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Influence of an arginine-containing toothpaste on bond strength of different adhesive systems to eroded dentin

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The aim of this study was to evaluate the bond strength of different adhesive systems to eroded dentin following toothbrushing with an arginine-containing toothpaste. Sixty standardized 3 × 3 × 2-mm fragments of root dentin (n = 10) were prepared. After all surfaces except the buccal surfaces were impermeabilized, specimens were subjected to an erosive wear protocol and stored for 24 hours at 37°C. The specimens underwent 1000 toothbrushing cycles with an arginine-containing toothpaste, an arginine-free toothpaste (positive control group), or artificial saliva (negative control group). Following application of a self-etching or an etch-and-rinse adhesive to the buccal surfaces of the specimens, 6-mm-high composite resin blocks were built up in 2-mm increments. After 24 hours' storage in 100% relative humidity, microtensile test specimens with an approximate area of 1 mm^2 were prepared. The test was performed at a speed of 0.5 mm/min until specimen fracture, and the failure patterns were evaluated using a stereoscopic loupe. Two-way analysis of variance revealed no significant difference between the toothpastes, the adhesive systems, or the interactions between toothpaste and adhesive system in terms of the bond strength to eroded dentin ($P > 0.05$). The predominant failure pattern was adhesive in all groups. It was concluded that a toothpaste containing arginine did not interfere with the bond between either the self-etching or the etch-and-rinse adhesive system and eroded dentin.

Received: May 13, 2014
Accepted: September 17, 2014

Key words: arginine, eroded dentin, microtensile bond strength

Dentinal sensitivity may be defined as pain or an exaggerated response by the pulp to exposure of the dentin to chemical, tactile, thermal, and osmotic stimuli from the intraoral environment, exposure that does not occur in a sound tooth.[1] Under normal circumstances, the dentin is protected by enamel and/or cementum and therefore is not subjected to direct stimulation.[2] Nonetheless, exposed dentinal tubules secondary to enamel loss caused by abrasion, erosion, or abfraction may produce strong dentinal sensitivity.[3-5] Additionally, patients retain teeth longer than in the past, increasing the risk for such lesions.[1,6]

Enamel loss is predominantly a process of wear resulting from erosion caused by exposure to intrinsic or extrinsic acids, which is generally followed by abrasion, attrition, and abfraction. Cervical lesions involving enamel loss and exposed dentin are more likely to occur when toothbrushing is performed together with abrasive compounds present in toothpaste as well as exposure to acidic substances (intrinsic and extrinsic).[5] When a tooth is exposed to acid, minerals are lost and, consequently, surface hardness is reduced. Therefore, if an abrasive challenge follows erosion, the softened tissue is easily removed before it has a chance to remineralize.[7,8]

The use of desensitizing agents to treat dentinal sensitivity has been advocated; the action of these agents is based on the occlusion of exposed dentinal tubules, which interrupts neural response to stimuli and thereby blocks the pain signal.[9] An arginine and calcium carbonate paste (utilizing Pro-Argin technology, Colgate-Palmolive Company) used to treat dentinal sensitivity has proved efficient in occluding dentinal tubules.[10-15] Studies have confirmed that arginine and calcium carbonate, when combined, accelerate the natural mechanism of occlusion by depositing dentinlike material, which is composed of calcium and phosphate within the dentinal tubules, thus forming a plug and a protective layer over the dentin surface.[10-15]

An alternative approach to management of noncarious cervical lesions is composite resin restorations. According to Grippo, unrestored lesions promote further deterioration of the dental structure.[16] It has been suggested that restoration of these lesions would reduce the concentration of tension in cervical exposed dentin and consequently halt the lesion progression.[16] Bonding of materials to eroded substrate is achieved via the establishment of a hybrid layer.[17-19] Furthermore, there is evidence that such a hybrid layer may act as a shock absorber for stresses between the dentin and the restorative material due to the elasticity of this hybrid layer.[20-23]

