

Effect of grape seed extract-containing phosphoric acid formulations on bonding to enamel and dentin

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Abstract: The aim was to evaluate the effect of 2% grape seed extract (GSE) containing phosphoric acid (PhA) on the bond strength to enamel and dentin. The control group was 37% PhA. The following three PhA formulations with 2% GSE and 20% ethanol were obtained: GSE5 = 5% PhA; GSE10 = 10% PhA; and GSE20 = 20% PhA. The enamel and dentin surfaces of molars were etched with the acid solutions, followed by Scotchbond Multi-Purpose adhesive and composite resin application. The tensile bond strength (TBS) test evaluated the bond to enamel after 24 h, and the microtensile bond strength (μ TBS) test evaluated the bond to dentin after 24 h and 12-month water storage. Etched enamel and dentin were observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM), respectively. The TBS data were submitted to one-way ANOVA, while μ TBS data were submitted to two-way ANOVA and Tukey's test ($\alpha = 0.05$). The TBS (MPa) to enamel did not significantly differ among the control (48.1 ± 15.7), GSE5 (46.1 ± 9.6), GSE10 (49.8 ± 13.6) and GSE20 (44.1 ± 11.9) groups ($p = 0.537$). The μ TBS (MPa) to dentin of the control (28.4 ± 14.4) and GSE20 (24.1 ± 8.1) groups were significantly higher than those of the GSE5 (16.8 ± 7.4) and GSE10 (17.5 ± 6.6) groups at 24 h ($p < 0.006$). After 12-month storage, only GSE5 (21.0 ± 7.8) and GSE10 (17.6 ± 8.0) did not show significantly decreased μ TBS ($p > 0.145$). SEM micrographs showed a shallower enamel etching pattern for GSE5. AFM images showed the formation of collagenous globular structures for GSE5 and GSE10. The different acid solutions did not influence the TBS to enamel, and the μ TBS to dentin was stable over time when dentin was etched with GSE5 and GSE10.

Keywords: Dental Enamel; Dentin; Acid Etching, Dental; Dental Bonding.

Introduction

To achieve a durable and stable bond in a hybrid tissue such as dentin, the adhesive monomers must be able to fully infiltrate and encapsulate the collagen fibrils that are exposed by acidic etching to form a homogeneous hybrid layer.^{1,2} However, the stability of this bond is affected by several factors, including inadequate envelopment of the collagen fibrils by the adhesive monomers as well as by hydrolytic and enzymatic processes that occur over time.^{3,4} Recent “in vitro” studies

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have indicated that bond durability is improved by the inhibition of endogenous collagenolytic activity^{3,5,6,7,8} and the addition of cross-linking agents, such as glutaraldehyde and derivatives of natural extracts rich in proanthocyanidins (PA),^{9,10} which may contribute to the integrity of the hybrid layer. These exogenous cross-linking agents have been suggested to maintain, restore, and enhance tissue function as well as produce a collagen with a support structure that is resistant to mechanical and enzymatic effects.^{3,10}

Grape seed extract (GSE), which is rich in PA, is an efficient collagen cross-linker,^{3,9,11,12} has low toxicity,¹³ and inhibits more than 90% of metalloproteinase (MMP)-2, MMP-8, and MMP-9 and approximately 75-90% of cysteine cathepsin B and K in dentin, proving to be more efficient than chlorhexidine.¹⁴ PA is known to stabilize and increase type-I collagen fibril cross-linking, improving the mechanical properties of the bonding interface, such as the modulus of elasticity and nanohardness.^{15,16} GSE significantly increased dentin resistance to collagenase digestion,^{12,17} being a promising clinical alternative for the improvement and durability of current adhesive systems,¹⁸ since PA binds to collagen in a short treatment time.¹¹

Pretreatment of demineralized dentin with PA increased the bond strength of total-etch adhesives to sound dentin,¹⁹ as well as carious dentin,²⁰ and produced resin-dentin interfaces less prone to nanoleakage.²¹ To simplify adhesive procedures, studies have evaluated the incorporation of PA in the adhesive,^{22,23} and a decrease in the initial bond strength to dentin was observed.²³ The incorporation of PA in the adhesive can negatively influence the degree of polymerization of the material.²⁴ Additionally, the dark brown color of PA might also cause an aesthetic issue.²³

Another strategy was the incorporation of PA in phosphoric acid. The addition of 2% GSE rendered phosphoric acid a collagen-stabilizing etchant. This effect occurred preferably at phosphoric acid concentrations less than 20% (5% and 10%).²⁵ Because the concentration of the GSE-containing phosphoric acid formulations is less than 37%,²⁵ it is important to evaluate the effect of these acids on both enamel and dentin, since restorative procedures involve

simultaneous etching of both dental tissues when the total-etch technique is applied. One study evaluated the composite resin bond strength of GSE-containing phosphoric acid formulations to enamel; however, only 10% phosphoric acid was tested.²⁶

Therefore, the aim of this study was to evaluate the use of 2% GSE-containing phosphoric acid with different acid concentrations (5%, 10% and 20%) on the bond strength to enamel at 24 h and to dentin after 24 h and 12-month storage. Complementary, the enamel etching pattern and dentin collagen fibril structures were also evaluated. This study tested the null hypotheses that grape seed extract-containing phosphoric acid formulations would not influence the resin composite TBS to enamel after 24 h (i) or the μ TBS to dentin after 24 h and 12-month storage (ii).

