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# Bond Stability of a Universal Adhesive System to Eroded/ Abraded Dentin After Deproteinization

MG Augusto • CRG Torres • CR Pucci • N Schlueter • AB Borges

### Clinical Relevance

Sodium hypochlorite deproteinization may be a viable technique to enhance bond strength and maintain stability to eroded and eroded/abraded dentin.


## SUMMARY

**Objective: Erosive/abrasive challenges can potentially compromise bonding to dentin. Aiming to improve the quality and stability of bonding to this substrate, this study investigated the combined effect of erosion and toothbrush abrasion on the microtensile bond strength (μTBS) stability to dentin using a universal adhesive system in total and self-etching modes, associated or not associated with deproteinization.**

**Methods: Bovine dentin specimens were divided into five groups according to the organic matrix condition (n=20): control (C); erosion (E); erosion + abrasion (EA); erosion + sodium hypochlorite (EH); erosion + abrasion + sodium hypochlorite (EAH). The groups were further divided (n=10) according to the mode of application (total or self-etching) of a universal adhesive. After the bonding procedure, composite blocks were built up, and the samples were cut to obtain sticks for μTBS testing. For each specimen, one-half of the sticks was immediately tested, and the other one-half was tested after artificial aging (5000 thermocycles, 5°C and 55°C).**

**Results: Three-way analysis of variance (α=5%) showed a significant difference for the triple**



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**interaction ($p$=0.0007). Higher μTBS means were obtained for the EH and EAH groups compared with the E and EA groups. The control group showed immediate μTBS values similar to that of the E and EA groups for both bond strategies.**

**Conclusions: Erosion and erosion/abrasion did not significantly influence the immediate μTBS to dentin. Artificial aging reduced μTBS values for the groups C, E, and EA using the total-etching mode. Deproteinization maintained the bond stability to artificially aged eroded and eroded/abraded dentin.**

## INTRODUCTION

In the last decades, the emergence of therapies based on the preventive properties of fluorides allowed a significant decline in the prevalence of caries and, consequently, an increase in tooth longevity.[1,2] However, the growing consumption of acidic beverages leads to an increase of erosive wear risk, especially in adolescents.[3,4]

Recurrent erosive challenges can result in the loss of enamel and exposure of dentin. In the case of dentin erosive wear, restorative procedures may be necessary to recover esthetics and/or function, protect the remaining tooth structure, and prevent hypersensitivity.[5] Since erosive lesions are normally shallow and flat surface defects,[6] retention of the restorations will be mainly determined by the bond strategies used.

The surface of eroded dentin lacks minerals and presents a mesh of collagen fibrils (organic matrix).[7] Studies have shown that this organic matrix is able to slow down erosion progression, meaning that, to some extent, it has a protective effect against further erosive episodes.[8] Interestingly, the organic matrix is notably resistant to toothbrushing abrasion.[9] Toothbrushing with forces up to 4 N is not able to remove the organic matrix, but as previously observed,[10] this collagen mesh undergoes compression as the intensity of the force increases.

The compression reduces the interfibular spaces between the collagen fibrils, thereby increasing the density of the organic matrix, which may act as a physical barrier for adhesive penetration. Over time, the collagen fibers that were not impregnated by the adhesive will be susceptible to hydrolysis, creating areas rich in water at the hybrid layer, which favors nonuniform stress distribution and the development of interfacial defects.[11]

The recently developed universal adhesive systems can be used with or without previous acid etching. Most of them contain acidic functional monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), that bind directly to calcium and phosphate ions from the dental hard tissues, forming heavily bound nanolayers.[12,13] This stronger chemical interaction may be able to improve the bonding stability to eroded dentin.

One possibility to cope with the problem of the barrier represented by the thick eroded organic matrix is to deproteinize the eroded dentin surface, allowing a direct interaction of the adhesive to the intact dentin. The classic deproteinization technique proposes the application of sodium hypochlorite after acid etching in order to remove exposed collagen fibrils, producing a mineral surface similar to enamel.[14] Therefore, the removal of the organic matrix of an eroded dentin surface would potentially improve the quality of the adhesive interface as well as the bonding durability.[15-17] Nevertheless, there is a lack of evidence supporting this hypothesis.

Thus, this study investigated the combined effect of erosion and toothbrush abrasion on the immediate and post-aging microtensile bond strength (μTBS) to dentin, using a universal adhesive system in total and self-etching modes, associated or not associated with deproteinization.

