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Influence of Matrix Metalloproteinase Synthetic Inhibitors on Dentin Microtensile Bond Strength of Resin Cements

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Clinical Relevance

Application of metalloproteinase synthetic inhibitors such as 2% chlorhexidine does not compromise immediate dentin bond strength of self-adhesive resin cements. The application of 24% ethylenediamine tetra-acetic acid (EDTA) gel increases dentin bond strength when self-adhesive resin cements containing methacrylated phosphoric acid ester monomers are used. Since EDTA is also considered a synthetic matrix metalloproteinase inhibitor, 24% EDTA gel might be a simple alternative to be used in conjunction with self-adhesive resin cements to improve their clinical performance.

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SUMMARY

This study evaluated the effect of dentin pretreatment with 2% chlorhexidine (CHX) or 24% ethylenediamine tetra-acetic acid gel (EDTA) on the dentin microtensile bond strength (μTBS) of resin cements. Composite blocks were luted to superficial noncarious human dentin (n=10) using two resin cements (RelyX ARC [ARC] and RelyX U100 [U100]) and three dentin pretreatments (without pretreatment–control, CHX, and EDTA). CHX was applied for 60 seconds on the acid-etched

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dentin in the ARC/CHX group, and for the same time on smear layer-covered dentin in the U100/CHX group. EDTA was applied for 45 seconds on smear-covered dentin in the U100/EDTA group, and it replaced phosphoric acid conditioning in the ARC/EDTA group for 60 seconds. After storage in water for 24 hours, specimens were prepared for microtensile bond strength testing. The results were submitted to two-way analysis of variance (ANOVA) followed by Tukey test. ARC produced significantly higher μTBS ($p<0.05$) compared to the U100, except when EDTA was used. For ARC, no pretreatment and CHX produced higher μTBS than EDTA. For U100, EDTA produced higher μTBS; no statistical difference occurred between CHX pretreatment and when no pretreatment was performed. While CHX did not affect immediate dentin bond strength of both cements, EDTA improved bond strength of U100, but it reduced dentin bond strength of ARC.

INTRODUCTION

The clinical success of indirect restorative procedures depends in part on the cementation technique used to create a stable link between the restoration and the different tooth structures. Resin cements have been widely used for this purpose, including fixation of inlays, onlays, crowns, posts, and veneers because of their enhanced mechanical properties, ease of handling, and good esthetic qualities. In this context, self-adhesive cements were recently introduced to simplify clinical practice as dentin acid-etching is not necessary. Currently, it is well accepted that a strong and stable union between the resin material and tooth substrate is highly important in determining the durability of the tooth-restoration complex.[1]

Despite many significant improvements in the past years, the bonded interface still remains the weakest area of the restorative complex.[2] Degradation of resin-dentin bonds, due to hydrolysis of the collagen fibrils, occurs over time[3-5] involving the participation of endogenous matrix metalloproteinases (MMP), which become entrapped within the dentin substrate during tooth development. When dentin is etched using phosphoric acid, latent MMP is denatured as rapidly as more proteases are exposed during dentin demineralization.[6] As a result, collagen fibrils that are not completely protected by resin monomers during dentin hybridization become highly susceptible to hydrolytic degradation over time, reducing bond strength. These gelatinolytic/collagenolytic enzymes can be produced by odontoblasts when a low pH environment is created along the dentin substrate.[7] Mild acids are known to activate MMP.[6] Low initial pHs are produced when resin adhesive materials composed of acidic monomers are coupled to dentin. An increase in MMP expression by the dentin pulp-complex,[7] as well as increased collagenolytic activity[8] to near-maximum levels,[6] can be expected when self-adhesive cements are bonded to dentin, similar to adhesive procedures involving self-etching adhesives, because residual unpolymerized acidic monomers might continue to etch the dentin substrate and activate MMP.[9] These events may contribute to resin-dentin bond degradation over time[6-8] when low pH adhesive resins such as self-adhesive resin cements are bonded to dentin. Even though most adhesive resin materials fulfill the necessity of a strong bond immediately after dentin coupling, bonding effectiveness naturally drops over time.[10-13]

Many approaches have been tested to increase the bonded interface durability by overcoming this self-degradation process. The use of matrix metalloproteinase synthetic inhibitors, such as chlorhexidine (CHX)[14-17] and ethylenediamine tetra-acetic acid (EDTA),[5,18] is a valid alternative as an attempt to prolong the resin-dentin bonding stability.[2,16] Nevertheless, little information involving self-adhesive resin cements and synthetic MMP inhibitors can be found. While CHX seems to impair self-adhesive resin cement bond strength to dentin,[19] EDTA does not affect dentin microtensile bond strength of self-adhesive resin cements.[20]