Regarding adhesive strategies, etch-and-rinse bonding agents rely on acid etching to dissolve hydroxyapatite crystals and expose the collagen mesh so that it can be permeated first by the adhesive components and then the composite resin.[24] Self-etching bonding systems, in contrast, are capable of demineralizing the outer layer of the dentin yet maintaining a residue of hydroxyapatite still attached to collagen.[25]

Noncarious cervical lesions may, therefore, generally cause dentinal sensitivity, which can be treated with desensitizers. Arginine deposited on dentin as a treatment for sensitivity may alter the substrate, which could subsequently receive a bonded restoration. However, the bond strength of adhesive systems to eroded dentin that has undergone toothbrushing with arginine has not yet been fully evaluated. The aim of the present study was to evaluate the bond strength of 2 adhesive materials to abraded dentin that underwent cycles of brushing with

Table 1. Composition of toothpastes and artificial saliva used in the toothbrushing cycles.

Material	Composition
Colgate Cavity Protection (batch 2336BR121J)	Water, calcium carbonate, sorbitol, sodium lauryl sulfate, sodium monofluorophosphate (1450 ppm fluoride), flavoring, cellulose gum, sodium bicarbonate, sodium silicate, sodium saccharin, xanthan gum, methylparaben, propylparaben, CI 74160/Blue No. 15 (CI 74160)
Colgate Pro-Relief (batch 2199BR12CB)	Active ingredients: 8% arginine, 1.10% sodium monofluorophosphate (1450 ppm fluoride) Other ingredients: calcium carbonate, water, bicarbonate, sorbitol, sodium lauryl sulfate, aroma, cellulose gum, sodium bicarbonate, potassium acesulfame, sodium silicate, xanthan gum, sucralose, titanium dioxide (CI 77891)
Artificial saliva	Described by McKnight-Hanes & Whitford[26] and modified by Amaechi et al[7]: sodium hydroxymethyl benzoate, sodium carboxymethyl cellulose, KCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot H_2O$, and K_2HPO_4, which simulates both the organic and inorganic contents of natural saliva

Abbreviation: CI, color index.

Table 2. Composition and application of adhesive systems and composite resin used in bond strength testing.

Material	Composition	Manufacturer's instructions
Clearfil SE Bond (batch 01628A)	Primer: MDP, HEMA, hydrophilic dimethacrylate, camphorquinone, *N,N*-diethanol-*p*-toluidine, water. Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, camphorquinone, *N,N*-diethanol-*p*-toluidine, silanized colloidal silica	Primer: Apply the primer and wait 20 seconds. Follow with gentle air drying. Bond: Apply the adhesive. Gently air dry and light cure for 10 seconds.
Adper Single Bond 2 (batch N368475BR)	Bis-GMA, HEMA, dimethacrylate, ethanol, water, a novel photoinitiator system, and a functional methacrylate copolymer of polyacrylic and polyalkenoic acids	Apply phosphoric acid for 15 seconds and rinse for 10 seconds. Remove excess water with absorbent paper. Apply 2 layers of adhesive for 15 seconds. Apply light air jet for 5 seconds, and light cure for 10 seconds.
Filtek Z100 XT (batch 1302400395)	Organic phase: Bis-GMA and TEGDMA Inorganic phase: zirconia/silica (71% volume)	Prepare cavity with adhesive. Place and adapt fine layers of resin in the cavity. Light cure for 20 seconds.

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate.

an arginine-containing toothpaste. The null hypothesis was that there was no difference in bond strength between different adhesive systems and eroded dentin that was treated with arginine-containing toothpaste.

Materials and methods

This study was approved by the Research Ethics Committee of the São Leopoldo Mandic Institute and Dental Research Center, Campinas, Brazil (protocol No. 2012/0253).

