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Research Article

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Pomegranate extract on eroded dentin: antioxidant action, bond strength and morphology of the adhesive interface after aging

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ABSTRACT

Objectives: This study aimed to evaluate the effect of pomegranate solution (*Punica granatum*) on eroded dentin through antioxidant action, shear bond strength (SBS) and interface morphology.
Materials and Methods: The 10% pomegranate peel extract was prepared by the lyophilization method. Punicalagin polyphenol was confirmed by high-performance liquid chromatography. Antioxidant activity was evaluated by capturing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. For the SBS, 48 dentin fragments were divided into sound or eroded, and subdivided according to the pretreatment ($n = 12$): water or *P. granatum*. The surfaces were restored with self-etch adhesive and a bulk-fill resin (Ecosite; DMG). The SBS was done immediately (24 hours) and after thermal cycling + water storage (12 months). For scanning electron microscopy, 48 dentin fragments (24 sound and 24 eroded) received the same treatments as for SBS ($n = 6$), and they were analyzed after 24 hours and 12 months.
Results: The *P. granatum* had antioxidant action similar ($p = 0.246$) to the phenolic standard antioxidants. After 24 hours, eroded dentin had lower SBS than sound dentin ($p < 0.001$), regardless of the pretreatment. After 12 months, *P. granatum* maintained the SBS of sound dentin (13.46 ± 3.42 MPa) and eroded dentin (10.96 ± 1.90 MPa) statistically similar. The lowest values were found on eroded dentin treated with water (5.75 ± 1.65 MPa) ($p < 0.001$). *P. granatum* on eroded dentin caused peritubular demineralization and hybrid layer with resin tags.
Conclusions: The pomegranate extract had antioxidant action and preserved the adhesive interface of the eroded dentin.

Keywords: Antioxidant; Dentin-bonding agent; *Punica granatum*

INTRODUCTION

Erosive tooth wear is related to acidic foods, erosive products, reduced salivary flow, and gastrointestinal disorders [1-4]. It is a chronic and irreversible disease associated with the loss of dental tissues (enamel or dentin) without bacterial involvement [1,5].

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The bond between restorative resin and dentin is associated with adhesive infiltration into the collagen matrix, which is exposed through acid etching or using an acidic adhesive/primer [6,7]. In the process of erosion, the tooth structure is in an acidic environment, causing demineralization of the tooth surface. This process causes mineral loss and the collapse of the collagen meshwork, which makes it difficult for the adhesive to penetrate the dentinal tubules and consequently adhesion to this substrate [7,8].

The endogenous proteases of oral saliva and dentin matrices are denominated matrix metalloproteinases (MMPs) [9]. In erosive conditions, salivary buffers have a lower pH, which can activate the host's proteases [10]. Upon demineralization, the collagen fibers of the organic matrix are exposed, activating MMPs that will degrade collagen fibers [10]. When triggered by a self-etching system, type I collagen fibrils' degradation influences the bond strength procedure [11].

Most of the natural extracts have antioxidant activity that plays an important role in the inhibition of MMPs. The antioxidant action of the natural extracts and their metabolites is related to their ability to scavenge superoxide radicals [12,13]. The free radicals come from metabolites produced by MMPs through the digestion of extracellular matrix proteins [12]. The role of antioxidants is related to the ability to eliminate free radicals locally [12,14].

In Restorative Dentistry, antioxidants have been used to inhibit MMPs and improve collagen stability prior to the restorative procedure [15]. For this reason, therapeutic strategies with compounds containing natural agents are widely studied to improve the wettability of dental substrate, modifying the surface for adhesive procedures [16,17].

Among the natural products used in dentistry, *Punica granatum* has an inhibitory effect on MMPs and antimetastatic potential against oral cancer cells, anti-inflammatory effect and antioxidant potential [18-22]. These favorable properties are due to flavonoids (catechins and anthocyanins) and tannins (punicalin, punicalagin, punic, gallic, and ellagic acids) present in the extract [23]. The high concentration of phenolic compounds is important for cellular protection against the harmful effects caused by free radicals that are responsible for inflammatory diseases, cellular aging, and alterations in the biological systems [22,24-26].

Studies on dental substrates bleached with high-concentrated hydrogen peroxide reported that the *P. granatum* solution could fully or partially restore the bond strength of the restorative material to enamel and dentin [13,27,28]. Unbleached dentin treated with 20% pomegranate solution increased the bond strength compared to the untreated control [29]. In addition, Peng *et al.* [18] demonstrated that the inhibitory migration of pomegranate extract in oral cells can be mediated by the inactivation of MMPs.