The null hypothesis tested was that dentin deproteinization with sodium hypochlorite does not affect immediate or post-aging μTBS to eroded or eroded/abraded dentin when a universal adhesive system is applied with either bonding strategy (total or self-etching mode).

## METHODS AND MATERIALS

### Sample-Size Calculation

A pilot study was conducted to obtain mean and standard deviation data used to calculate the effect size ($f$). With the parameters of 0.05 as the significance level and 0.8 as the power test, using the G Power 3.1 statistical analysis software (Heinrich-Heine-Universität, Düsseldorf, Germany); a total of nine specimens per group was determined necessary. Taking into account the potential losses during the experiment, 10 specimens per group were prepared.

### Specimen Preparation

One hundred recently extracted bovine incisors stored in 0.1% thymol solution, pH 7.0, at 4°C, were used. The roots were removed using a diamond disc coupled to a low-speed handpiece, and the crowns were embedded in self-curing acrylic resin (Jet Classico, São Paulo, Brazil) inside polyvinyl chloride rings. The labial surfaces were ground flat using silicon carbide sandpaper P120 grit (Extec Corp, Enfield, CT, USA) coupled to a circular polishing machine (Panambra, São Paulo, Brazil) under constant water cooling, until an area of approximately 6 $mm^2$ of dentin was exposed. In order to standardize the surface roughness and smear layer, the specimens were polished in the circular polishing machine at a speed of 600 rpm for one minute using silicon carbide sandpaper P600 grit (Extec Corp) under constant water cooling, with a load of 6N (ISO/TS 11405, 2015).

### Experimental Group Division

The specimens were randomly assigned into five groups according to the organic matrix condition (n=20): control (C); erosion (E); erosion + abrasion (EA); erosion + sodium hypochlorite (EH); erosion + abrasion + sodium hypochlorite (EAH). The specimens of the control group remained in artificial saliva, four hours a day, for five days. During the intervals of the procedures, all specimens remained stored in 100% relative humidity.

### Erosion

The eroded groups were exposed to an erosive cycling procedure consisting of immersion in 0.5% citric acid (natural pH 2.6) for five minutes, four times a day, for five consecutive days. After each immersion, the specimens were rinsed with deionized water for 20 seconds and stored in artificial saliva for 1 hour. Artificial saliva was composed of 0.002 g/L ascorbic acid; 0.030 g/ L glucose; 0.580 g/L NaCl; 0.225 g/L $CaCl_2 \cdot H_2O$; 0.160 g/L $NH_4Cl$; 1.270 g/L KCl; 0.160 g/L NaSCN; 0.330 g/L $KH_2PO_4$; 0.200 g/L urea; and 0.426 g/L $Na2HPO_4 \cdot 2H_2O$.[18]

### Abrasion

The abrasion was performed twice daily (once after the first erosive impact and again after the last one), 15 seconds per specimen, using an automatic toothbrushing machine (SEM-2T, Odeme Dental Research, Luzerna, Brazil) that performed 30 strokes at a frequency of 2 Hz. A slurry containing fluoride-free toothpaste (Tom's of Maine, Kennebunk, ME, USA) and artificial saliva at a ratio of 1:3 (w/w) was used.[10] A brushing force of 2 N was applied.[19] Soft toothbrushes (Sanifill Ultra Profissional, Hypermarcas, São Paulo, Brazil) with nylon bristles and rounded tips were used. The brushes were fixed in the machine so that the long axes of the toothbrushes were at an angle of 12° to the direction of brushing, avoiding the formation of tracks induced by the bristles on the dentin surface.[20]

### Bonding Procedures

The groups were further divided into subgroups (n=10) according to the mode of application of the adhesive system: total-etching or self-etching. The universal adhesive system FuturaBond M+ (Voco, Cuxhaven, Germany) was applied in all groups. For the total-etching mode, a 37% phosphoric acid gel (Condac 37, FGM, Joinville, Brazil) was applied for 15 seconds and rinsed for 15 seconds; the dentin surface was then blot-dried using absorbent paper. For the self-etching mode, no additional etching was used.