Dentin pretreatment with MMP synthetic inhibitors would benefit the dentin bond of self-adhesive resin cements over the course of time, as long as dentin bond strength is not immediately impaired. The purpose of this study was to assess the influence of a higher concentration of EDTA and 2% chlorhexidine digluconate, on the dentin microtensile bond strength of a self-adhesive resin cement and a conventional dual-cure resin cement. The hypotheses to be tested were: 1) 2% CHX has no influence on the immediate dentin bond strength of both a self-adhesive resin cement and a conventional resin cement; 2) 24% EDTA gel increases self-adhesive resin cement dentin bond strength and; 3) substitution of phosphoric acid etching with 24% EDTA gel does not affect dentin bond strength of conventional resin cements.

MATERIALS AND METHODS

Tooth Preparation

Sixty recently extracted human noncarious lower third molars stored at 4°C in saline solution with 0.2% sodium azide for up to one month were obtained after patient informed consent under a protocol analyzed and approved by the Ethical Committee of the Federal University of Uberlândia, Brazil. After disinfection and removal of soft tissues, a flat coronal dentin surface, perpendicular to the tooth's longitudinal axis, was ground flat using 180-grit SiC paper (Norton, Saint-Gobain Abrasives, Garulhos, SP, Brazil) and standardized with 600-grit SiC paper (Norton, Saint-Gobain Abrasives) for one minute under water cooling.

Cylindrical composite blocks were prepared using a nanofilled light-activated resin composite (Filtek Supreme Z-350, 3M ESPE, St Paul, MN, USA). Incremental layers measuring no more than 2 mm in thickness of composite were placed into a Teflon mold (5 mm in thickness and 10 mm in diameter) and individually light-cured using a quartz-tungsten halogen (QTH) unit (3M Curing Light, 3M ESPE) with irradiance of 550 mW/cm^2. To improve double-bond conversion, the cylindrical blocks were heat treated at 110°C for five minutes inside an inlay composite chamber (Fotoceram, Goiânia, GO, Brazil). One side of the composite block was abraded with 600-grit SiC paper (Norton, Saint-Gobain Abrasives) under water cooling to create a flat surface with standardized roughness and air-abraded with 50-μm aluminum oxide particles (Bioart, São Carlos, SP, Brazil) for 10 seconds, at four bars pressure and 10 mm away from the composite surface. The composite blocks were ultrasonically cleaned in distilled water for 10 minutes, rinsed with running water, completely air dried, treated with a prehydrolyzed silane solution (Prosil, FGM, Joinville, SC, Brazil), and blow dried before bonding.

Luting Procedures

Two resin cements were used: one self-adhesive luting cement that requires no substrate pretreatment (RelyX U100, 3M-ESPE) and one conventional dual-cured cement requiring previous dentin etching and application of an adhesive system prior to the luting procedure (RelyX ARC, 3M-ESPE) (Table 1). Teeth were randomly assigned to six groups (n=10): 1) ARC, 2) ARC/CHX, 3) ARC/EDTA, 4) U100, 5) U100/CHX, and 6) U100/EDTA. The groups were divided according to the following factors: resin cement (ARC or U100) and dentin pretreatment (CHX, EDTA or distilled water). Control groups were treated with distilled water instead of CHX or EDTA before dentin hybridization or luting with the self-adhesive cement. In all groups, moisture control was performed with sterilized lint-free 1-cm diameter absorbent papers (Mellita Clássico, Celupa Industrial Cellulose e Papel Guaíba LTDA, RS, Brazil) before and after dentin pretreatments: absorbent papers were gently placed on top of the flat dentin surface and replaced after five seconds until visible water was no longer absorbed and a dentin surface with a slightly glossy appearance was observed. This step was performed to remove excess moisture while ensuring adequate conditions for bonding of both resin cements. Adhesive procedures were then immediately carried out in a controlled environment with a temperature of 24°C ± 1°C and a relative humidity of 50% ± 5% using the same QTH unit used for the photocuring of the indirect restorations.