Methodology

Thirty-four human third molars were collected from adults aged between 17 and 30 years after approval from the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (47845315.5.0000.5336). The teeth were cleaned of gross debris and disinfected in 0.5% Chloramine-T solution for 24 h. After that, the teeth were stored in distilled water at 4°C. The water was changed every week and the teeth were used within three months. The roots were mounted in self-cured acrylic resin and the occlusal, buccal, and lingual enamel surfaces were removed using a water-cooled low-speed diamond saw (Extec Corp., London, UK). The buccal and lingual fragments were used for the methodologies applied on enamel, and the exposed occlusal dentin surfaces were used for the methodologies applied on dentin (Figure 1). On the occlusal surface, the cut was performed in a such way that enamel islands remained on the surface, which were later removed with 400-grit silicon carbide abrasive paper under water.

Three GSE-containing formulations (GSE20, GSE10, and GSE5) were prepared by mixing GSE powder (MegaNatural Gold, Madera, USA), ethanol, deionized water, and 85% phosphoric acid to final concentrations (weight percentage with respect to total mass) of 2% GSE, 20% ethanol, and 20% (GSE20), 10% (GSE10), and 5% (GSE5) phosphoric acid.²⁵ The

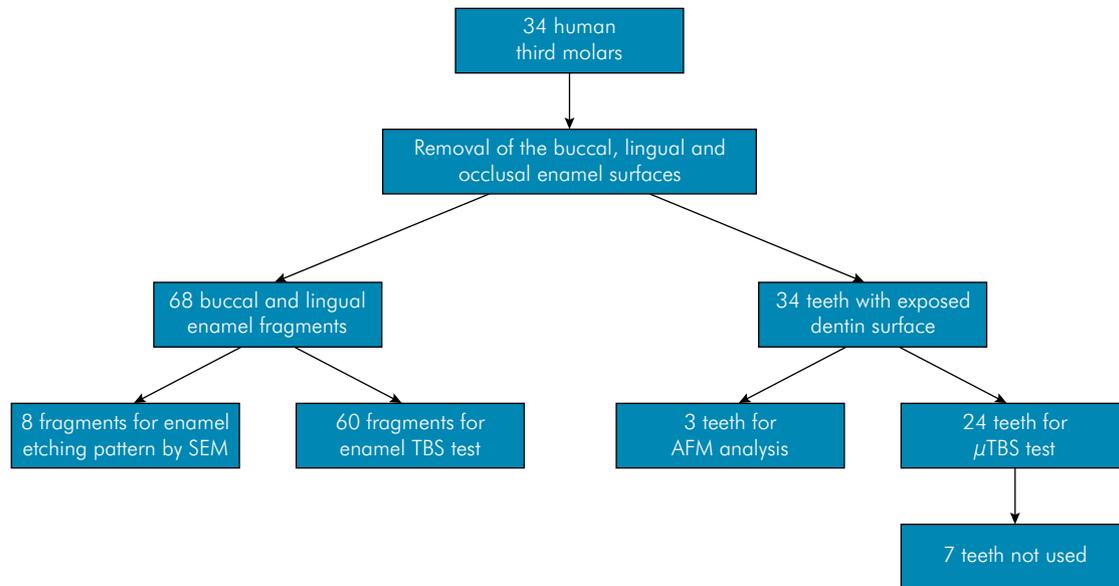


Figure 1. Schematic diagram of the study design.

control group was 37% phosphoric acid (Dentsply, Petrópolis, Brazil).

The pH of the GSE-containing phosphoric acid formulations was measured in a W3B pH meter (Bel Engineering, Monza, Italy). For each formulation, three pH measurements were obtained and the mean was calculated.

Enamel etching pattern by scanning electron microscopy (SEM)

The surfaces of the buccal and lingual enamel specimens were ground on 800-grit silicon carbide abrasive paper under water. Eight specimens were randomly assigned into four groups according to the acid formulations applied to the enamel surfaces ($n = 2$). The control group was etched with 37% phosphoric acid for 30 s, followed by rinsing for 30 s and drying with air jet. The GSE5, GSE10, and GSE20 groups were etched with the respective acid solutions using a disposable applicator with scrubbing for 30 s, followed by rinsing for 30 s and drying with air jet. The specimens were dried in a vacuum dehumidifier for three days. After this period, the specimens were gold-sputter-coated and observed using a scanning electronic microscope (JSM 6060 LV, JEOL, Tokyo, Japan) at 5,000 \times magnification operated at 20.00 kV and a WD of 10 mm.