When associated with the self-etching mode, the deproteinization was performed before the adhesive application. When associated with the total-etching mode, the deproteinization was performed after the acid etching. The 10% w/v sodium hypochlorite solution (pH 12) was actively applied and rubbed with a disposable applicator (Microbrush, KG Sorensen, Barueri, Brazil) for 60 seconds. The specimens were rinsed for 15 seconds and blot-dried using absorbent paper.[21]

The adhesive was actively applied for 20 seconds, following the manufacturer's instructions, air-dried for 10 seconds, and light-cured using a light-emitting diode device (Radii-cal, SDI, Bayswater, Australia) with an irradiance of 800 $mW/cm^2$ for 10 seconds.[17] Figure 1 shows the distributions in the experimental groups.

Blocks of composite (GrandioSO, Voco) were built up in two increments on the specimens' surfaces using a silicone matrix (6×6×4 mm). Each increment was light-cured for 40 seconds. After removal of the matrix, additional light-curing was performed for 40 seconds in two opposed lateral sides of the blocks. The specimens were stored in deionized water for 24 hours at 37°C to allow the postcuring of the composite. Table 1 shows the specifications of the materials used for the restorative procedures.

### Artificial Aging

In order to obtain sticks with a sectional area of approximately 1 × 1 mm, specimens were mounted

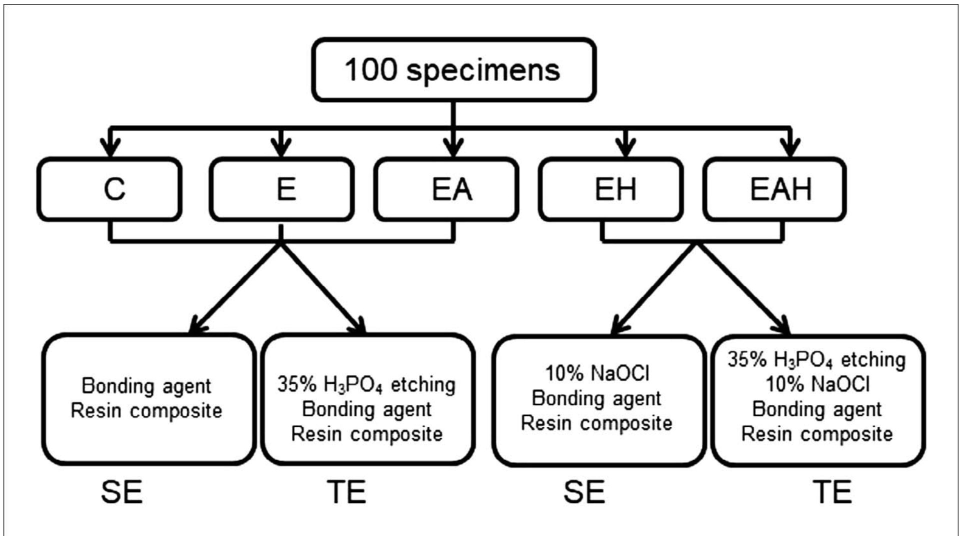


Figure 1. *Division of the samples according to each subgroup (n=10 each). C: control; E: erosion, EA: erosion + abrasion; EH: erosion + sodium hypochlorite; EAH: erosion + abrasion + sodium hypochlorite.*

in a serial cutting machine (Labcut 1010, Extec Corp) and sectioned using a water-cooled diamond disc.

One-half of the sticks obtained from each specimen were submitted to thermocycling (5000 cycles, 5°C-55°C, with the dwell time set at 15 seconds) before the microtensile test in order to simulate approximately 6 months of aging.[22] If an odd number of sticks was obtained from one specimen, the supernumerary stick was forwarded to the artificial aging one-half.

## Microtensile Bond Strength Test

The areas of the adhesive interfaces were measured with a digital caliper (accuracy: 0.001 mm; Mitutoyo, Kawazaki, Japan) and the values were used to calculate the bond strength values in MPa. The sticks were individually fixed on metallic holders using cyanoacrylate gel (Loctite 454, Henkel, Düsseldorf, Germany). The microtensile test was performed in a universal testing machine (DL-200MF, EMIC, São José dos Pinhais, Brazil), with a 10 kg load cell, at a constant speed of 1 mm/min.

## Failure Mode

Failures were classified as adhesive, cohesive in dentin, cohesive in resin, or mixed through optical stereomicroscopic observation with 60× magnification (Discovery V20, Karl Zeiss, Jena, Germany). Specimens that debonded during preparation or artificial aging (premature failures) were considered for the failure mode analysis but not for the mean μTBS values.

