

Three-Year Effects of Deproteinization on the *In Vitro* Durability of Resin/Dentin-Eroded Interfaces

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Clinical Relevance

The use of NaOCl at the interface of resin and eroded dentin may be a viable alternative to minimize the degradation of the etch-and-rinse and self-etch resin–dentin interfaces.

SUMMARY

Objective: To evaluate the effect of sodium hypochlorite on the immediate and three-year bonding properties of a resin-eroded dentin interface produced by one of two adhesive strategies.

Methods and Materials: Forty-eight molars were randomly assigned to six experimental groups, according to the combination of the adhesive strategy (etch-and-rinse and self-etch) and the dentin surface (control groups without erosion, eroded dentin surface [ED], and eroded dentin surface + NaOCl 5.2% [ED + NaOCl]).

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After completing restoration, specimens were stored in water (37°C) for 24 hours and then sectioned into resin–dentin beams (0.8 mm²) to be tested under tension (0.5 mm/min) immediately thereafter or after three years of water storage. To assess nanoleakage (NL), specimens were immersed in silver nitrate solution and examined by scanning electron microscopy at both time points. The dentin-etching pattern was examined under a scanning electron microscope. Data were subjected to appropriate statistical analysis ($\alpha=0.05$)

Results: In both strategies, a more pronounced and significant reduction of the microtensile

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bond strength (μ TBS) values was observed for the ED groups ($p=0.0001$) after three years. However, in the ED + NaOCl group, μ TBS values were maintained after three years of water storage. Furthermore, application of NaOCl to eroded dentin significantly reduced the immediate NL values and also preserved these values after three years of water storage for both adhesive strategies ($p>0.05$). When considering the ED group, a superficial removal of the smear layer and enlarged lumen tubules in comparison to control were present. However, for ED + NaOCl, there was a total removal of the smear layer and significant numbers of collagen fibrils were exposed.

Conclusion: The use of NaOCl may maintain the long-term stability of a resin-eroded dentin interface formed by etch-and-rinse and self-etch adhesives.

INTRODUCTION

Dental erosion is characterized by the loss of tooth structure due to the action of acids of nonbacterial origin in the short and long term. The prevalence of dental erosion has been increasing due to the increased consumption of acidic drinks, soft drinks, wine, and tea.^{1,2} Although erosion wear is typically restricted to the enamel,^{3,4} it can result in dentin exposure over time if the etiological factors are not controlled,⁵ leading to the necessity for restorative treatment.⁶

An eroded dentin surface has characteristics that differ from those of sound dentin.^{7,8} Erosion can remove dentinal plugs and organic intertubular dentin, resulting in increased tubule diameters. In addition, the eroded dentin surfaces have more exposed collagen fibrils and a reduced mineral content in the outermost layer,^{7,8} which represent additional challenges for dental adhesion. Furthermore, the superficial layer of exposed collagen is often inadequately infiltrated by resin monomers.⁷ When the hybrid layer formed during restoration involves incomplete infiltration by adhesive, the durability of the bonding interface may be jeopardized.⁹⁻¹¹

Although only a few alternatives for improving the longevity of eroded dentin have been tested,^{7,12,13} none of them have evaluated the removal of collagen fibrils by a deproteinization substance.¹⁴⁻¹⁷ Sodium hypochlorite (NaOCl) solution is the substance most commonly evaluated for its nonspecific deproteinizing effect.¹⁸ It is capable of producing chemical alterations that change the structure of demineralized dentin,

such as altering the dentin composition to make it more similar to enamel (hydroxyapatite rich).¹⁹ In addition, removal of unsupported collagen could have a beneficial effect on the spread and diffusion of primers and adhesives throughout the dentin²⁰ and may provide a more permeable substrate.

To date, the long-term stability of resin-eroded dentin interfaces after application of NaOCl has not been examined. The aim of the present study was therefore to compare the dentin microtensile bond strength (μ TBS) and the nanoleakage (NL) after application of sodium hypochlorite both in the short term (immediately) and in the long term (after three years) and the dentin-etching pattern. The null hypotheses tested were that pretreatment with NaOCl solution would not influence 1) the immediate and three-year μ TBS or 2) the immediate and three-year NL in eroded dentin.

METHODS AND MATERIALS

Selection and Preparation of Teeth

Forty-eight extracted, caries-free human molars were used. The ethics committee of the local university approved this research project (protocol no. 667.240). The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within six months after extraction.

The teeth were sectioned parallel to the occlusal surface using a low-speed diamond saw (Isomet, Buehler, Evanston, IL, USA) under water cooling to expose the mid-coronal dentin. All specimens had a smear layer standardized by polishing the flat dentin surface with 600-grit SiC paper under running water for 60 seconds.

Experimental Design

The teeth were then randomly assigned to six groups (42 dentin specimens to μ TBS and NL and 6 to the dentin-etching pattern) based on the combination of the main variables, that is, the adhesive strategies and the dentin surface. The adhesive strategies used were an etch-and-rinse adhesive (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA) and a self-etch adhesive (Scotchbond Universal Adhesive, 3M ESPE [also known as Single Bond Universal in some countries]) (Table 1). The dentin surfaces tested included a control group without erosion, an eroded dentin surface (ED), and an eroded dentin surface treated with NaOCl 5.2% (ED + NaOCl). In the control group, after preparation, all teeth were ready for restoration. In the other groups, an erosive protocol was performed prior to restoration.