Enamel TBS test

The buccal and lingual enamel specimens were included in self-cured acrylic resin. Sixty specimens were randomly assigned into four groups ($n = 15$).²⁷ Each specimen was etched with its respective acid formulation as described for the SEM analysis, followed by an unsolvated bonding resin (Adper Scotchbond Multi-Purpose Adhesive, bottle 3, 3M ESPE, St. Paul, USA). An inverted cone of composite resin Filtek Z250 (3M ESPE St. Paul, USA) was built on the adhesive using a metallic split mold with a 3-mm-diameter orifice at the bottom, 5-mm-diameter orifice at the top and 4-mm-high. The composite resin was light-cured for 40 s with the Radii Cal LED curing unit (SDI, Bayswater, VIC, Australia) with a light intensity of 1,000 mW/cm², which was controlled by a radiometer (LED Curing Radiometer, Demetron, USA). The specimens were stored in distilled water at 37°C for 24 h and then submitted to TBS test in a universal testing machine (EMIC DL-2000, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min using a cell load of 500 N. The TBS values were obtained in MPa. The fractured surfaces of the specimens were observed with a stereomicroscope (Olympus Corp., Tokyo, Japan) at 20X. The failures were classified as adhesive (rupture between enamel and adhesive), cohesive (cohesive failure in the enamel

or in the composite resin cone), or mixed (adhesive and cohesive failure).

Dentin collagen fibril structure by atomic force microscopy (AFM)

A sample from the middle third of the coronal dentin (~3 mm thickness and ~8 mm diameter) of three teeth was obtained by a water-cooled low-speed diamond saw. The dentin surfaces were finished with 600-, 1,200- and 1,500-grit silicon carbide abrasive paper under water and then polished with 0.5- and 0.3- μm grit alumina paste on a felt disk. The samples were ultrasonically cleaned with deionized water. Half of the dentin of each tooth was etched with phosphoric acid formulation without GSE (AC5, AC10 or AC20) for 30 s and then rinsed with deionized water. The excess water was removed with absorbent paper, leaving a moist surface. The other half of the dentin was etched with its corresponding acid formulation with GSE (GSE5, GSE10 or GSE20) in the same way. The specimens were immediately observed by AFM (Dimension Icon, Bruker, Camarillo, USA) in Peak Force Tapping mode. A TAP 150A probe (Bruker, Camarillo, USA) was used, and the scan resolution was 256 x 256 pixels per image with a drive amplitude of 100 mV. The images of the etched dentin surfaces were obtained at room temperature in a non-aqueous medium. The 10 μm x 10 μm images were used to locate the areas of intertubular dentin, where 3 μm x 3 μm images were taken; from these images, 1 μm x 1 μm images were obtained.

Dentin μTBS test

The exposed superficial dentin from 24 teeth was finished with 600-grit silicon carbide abrasive paper under water for 30 s. The teeth were randomly divided into four groups (n = 6). In the control group, the dentin was etched with 37% phosphoric acid (Dentsply, Petrópolis, Brazil) for 15 s, followed by rinsing for 15 s. The excess water was removed with cotton buds. A layer of Primer (Adper Scotchbond Multi-Purpose, bottle 2, 3M ESPE, St. Paul, USA) was applied, followed by gentle air-drying for 5 s. Subsequently, the adhesive was applied with a microbrush and light-cured for 10 s. In the GSE5, GSE10, and GSE20 groups, the acidic formulations were applied to the dentin for 30 s with a disposable applicator under friction, rinsed for 30 s,

and the excess water was removed with cotton buds. The Adper Scotchbond Multi-Purpose adhesive was applied as described for the control group. Then, two 2 mm increments of composite resin Filtek Z250 were applied, and each was light-cured for 40 s. After storage in distilled water at 37°C for 24 h, the six tooth/resin composite sets per group were sectioned perpendicular to the bonding surface using a laboratory-cutting machine (Labcut 1010, Extec Corp., London, UK) with a diamond disk under water-cooling. The specimens were cut into approximately 0.90 x 0.90 mm² transverse sections and measured with a digital caliper (Mitutoyo Sul Americana Ltda., Suzano, Brazil). Eight beams from the central region of each tooth were obtained. The beams that originated from the same tooth were randomly divided for immediate (n = 24) or 12-month testing (n = 24).²⁸ The specimens for the 12-month group were stored in distilled water at 37°C.

The specimens were fixed with cyanoacrylate glue (Loctite, São Paulo, Brazil) to the microtensile testing device. This device has two stainless steel grips with an area of 8 x 10 mm and sliding shafts that prevent torsion movements during the tests. These shafts have a fixing screw that prevents the specimen from moving during bonding. The specimens were stressed at a crosshead speed of 0.5 mm/min until failure in a universal testing machine (EMIC DL-2000) using a cell load of 50 N. The μTBS was expressed in MPa. The fractured surfaces of all specimens were observed by scanning electron microscopy (SEM) (EVO LS15, Zeiss, Germany). The failures were classified as interfacial [adhesive (between adhesive and dentin) and cohesive in adhesive], cohesive in dentin (failure inside the dentin), cohesive in composite resin (failure in composite resin), mixed I (interfacial + cohesive in dentin), or mixed II (interfacial + cohesive in composite resin).