## Statistical Analysis

The statistical unit was considered the mean bond strength value obtained from the sticks of each tooth. Thus, statistical analysis was based on the mean bond strength of all the teeth from each subgroup. The results indicated that the residuals were normally distributed and, by plotting against pre-

Table 1: *Materials Used in Restorative Procedures*

| Product | Composition | Manufacturer | Batch Number |
|---|---|---|---|
| Condac 37 | Phosphoric acid gel 37% | FGM, Joinville, Brazil | 131213 |
| Futurabond M+ | UDMA, HEMA, MDP, CQ, BHT, and ethanol. | Voco, Cuxhaven, Germany | 503138 |
| GrandioSO | Resin matrix: Bis-GMA, BisEMA, TEGDMA, CQ, Amina, BHT. | Voco, Cuxhaven, Germany | 427541 |
| | Inorganic content: Nanoparticles of $SiO_2$: 20-40 nm; Glass ceramic: 1 μm | | |
| | Filler content: 89% (w/w) and 73% (v/v) | | |
| 10% sodium hypochlorite | 10% Sodium hypochlorite | Quimesp Química Ltda, Guarulhos, Brazil | 59146 |

*Abbreviations: BHT, butylated hydroxytoluene; BisEMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; CQ, camphorquinone; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogenphosphate, TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.*

dicted values, the uniformity was checked. None of the analysis of variance (ANOVA) assumptions were violated. Thus, three-way ANOVA was applied to evaluate the effects of the organic matrix condition, bond strategy, and artificial aging on μTBS, as well as their interaction. Post hoc pairwise comparisons were performed using the Tukey test. GraphPad Software (version 6.01, 2012) was used for the calculations, with a significance level of 5%.

### Scanning Electronic Microscopy

In order to provide a qualitative analysis of the dentin surfaces, additional specimens of each subgroup were prepared for scanning electron microscopy evaluation. Crowns of two bovine incisors were used, and five dentin specimens were obtained from each crown (one for each tested group). Their labial surfaces were polished with P600 silicon carbide sandpaper (Extec Corp) and treated as previously described. On the specimens of one crown, the adhesive system was applied according to the total-etching mode, and on the specimens of the other crown the self-etching mode was used. No light-curing was performed. For complete removal of the adhesive, the specimens were placed in plastic tubes and immersed in 2 mL of acetone for 10 minutes in an ultrasonic cube, with one change of the acetone after 5 minutes. The specimens were kept in a desiccation chamber for one week to remove any remaining water. Then, they were placed on aluminum stubs, sputter coated with gold (Emitech SC7620 Sputter Coater, Moorestown, NJ, USA), and observed in a scanning electron microscope (Inspect S50, FEI, Hillsboro, OR, USA) operating at 15-20 KV.

## RESULTS

### Microtensile Bond Strength

Three-way ANOVA showed significant bond strength differences for the organic matrix condition ($p=0.0001$), artificial aging ($p=0.0001$), and the interaction between them ($p=0.0007$). It did not show a significant difference for the bond strategy ($p=0.7843$).

The mean μTBS data (MPa, standard deviations in parenthesis) of the tested groups and results of the Tukey test for the triple interaction are shown in Table 2.

It is possible to observe that erosion associated or not associated with toothbrush abrasion, although presenting overall lower numerical μTBS means, did not statistically differ from that of the control group. Deproteinization with 10% sodium hypochlorite increased the bond strength of these groups.

Before aging, the bond strength means of each subgroup were similar for the total-etching and self-etching modes. Artificial aging decreased the bond strength values of groups C, E, and EA using the total-etching mode but not after deproteinization (EH and EAH).

### Failure Analysis

In groups C, E, and EA, the failure mode was predominantly adhesive, followed by mixed and cohesive in dentin. In groups EH and EAH, the failure mode was predominantly cohesive in dentin, followed by adhesive and mixed. After artificial aging, an increased number of premature failures was observed (Figures 2 and 3), especially in groups E and EA.

### Scanning Electron Microscopy

When the universal adhesive was applied using the self-etching mode, it is noticeable that the dentinal tubules are occluded by a smear layer in the control group (Figure 4A) and opened in the other subgroups (Figures 4B-E). When using the total-etching mode, the dentinal tubules are opened in all subgroups (Figures 4F through J). Regardless of the adhesive mode used, the deproteinized groups presented a characteristic surface pattern of a funnel shape around the dentinal tubules.