Although some studies clarify the benefits of *P. granatum* in Dentistry, only a few used *P. granatum* extract to treat tooth surfaces before resin composite application, mainly in bleached surfaces [13,18,20,27,28,30-32]. Besides, to the best of our knowledge, there is only 1 that investigated the *P. granatum* on eroded dentin and 2 that assessed the bond strength of resin composite to dentin pretreated with *P. granatum* extract [17,29,33]. Incorporating a pomegranate extract in the demineralized dentin could inactivate matrix MMPs by the antioxidant action, increase the mechanical strength and raise the degradation time of the adhesive interface.

This study investigates the effect of *P. granatum* 10% on the shear bond strength (SBS) and interface morphology of the resin composite to eroded dentin substrate. The alternative hypotheses were: 1) After interface degradation, the SBS of the specimens treated with *P. granatum* will be higher than those treated only with water, regardless of the substrate (sound or eroded); 2) Surface pretreatment with *P. granatum* will provide a better interface morphology that surface that received water, mainly on eroded dentin.

MATERIALS AND METHODS

Estimation of sample size

A pilot study with *n* = 3 was done for the SBS test to estimate the number of dental specimens required to find a difference among at least 1 experimental group. Power analysis was done using G*Power software with the following parameters: 2-tailed test, α error = 0.05, power (1−β) = 0.80, and effect size = 0.5. A minimum of 12 specimens was found for each group.

Experimental design

In the antioxidant action test, pomegranate peel patterns were used and tested in triplicate, in different models modified to MOHA (Sigma-Aldrich, St. Louis, MO, USA). The variable response was tested by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical capture method. For the SBS, 48 bovine dentin fragments were divided into 2 levels: sound (*n* = 12) and eroded (*n* = 12), then subdivided into 2 subgroups according to surface pre-treatment: distilled water (control) and *P. granatum* (12 teeth for each subgroup), the same sample was used for the analysis after aging. An immediate analysis was performed (24 hours after the restorative protocol) and a long-term analysis was performed after thermocycling and water storage for 12 months. In the morphological analysis, 48 dentin fragments (*n* = 6) were used, subdivided, and treated in the same way as the bond strength.

Sample selection and solution preparation

Granatum solutions were prepared from pomegranate fruits obtained from the same plantation. Approximately 10 ripe fruits were harvested and sanitized with running filtered water and a brush. Each fruit was cut in half and the peel and pulp (seed and juice) were separated. The pulp was sieved until all the juice was extracted from the seed. The juice extract was not used due to its high sugar content to avoid changes in the result.

The peel samples were weighed on an analytical balance (Shimadzu, AUW, Baureri, SP, Brazil) and dried by lyophilization method (Nova analytical, São Paulo, SP, Brazil) at −80ºC for 48 hours. The diluent used to prepare the extract (50% ethanol) was previously analyzed for antimicrobial activity at several hydroalcoholic concentrations, and 50% ethanol did not inhibit microbial growth [34].

After extraction, the samples were taken to total evaporation by controlled heating and vacuum. The pure powder of the fruit peel was obtained, identified and stored at 4ºC.

The solutions were prepared from 1g of powder, diluted in 10 mL of 50% ethanol (Merck KGaA, Darmstadt, HE, Germany) + distilled water (ethanol and water 1:1), thus obtaining a 10% hydroalcoholic extract. The concentration was determined in a previous study of our group, in which the 10% pomegranate peel extract had lower microbial growth [34].

The samples were then subjected to an ultrasonic bath (Cientec, Belo Horizonte, MG, Brazil) for 60 minutes at 15,000 rpm. The extract was filtered in a rotary evaporator (ATX, Odontobras, Ribeirão Preto, SP, Brazil) until obtaining a clear aspect.

Detecting the punicalagin tannin in the extract by the high-performance liquid chromatography (HPLC) technique

The extract was characterized by HPLC. The tannin punicalagin was chosen, which is a polyphenol classified as a high molecular weight hydrolyzable tannin (ellagitannin) with recognized antioxidant, anti-inflammatory and antimicrobial activities, standing out as a promising molecule with several beneficial health functions [23].

The samples were analyzed in an Acquity UPLC H-Class system (Waters Corporation, Milford, MA, USA) of ultra-performance liquid chromatography coupled to diode array detectors (Diode Array Detector) and the Xevo TQ-S mass spectrometer (Waters Corporation) with Z-Spray ionization source in negative analysis mode.