In group ARC/CHX, the flat dentin surface was etched with 37% phosphoric acid (Scotchbond Etchant, 3M ESPE) for 15 seconds and rinsed with water for 30 seconds; excess moisture was removed with absorbent paper. CHX pretreatment was performed and consisted of light-pressure circular rubbing movements of 2% chlorhexidine digluconate (Clorhexidina s, FGM) for 60 seconds, using a cavity brush (Cavibrush, FGM). Excess moisture was removed once again with absorbent paper, and one coat of primer (Adper Scotchbond Multi-Purpose, 3M ESPE) was applied actively for 10 seconds and gently blow-dried; this was followed by active application of one coat of adhesive (Adper Scotchbond Multi-Purpose, 3M ESPE) for 10 seconds and then light-activation for 10 seconds. The indirect restoration was then luted with RelyX ARC. In group U100/CHX, moisture control was performed and CHX was applied on the smear layer-covered dentin for 60 seconds with a cavity brush. Excess moisture was removed with absorbent paper and the indirect restoration was luted with RelyX U100.

For group U100/EDTA, moisture control was performed, EDTA 24% gel (E.D.T.A. Gel, Biodinâmica, Ibiporã, PR, Brazil) was carefully applied for 30 seconds on the smear layer-covered dentin with circular rubbing movements using a cavity brush and rinsed for 30 seconds. Excess moisture was removed and the indirect restoration was luted with RelyX U100. In group ARC/EDTA, moisture control was performed, EDTA was applied for 60 seconds with circular rubbing movements replacing the phosphoric acid-etching step and rinsed for 30 seconds. Excess moisture was removed and the

Table 1: *Material Brand Name, Composition, and Manufacturer*

Brand Name	Composition	Manufacturer
Scotchbond Etchant	37% phosphoric acid	3M ESPE Dental Products, St Paul, MN, USA
Adper Scotchbond	Primer: HEMA, polyalkenoic acid methacrylate copolymer Adhesive: Bis-GMA, HEMA, photo-initiators	3M ESPE Dental Products, St Paul, MN, USA
RelyX U100	Phosphoric acid methacrylates, dimethacrylates, inorganic fillers (72 wt %), fumed silica, initiators	3M ESPE Dental Products, St Paul, MN, USA
RelyX ARC	TEGDMA, Bis-GMA, zirconia/silica filler (67.5 wt%, initiators	3M ESPE Dental Products, St Paul, MN, USA
Filtek Z-350	Bis-GMA, UDMA, TEGDMA, ethyl methacrylates, inorganic fillers, photo-initiators	3M ESPE Dental Products, St Paul, MN, USA
E.D.T.A. Gel	24% EDTA	Biodinâmica, Ibiporã, PR, Brazil
Clorhexidina s	2% Chlorhexidine	FGM, Joinville, SC, Brazil

Abbreviations: Bis-GMA, bisphenol A-glycidyl methylmethacrylate; HEMA, hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate,UDMA, urethane dimethacrylate.

dentin hybridization and luting procedures were done as described for the ARC/CHX group.

In the control groups (ARC and U100), no dentin pretreatment with MMP synthetic inhibitors was performed: distilled water was applied to dentin to serve as a control treatment. Since both CHX and EDTA have water in their chemical compositions, we found it reasonable to submit control groups to this moisture factor to keep the experiment in a more controlled condition. An additional removal of excess water happened, returning the dentin to the same initial condition. In the ARC group, the same bonding procedures were carried out as in the ARC/CHX group except that CHX application was also replaced with distilled water for 60 seconds. Moisture control with absorbent paper, adhesive system application and luting with RelyX ARC were the same. In the U100 group, moisture control was performed, distilled water was applied on the smear layer-covered dentin for 60 seconds, moisture control was performed again, and the indirect restoration was luted with RelyX U100.

In all groups, following the application of the respective resin cement on the dentin surface, the composite resin blocks were placed on top of the flat dentin surfaces and received a constant seating pressure of 3 kg for three minutes[19] after which excess cement was removed and then light-activation was performed from four different directions for 40 seconds along the cement interface.

Microtensile Bond Test

After storage in distilled water at 37°C for 24 hours, the bonded teeth were sectioned (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) occlusogingivally into serial slabs and further into 0.9 mm $\times$ 0.9 mm composite-dentin sticks. Ten restored teeth were used in each group. Only central sticks were selected as peripheral sticks may not have had the same dentin thickness. Six central sticks were individually attached to a metallic grip with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and submitted to the microtensile bond test on a mechanical testing machine (DL2000, EMIC, São José dos Pinhais, PR, Brazil) at crosshead speed of 0.5 mm/min until failure using a Geraldeli device. The bond strength value for each tooth was determined by the microtensile bond strength average values including the six sticks from each tooth. After testing, the specimens were carefully removed from the grips with a scalpel blade, and the cross-sectional area at the site of the fracture was measured to the nearest 0.001 mm using a digital micrometer (Digimatic

Micrometer, Mitutoyo, Japan). Microtensile bond strength test values were expressed in MPa, and the data were submitted to two-way analysis of variance (ANOVA) followed by Tukey test. Statistical significance was set in advance at α=0.05, considering the tooth as the statistical unit. Sticks with pretest failures were recorded as null bond strengths, and those values were included in the statistical analysis.