Table 1: Adhesive System (Batch Number), Composition, and Application Mode

Adhesive System/Manufacturer (Batch Number)	Composition	Groups	Application Mode
Adper Single Bond 2 (ER) 3M ESPE, St Paul, MN, USA (395127BR)	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly (itaconic) acids, 10% by weight of 5-nm-diameter spherical silica particles	Control	<ol style="list-style-type: none"> 1. 37% phosphoric acid (Condac, FGM Dental Products, Joinville, SC, Brazil) for 15 s 2. Rinsing for 15 s and air-drying for 30 s 3. Apply two to three consecutive coats of adhesive for 15 s with gentle agitation 4. Gently air-dry for 5 s. 5. Light cure for 10 s at 1200 mW/cm²
		Eroded dentin (ED)	
Scotchbond Universal (SE) 3M ESPE(504834)	MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Control	<ol style="list-style-type: none"> 1. Apply the adhesive with a microbrush and rub it in for 20 s^a 2. Gently air-dry for 5 s 3. Light cure for 10 s at 1200 mW/cm²
		Eroded dentin + NaOCl 5.2% (ED + NaOCl)	
		Eroded dentin + NaOCl 5.2% (ED + NaOCl)	<ol style="list-style-type: none"> 1. Actively apply NaOCl for 40 s, washing, and drying 2. Apply the adhesive with a microbrush and rub it in for 20 s^a 3. Gently air-dry for 5 s 4. Light cure for 10 s at 1200 mW/cm²

Abbreviations: Bis-GMA, bisphenolglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, methacryloyloxydecyl dihydrogen phosphate.
^a The materials were applied according to the recommendations of their respective manufacturers.

Erosive Protocol

For eight days, teeth were exposed to erosive cycles by immersion in a demineralization solution of citric acid for five minutes, followed by immersion in a remineralization solution for 3.5 hours, repeated six times per day; teeth were then rinsed with demineralized water.⁷ The pH of the solutions was monitored periodically with the aid of a pH meter. The compositions of the demineralization and remineralization solutions are listed in Table 2.

Restorative Procedures

All step-by-step bonding procedures are described in Table 1. Composite resin buildups (Filtek Z350, 3M ESPE) were constructed incrementally (three increments of 1.5 mm each) and each increment light

activated for 40 seconds using a light-emitting-diode light-curing unit set at 1200 mW/cm² (Radii, SDI, Bayswater, Australia). A single operator carried out all bonding procedures. Seven teeth were used for each experimental group.

After storage in distilled water at 37°C for 24 hours, the specimens were sectioned longitudinally in mesial-to-distal and buccal-to-lingual directions across the bonded interface, using a low-speed diamond saw (Isomet) to obtain resin-dentin bonded sticks with a cross-sectional area of approximately 0.8 mm² as measured with digital calipers (Digimatic Caliper, Mitutoyo, Tokyo, Japan). The number of sticks showing premature failure during specimen preparation was recorded for each tooth. The bonded sticks originating from the same tooth were randomly assigned for immediate testing or

Table 2: Composition of Demineralization and Remineralization Solution (pH Cycling)^a

Solution (at 37°C)	Composition
Demineralization	0.5% citric acid with pH of 3.5
Remineralization	0.004 g ascorbic acid; 1.16 g NaCl; 0.34 g CaCl ₂ ; 0.32 g NH ₄ Cl; 2.54 g KCl; 0.38 g NaSCN; 0.64 g KH ₂ PO ₄ ; 0.64 g Na ₂ HPO ₄ dissolved in 1 l of demineralized water; pH is set to 6.4 with HCl

^a Adapted in accordance with Zimmerli.

testing after three years of storage in distilled water at 37°C. The distilled water was changed monthly.²¹

Microtensile Bond Strength

Resin–dentin bonded sticks were attached to a Geraldeli jig²² with cyanoacrylate adhesive and tested under tension (Kratos Dinamometros, Cotia, Brazil) at 0.5 mm/min until failure. The μ TBS values (MPa) were calculated by dividing the load at failure by the cross-sectional bonding area.

The failure mode of the resin–dentin bonded sticks was classified as cohesive (C; failure exclusively within the dentin or the resin composite) or adhesive/mixed (A/M; failure at the resin–dentin interface or failure at the resin–dentin interface with partial cohesive failure of the neighboring substrates). This classification was performed under a stereomicroscope at 100 \times magnification (SZ40, Olympus, Tokyo, Japan). Specimens with premature failures were not included in the tooth mean for statistical analysis.

Nanoleakage Evaluation

Three bonded sticks from each tooth that were not used in the microtensile test were evaluated for nanoleakage at each time point. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay and others.²³ The sticks were placed in ammoniacal silver nitrate solution in the dark for 24 hours, rinsed thoroughly in distilled water, and immersed in photo-developing solution for eight hours under a fluorescent light to reduce silver ions to metallic silver grains within spaces along the bonded interface. Specimens were polished with wet 600-, 1000-, 1200-, 1500-, 2000-, and 2500-grit SiC paper and 1- and 0.25-mm diamond paste (Buehler) using a polishing cloth. They were ultrasonically cleaned, air-dried, mounted on stubs, and coated with carbon-gold (MED 010, Balzers Union, Balzers, Liechtenstein). Resin–dentin interfaces were analyzed in a field-emission scanning electron microscope operated in the backscattered mode (LEO 435 VP, LEO Electron Microscopy Ltd, Cambridge, UK).

Three images of each resin–dentin bonded stick were captured.²⁴ The relative percentage of NL within the adhesive and hybrid layers in each specimen was measured in all images using the UTHSCSA ImageTool 3.0 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA) by a blinded researcher. The mean NL of all sticks from the same tooth was averaged for statistical purposes.