Experimental design

In this study, 60 eroded dentin fragments were abraded by toothbrushing with an arginine-containing toothpaste (Colgate Pro-Relief, Colgate-Palmolive Company), an arginine-free toothpaste (Colgate Cavity Protection, Colgate-Palmolive Company), or artificial saliva and restored using a conventional etch-and-rinse (Adper Single Bond 2, 3M ESPE) or self-etching bonding system (Clearfil SE Bond, Kuraray America, Inc) and composite resin (Filtek Z100, 3M ESPE). The outcome variable was bond strength via microtensile testing.

The factors under analysis were toothpaste at 3 levels (negative control, no toothpaste [artificial saliva]; positive control, arginine-free toothpaste; and experimental, arginine-containing toothpaste) and bonding systems at 2 levels (etch-and-rinse and self-etching). The factors under analysis were designated to the experimental units randomly, constituting a 3 × 2 factorial, forming 6 experimental groups. Table 1 describes the composition of the toothpastes and artificial saliva used in the toothbrushing cycles. Table 2 describes the main components of the adhesive systems and composite resin as well as steps for their application. Figure 1 presents a summary of the experimental steps.

Selection of teeth and preparation of the dentin fragments

Sixty extracted third molars were selected from the tooth bank of the São Leopoldo Mandic Institute and Research Center. They were free of carious lesions, restorations, and cracks. They were stored in 0.1% thymol, cleaned with periodontal curettes (Duflex, SS White), and polished with a polishing stone (SS White) and a Robinson brush (Microdont).

The human third molars were cut at the cementoenamel junction with a diamond disc mounted on a precision