Statistical analysis

According to Kolmogorov-Smirnov test, the TBS and μTBS data followed a normal distribution (p > 0.05). The mean enamel TBS was analyzed using one-way ANOVA and Tukey's test. The mean dentin μTBS was analyzed using two-way ANOVA (surface treatment x storage time) and Tukey's test. P \leq 0.05 was considered significant. The software used was SPSS v10.0 (IBM SPSS, Chicago, USA).

Results

The pH values of the modified phosphoric acids were 1.08 for GSE20, 1.25 for GSE10, and 1.38 for GSE5. The pH of 37% phosphoric acid was 0.2 according to the manufacturer's information.

Enamel etching pattern by SEM

Phosphoric acid at 37% caused a predominant dissolution of enamel prism core, with simultaneous conservation of the marginal area (Figure 2A). Similar

etching pattern was observed for GSE5 (Figure 2B), GSE10 (Figure 2C), and GSE20 (Figure 2D). However, GSE5 caused the shallowest etching pattern.

Enamel TBS

There was no significant difference in the mean TBS among the groups ($F = 0.73$, $p = 0.5370$) (Table 1). There were predominantly mixed failures for the control group and the GSE20 group. In the GSE5 and GSE10 groups, there were predominantly adhesive failures.

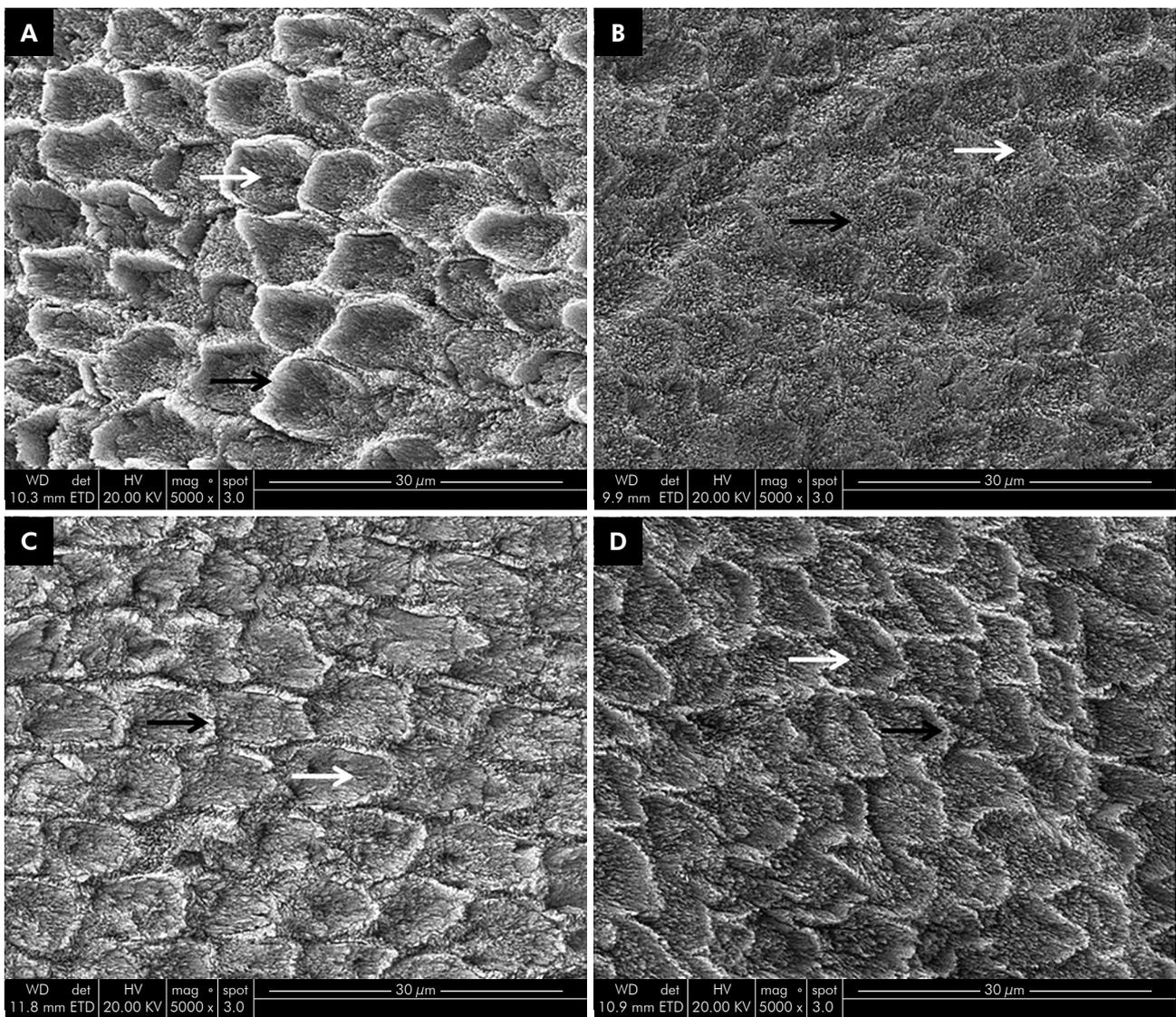


Figure 2. SEM micrographs (5,000x) of the enamel surfaces etched with 37% phosphoric acid (A), GSE5 (B), GSE10 (C), and GSE20 (D). The predominant dissolution of the enamel prism core (white arrows) with simultaneous conservation of the marginal area (black arrows) is shown for the four acid solutions. However, this etching pattern is shallower for GSE5 and more evident for 37% phosphoric acid.

