 ± 1.5	16.3 ± 1.6	14.4 ± 1.3	15.3 ± 1.4 A
PBE	9.3 ± 1.3	9.4 ± 0.8	10.2 ± 0.9	9.5 ± 0.8	9.6 ± 1.0 B
Main factor surface treatment	12.4 ± 1.2 a	12.3 ± 1.3 a	13.3 ± 1.3 a	12.0 ± 1.1 a	—

Abbreviations: EDTA, ethylene diamine tetra-acetic acid; PBE, Prime & Bond Elect; SBU, Scotchbond Universal Adhesive.

[a] *Uppercase letters indicate comparison of the main factor adhesive, and lowercase letters indicate comparison between the main factor surface treatments. Different uppercase letters indicate groups statistically different (analysis of variance, Tukey's test, $p<0.05$). Similar lowercase letters indicate groups statistically similar (analysis of variance, Tukey's test, $p>0.05$).*

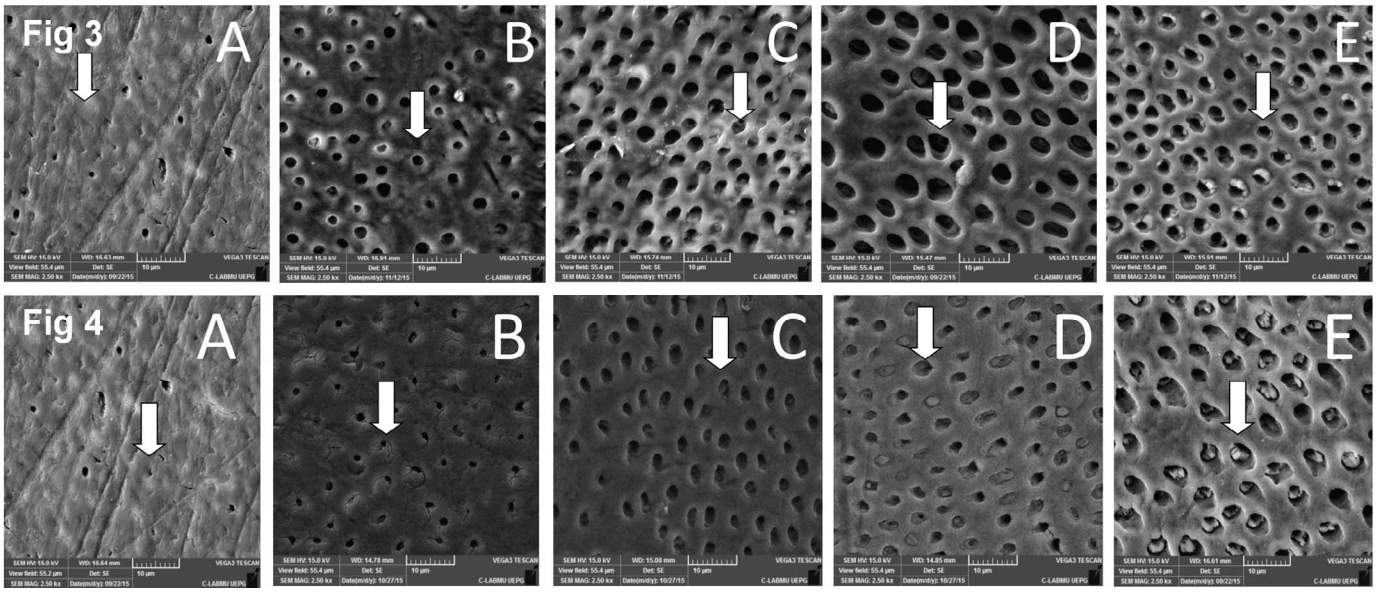

Figure 3. *Etching pattern of sclerotic dentin after the different protocols of bonding with the Scotchbond Universal Adhesive (SBU). In (A), we can observe the sclerotic dentin surface without any type of dentin treatment. There are a high number of obliterated dentin tubules (white arrow) in the dentin substrate. In (B), the sclerotic dentin surface was treated only with the universal adhesive in the self-etch mode. Although the dentin tubules are more evident, they are still obliterated by mineralized deposits (white arrow). In (C), the sclerotic dentin was treated with ethylene diamine tetra-acetic acid (EDTA) for 30 seconds with manual application before adhesive application. Dentin tubules are opened, but there are mineralized sclerotic deposits (white arrow) inside all of the dentin tubules. In (D), the sclerotic dentin was treated with EDTA for two minutes before adhesive application. The dentin tubules are much more opened, and only a few of them are obliterated by mineralized deposits (white arrow). In (E), dentin was treated with EDTA for 30 seconds with sonic application. This surface looks like the EDTA two-minute group with dentin tubules opened and less mineralization inside the dentin tubules (white arrow).*

Figure 4. *Etching pattern of sclerotic dentin after the different protocols of bonding with Prime & Bond Elect (PBE). In (A), we can observe the sclerotic dentin surface without any type of dentin treatment. There is a high number of obliterated dentin tubules (white arrow) in the dentin substrate. In (B), the sclerotic dentin surface was treated only with the universal adhesive in the self-etch mode. Although the dentin tubules are more evident, they are still obliterated by mineralized deposits (white arrow). In (C), the