Dentin-Etching Pattern: Occlusal and Lateral Surfaces

For this part of the study, six teeth were used. A flat dentin surface was exposed on each tooth after wet grinding the occlusal enamel with 180-grit SiC paper. The enamel-free, exposed dentin surfaces were further polished with 600-grit silicon-carbide paper for 60 seconds to standardize the smear layer. The crowns of teeth were longitudinally sectioned in a buccal-to-lingual direction with a water-cooled low-speed diamond saw (Isomet). After that, each third was transversely sectioned in a buccal-to-lingual direction to obtain three slices per tooth (n=18 specimens). The unique difference was that for lateral analysis of the dentin-etching pattern, in half the teeth a precut groove was made on the pulpal side to allow segmentation.

Specimens from each tooth were divided according to control group without erosion, ED, and ED + NaOCl. Then the surfaces were rinsed with tap water for 30 seconds and air-dried with an air spray for five seconds, keeping the dentin wet. The specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 hours at 4°C, rinsed with 20 mL (outer diameter) of 0.2 M sodium cacodylate buffer at pH 7.4 for one hour, and dehydrated in ascending grades of ethanol: 25% (20 minutes), 50% (20 minutes), 75% (20 minutes), 95% (30 minutes), and 100% (60 minutes).²⁵

The specimens were segmented and sputter coated with gold-palladium in a vacuum evaporator (SCD 050, Balzers). The entire surface was examined under a scanning electron microscope (MIRA3 LM, Tescan Orsay Holding, Warrendale, PA, USA). Three photomicrographs of representative surface

Table 3: Number of Specimens According to Fracture Pattern Mode for Each Group^a

	Etch-and-Rinse Adhesive		Self-Etch Adhesive	
	Immediate	3 y	Immediate	3 y
Control	51/2/0	59/0/3	62/3/1	58/1/3
ED	50/0/3	62/1/6	57/0/3	61/2/5
ED + NaOCl	58/2/2	59/1/5	63/0/1	59/1/3

^a The numbers represent the number of bonded sticks that showed adhesive-mixed/cohesive/premature failures for all experimental conditions.

areas were taken at 2500× and 20,000× magnification.

Statistical Analysis

The experimental unit in the current study was the hemitooth since half the sample was tested immediately and the other half after three years. The μ TBS (MPa) and NL (%) values of all sticks from the same tooth were averaged for statistical purposes. The μ TBS and NL means for every test group were the average of the seven hemiteeth used per group. Data were compared by three-way repeated measures analysis of variance (adhesive strategies vs. surface treatment vs storage time) and the Tukey *post hoc* test for pairwise comparisons ($\alpha=0.05$).

RESULTS

Microtensile Bond Strength

The majority of specimens were classified as having had adhesive/mixed failures (93.6%). A low percentage of cohesive failures (1.54%) in the dentin and the resin composite occurred for both adhesives after three years. Although the number of specimens with pretest failures increased after three years, it did not reach statistical significance for any of the tested conditions (Table 3, data not shown).

The cross-product interaction was statistically significant ($p=0.0001$; Table 4). The control group for the etch-and-rinse adhesive showed the highest μ TBS values. On the other hand, for self-etch adhesive, higher μ TBS values were observed for

the eroded dentin after NaOCl application ($p=0.0001$; Table 4).

In the long term, for both adhesives, a more pronounced and significant reduction in the μ TBS values were observed in the ED groups ($p=0.0001$; Table 4). However, when NaOCl was applied to the eroded dentin, the μ TBS values were maintained after the three-year water storage period for both adhesive strategies tested ($p>0.05$; Table 4).

Nanoleakage Evaluation

The cross-product interaction was statistically significant ($p=0.001$; Table 5). When immediate NL values were observed, statistically significant differences in NL values were observed for the ED groups in both adhesive approaches as compared to control groups ($p=0.001$; Table 5). The application of NaOCl to the eroded dentin significantly diminished the immediate values of NL ($p=0.001$; Table 5) and also preserved the NL values after three years of water storage ($p>0.05$; Table 5). Application of NaOCl to the eroded dentin showed similar NL values to the control group for both adhesive strategies evaluated ($p>0.05$; Table 5).

Figure 1 shows scanning electron microscopic images representative of NL for all groups after immediate and three years. The amount of NL was lower and practically limited to the hybrid layer in the control group and the ED + NaOCl group. For the ED group, in contrast, the amount of NL was higher, with silver nitrate uptake occurring virtually throughout the entire thickness of the hybrid layer.

Dentin-Etching Pattern: Occlusal and Lateral Surfaces

Scanning electron microscopic images of the dentin surface of different groups are shown in Figure 2. For the control group (A and B), an irregular smear layer was observed on the dentin surface without any change in the tubular diameter. For the lateral view (C), it was possible to observe the presence of a smear layer in the lumen of the tubule. For the ED group, superficial demineralization of dentin was found with

Table 4: Mean Values (MPa) and Standard Deviations of Microtensile Bond Strength for Each Experimental Group^a

	Etch-and-Rinse Adhesive		Self-Etch Adhesive	
	Immediate	3 y	Immediate	3 y
Control	53.9 ± 3.4 A	45.7 ± 5.9 B	32.5 ± 2.0 D	30.1 ± 5.8 DE
ED	35.0 ± 3.7 C	26.2 ± 6.1 EF	34.5 ± 5.4 CD	21.4 ± 5.4 F
ED + NaOCl	33.4 ± 4.4 CD	27.3 ± 6.1 DE	42.9 ± 3.3 B	40.2 ± 3.1 B

^a Different letters show statistically significant differences ($p<0.05$).