## DISCUSSION

The main finding of this study was that deproteinization can improve bond strength and maintain the bond stability to eroded and eroded/abraded dentin, thus rejecting the null hypothesis.

Physical and chemical mechanisms are associated with the improved bond strength values observed in groups EH and EAH. The original deproteinization technique (acid etching + sodium hypochlorite) increased the bond strength values[23,24] due to the ability of sodium hypochlorite to remove the collagen network exposed by the acid etching and the organic content from the mineralized underlying dentin. This exposes an extensive labyrinth of secondary tubules and anastomoses that can be penetrated by the adhesive,[16,25] increasing the substrate area of interaction and, consequently, improving the micromechanical retention of the adhesive.[17,26]

The tubule entrances of etched and deproteinized dentin have a characteristic funnel shape. This occurs because the peritubular dentin presents a

Table 2: *Mean Microtensile Bond Strength Data (Standard Deviation) and Results of the Tukey Test for All Subgroups[a]*

| | C | E | EA | EH | EAH |
|---|---|---|---|---|---|
| Immediate | | | | | |
| SE | 21.87 (5.20)AB ab | 19.45 (4.80)AB b | 18.17 (3.66)A b | 29.98 (2.88)A a | 30.29 (6.59)A a |
| TE | 25.74 (7.03)A ab | 26.42 (6.53)A ab | 20.12 (5.83)A b | 29.65 (5.79)A a | 29.28 (5.05)A a |
| After aging | | | | | |
| SE | 15.99 (3.15)B bc | 13.10 (4.04)BC c | 12.98 (4.49)AB c | 23.62 (2.91)A ab | 24.56 (5.62)A a |
| TE | 13.95 (5.56)B b | 7.10(5.03)C b | 6.87(3.97)B b | 25.85 (6.43)A a | 25.28 (5.14)A a |

*Abbreviations: C, control; E, Erosion; EA, erosion + abrasion; EAH, erosion + abrasion + sodium hypochlorite; EH, erosion + sodium hypochlorite; SE, self-etching; TE, total-etching.*
*[a] Different uppercase letters indicate differences between adhesive applications and aging conditions (in columns). Different lowercase letters indicate differences between organic matrix conditions (in rows) ($p<0.05$).*

higher mineralization than the intertubular dentin.[27] When acid etching is performed, the peritubular dentin is completely dissolved; the intertubular dentin, near the tubule's entrance, is also demineralized. When sodium hypochlorite is applied, it dissolves the exposed collagen around the tubules, creating the funnel shape.[26] In Figure 4 it is possible to see the funnel shape in both the EH and EAH groups. Although phosphoric acid was not applied in the self-etching mode, the dentin surface was previously etched during erosive challenges, creating a similar aspect.

The dentin deproteinization exposes numerous hydroxyapatite crystals that can potentially react with the adhesive monomer. The tested universal adhesive contains the functional monomer 10-MDP, which has a high affinity to deproteinized dentin and forms 10-MDP-Ca salts.[13] This chemical interaction may be one of the factors responsible for providing bond stability when using the universal adhesive in both modes.

Limited information is available regarding bonding to eroded dentin, and controversial results have been found. Previous studies observed lower bond strength values to eroded dentin than to sound dentin.[28-31] However this finding was not observed in the results of the present study, which is in agreement with the findings of other studies.[32,33] This divergence may be related to the differences in the aggressiveness of the erosive challenge (exposure time, pH of acid solution, type, and concentration of acid), storage time in the remineralizing solution, composition of the remineralizing solution, and adhesive system used. Moreover, the presence of the organic matrix depends on the moment that the last erosive challenge occurred. If it occurred shortly before the adhesive application, the collagen matrix will be present, but over time, the collagen fibrils can be partially or completely degraded by dentin-derived proteolytic enzymes[34] and nonspecific enzymes[35] (digestive enzymes like pepsin or trypsin), leading to an increase of erosion progression.