Table 1. Mean enamel tensile bond strengths \pm standard deviation (SD) and mode of failure (%).

Groups	Tensile bond strength (MPa) and SD	Mode of failure (%)			
		A	CE	CC	M
Control	48.1 \pm 15.7	20.0	0.0	26.6	53.4
GSE20	44.1 \pm 11.9	26.6	0.0	13.4	60.0
GSE10	49.8 \pm 13.6	53.4	0.0	6.6	40.0
GSE5	46.1 \pm 9.6	60.0	0.0	13.4	26.6

There were no significant differences between groups (Tukey's test; $p > 0.05$). A: Adhesive; CE: Cohesive in enamel; CC: Cohesive in composite resin; M: Mixed (adhesive + cohesive in composite resin).

Dentin collagen fibril structure by AFM

AC5, AC10, and AC20 (Figures 3A, 3B and 3C, respectively) caused dentin demineralization and collagen fibril (arrow) periodicity (gap zones and overlap zones) was observed. In GSE5 (Figure 3D), the collagen fibrils formed globular structures (arrow). These globular structures are also visualized in GSE10 (arrow) (Figure 3E), in which the original periodicity pattern is no longer visualized. However, this change is less significant in the dentin treated with GSE20 (Figure 3F) because the collagen fibrils are not as bulky and agglomerated as the collagen fibrils

of GSE5 and GSE10. Comparing GSE5 and GSE10, the globular structures are more evident in GSE5.

Dentin μ TBS

The surface treatment factor ($p = 0.007$), storage time factor ($p = 0.013$), and interaction between the factors ($F = 7.219$, $p = 0.0001$) were significant. At the 24 h-storage, the control group obtained the highest mean μ TBS, which did not significantly differ from the GSE20 group ($p = 1.000$) (Table 2). Both groups resulted in a significantly higher mean μ TBS than the other groups ($p < 0.006$). The mean μ TBS for the GSE10 and