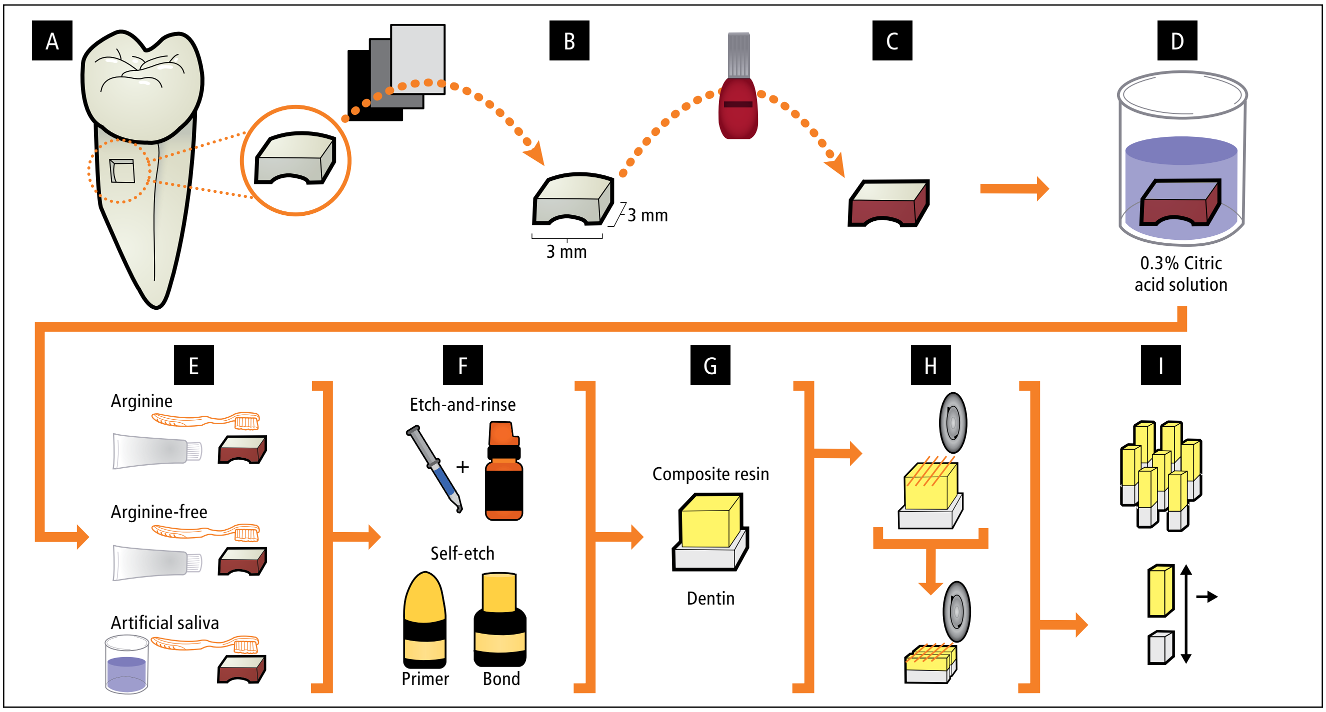

Fig 1. Experimental steps. A. Preparation of fragments of root dentin. B. Polishing of the buccal surface of 3 × 3 × 2-mm fragment. C. Impermeabilization of all fragment surfaces except the buccal aspect. D. Erosive procedures. E. Toothbrushing cycles. F. Application of the adhesive systems. G. Composite resin restoration. H. Preparation of dentin–bonded composite resin sticks for microtensile testing. I. Microtensile testing performed on a universal testing machine until fracture.

electric saw (IsoMet 1000 Precision Diamond Saw, Buehler). Sectioning resulted in 60 root dentin slabs with a dimension of 3 × 3 × 2 mm. The fragments were subsequently planed and polished with a rotating polisher (Aropol 2V, Arotec SA) and aluminum oxide sandpaper (Imperial Wetordry, 3M ESPE), in order of increasing fineness (400, 600, and 1000 grit).

Erosive wear protocol

All surfaces of the samples except the buccal surface were impermeabilized with nail varnish prior to the wear stage. Erosive wear was simulated according to the protocol proposed by Vanuspong et al, in which teeth were immersed in a 0.3% citric acid solution, buffered to a pH of 3.2.[27] Each specimen was individually immersed in 10 mL of the solution under magnetic agitation for 30 minutes. The specimens were then rinsed in distilled water, dried with absorbent paper, and stored in artificial saliva (remineralizing solution) for 24 hours at 37°C.

Toothbrushing cycles

The total number of brushing cycles was 1000 per test specimen. According to Goldstein & Lerner, 10,000 cycles are equivalent to 1 year of toothbrushing.[28] However, for abraded dentin surfaces, 1000 cycles are sufficient.[29] The load applied was 200 g, simulating the force applied during oral hygiene procedures. One toothbrush (batch 166605; Johnson & Johnson) was used for each test specimen. The toothbrush had an angulated head and handle and soft, rounded tufts.

The toothbrushing cycles were performed in a brushing machine (Equilabor) on the buccal surfaces of the specimens. The appropriate slurry for the assigned group was used. For each specimen in the arginine-containing and arginine-free toothpaste groups, the slurry consisted of 50 g of toothpaste and 150 g of distilled water (ie, a 1:3 ratio), which is similar to that used daily in the intraoral environment.[29-31] For the negative control group, the toothpaste slurry was replaced with 200 mL of artificial saliva (Table 1).

Restoration procedure

Following completion of the brushing cycles, the adhesive systems were applied to the buccal surfaces of the specimens according to their experimental group and as directed by the manufacturer's instructions (Table 2). A 6-mm-high block of composite resin (Filtek Z100) was then built up in increments of 2 mm. Each increment was light cured for 40 seconds with a halogen curing light (Demetron Research Corp) at 450 mW/cm^2, as measured by a radiometer (Newdent). The specimens were then stored in an incubator at 100% relative air humidity for 24 hours.

Preparation of the test specimens for microtensile testing

The prepared test specimens were individually fixed to acrylic plates (5 × 5 × 4 mm), using first an adhesive glue (Loctite Super Bonder, Henkel Corporation) and then tacky wax (Asfer Indústria Química). This test set was appropriately fixed to a precision saw (IsoMet 1000 Precision Diamond

Saw), and a high-concentration diamond disc was used to serially cut the specimens from the composite resin to the dentin perpendicular to its long axis, at both the x-axis and y-axis, with a distance of 1 mm between sections. The specimens were then removed from the precision saw and the acrylic plate so that the dentin–bonded composite resin specimens (sticks) of 1 mm^2 could be selected. On sectioning, 4-6 sticks were obtained from each fragment.