SEM Failure Mode Analysis

After bond strength testing, fractured sticks were mounted on aluminum stubs, gold-sputtered under high-vacuum (MED 010, Balzers Union, Balzers, Liechtenstein) and analyzed using a scanning electron microscope (LEO 435 VP; LEO Electron Microscopy Ltd, Cambridge, UK). The work distances ranged between 18 and 22 mm, according to specimen height. Each specimen was classified according to the predominant remaining structure upon the dentin surface following the described failure mode classification:[19] 1: adhesive failure along the cement/dentin interface, 2: adhesive failure along the cement/composite interface, 3: cohesive failure within resin cement, 4: mixed failure of 1 and 3, and 5: mixed failure of 2 and 3.

RESULTS

ANOVA two-way analysis of variance revealed that resin cement ($p<0.001$), as well as the interaction between dentin pretreatment and resin cement ($p<0.001$), had significant effects on the microtensile bond strengths. RelyX ARC produced significantly higher microtensile values ($p<0.05$) when compared to the groups luted with RelyX U100, except when EDTA was used (Table 2). CHX pretreatment did not have significant effects on the dentin microtensile bond strengths ($p<0.05$) regardless of the resin cement used. In contrast to the distilled water and CHX pretreatment, dentin pretreatment with 24% EDTA gel significantly reduced dentin microtensile values ($p<0.05$) when Relyx ARC was used, and it increased bond strength ($p<0.05$) when dentin was luted with RelyX U100.

Cohesive failures within the resin composite or dentin substrate were not observed in any of the groups. Representative failure modes for RelyX ARC can be observed in Figure 1. In group ARC, the prevalent type of failure was adhesive along the cement/composite interface (2), followed by a mixed pattern of adhesive failure along the cement/composite interface and cohesive failure along resin cement (5), which were the same predominant failures observed in group ARC/CHX (Table 2). Fractured resin tags tightly occluding the enlarged dentinal tubule entrances after H_3PO_4-etching were observed when adhesive failure occurred along the cement/dentin interface (Figure 1F). The group ARC/EDTA presented predominately a mixed pattern of adhesive failure along the cement/dentin interface and cohesive failure along resin cement (4), followed by adhesive failures of the cement/dentin interface (1). Sparse enlarged tubule entrances were evident when adhesive failure along cement dentin interface occurred.

Table 2: *Overall Means and Standard Deviations of Microtensile Bond Strength Values for All Groups (MPa ± SD) and Fracture Modes. The Tooth Was Considered the Statistic Unit (n=10)[a]*

Pretreatment	RelyX ARC	RelyX U100
Distilled water	42.72^{aA} ± 4.3 (1/21/15/3/20)	12.98^{bB} ± 3.4 (6/1/26/22/2) N_0=3
Chlorhexidine	39.19^{aA} ± 7.7 (3/24/10/2/21)	11.56^{bB} ± 2.6 (10/1/14/29/1) N_0=5
EDTA	22.78^{bA} ± 5.6 (8/14/6/13/19)	18.61^{aA} ± 4.6 (3/4/30/17/4) N_0=2

Abbreviation: EDTA, ethylenediamine tetra-acetic acid.
[a] Values with different lowercase letters indicate significant difference according to Tukey test (p<0.05) when analyzed per column. Different capital letters indicate significant difference according to Tukey test (p<0.05) when analyzed per row. Numbers in parentheses are the number of specimens classified into five fracture modes in sequence (1/2/3/4/5): 1) adhesive failure along the cement/dentine interface; 2) adhesive failure along the cement/composite interface, 3) cohesive failure within resin cement, 4) mixed failure of 1 and 3; and 5) mixed failure of 2 and 3 (N_0=the number of premature failures).