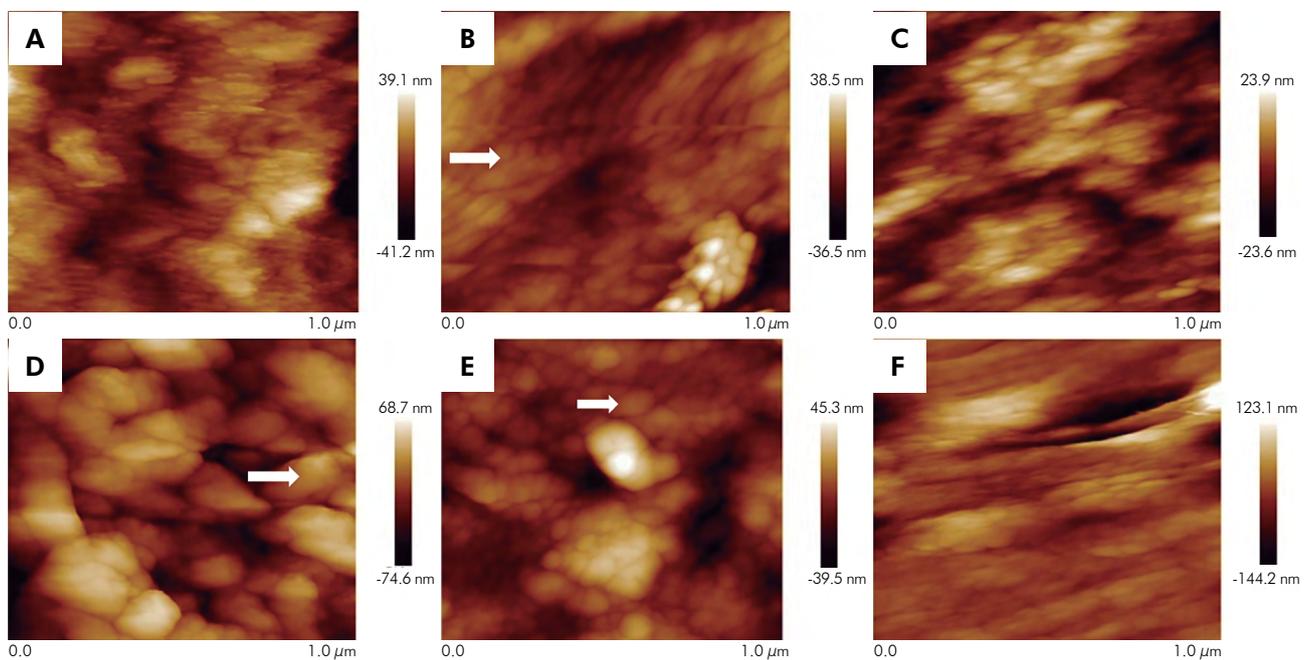


Figure 3. Two-dimensional images of the dentin surfaces etched with the different solutions using AFM. (A) 5% phosphoric acid (AC5). (B) 10% phosphoric acid (AC10). (C) 20% phosphoric acid (AC20). (D) GSE5. (E) GSE10. (F) GSE20. The periodicity of the collagen fibrils (arrow) is observed in AC10. In GSE5, GSE10, and GSE20, the collagen fibrils formed globular structures (arrows), which are more evident in GSE5.

GSE5 groups did not differ significantly from each other ($p = 1.000$). After 12-month storage, there was no significant difference in the mean μ TBS between any pair of groups ($p > 0.681$). Additionally, after 12-month storage, there was a significant decrease in the mean μ TBS for the control ($p = 0.001$) and GSE20 ($p = 0.004$) groups. There was no significant decrease in the mean μ TBS for the GSE5 ($p = 0.145$) and GSE10 ($p = 0.978$) groups. There was a prevalence of interfacial failure for all groups at 24 h and 12-month storage (Figures 4A and 4B). The mixed failures observed were mixed II type (interfacial and cohesive in composite resin) (Figures 4C and 4D) (Table 2).

Discussion

Different concentrations of GSE-containing phosphoric acid solutions were used in this study to analyze their potential as an etching agent for enamel and dentin, as well as the potential to inhibit degradation at the dentin-resin interface over time.

There were no significant differences in the mean enamel TBS among the four concentrations of phosphoric acid. Therefore, the first null hypothesis was not rejected. The results of the present study are in agreement with Hass et al.²⁶ Erickson et al.²⁹ reported that higher concentrations of phosphoric acid, such as 40%, allowed resin penetration around the enamel prism, while lower concentrations (2.5%) showed resin only partially surrounding the prisms, but still penetrating the interprismatic spaces. The patterns on intercrystallite resin infiltration within the prism and deeper penetration into the interprismatic spaces seem to be the morphology that results in

the highest bond strengths.²⁹ However, studies have found no significant difference in the bond strength for phosphoric acid concentrations from 65% to 3%³⁰ and from 2.5% to 40%.²⁹

Comparing the SEM micrographs of the enamel etched by the four acids, GSE5 caused an etching pattern that was shallower than the other concentrations. This finding corroborates other studies that have shown that lower percentages of phosphoric acid cause low enamel loss³¹ and reduced etching depth.³² GSE10 and GSE20 etched enamel in a similar way, whereas the etching pattern for 37% phosphoric acid was more evident. This microscopic finding corroborates the lower pH value of the 37% phosphoric acid ($pH = 0.2$ according to manufacturer's information) compared with the pH of the 2% GSE-containing phosphoric acid solutions that ranged from 1.08 for GSE20 to 1.38 for GSE5.

By correlating the acid etching pattern with the failure modes obtained after the TBS test, GSE5 and GSE10 showed more adhesive failures. Studies have shown that resin infiltration decreases with decreasing phosphoric acid concentrations.^{29,30} The shorter resin tag lengths obtained by GSE5 and GSE10 may justify the adhesive failures observed in these groups, since they have a concentration of 5% and 10% phosphoric acid, respectively. Regardless of the differences in the etching patterns, GSE5, GSE10, and GSE20 would be effective on the enamel during a 24-h evaluation period.

In the present study, the enamel bond strength after storage was not evaluated since other studies have shown that bonding to enamel does not deteriorate over time as occurs with dentin.^{26,33}

Table 2. Mean dentin microtensile bond strengths (μ TBS) \pm standard deviation (SD) and mode of failure (%).

Groups	μ TBS (MPa) and SD		Mode of failure (%)							
	24 h	12 months	I ₀	CD ₀	CC ₀	M ₀	I ₁₂	CD ₁₂	CC ₁₂	M ₁₂
Control	28.4 ^{Aa} \pm 14.4	17.7 ^{Ab} \pm 8.6	88.7	6.8	0.6	3.9	76.8	7.6	0.9	14.7
GSE5	16.8 ^{Bo} \pm 7.4	21.0 ^{Aa} \pm 7.8	93.8	2.5	1.2	2.5	85.6	0.5	8.7	5.2
GSE10	17.5 ^{Bo} \pm 6.6	17.6 ^{Aa} \pm 8.0	86.8	3.5	5.4	4.3	85.5	2.6	2.6	9.3
GSE20	24.1 ^{Aa} \pm 8.1	18.5 ^{Ab} \pm 6.3	88.5	0.0	9.7	1.8	68.3	21.0	0.0	10.7

Means followed by the same capital letter in columns and by the same lowercase letter in lines do not present significant differences (Tukey's test; $p > 0.05$). 24 h: I₀: Interfacial; CD₀: Cohesive in dentin; CC₀: Cohesive in composite resin; M₀: Mixed II. 12 months: I₁₂: Interfacial; CD₁₂: Cohesive in dentin; CC₁₂: Cohesive in composite resin; M₁₂: Mixed II.