A 5 µL volume of sample was injected onto an Ascentis® Express C18 column (100 × 4.6 mm, 2.7 µm particle diameter) (Supelco-53829U MSDS). The mobile phase consisted of 0.1% formic acid (solvent A) and methanol + 0.1% formic acid (solvent B). Separation of compounds started in gradient mode with 10% methanol, then increased to 90% methanol in 4 minutes, remaining there for 6 minutes, and returning to initial conditions with 10% methanol within the next 6 minutes. The total analysis time was 12 minutes with a flow rate of 500 µL/min.

The operating parameters used in the Z-spray source were: capillary voltage = 2.50 kV, cone voltage = 40 V, source temperature = 150°C, desolvation gas temperature = 400°C, and dissolving gas = 900 L/h. The analysis was performed in selected ion recording mode. The concentration of punicalagin in the experimental extract was compared with that of the standard punicalagin (Sigma-Aldrich, Louis, MO, USA). These extracts were stored in closed flasks and refrigerated at 4°C. The pH of the extract was measured at 4.75 [34].

The standard curves for the identification of punicalagin (α and β isomers) were constructed with concentrations based on estimates of the levels of the fruit. Seven dilutions were made from a stock solution in methanol containing punicalagin, starting from a concentration of 1,000 µg/mL to a concentration of 0.05 µg/mL. For the *P. granatum* solution, 6 dilutions were made, starting from 1,000 µg/mL to 0.1 µg/mL.

It was verified that the peaks of the isomers of the punicalagin standard (α = 3.05 µg/mL and β =3.25 µg/mL) and of the experimental extract of *P. granatum* (α = 3.05 µg/mL and β =3.26 µg/mL) were similar. The total amount of punicalagin was 6.31 µg/mL (α + β) for the experimental extract.

Antioxidant potential of the experimental extract

The evaluation of the antioxidant potential was performed by the DPPH free radical scavenging method [34]. 10 µL of hydroalcoholic extract of pomegranate peel was used, which were tested in triplicate at different concentrations (10, 15, 20, 30, 40, and 50 µg/mL). Then, to the extracts, 1.0 mL of 0.1 M acetate buffer (pH 5.5), 1.0 mL of PA ethanol and 0.5 mL of 250 µM DPPH ethanolic solution were added. (Merck KGaA).

The change in absorbance was measured after 20 minutes at 515 nm at 25°C. The antioxidant activity of the specimens was compared with synthetic standards that prevent oxidation and

scavenge free radicals. The standards used were 2(3)-tert-butyl-4-hydroxyanisole (BHA) and butylhydroxytoluene (BHT).

Results were expressed as IC50 (µg/mL), which indicates the concentration of solution required to produce a 50% reduction in DPPH free radical and presented as the mean and standard deviation obtained in triplicate measurements. Free radical scavenging was calculated by the formula: %AA = 100 − [(A (control) − A (sample) / A (control)] × 100, where A (control) represents the absorbance of the DPPH solution without the sample and A (sample) represents the absorbance of the sample with the DPPH [34].

Artificial erosion lesions
Bovine incisors preserved in 0.1% thymol solution at 9°C were washed in running water for 24 hours to remove residues of thymol solution and examined macroscopically with the aid of a stereoscopic magnifying glass (Leica Microsystems, Wetzlar, HE, Germany) at 20× magnification. Sixty incisors without fracture lines or crown fissures were selected. The bovine teeth were submitted to prophylaxis with a pumice stone for 60 seconds, transversely sectioned 2 mm at the cemento-enamel junction in a cutting machine (Isomet 1000, Buehler, Düsseldorf, Germany). The specimens were standardized in dimensions (5 × 5 × 3 mm). Subsequently, they were polished using #1200 sandpaper (Panambra, São Paulo, SP, Brazil) for 60 seconds.

Each specimen was immersed in 20 mL of 0.3% citric acid (pH = 3.2) individually and placed on the shaking table (CT155, Cientec) under constant agitation at 50 rpm for 2 hours to induce erosion. After, the specimens were washed with distilled water and stored individually in Eppendorf tubes containing artificial saliva at 37°C for 24 hours. Artificial saliva was composed of methylparaben (2.0 g), sodium carboxymethyl cellulose (10.0 g), KCl (0.625 g), $MgCl\ 2.6H_2O$ (0.059 g), $CaCl_2.2H_2O$ (0.166 g), K_2HPO_4 (0.804 g), and KH_2PO_4 (0.326 g) in 1L of distilled water [35].