Microtensile testing

The specimens were fixed by their ends to the grip device of a universal testing machine (EMIC DL2000, Instron Brasil Equipamentos Científicos Ltda), aided by cyanoacrylate glue. Traction was applied at a speed of 0.5 mm/min until failure. The strength values were recorded in kilograms-force. The load needed to fracture the specimens was calculated in megapascals after the adhesive area was measured with a digital gauge (Starrett 727-6/150, The L.S. Starrett Company).

Evaluation of the failure pattern and interface via scanning electron microscopy

The specimen surfaces were visually examined with a stereoscopic loupe (Eikonal do Brasil) to classify the failure pattern: type 1, adhesive failure between the adhesive and the dentin; type 2, partial adhesive failure between the adhesive and the dentin as well as partial cohesive failure in the adhesive; type 3, total cohesive failure of the adhesive system; type 4, partially cohesive failure in the dentin; type 5, partially cohesive failure in the composite resin.

Two slices of each tooth were kept for scanning electron microscopy (SEM) to allow characterization of the tooth-restoration interface. The slices were polished with water-cooled sandpaper in order of increasing fineness (400, 600, and 1200) followed by diamond paste in order of decreasing particle size (6.0, 3.0, 1.0, and 0.5 μm) on a mineral oil–cooled cotton cloth wheel. The specimens were thoroughly rinsed and demineralized in 6 N hydrochloric acid for 30 seconds, rinsed again, deproteinized in 2.5% sodium hypochlorite for 10 minutes, and dehydrated in a series of alcohol solutions at 25%, 50%, 75%, and 100%. The specimens were then chemically dried in HMDS (hexamethyldisilazane) for 10 minutes, mounted on aluminum stubs, gold coated, and examined under a scanning electron microscope (JEOL 5900LV, JEOL Ltd).

Statistical analysis

The data relating to bond strength were analyzed with a 2-way analysis of variance. The failure patterns were reported descriptively. The significance level adopted was 5%, and the statistical calculations were performed with SPSS statistical software, version 20 (IBM Corporation).

Results

Statistical analysis of the mean microtensile bond strengths of the groups did not reveal any significant differences ($P > 0.05$) between the toothpastes, adhesive systems, or interactions between toothpaste and adhesive in bond strength to eroded dentin (Table 3). Adhesive failures were predominant in all groups.

Table 3. Mean (SD) bond strengths (in MPa) of the experimental groups.

Brushing group	Adhesive system	Tensile bond strength
Artificial saliva	Etch-and-rinse	13.98 (7.73)
Artificial saliva	Self-etch	13.25 (6.80)
Arginine-free toothpaste	Etch-and-rinse	12.66 (5.17)
Arginine-free toothpaste	Self-etch	14.49 (6.33)
Arginine-containing toothpaste	Etch-and-rinse	14.22 (7.47)
Arginine-containing toothpaste	Self-etch	11.91 (3.81)

There are no statistically significant differences between the toothpastes, adhesive systems, or interactions between toothpaste and adhesive ($P > 0.05$; 2-way analysis of variance).

Figures 2 and 3 represent SEMs from the hybrid layer at the tooth-restoration interface following treatment of eroded dentin with an arginine-containing toothpaste, an arginine-free toothpaste, or artificial saliva. The SEM images from the groups restored with an etch-and-rinse adhesive system confirmed the formation of a uniform hybrid layer with resin plugs and numerous tags (Fig 2). The SEM images from the groups restored with a 2-step self-etching adhesive system revealed the presence of resin tags and lateral resin extensions (Fig 3).