sclerotic dentin was treated with ethylene diamine tetra-acetic acid (EDTA) for 30 seconds with manual application before the adhesive protocol. Dentin tubules are opened, and sclerotic mineralized deposits can be seen practically in all dentin tubules (white arrow). In (D), the sclerotic dentin was treated with EDTA for two minutes before adhesive application. The dentin tubules are much more opened (white arrow). In (E), dentin was treated with EDTA for 30 seconds with sonic application. Dentin tubules are evident and opened, although some mineralized deposits can still be seen, but they are located deeper in the dentin tubules (white arrow).*

EDTA without compromising the bond strength values to this substrate.

The higher μTBS values of the EDTA-treated group can be attributed to the structure of the chelating ability of the EDTA molecule. The presence of four carboxylic acid groups produces the sequestration of metal ions of dental substrates and causes the selective dissolution of hydroxyapatite.[14] In dentin, EDTA removes the surface smear layer, which is a natural barrier to the penetration of acidic primers,[19,41] and creates a cleaner substrate, with a more retentive etching pattern (Figures 3 and 4) than that produced by the self-etch without previous EDTA application. This allows for better interaction of the self-etch adhesive with the sclerotic dentin substrate.

Unlike phosphoric acid etching (which also removes the smear layer but leaves collagen fibers exposed and perhaps prone to degradation), use of EDTA produces only a partial dissolution of hydroxyapatite. EDTA leaves residual apatite crystals in the collagen matrix and may make collagen more resistant to denaturation,[14,42] producing dentin-bonded interfaces that are less prone to degradation over time.[14,15]

Interestingly, the present study demonstrated that the application of EDTA for only 30 seconds with the aid of a sonic device produced a higher μTBS than the other EDTA protocols. Sonic vibration propagates pressure waves because of the stimulation of the EDTA molecules. The agitated molecules are able to reach areas beyond those where the bristles of the microbrush can touch. The high-speed vibration of the microbrush creates pressure waves and shear forces in the adhesive.[27] It also generates microscopic bubbles that are forcefully propelled against surfaces to which the adhesive solution is applied, increasing the dissolution of the smear layer, due to the fluid dynamics of