Restorative procedure
The experimental extract was applied according to the protocol: drip 3 mL for 2 minutes, actively rub with a microbrush for 10 minutes, and lightly dry with absorbent paper [34]. Then, self-etching adhesive (Ecosite Bond DMG, Hamburg, Germany) was applied and light-cured according to the manufacturer's instructions. The dentin surfaces were restored with Ecosite Bulk Fill resin composite (Universal shade, Ecosite Bond DMG) using Teflon molds (3 mm in diameter and internal 4 mm in height), stabilized with wax, to obtain resin composite fragments with the measurements mentioned above. The resin composite was included in increments (3 mm to 4 mm) and polymerized using a light-emitting diode source (Ultradent Products Inc., South Jordan, UT, USA) for 20 seconds. Then, the wax was removed with a scalpel, the split matrix was opened and additional light curing was performed. The dentin/restorative material set was stored in relative humidity at 37°C for 24 hours.

Hydrolytic degradation of the adhesive interface
Half of the specimens from each group were submitted to thermal and hydrolytic degradation of the adhesive interface. The samples were subjected to water storage in relative humidity for 12 months (37°C) and thermal cycling in a thermocycler machine (Ética Odontológica, São Paulo, SP, Brazil) at 5°C and 55°C in water. The immersion time in each bath was 30 seconds and the transfer time between baths was 2 seconds. The samples were subjected to 1,000 thermal cycles per month (twice a month) and were kept in distilled water at 37°C in intervals, totaling 12,000 cycles and 12 months of water storage. Distilled water was changed every week [28].

SBS

After 24 hours, the samples were positioned in a universal test machine (Instron Corporation, Canton, MA, USA) with a load cell of 2 kN. The force was applied with a rectangular stainless steel tip at a speed of 0.5 mm/min until the displacement of the restoration.

Failure patterns were analyzed using a ×40 stereomicroscope (Leica Microsystems) and were classified: adhesive, when the dentin surface was covered by a thin layer of adhesive material; cohesive of the material, when the area was covered with resin composite; cohesive of the substrate, when the failure occurred in dentin; and mixed, a combination of adhesive and cohesive.

Morphological surface assessment by scanning electron microscopy (SEM)

Forty-eight samples were prepared for adhesive interface analysis, half destined for each period: immediately after 24 hours or aged after 12 months ($n = 6$). The dentin fragment (sound or eroded) was treated with different protocols. The specimens were restored as previously described for the shear strength test. The sections were ground with 600 and 1,200-grit sandpapers (Norton, Lorena, SP, Brazil) for 30 seconds each and with alumina 0.3 µm and 0.05 µm for 5 minutes. After polished, the sections received 37% phosphoric acid (Villevie, Joinvile, SC, Brazil) for 20 seconds, followed by rinsing and ultrasonically cleaned for 10 minutes. Then, the samples were dehydrated in 25%, 50%, 75%, and 95% ethanol by immersion for 20 minutes in each solution and then immersed in 100° ethanol for 1 hour and finally, were on hexamethyldisilazane for 10 minutes. They were fixed in metallic stubs (SCD 050 Bal-Tec, New York, NY, USA) and analyzed under a scanning electron microscope (Zeiss EVO 50, Carl Zeiss, Cambridge, MA, England). The most representative areas were photographed.

Statistical analysis

Statistical analysis was conducted using Statistical Package for Social Science software (SPSS 25, IBM Corp., Armonk, NY, USA) with a pre-set alpha of 0.05. Antioxidant action data (µg/mL) were analyzed by the non-parametric chi-square test. SBS (MPa) were subjected to normality and homogeneity tests, showing normal (Shapiro-Wilk test) and homogeneous (Levene test) distribution. Then, they were analyzed by 3-way analysis of variance, considering the substrate (sound or eroded), treatment (experimental or control) and time (immediate or aged) as independent factors. Failures were analyzed using the chi-square test. Photomicrographs were analyzed qualitatively by 3 calibrated examiners (kappa ≥ 0.8).

RESULTS

Antioxidant potential of the *P. granatum* solution

The experimental extraction was performed with the synthetic standards BHA and the antioxidant expression of the extract (%AA). There was no significant difference in the values of BHA, BHT, and *P. granatum* 10% ($p = 0.246$) (**Table 1**), evidencing the antioxidant potential of the experimental extract.