Table 5: Mean Values (%) and Standard Deviations of Nanoleakage (NL) for Each Experimental Group^a

	Etch-and-Rinse Adhesive		Self-Etch Adhesive	
	Immediate	3 y	Immediate	3 y
Control	6.5 ± 2.5 A	12.3 ± 2.9 AB	9.3 ± 2.7 AB	11.3 ± 3.6 AB
ED	21.3 ± 3.3 c	43.8 ± 4.5 D	40.9 ± 2.9 D	46.4 ± 4.0 D
ED + NaOCl	11.9 ± 2.9 AB	14.2 ± 2.8 B	10.5 ± 2.5 AB	12.1 ± 3.0 AB

^a Different letters show statistically significant differences ($p \leq 0.05$).

absence of dentinal plugs, enlarged tubules, and a fibrous intertubular collagen network (A and B). The lateral view revealed the presence of a higher portion of the collagen fibril layer collapsed, and the border of the demineralized and the mineralized tissue (C). For the ED + NaOCl group, it was evident that the majority of the collagen network covering the intertubular dentin and the peritubular dentin was removed. This was also observed in the lateral view (C). An enlargement in the tubular diameter was also noted when compared to ED group (A and B).

DISCUSSION

The composition of eroded dentin differs structurally and chemically from that of sound dentin because of the mineral/organic composition.^{26,27} Eroded dentin is characterized by dissolution of the mineral component and the presence of a zone comprising a dense fibrous collagen network.²⁸ The presence of this highly disorganized collagen on the surface hinders adequate infiltration of the adhesive into the underlying dentin, affecting the quality of bonding.^{29,30}

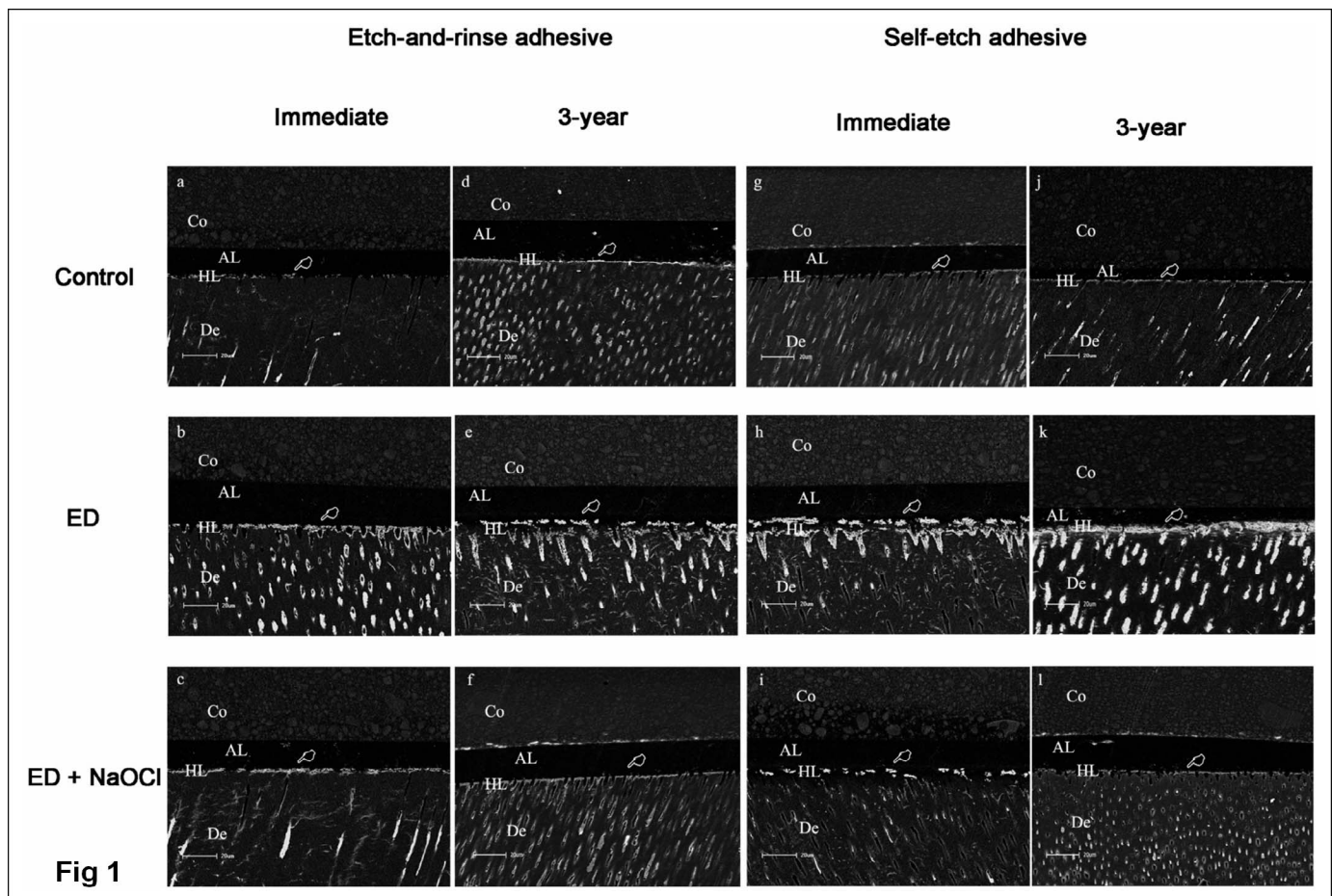


Figure 1. Backscatter scanning electron microscopic micrographs of the adhesive interface of the experimental groups. Silver nitrate deposit (white hands) in all groups is mainly within the hybrid layer. However, after three years of the water storage, this deposition was more pronounced in ED (e and k) when compared to control (d and j) and ED + NaOCl (f and l). Co, composite resin; AL, adhesive layer; HL, hybrid layer; De, dentin.