Discussion

Dentinal sensitivity presents as a short, sharp pain that results from a pulpal response to stimuli at the exposed dentin and that cannot be attributed to any other dental pathosis. The stimuli could be thermal, tactile, osmotic, or chemical.[32,33] The pain generally occurs when the root surface is exposed by gingival recession, which may be a natural, age-related phenomenon; however, the exposure is typically associated with either aggressive toothbrushing or periodontal disease.[2] According to Brännström, dentinal sensitivity features fluid movement within the dentinal tubules, leading to sensory activation of nerve cells in the pulp and thereby causing pain.[34,35]

The treatment of dentinal sensitivity includes the use of desensitizers, which is based on the interruption of the neural response to stimuli; this interruption is accomplished through occlusion of the exposed dentinal tubules, which inactivates the pain signals.[9] Pro-Argin technology, which uses 8% arginine and calcium carbonate, has proven effective in occluding the dentinal tubules.[10-15] Such technology may yield good results, since arginine and calcium occur naturally in saliva, and the combination of the 2 accelerates deposition of calcium and phosphate, and therefore occlusion of the tubules, creating a fine protective layer over the dentin and effectively reducing sensitivity.[36-42]

An alternative treatment for sensitive dentin is restoration of lesions with composite resin.[17-19] The present study aimed to investigate the bond strength

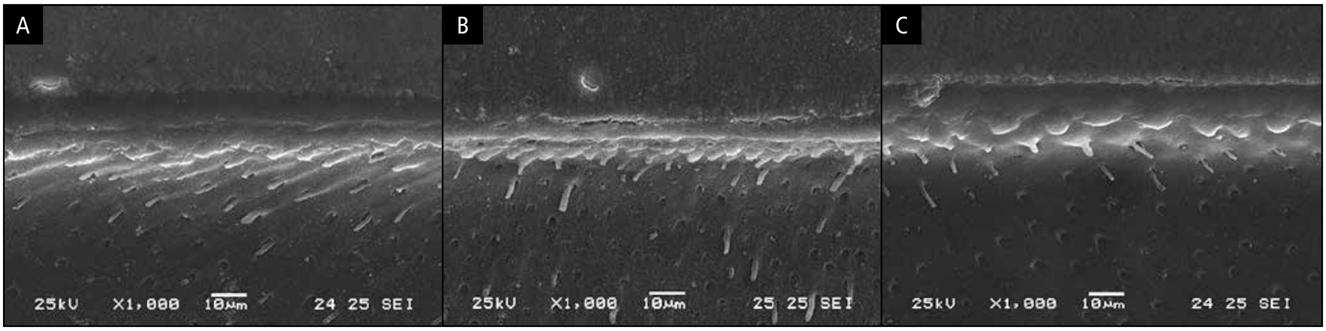

Fig 2. Scanning electron micrographs of groups restored with the etch-and-rinse system (Adper Single Bond) confirm the formation of a uniform hybrid layer, resin plugs, and tags, regardless of the toothpaste used. A. Brushing with artificial saliva. B. Brushing with arginine-free toothpaste. C. Brushing with arginine-containing toothpaste.