The IC50 (µg/mL) of each solution was calculated, which represents the amount of a substance needed to inhibit a given biological process by 50% (in this study, the DPPH radical). The respective IC50 values for BHA, BHT, and *P. granatum* were: 2.97 µg/mL, 3.01 µg/mL and 3.49 µg/mL.

Table 1. Means and standard deviations (triplicates) of the inhibitory concentration of the 2,2-diphenyl-1-picrylhydrazyl radical (%AA) for the antioxidant activity of the 2(3)-tert-butyl-4-hydroxyanisole (BHT), butylhydroxytoluene (BHA), and *P. granatum* extract standards

Samples	BHT	BHA	*P. granatum* 10%
1	88.20 ± 0.24	85.81 ± 0.82	83.05 ± 0.52
2	81.69 ± 1.29	83.40 ± 0.66	83.60 ± 1.44
3	73.26 ± 1.67	76.30 ± 1.38	80.79 ± 2.65
4	64.67 ± 0.63	60.24 ± 1.26	67.47 ± 4.98
5	42.78 ± 1.68	35.46 ± 0.38	45.85 ± 9.96
6	27.78 ± 1.80	21.93 ± 3.46	32.41 ± 5.40
Total	63.06 ± 0.64*	60.52 ± 1.10*	65.52 ± 3.42*

*No significant difference between the mean values of BHA, BHT, and *P. granatum* (Chi-square test, p = 0.246).

Table 2. Shear bond strength means and standard deviations (MPa) of the restorative material to dentin, considering substrate and surface treatment (immediate analysis)

Pretreatment	Sound dentin	Eroded dentin
Water (control)	(17.70 ± 2.40) Aa	(12.27 ± 2.28) Ab
10% *P. granatum*	(16.95 ± 4.60) Aa	(11.96 ± 3.24) Ab

Different capital letters indicated significant difference within the same column (treatment).
Different small letters indicated significant difference within the same line (substrate).

SBS analysis

Data analysis showed a significant difference for the 3 tested factors: *substrate* (p < 0.001), *treatment* (p = 0.008), and *time* (p < 0.001). The sound dentin had higher SBS than the eroded dentin. Dentin pretreatment with *P. granatum* provided higher SBS than water pretreatment (control). The immediate SBS data was higher than the 12-months data, thus the degradation of the adhesive interface decreased the SBS.

The interactions between *treatment × time* (p < 0.001) and *treatment × time × substrate* (p < 0.001) also had significant differences. No significant interactions were found for *treatment × substrate* (p = 0.183) and *substrate × time* (p = 0.084).

Table 2 shows the means and standard deviations of the immediate SBS, considering the substrate and surface treatment. In the immediate analysis, eroded dentin had lower SBS than sound dentin, regardless of the dentin pretreatment (*P. granatum* or water).

Table 3 shows the means and standard deviations of the SBS after 12 months, considering the substrate and surface treatment. After degradation, the *P. granatum* applied on the eroded dentin kept the bond strength stable. The lower adhesion values were found after 12 months on eroded dentin treated with water.
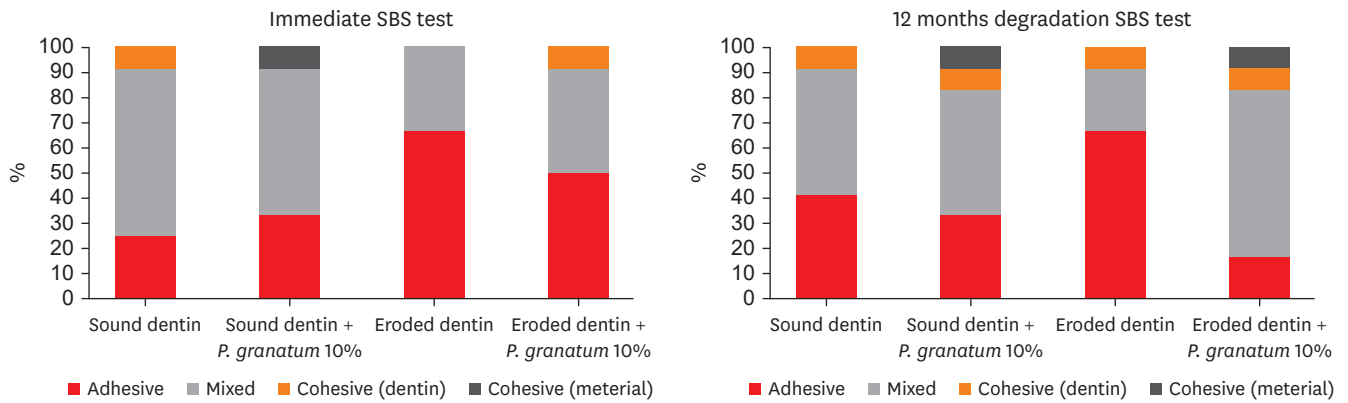
Failure modes