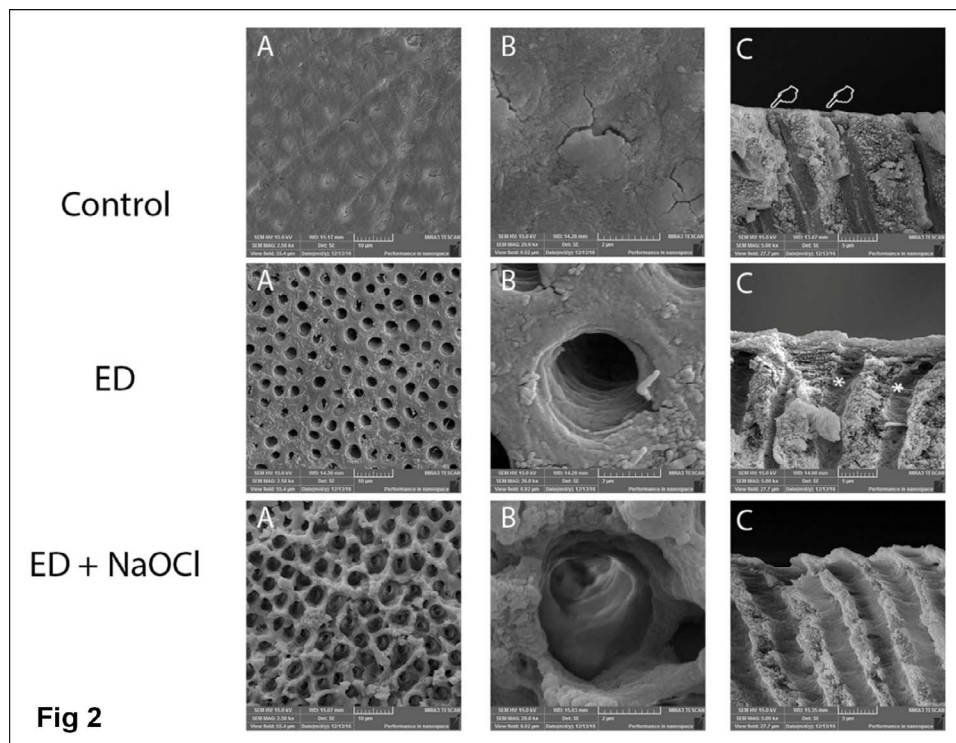


Figure 2. Scanning electron microscopic images of the dentin surface of the experimental groups. For the control group, a smear layer is observed covering all surfaces (A and B). It was possible to observe the presence of a smear layer in the lumen of the tubule (white arrows in C). For the ED group, decalcified organic matrix was found with enlarged lumen tubules (A and B). The lateral view revealed the presence of a higher portion of the collagen fibril layer collapsed and the border of the demineralized and the mineralized tissue (asterisk in C). For the ED + NaOCl group, the removal of the majority of collagen network covering the intertubular dentin and the peritubular dentin occurred, this was also observed in the lateral view (A-C).

In the present study, the appropriate hybridization of resin with the deeply eroded dentin surface, in the absence of NaOCl treatment, appeared difficult regardless of the adhesive strategy used, as confirmed by the significantly reduced μ TBS values and increased NL after three years of water storage. These findings are in accordance with those of Zimmerli and others.⁷ In their study, after one year of water storage, μ TBS values were significantly reduced for an eroded surface.⁷ This can most likely be attributed to the collapse of the demineralized collagen fibrils and to the higher water content, which prevented the adhesive from infiltrating fully as well as polymerizing properly. Such inefficient hybridization enhanced NL and consequently accelerated bond degradation.^{7,9,10}

It is known that dentin erosion can anticipate or potentiate proteolytic activity in dentin, similar to the process of carious lesion progression.³¹⁻³⁴ Metalloproteinases represent a family of zinc- and calcium-dependent endopeptidases present in the dentin and saliva that are capable of degrading extracellular matrix components, including collagen, in their native and denatured forms.³⁵ These enzymes are exposed and activated when dentin is solubilized.³⁵ This may also have contributed to the decrease in μ TBS values after three years of water storage in the eroded dentin groups. Thus, the poor infiltration of demineralized collagen and the increased activity of

the metalloproteinases in eroded dentin may have contributed to increased NL both immediately and after three years of storage.

On the other hand, when use of NaOCl was incorporated into the bonding protocol, the immediate and three-year performance of resin-eroded dentin interfaces was maintained or improved, leading to rejection of both null hypotheses. Sodium hypochlorite is a nonspecific proteolytic agent that effectively promotes dissolution of collagen and proteoglycans, thus facilitating the intertubular and intratubular infiltration of resin, which minimizes the degradation of the denuded collagen matrix.^{18,36-38} For this reason, NaOCl is usually indicated for use with an etch-and-rinse adhesive, particularly after applying the etch acid.^{18,36-38} The use of NaOCl also significantly alters the mineral content of dentin, leaving a smear layer of mineralized tissue, which increases the Ca/P ratio on the dentin surface,^{39,40} and yielding a dentin surface with characteristics similar to those of enamel.

However, it is noteworthy that the bonding efficacy to eroded dentin did not depend on the adhesive strategies applied when NaOCl was used in the bonding protocol. The etch-and-rinse adhesive maintained effective bonding at the resin-eroded dentin interface after three years of water aging. Similarly, when the self-etch adhesive was used,

although the performance was slightly better than that of the etch-and-rinse adhesive, the bond strength was also maintained after three years of water storage if NaOCl solution was first applied to the eroded dentin.