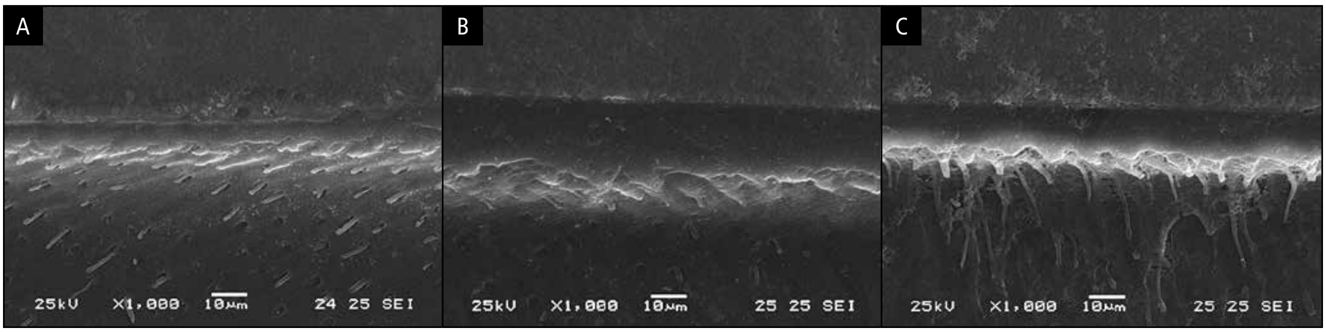

Fig 3. Scanning electron micrographs of groups restored with a 2-step self-conditioning adhesive system (Clearfil SE Bond) confirm the formation of resin tags and lateral extensions, regardless of the toothpaste used. A. Brushing with artificial saliva. B. Brushing with arginine-free toothpaste. C. Brushing with arginine-containing toothpaste.

of different adhesive strategies to eroded dentin. The study protocol included simulation of lesions that cause dentinal sensitivity and subsequent cycles of brushing with an arginine-containing toothpaste.[27] Dentin exposure to citric acid at pH 3.2, with or without magnetic agitation, results in exposed dentinal tubules. Furthermore, exposing dentin to artificial saliva, even for periods longer than 24 hours, does not lead to the occlusion of the tubules.[27] The process of tooth wear used by Absi et al resulted in a dentin surface similar to that of sensitive dentin in vivo.[43,44] The present study used the same protocol to guarantee that the substrate used was in fact eroded dentin, thus simulating the phenomenon of dentinal sensitivity.

The results of the present study did not demonstrate that arginine-containing toothpaste had a significant effect on the bond strength of eroded dentin compared to arginine-free toothpaste and artificial saliva. Therefore, the null hypothesis was accepted. These findings corroborate those of other studies both in human dentin and enamel.[45,46]

Likewise, no difference in performance was observed between an etch-and-rinse adhesive and a self-etching adhesive systems. Etch-and-rinse adhesive systems that use phosphoric acid remove the mineral phase of dentin.[47] Despite the moderate acidity of the etch-and-rinse adhesive system used (pH 4.3), the etching stage removed the smear layer, demineralized the superficial dentin, and exposed the collagen matrix.[48-50] This process also removed the arginine layer that had been deposited over the dentin, as demonstrated by the penetration of resin monomers in the tubules. The SEMs of specimens prepared with the conventional system illustrated and confirmed the formation of a uniform hybrid layer with resin plugs and numerous tags, which are characteristics of such systems (Fig 2).

The self-etching system used in the present study, Clearfil SE Bond, features moderate acidity (pH 2.1), which is capable of demineralizing a superficial layer of dentin, creating a porous surface needed for hybridization by micromechanical interlocking.[49] Additionally, the presence of the monomer 10-methacryloyloxydecyl dihydrogen phosphate optimizes a chemical interaction with free calcium (Ca^{2+}), which may have contributed toward the maintenance of bond strength values. The likely solubilization of the arginine and calcium carbonate layer may have reopened the dentinal tubules, thus allowing the formation of resin tags.[25] It is suggested that such characteristics may have been responsible for the similar values of bond strength to those of the conventional adhesive in this study as well as the bonding to the eroded substrate that had undergone brushing with arginine-containing toothpaste. Other authors have also reported similar values between both adhesive strategies (etch-and-rinse and self-etching), despite the difference in the substrate used.[51,52] The SEMs revealed that the 2-step self-etching adhesive system promoted the formation of resin tags and lateral extensions, typical of this bonding system (Fig 3).

The failure patterns observed were predominantly adhesive in all groups, corroborating previous findings.[53] In the earlier study, failures of bonding to eroded substrate were also predominantly of the adhesive type, although the pretreatment used was different from the pretreatment used in the present study. It is believed that eroded dentin promotes predominantly adhesive failure.

Conclusion

An arginine-containing toothpaste did not interfere with the bond strength of either a self-etching or a conventional etch-and-rinse adhesive system to eroded dentin, providing evidence that use of an arginine-containing toothpaste for the treatment of eroded dentin would not affect restorations placed clinically.

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