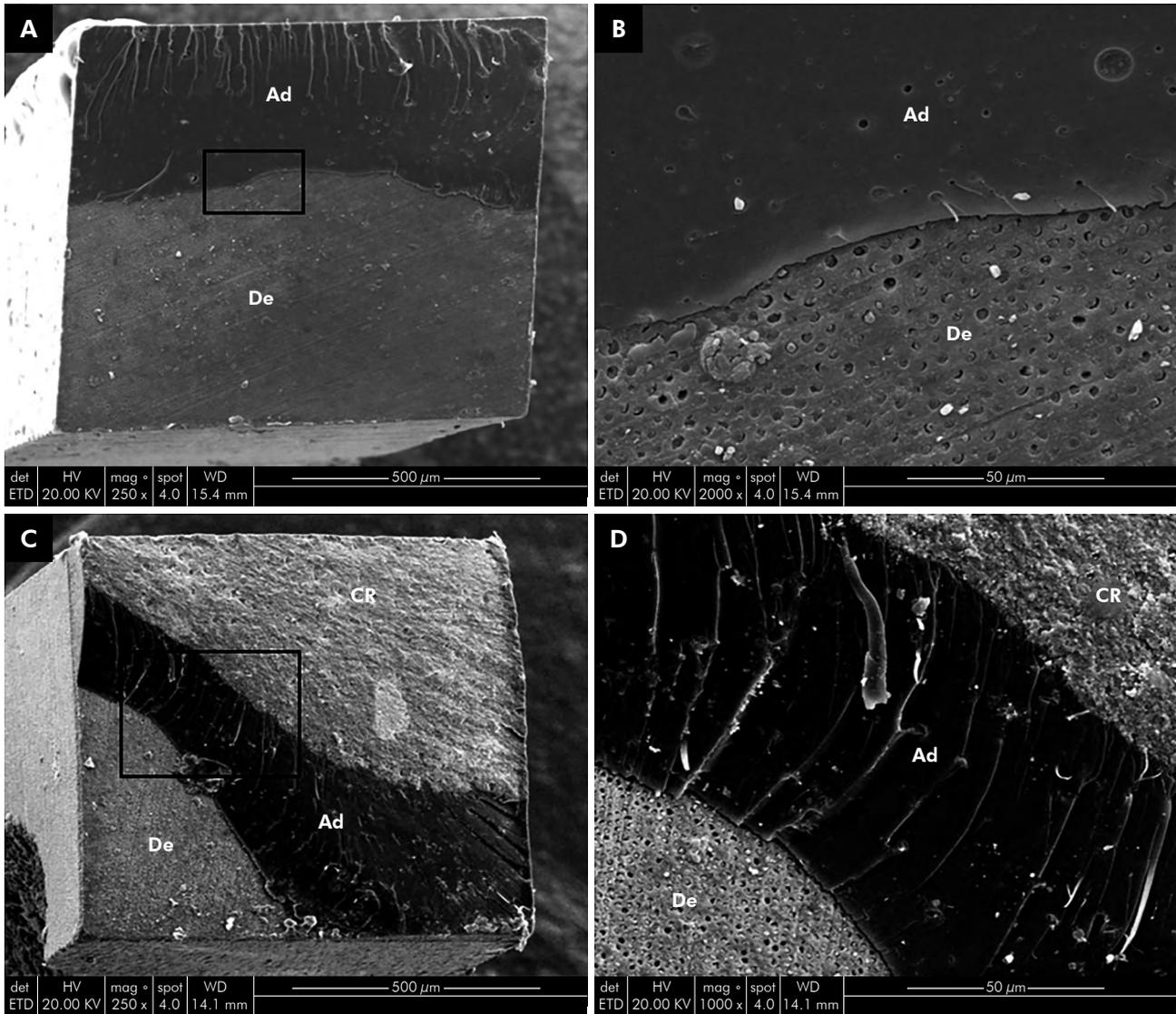


Figure 4. SEM micrographs of the predominant failures obtained after the μ TBS test. (A) Interfacial failure (250x): Ad – adhesive; De – dentin. (B) Magnification of the delimited area in (A) (2,000x). (C) Mixed II failure (250x): Ad – adhesive; De – dentin; CR – composite resin. (D) Magnification of the delimited area in (C) (1,000x).