It has been reported that when NaOCl solution was applied to a smear layer-covered dentin, the mineral ratio increased and the smear layer became thinner due to dissolution of its collagen portion.⁴¹ This NaOCl-treated smear layer with fewer organic components may improve the bonding performance of adhesive systems.⁴²⁻⁴⁴

However, it is worth mentioning that a significant reduction of immediate μ TBS for the etch-and-rinse adhesive was observed when compared to the control group. The etch-and-rinse adhesive requires an acid pre-etching with phosphoric acid to demineralize the 6- to 9- μ m surface of the intertubular dentin and create porosities within the underlying collagen fibrillary matrix.⁴⁵⁻⁴⁷ It is known that NaOCl is able to remove the majority of collagen fibrils^{18,36,37} and transform a demineralized substrate to a more mineralized and porous substrate. Thus, we hypothesized that the lower amount of intertubular dentin created by the acid etching following the NaOCl pretreatment promoted the lower μ TBS. Furthermore, the excessive etching associated with the erosion, before the acid etching and NaOCl pretreatment, caused deeper demineralization of the intertubular and peritubular dentin, which challenged complete infiltration by the etch-and-rinse adhesive.⁴⁸⁻⁵⁰

On the other hand, the NaOCl pretreatment is capable of partially removing the organic components and thinning the smear layer, as observed in the scanning electron microscopic images.^{41,51} This might enhance the bonding performance of the self-etch adhesive system.^{44,52} A thinner smear layer with a reduced organic component might promote the infiltration self-etch adhesives into the smear layer and the underlying dentin, leading to higher bond strengths.^{43,44}

In addition, the compositional difference between adhesives may play an important role in adhesive performance. The self-etching adhesive used in the present study contained a 10-methacryloyloxydecyl dihydrogen phosphate monomer capable of chemically interacting with hydroxyapatite by forming a stable nanolayered adhesive interface.⁵³⁻⁵⁵ Furthermore, it contains a polyalkenoic acid copolymer in its formulation. Although these molecules may compete by binding to the calcium in hydroxyapatite,⁵⁴ they are usually associated with improved adhesive

performance,^{56,57} which may explain the good performance of this adhesive in the present study, both immediately and after three years of storage, in eroded dentin to which NaOCl was applied.

The slight increase in NL after three years of water storage in the control group and eroded dentin after application of NaOCl can be attributed to polymer degradation. Over time, water sorption and swelling of polymers facilitate leaching of hydrophilic monomers within the adhesive blends,^{58,59} thus contributing to an increase in the number of spaces within the polymer network^{24,58} and exposing additional collagen fibrils to activated MMPs.

Although the results observed in the present study are promising, when associating ethylenediaminetetraacetic acid and NaOCl or other chemical agents, promising results are usually shown in terms of bond strength to dentin.^{60,61} However, Dikmen and others⁶⁰ and Chauhan and others⁶¹ evaluated different chemical agents in noneroded dentin. Future studies are needed to compare the association of different chemical agents with NaOCl in the adhesive performance of eroded dentin.

Even considering the limitations of the *in vitro* methodology, which only partially simulated intraoral conditions, the long-term benefits of deproteinization presented herein are encouraging and can justify this as an additional step in clinical practice. However, the benefits of NaOCl may need verification by longitudinal clinical trials.

CONCLUSION

The use of NaOCl at the interface of resin and eroded dentin minimized the degradation of the etch-and-rinse and self-etch resin-dentin interfaces after three years of water storage.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the State University of Ponta Grossa. The approval code for this study is 667.240/2102.