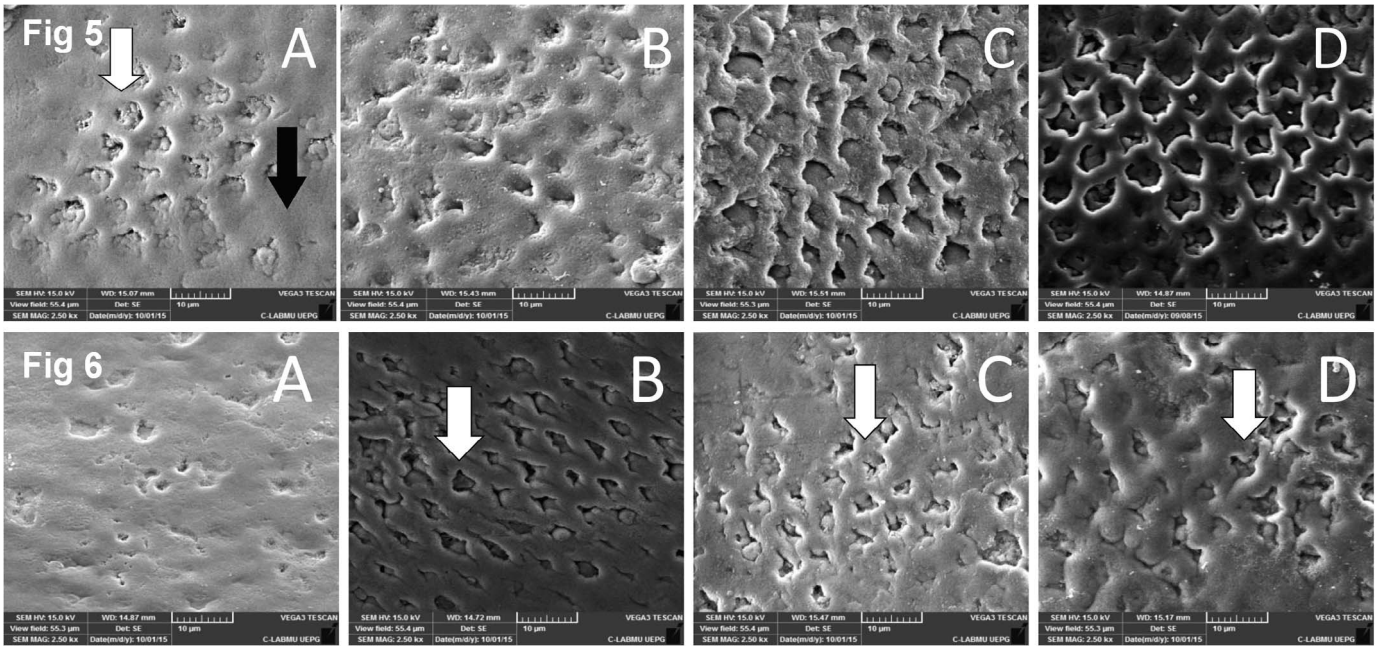

Figure 5. *Etching pattern of enamel after the different protocols of bonding with the Scotchbond Universal Adhesive (SBU). In (A), the adhesive was applied without any pretreatment. One can observe a very smooth selective etching of prism cores (white arrow, type 1 pattern), with some areas without selective demineralization (black arrow). In (B), ethylene diamine tetra-acetic acid (EDTA) was manually applied for 30 seconds. The etching pattern is somewhat better than that of image A, with a shallow demineralization of the prism cores and just some islands without selective enamel etching. In (C), EDTA was applied for two minutes before adhesive application. The same type of etching pattern was observed, but the demineralization of the prism cores is deeper, and there is no area of unetched enamel. In (D), EDTA was applied for 30 seconds with a sonic device. Some prism cores were demineralized deeper, similar to the EDTA two-minute group.*

Figure 6. *Etching pattern of enamel surface after the different protocols of bonding with the Prime & Bond Elect (PBE). In (A), the adhesive was applied without any pretreatment. One can observe a very smooth surface without any selective pattern of etching. In (B), ethylene diamine tetra-acetic (EDTA) was manually applied for 30 seconds. In (C), EDTA was applied for two minutes before adhesive application. In (D), EDTA was applied for 30 seconds with a sonic device. One can observe in all groups where EDTA was applied a very shallow demineralization of the prism cores (white arrow) and some islands of unetched enamel.*

acid on the sclerotic surface.[27] This allows better removal of the mineralized deposits inside the dentin tubules than the application of EDTA for 30 seconds with manual application. Use of the sonic application device generated the highest μTBS values.