Mixed failures (combination of adhesive and cohesive) were found in the immediate analysis (24 hours) for sound dentin, regardless of treatment. Adhesive failures were more significant in eroded dentin, regardless of the treatment. The number of cohesive failures (dentin and material) was low. After aging, there was a predominance of mixed failures in sound dentin

Table 3. Shear bond strength means and standard deviations (MPa) of the restorative material to dentin, considering substrate and surface treatment (after 12 months)

Pretreatment	Sound dentin	Eroded dentin
Water (control)	(11.47 ± 1.82) Aa	(5.75 ± 1.65) Bb
10% *P. granatum*	(13.46 ± 3.42) Aa	(10.96 ± 1.90) Aa

Different capital letters indicated significant difference within the same column (treatment, p = 0.008).
Different small letters indicated significant difference within the same line (substrate, p < 0.001).

Figure 1. Failure modes after immediate bonding test and adhesive interface aging. SBS, shear bond strength.

pretreated with water or *P. granatum* and in the eroded dentin pretreated with *P. granatum*. However, the predominance of adhesive failures in eroded dentin that received only water was observed (**Figure 1**).

Surface morphology by SEM

Overall, both pretreatments (water and *P. granatum*) provided a uniform adhesive layer. The immediate analysis of the sound dentin pretreated with water or *P. granatum* had resin tags and a good adhesive interface. A reduction in resin tags, peritubular and intertubular demineralization was observed in the eroded dentin pretreated with water. (**Figure 2**).
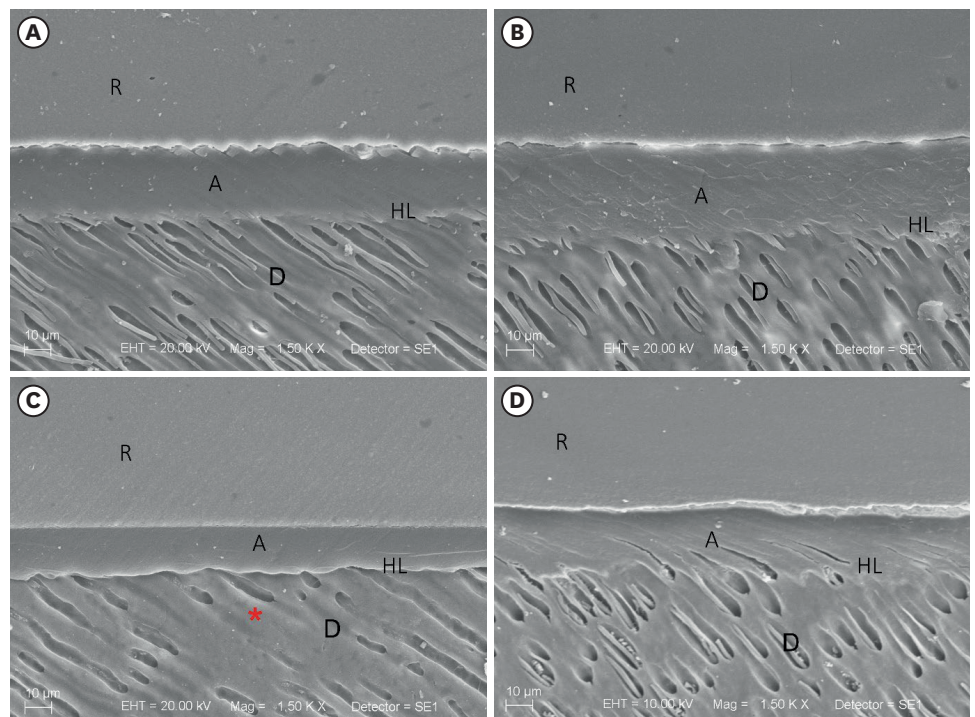
Figure 2. SEM of the interface of the restorative procedure (no degradation after the restorative procedure). (A) Sound dentin (control), (B) sound dentin + 10% *P. granatum*, (C) eroded dentin, and (D) eroded dentin + 10% *P. granatum*. Demineralized dentin without resin tags (red asterisk).
R, resin; A, adhesive; HL, hybrid layer; D, dentin; SEM, scanning electron microscopy.
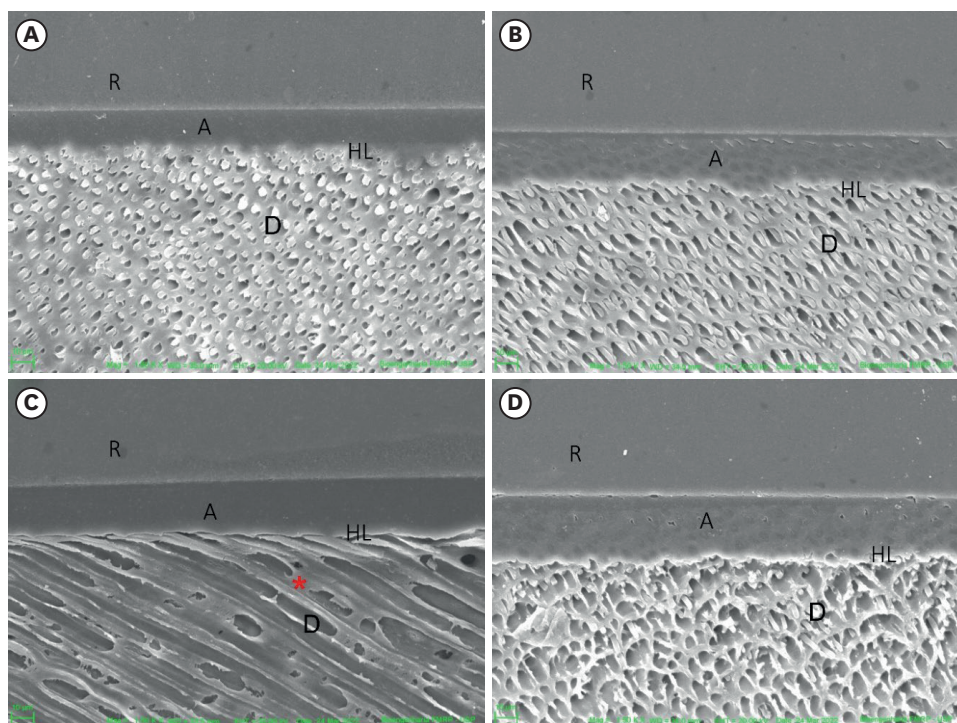