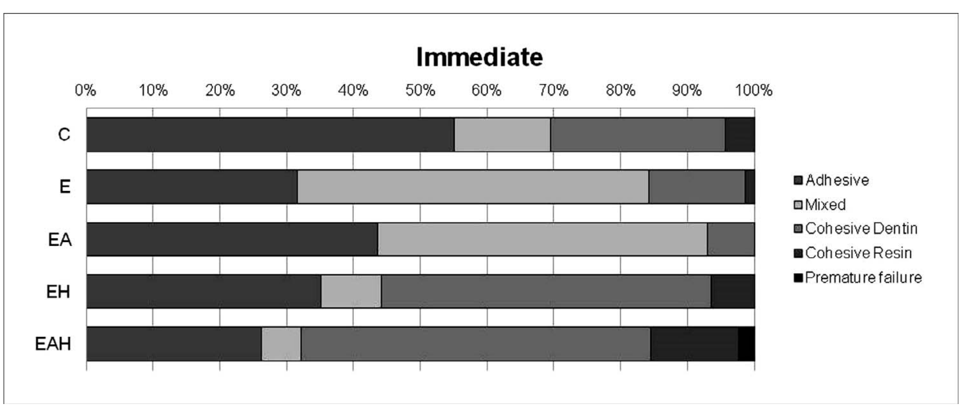


Figure 2. *Bar graph of the failure analysis results for the immediate groups.*

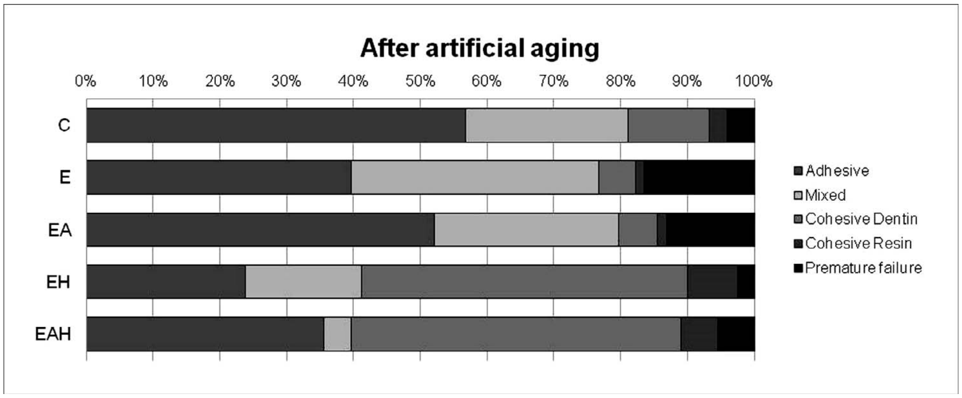


Figure 3. *Bar graph of the failure analysis results for the aged groups.*

Comparing the eroded and eroded/abraded groups, it is noticeable that toothbrushing abrasion in the conditions evaluated had no significant effect on μTBS values. This study was intended to simulate normal conditions, so the specimens were brushed with a 2 N load.[19] Although brushing with forces up to 4 N leads to compression of the organic matrix on the eroded dentin surface,[10] lower forces like those used in this study may not promote significant structural changes in this organic mesh.[9]

There is still some debate about which mode of application (total-etching or self-etching) is the most suitable when using universal adhesives in dentin, but it has been generally found that both techniques promote similar immediate bond strength values.[36-38] However, self-etching may be preferable in long-term *in vitro* studies.[39,40] Nevertheless, this has not been confirmed in clinical studies[41,42] and further long-term studies are necessary.

A previous study comparing the bond strength of a universal adhesive system applied by total-etching and self-etching modes found that the acid etching on sound dentin did not reduce the immediate μTBS values. However, it was observed by transmission electron microscopy that there were areas of low-quality hybridization after the total-etching approach, in particular in the form of a porous and poorly resin-infiltrated collagen mesh.[39] Thus, it was expected that over time the bond strength in the total-etching group would be lower than that of the self-etching group. Indeed, this is in agreement with this study's results, since lower bond strength values were observed after artificial aging in the C, E, and EA groups when the total-etching mode was used.

The inability of adhesive monomers to penetrate the whole depth of the organic matrix exposed by acid etching, associated or not with erosive challenges, creates areas rich in water and poor in adhesive monomers in the hybrid layer.[11] Variations in temperature during thermocycling cause mechanical stresses in these areas due to different coefficients of linear thermal expansion.[22] The stresses can directly induce crack propagation through the bonded interface and may be responsible for the lower μTBS values observed in total-etching and eroded groups. The degradation of the hybrid layer in these groups may be even greater since the phosphoric acid etching[43] and the erosive challenges[34] activate the metalloproteinases and cathepsins from dentin, which can hydrolyze the collagen fibrils and thus reduce the bond strength. A study comparing the bond stability of a universal adhesive by total-