The GSE-containing phosphoric acid solutions were also applied on dentin as a dentin biomodification agent. The aim of the dentin biomodification studies was to reinforce the dentin collagen network exposed by acid etching and control the degradation of extracellular matrix components.^{14,34}

On the dentin substrate, the highest immediate mean μ TBS was obtained with the control group and the GSE20 group. GSE10 and GSE5 resulted in significantly lower mean μ TBS values. Dentin etching with concentrations of phosphoric acid above 10% for 15 s causes the removal of the smear

layer and smear plugs, the opening of the dentin tubules, and intertubular demineralization, with a significant correlation between the depth of etching and pH.³⁵ Liu et al.²⁵ verified the depth of dentin demineralization with the same GSE-containing phosphoric acid solutions used in the present study. The solutions were applied for 30 s, obtaining depths of 3.8 μ m for GSE5, 5.0 μ m for GSE10, and 6.6 μ m for GSE20. Therefore, the lower pH of GSE20 (1.08) compared to GSE10 (1.25) and GSE5 (1.38) tends to cause a greater dentin demineralization depth and, as a consequence, greater thickness of the hybrid

layer. However, bond strength and its durability depend on the quality of the hybrid layer rather than the thickness or morphology of the hybrid layer and the resin tags.³⁶

After 12-month storage, the control and GSE20 groups had a significant decrease in their mean μ TBS, whereas the GSE10 and GSE5 groups maintained the mean dentin μ TBS. Therefore, the second null hypothesis was rejected. This finding is in agreement with another study.²⁶ According to Liu et al.,²⁵ the penetration of the phosphoric acids with GSE into the dentin is not synchronized. GSE is primarily composed of (epi)catechin monomers and oligomers, which are intrinsically more hydrophobic (indicated by involvement in hydrophobic interactions) and less mobile (higher molecular weight) than the small hydrophilic phosphoric acid. As such, GSE diffuses slower into the intratubular space than phosphoric acid. Thus, GSE20 etches the dentin too fast for GSE to catch up, which leaves the collagen fibrils at the etching front not fully protected as the top of the demineralized dentin.²⁵

The collagen fibril layer of the dentin base tends not to be completely enveloped by the adhesive monomers, which favors hydrolytic degradation and enzymatic degradation,⁸ which would justify the decrease of the mean μ TBS after 12-month storage for GSE20. Otherwise, it is reasonable to think that GSE10 and GSE5 caused demineralization at less depth and not as fast as GSE20, allowing greater interaction of the PA contained in the GSE with collagen fibrils at the level of the hybrid layer base that tends not to be surrounded by adhesive monomers.

AFM images showed structural changes of the specimens treated with GSE-containing phosphoric acid solutions. For GSE5 and GSE10, in particular, the formation of globular structures was observed, being more intense than GSE20. Even under a short treatment time (30 s) with extensive rinsing (30 s), it is believed that these globular structures are a consequence of the PA interaction with collagen. PA induce covalent-like bonds in type I collagen, increasing the interaction forces between collagen fibrils.³⁷ The building blocks of PA are catechins, which can be linked by an additional ether (C-O) or one or more C-C bonds to form oligomers and polymers.³⁸ Thus, GSE, which is a mixture of different

oligomeric structures of PA, may result in a similar increase in collagen-collagen interaction forces for both molecules and fibrils, supporting the hypothesis that PA interactions with collagen occur at different hierarchical fibril organization levels.³⁷ Higher oligomeric forms of PA mediate both intramolecular cross-links, which provide biostability to the collagen molecule, and intermolecular and intermicrofibrillar cross-links, which enhance mechanical properties.³⁸ The AFM images from the present study showed that the collagen fibrils aggregated and formed much larger globular structures with GSE5 and GSE10. This is an important finding because it shows that the structural changes that occur in the collagen fibrils, as a function of the presence of GSE, are dependent on the concentration of the phosphoric acid.

Therefore, as follows, several factors may explain the stability of μ TBS for GSE5 and GSE10: (1) PA contained in GSE altered the mechanical properties of dentin collagen, increasing the modulus of elasticity and stiffness of collagen fibrils,^{9,12} making the collagen fibrils more resistant to enzymatic challenges;³⁹ (2) PA favored MMP inhibition that is activated by the low pH of the acids;¹⁴ and (3) the acid solutions were able to modify the contact angle of the exposed collagen fibrils, facilitating solvent volatilization after the use of adhesive systems, which results in a less hydrophilic dentin and contributes to a better stability of the resin-dentin interface.⁴⁰ The sum of these factors, acting synergistically, could have favored bond stability at the resin-dentin interface over the 12-month storage period.

The failure mode of the specimens submitted to μ TBS was observed by SEM, and there was a predominance of interfacial failure at 24 h, as well as after 12-month storage. In this way, the interface of interest (adhesive-dentin) was measured in most specimens.

In the present study, the acid solutions were applied for 30 s, instead of 15 s used clinically for the 37% phosphoric acid. The etching time for 30 s, based on the study by Liu & Wang,¹¹ showed optimal protection for dentin collagen against collagenase digestion with $\geq 2\%$ GSE applied for 30 s. However, future studies should evaluate, under short- and long-term storage, the bond strength after 15 s

etching with the GSE-containing phosphoric acid solutions to verify whether the results are similar to 30 s of etching.

Conclusions

The bond strength to enamel was similar for all acid solutions.

GSE5 and GSE10 promoted the stability of the bond strength to dentin after 12-month storage.

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