Figure 3. SEM of the interface after degradation of the adhesive interface (12 months). (A) Sound dentin (control), (B) sound dentin + 10% *P. granatum*, (C) eroded dentin, (D) eroded dentin + *P. granatum* 10%. Demineralized dentin without resin tags (red asterisk).
R, resin; A, adhesive; HL, hybrid layer; D, dentin; SEM, scanning electron microscopy.

After 12 months, the hybrid layer, resin tags and good adhesive interface were observed in sound dentin (control) pretreated with water (**Figure 3A**), as well as in the dentin pretreated with *P. granatum* (**Figure 3B**).

In eroded dentin, it was observed peritubular and intertubular demineralization (**Figure 3C and 3D**). The number of resin tags was reduced in the eroded dentin treated with water and *P. granatum*.

DISCUSSION

Exposed dentin from erosive wear needs restorative treatment, protecting dentinal tubules to reduce sensitivity and restoring severe wear through major reconstructions or indirect composites [36]. However, the bond strength of the resin composite to the eroded substrate over time has been described as low [37]. This fact can be explained by the degradation process of the hybrid layer mainly due to the action of MMPs, which remain trapped inside the extracellular matrix of dentin in an inactive form and are activated by the acid challenge [38].

Pretreatment with modifying substances, such as natural extracts, has been suggested to improve bond strength on the dentin surface, as they decrease the degradation of collagen fibrils [16,17,28]. *In vitro* study demonstrated antioxidant activity and a high concentration of flavonoids in the hydroalcoholic *P. granatum* [14].

This study identified the antioxidant potential of an experimental hydroalcoholic extract of 10% *P. granatum,* and analyzed its effect on the adhesive strength of the resin material to the eroded dentin

substrate. The lyophilization method was used to prepare the hydroalcoholic extract of *P. granatum*. This process consists of removing water from the material by sublimation at low temperatures without chemical change (loss of volatile constituents) by heat [39]. The lyophilized product is resistant to microorganisms due to the minimal residual water concentration and allows proper handling of the concentrations necessary for the experiment [39].