Conflict of Interest

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

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REFERENCES

1. Jaeggi T, & Lussi A (2014) Prevalence, incidence and distribution of erosion *Monographs in Oral Science* **25** 55-73, DOI: 10.1159/000360973.
2. Schlueter N, & Tveit AB (2014) Prevalence of erosive tooth wear in risk groups *Monographs in Oral Science* **25** 74-98, DOI: 10.1159/000359938.
3. Murakami C, Oliveira LB, Sheiham A, Nahas Pires Correa MS, Haddad AE, & Bonecker M (2011) Risk indicators for erosive tooth wear in Brazilian preschool children *Caries Research* **45**(2) 121-129, DOI: 10.1159/000324807.
4. Dugmore CR, & Rock WP (2004) The prevalence of tooth erosion in 12-year-old children *British Dental Journal* **196**(5) 279-282; discussion 273, DOI: 10.1038/sj.bdj.4811040.
5. Jaeggi T, Gruninger A, & Lussi A (2006) Restorative therapy of erosion *Monographs in Oral Science* **20** 200-214, DOI: 10.1159/000093364.
6. Wang X, & Lussi A (2010) Assessment and management of dental erosion *Dental Clinics of North America* **54**(3) 565-578, DOI: 10.1016/j.cden.2010.03.003.
7. Zimmerli B, De Munck J, Lussi A, Lambrechts P, & Van Meerbeek B (2012) Long-term bonding to eroded dentin requires superficial bur preparation *Clinical Oral Investigations* **16**(5) 1451-1461, DOI: 10.1007/s00784-011-0650-8.
8. Prati C, Montebugni L, Suppa P, Valdre G, & Mongiorgi R (2003) Permeability and morphology of dentin after erosion induced by acidic drinks *Journal of Periodontology* **74**(4) 428-436, DOI: 10.1902/jop.2003.74.4.428.
9. Hashimoto M, Ohno H, Sano H, Kaga M, & Oguchi H (2003) Degradation patterns of different adhesives and bonding procedures *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **66**(1) 324-330, DOI: 10.1002/jbm.b.10010.
10. Sano H, Ciucchi B, Horner JA, Matthews WG, & Pashley DH (1995) Nanoleakage: Leakage within the hybrid layer. *Operative Dentistry* **20**(1) 18-25.
11. Vaidyanathan TK, & Vaidyanathan J (2009) Recent advances in the theory and mechanism of adhesive resin bonding to dentin: A critical review *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **88**(2) 558-578, DOI: 10.1002/jbm.b.31253.
12. Francisconi-dos-Rios LF, Casas-Apayco LC, Calabria MP, Francisconi PA, Borges AF, & Wang L (2015) Role of chlorhexidine in bond strength to artificially eroded dentin over time *Journal of Adhesive Dentistry* **17**(2) 133-139, DOI: 10.3290/j.jad.a34059.
13. Ramos TM, Ramos-Oliveira TM, de Freitas PM, Azambuja N Jr, Esteves-Oliveira M, Gutknecht N, & de Paula Eduardo C (2015) Effects of Er:YAG and Er,Cr:YSGG laser irradiation on the adhesion to eroded dentin *Lasers in Medical Science* **30**(1) 17-26, DOI: 10.1007/s10103-013-1321-6.
14. Pimenta LA, Amaral CM, Bedran de Castro AK, & Ritter AV (2004) Stability of dentin bond strengths using different bonding techniques after 12 months: Total-etch, deproteinization and self-etching *Operative Dentistry* **29**(5) 592-598.
15. Toledano M, Perdigao J, Osorio R, & Osorio E (2000) Effect of dentin deproteinization on microleakage of class V composite restorations *Operative Dentistry* **25**(6) 497-504.
16. Perdigao J, Lopes M, Geraldini S, Lopes GC, & Garcia-Godoy F (2000) Effect of a sodium hypochlorite gel on dentin bonding *Dental Materials* **16**(5) 311-323.
17. Barbosa de Souza F, Silva CH, Guenka Palma Dibb R, Sincler Delfino C, & Carneiro de Souza Beatrice L (2005) Bonding performance of different adhesive systems to deproteinized dentin: Microtensile bond strength and scanning electron microscopy *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **75**(1) 158-167, DOI: 10.1002/jbm.b.30280.
18. Marshall GW, Yücel N, Balooch M, Kinney JH, Habelitz S, & Marshall SJ (2001) Sodium hypochlorite alterations of dentin and dentin collagen *Surface Science* **491**(3) 444-445.
19. Tanaka J, & Nakai H (1993) Application of root canal cleaning agents having dissolving abilities of collagen to the surface treatment for enhanced bonding of resin to dentin *Dental Materials Journal* **12**(2) 196-208.
20. Prati C, Chersoni S, & Pashley DH (1999) Effect of removal of surface collagen fibrils on resin-dentin bonding *Dental Materials* **15**(5) 323-331.
21. Skovron L, Kogeo D, Gordillo LA, Meier MM, Gomes OM, Reis A, & Loguercio AD (2010) Effects of immersion time and frequency of water exchange on durability of etch-and-rinse adhesive *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **95**(2) 339-346, DOI: 10.1002/jbm.b.31718.
22. Perdigao J, Geraldini S, Carmo AR, & Dutra HR (2002) In vivo influence of residual moisture on microtensile bond strengths of one-bottle adhesives *Journal of Esthetic and Restorative Dentistry* **14**(1) 31-38.
23. Tay FR, Pashley DH, Suh BI, Carvalho RM, & Itthagarun A (2002) Single-step adhesives are permeable membranes *Journal of Dentistry* **30**(7-8) 371-382.
24. Reis A, Grande RH, Oliveira GM, Lopes GC & Loguercio AD (2007) A 2-year evaluation of moisture on microtensile bond strength and nanoleakage *Dental Materials* **23**(7) 862-870, DOI: 10.1016/j.dental.2006.05.005.
25. Kenshima S, Francci C, Reis A, Loguercio AD, & Filho LE (2006) Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer *Journal of Dentistry* **34**(10) 775-783, DOI: 10.1016/j.jdent.2006.03.001.
26. Lussi A, Schlueter N, Rakhmatullina E, & Ganss C (2011) Dental erosion—An overview with emphasis on chemical and histopathological aspects *Caries Research* **45**(Supplement 1) 2-12, DOI: 10.1159/000325915.
27. Zavgorodny AV, Rohanizadeh R, & Swain MV (2008) Ultrastructure of dentine carious lesions *Archives of Oral Biology* **53**(2) 124-132, DOI: 10.1016/j.archoralbio.2007.08.007.