Although there is no study that evaluated the EDTA application on sclerotic dentin with this sonic device, which prevents us from further comparison with the literature, an earlier study reported that the application of EDTA as an endodontic irrigant, with the sonic device, was more efficient at removing more of the smear layer inside the root canal than the conventional application.[43] In coronal dentin, there are reports that the use of this sonic device increases the resin-dentin μTBS of adhesive systems[27,44] and also reduces the permeability of the adhesive layer.[27]

Apart from the increases in the resin-dentin μTBS in the present and earlier studies by EDTA etching,[14,15,41] previous authors demonstrated that 17% EDTA applied for two minutes can reduce the activity of MMPs of human dentin.[16] The chelating activity of EDTA promotes the sequestration of zinc and calcium ions that act as potential activators of MMPs,[45,46] thereby minimizing its action on the hybrid layers.

The benefits of EDTA pretreatment in dentin were also confirmed in a recent randomized clinical trial. A higher retention rate of composite resins was observed in noncarious cervical lesions bonded with a self-etch adhesive when the dentin was pretreated with EDTA 17% for two minutes.[13]

In the current study, EDTA pretreatment of enamel did not show any benefits. None of the different types of surface treatment affected the μSBS values of either adhesive, leading us to accept the second null hypothesis. This finding is in agreement with previous studies[18,19] that observed that EDTA pretreatment in enamel was not effective at improving the bond strength of self-etch adhesives.

Although a more retentive etching pattern was observed in enamel after EDTA pretreatment, in

none of the conditions was this pattern similar to that produced by phosphoric acid etching,[47,48] which is considered the gold standard etchant for enamel. The EDTA pretreatment and self-etch application allowed for some selective demineralization of the enamel, with preferential dissolution of the enamel prism cores. However, this procedure did not produce microporosities within the prism cores and peripheries, as seen with phosphoric acid etching.

For both enamel and dentin, clear differences among the adhesives were observed. Most currently available universal adhesives contain at least one functional acidic monomer, which has chemical bonding potential. According to the manufacturer's information, SBU contains two components with this potential: methacryloxydecyl phosphate (MDP)[49] and methacrylate-modified polyalkenoic acid copolymer,[31] while PBE contains only dipentaerythritol-penta acrylate monophosphate as the functional monomer. This twofold mechanism of demineralization of the substrates may be responsible for the better etching potential of SBU than PBE in enamel and dentin in the present study as well as in previous studies.[31,50]

Although monomers with potential bonding to calcium are also presented in the composition of PBE,[51] chemical bonding alone is not enough to provide a strong bonded interface; the calcium salt produced by this chemical interaction should also be stable in an aqueous environment, such as that produced by MDP.[49,52] Furthermore, the interaction of MDP with hydroxyapatite is significantly stronger and within a clinically reasonable application time. This is reflected in the higher microtensile strength to dentin and enhanced sealing potential for the prevention of nanoleakage and thus extend bonding longevity.[53] Hydroxyapatite, however, needs to remain available at the partially demineralized dentin surface; this may also explain the greater difficulty with the effectiveness of self-etching adhesive in sclerotic dentin.

One should not deny that EDTA preconditioning includes an extra clinical step to the already complicated bonding protocol, but this study showed that with the aid of a sonic application, the extra time for EDTA preconditioning may be reduced even further than the application times recommended so far while keeping the benefits of this protocol. However, when available for purchase, the sonic device will add some additional cost to clinicians, which may be seen as a clinical limitation to the implementation of its use.

Further clinical trials should be performed to validate the results of the current *in vitro* study.

CONCLUSIONS

In sclerotic dentin, a shorter EDTA application time of 30 seconds can yield similar results to those produced by the conventional two-minute EDTA application in dentin. However, a 30-second application of EDTA in combination with a sonic device produced the highest resin-bond strengths to sclerotic dentin. The more visibly retentive etching pattern produced in enamel after EDTA pretreatment did not result in improved bond strengths to enamel.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local oversight committee guidelines and policies of UEPG. The approval code for this study is 110.234.

Conflict of Interest

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

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