Lyophilized powder from the fruit peel was used [40,41]. The concentration of the experimental extract was standardized at 10% to potentiate the effects of flavonoids since the pomegranate solution at low concentrations did not provide promising results in bond strength [17,28].

The punicalagin was quantified in the pomegranate peel extract by HPLC [42]. This technique performs quantitative analysis of compounds from various samples with high efficiency [41,42]. This study found that the peaks of punicalagin isomers from the *P. granatum* extract and the standard were close, evidencing the presence of this antioxidant tannin in the experimental extract.

DPPH is a violet-colored stable nitrogen-free radical with 515 and 520 nm absorption. The reduction of the DPPH radical is monitored by the decrease in absorbance during the reaction [43]. Furthermore, the DPPH radical scavenging method is used to evaluate the antioxidant activity of extracts and has been widely used in the food industry [44]. In our study, *P. granatum* extract was shown to be as effective in scavenging DPPH free radicals as the synthetic standards BHA and BHT. This process can be explained by tannins such as punicalagin in the fruit peel [40,41]. Besides, punicalagin in *P. granatum* has DPPH free radical scavenging activity, and antioxidants stabilize electrons so that the surface to be restored is uniform and non-porous, favoring adhesive procedures [13,24,45,46].

The first alternative hypothesis was accepted since the experimental pomegranate-based solution increased the SBS compared to the control without treatment. The pretreatment of eroded dentin with *P. granatum* maintained the SBS after aging. This fact can be explained as flavonoids in natural extracts can induce collagen molecular bonds, improve dentin's mechanical properties, inhibit MMPs and prevent early degradation of the adhesive interface [15,17,47]. Natural bioactive compounds improve the wettability of the dental substrate and leave the surface chemically prepared for adhesive procedures [35].

A previous study of our research group did not find promising results with the pomegranate solution, probably because we used the standard method of preparing a solution (commercial powder heated in water), which caused residues of the solution in the dentin (checked by SEM) [17]. Besides, we used pomegranate solution at 0.5%, a low concentration to achieve the desired effect. Sajana *et al.* [29] verified that *P. granatum solution at* 20% increased bond strength of resin in sound dentin.

Regarding failures, the adhesive type was predominant in the immediate analysis of eroded dentin treated with water or *P. granatum*. These findings agree with previous studies that reported a higher prevalence of adhesive failures in eroded teeth, suggesting fragility in the bond between the material and substrate [37,48]. In the 12-month analysis, a higher rate of mixed failures was observed in sound dentin pretreated with water, and in both dentin (sound and eroded) pretreated with *P. granatum*. A higher percentage of mixed failures represents a better adhesive interface, which reflects superior bond strength values [49].

The adhesive interface was qualitatively analyzed by SEM [50]. The erosive process caused demineralization of the peri and intertubular dentin, and the matrix degradation probably reduced the resin tags in the specimens treated only with water. These findings agree with previous studies on dentin, which suggest that morphological changes caused by erosion may reflect lower values of bond strength of the restorative material [10,51].

The reduction of resin tags in the eroded groups reinforces the idea that the organic dentin matrix subjected to an erosive process makes it difficult for the adhesive system to penetrate due to the fragility of the fibers, which impairs hybridization and accelerates the degradation of the interface [8]. The specimens generally showed a good adhesive interface, suggestive of a uniform hybrid layer and with resinous tags. The favorable adhesive interface observed in this study corroborates the high bond strength obtained in the SBS. Considering the above-mentioned facts, the second alternative hypothesis cannot be accepted.

Overall, this study elucidated some advantages of the 10% *P. granatum* solution applied to eroded dentin, such as antioxidant potential and durability of resin bonds over time. The main limitation is that this solution was not tested as a dental pretreatment in a clinical trial. Besides, its light brown color could stain the tooth surface. Our study supports new laboratory and randomized clinical research to clarify the benefits of using *P. granatum* in the dental substrate.

CONCLUSIONS

Based on our results, it can be concluded that the experimental extract of *P. granatum* showed antioxidant potential compared to synthetic standards BHT and BHA, adhesive interface with resin tags, preserved the integrity of the adhesive interface on eroded dentin after thermocycling and 12-months storage.

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