28. Breschi L, Gobbi P, Mazzotti G, Falconi M, Ellis TH, & Stangel I (2002) High resolution SEM evaluation of dentin etched with maleic and citric acid *Dental Materials* **18**(1) 26-35.
29. Pashley DH, Ciucchi B, Sano H, & Horner JA (1993) Permeability of dentin to adhesive agents *Quintessence International* **24**(9) 618-631.
30. Wang Y, & Spencer P (2002) Analysis of acid-treated dentin smear debris and smear layers using confocal Raman microspectroscopy *Journal of Biomedical Materials Research* **60**(2) 300-308.
31. Hebling J, Pashley DH, Tjaderhane L, & Tay FR (2005) Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo *Journal of Dental Research* **84**(8) 741-746.
32. Tjaderhane L, Buzalaf MA, Carrilho M, & Chaussain C (2015) Matrix metalloproteinases and other matrix proteinases in relation to cariology: The era of "dentin degradomics" *Caries Research* **49**(3) 193-208, DOI: 10.1159/000363582.
33. Nascimento FD, Minciotti CL, Geraldini S, Carrilho MR, Pashley DH, Tay FR, Nader HB, Salo T, Tjaderhane L, & Tersariol IL (2011) Cysteine cathepsins in human carious dentin *Journal of Dental Research* **90**(4) 506-511, DOI: 10.1177/0022034510391906.
34. Komori PC, Pashley DH, Tjaderhane L, Breschi L, Mazzoni A, de Goes MF, Wang L, & Carrilho MR (2009) Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentin *Operative Dentistry* **34**(2) 157-165, DOI: 10.2341/08-55.
35. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjaderhane L, Mazzoni A, Breschi L, Foulger S, & Pashley DH (2009) Host-derived loss of dentin matrix stiffness associated with solubilization of collagen *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **90**(1) 373-380, DOI: 10.1002/jbm.b.31295.
36. Toledano M, Osorio R, Perdigao J, Rosales JI, Thompson JY, & Cabrerizo-Vilchez MA (1999) Effect of acid etching and collagen removal on dentin wettability and roughness *Journal of Biomedical Materials Research* **47**(2) 198-203.
37. de Castro AK, Hara AT, & Pimenta LA (2000) Influence of collagen removal on shear bond strength of one-bottle adhesive systems in dentin *Journal of Adhesive Dentistry* **2**(4) 271-277.
38. Inai N, Kanemura N, Tagami J, Watanabe LG, Marshall SJ, & Marshall GW (1998) Adhesion between collagen depleted dentin and dentin adhesives *American Journal of Dentistry* **11**(3) 123-127.
39. Baumgartner JC, & Mader CL (1987) A scanning electron microscopic evaluation of four root canal irrigation regimens *Journal of Endodontics* **13**(4) 147-157.
40. Baumgartner JC, & Cuenin PR (1992) Efficacy of several concentrations of sodium hypochlorite for root canal irrigation *Journal of Endodontics* **18**(12) 605-612, DOI: 10.1016/S0099-2399(06)81331-2.
41. Mountouris G, Silikas N, & Eliades G (2004) Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin *Journal of Adhesive Dentistry* **6**(3) 175-182.
42. Montagner AF, Skupien JA, Borges MF, Krejci I, Bortolotto T, & Susin AH (2015) Effect of sodium hypochlorite as dentinal pretreatment on bonding strength of adhesive systems *Indian Journal of Dental Research* **26**(4) 416-420, DOI: 10.4103/0970-9290.167633.
43. Fawzy AS, Amer MA, & El-Askary FS (2008) Sodium hypochlorite as dentin pretreatment for etch-and-rinse single-bottle and two-step self-etching adhesives: Atomic force microscope and tensile bond strength evaluation *Journal of Adhesive Dentistry* **10**(2) 135-144.
44. Taniguchi G, Nakajima M, Hosaka K, Iwamoto N, Ikeda M, Foxton RM, & Tagami J (2009) Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives *Journal of Dentistry* **37**(10) 769-775, DOI: 10.1016/j.jdent.2009.06.005.
45. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, & Tezvergil-Mutluay A (2011) State of the art etch-and-rinse adhesives *Dental Materials* **27**(1) 1-16, DOI: 10.1016/j.dental.2010.10.016.
46. Yousry MM (2012) Effect of re-etching oxalate-occluded dentin and enamel on bonding effectiveness of etch-and-rinse adhesives *Journal of Adhesive Dentistry* **14**(1) 31-38, DOI: 10.3290/j.jad.a22744.
47. Kanca J III (1992) Resin bonding to wet substrate. 1. Bonding to dentin *Quintessence International* **23**(1) 39-41.
48. Perdigao J (2001) The effect of etching time on dentin demineralization *Quintessence International* **32**(1) 19-26.
49. Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, & Oguchi H (2000) The effect of hybrid layer thickness on bond strength: Demineralized dentin zone of the hybrid layer *Dental Materials* **16**(6) 406-411.
50. Hashimoto M, Ohno H, Kaga M, Sano H, Tay FR, Oguchi H, Araki Y, & Kubota M (2002) Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems *Journal of Dentistry* **30**(2-3) 99-105.
51. Montes MA, de Goes MF, & Sinhoreti MA (2005) The in vitro morphological effects of some current pre-treatments on dentin surface: A SEM evaluation *Operative Dentistry* **30**(2) 201-212.
52. Hiraishi N, Kitasako Y, Nikaido T, Nomura S, Burrow MF, & Tagami J (2003) Effect of artificial saliva contamination on pH value change and dentin bond strength *Dental Materials* **19**(5) 429-434.
53. Yoshihara K, Yoshida Y, Nagaoka N, Fukegawa D, Hayakawa S, Mine A, Nakamura M, Minagi S, Osaka A, Suzuki K, & Van Meerbeek B (2010) Nano-controlled molecular interaction at adhesive interfaces for hard tissue reconstruction *Acta Biomaterialia* **6**(9) 3573-3582, DOI: 10.1016/j.actbio.2010.03.024.
54. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, & Meerbeek BV (2012) Self-assembled nano-layering at the adhesive interface *Journal of Dental Research* **91**(4) 376-381, DOI: 10.1177/0022034512437375.
55. Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, Sano H, & Van Meerbeek B (2005) Hydrolytic

- stability of self-etch adhesives bonded to dentin *Journal of Dental Research* **84**(12) 1160-1164.
56. Perdigao J, Sezinando A, & Monteiro PC (2013) Effect of substrate age and adhesive composition on dentin bonding *Operative Dentistry* **38**(3) 267-274, DOI: 10.2341/12-307-L.
57. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, & Van Landuyt KL (2011) State of the art of self-etch adhesives *Dental Materials* **27**(1) 17-28, DOI: 10.1016/j.dental.2010.10.023.
58. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials* **24**(1) 90-101, DOI: 10.1016/j.dental.2007.02.009.
59. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation *Journal of Dental Research* **90**(8) 953-968, DOI: 10.1177/0022034510391799.
60. Dikmen B, Gurbuz O, Ozsoy A, Eren MM, Cilingir A, & Yucel T (2015) Effect of different antioxidants on the microtensile bond strength of an adhesive system to sodium hypochlorite-treated dentin *Journal of Adhesive Dentistry* **17**(6) 499-504, DOI: 10.3290/j.jad.a35257.
61. Chauhan K, Basavanna RS, & Shivanna V (2015) Effect of bromelain enzyme for dentin deproteinization on bond strength of adhesive system *Journal of Conservative Dentistry* **18**(5) 360-363, DOI: 10.4103/0972-0707.164029.