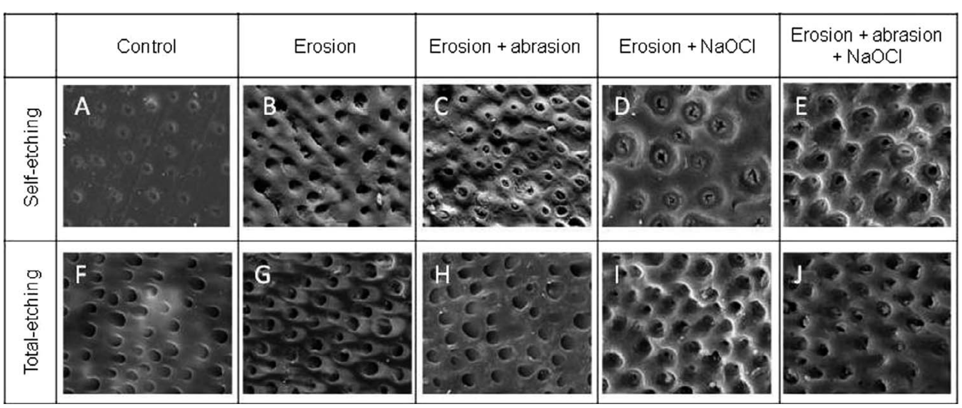


Figure 4. *Scanning electron microscopy images of the different organic matrix conditions when applying the universal adhesive using both adhesive techniques. Note that when using the self-etching mode, the dentinal tubules are occluded by smear layer in the control (A) and opened in the other subgroups (B-E). When using the total-etching mode, the dentinal tubules are opened in all subgroups (F-J). The deproteinized groups (D, E, I, J) present a characteristic funnel shape around the tubules.*

etching and self-etching modes showed that metalloproteinase activation occurs irrespective of the technique employed. However, higher bond strength values and lower nanoleakage have been reported with the self-etch mode.[40]

In deproteinized groups, a higher number of dentin cohesive failures were observed.[44] This may be related to the absence of a hybrid layer and consequent reduction of interfacial defects, which increases the bond strength and makes it closer to the cohesive strength of dentin. After artificial aging, a higher number of premature failures were observed in the E and EA groups, which is probably related to the presence of an increased amount of interfacial defects in these groups compared with the deproteinized ones. This finding reinforces the idea that deproteinization may be a viable alternative to improve not only the bond strength values but also the durability of the adhesion to eroded dentin.

Use of the deproteinization technique on eroded dentin seems to be promising and should be thoroughly investigated. Although some concern exists related to pulp toxicity, the outward dentinal fluid movement has a protective effect against the penetration of substances applied on the dentin surface,[45] reducing the possible cytotoxic effects of sodium hypochlorite. In addition, the remaining dentin thickness acts as a barrier contributing to the cytotoxicity reduction.[46,47] Indeed, this technique has promoted satisfactory clinical results with no significant difference in postoperative sensitivity compared with conventional adhesive technique, with up to 5 years of recall.[48,49]

However, it is important to consider some limitations of the sodium hypochlorite application. First, the stability of this agent is affected by storage conditions, such as temperature, time, and ultraviolet light.[50] Second, it increases the adhesive procedure duration, since 10% sodium hypochlorite must be applied for 1 minute because using lower concentrations of the solution or reduced a time resulted in incomplete collagen removal, leaving denaturated and disorganized organic residues on the surface, thus impairing the adhesive penetration.[51]

The results obtained in this study indicate that sodium hypochlorite is a promising technique to enhance bond strength to eroded dentin. However, it is important to keep in mind that additional clinical studies are needed to evaluate the long-term effect of the organic matrix on adhesion under *in vivo* conditions before adopting this protocol as a restorative therapy for eroded dentin lesions.

## CONCLUSION

Considering the limitations of this *in vitro* study, it was concluded that the combined effect of erosion and toothbrushing abrasion did not significantly influence the immediate μTBS values to dentin when using a universal adhesive in total-etching and self-etching modes. Artificial aging negatively affected bond strength to sound, eroded, and eroded/abraded dentin when the total-etching mode was used. The deproteinization technique using 10% sodium hypochlorite increased the μTBS values and maintained the bond stability to eroded and eroded/abraded dentin.

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### Regulatory Statement

This study was conducted in accordance with all the provisions of the local oversight committee guidelines and policies of the Institute of Science and Technology, São Paulo State University.

### Conflict of Interest

The authors of this manuscript 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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