Representative failure modes for RelyX U100 can be observed in Figure 2. In group U100, the prevalent mode of fracture occurred cohesively within the resin cement (3), which also was observed in group U100/EDTA. When CHX pretreatment was performed, RelyX U100 presented mainly a mixed pattern between adhesive failure in the cement/dentin interface and cohesive failure along resin cement (4), followed by cohesive failure of resin cement (3). When adhesive failures along the cement/dentin interface occurred for RelyX U100, no open dentinal tubules were evident irrespective of the dentin pretreatment. When CHX pretreatment was performed, a high number of bubbles could be observed when adhesive failure along the dentin/cement interface occurred (Figure 2F).

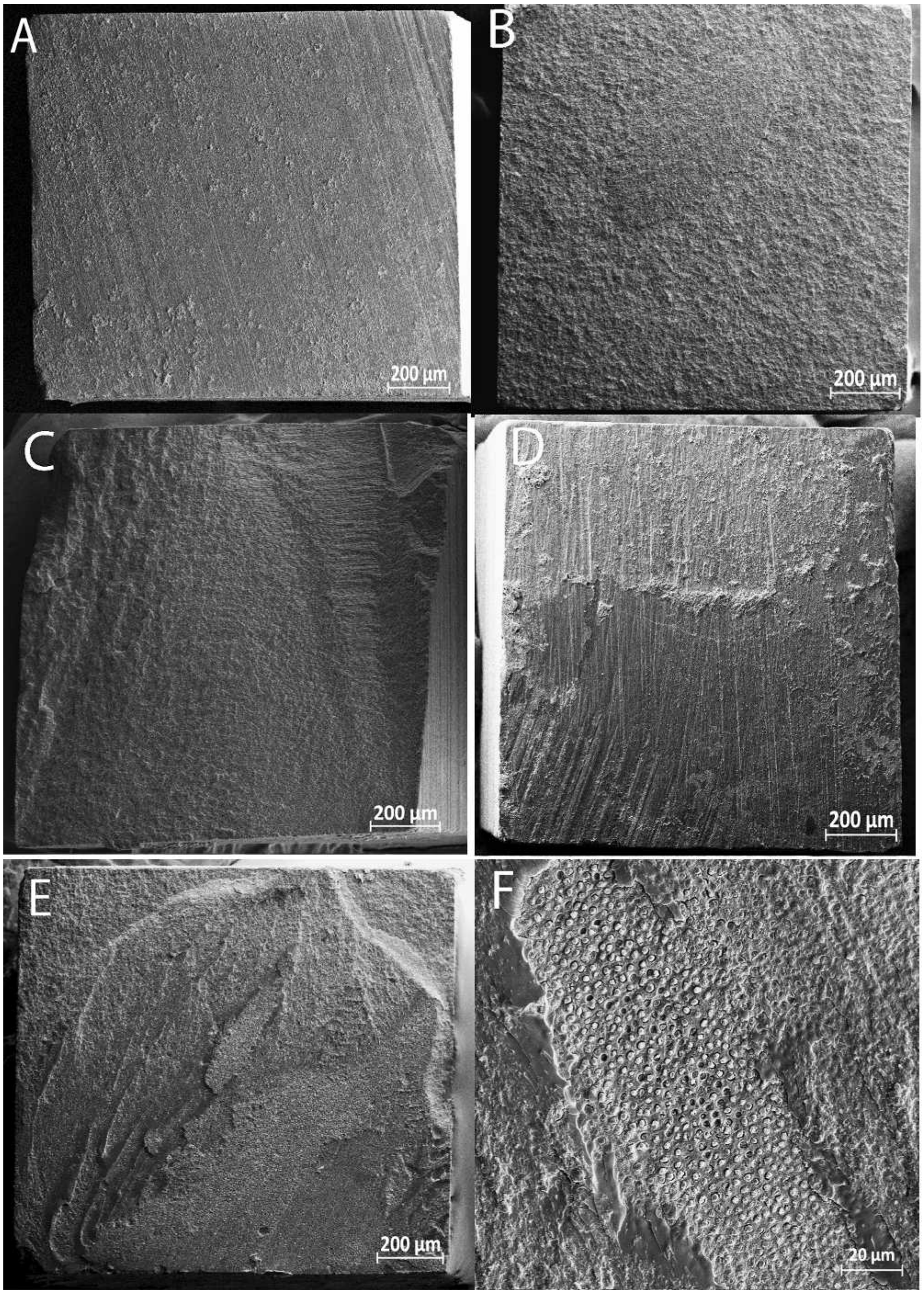

Figure 1. *Representative scanning electron micrographs for RelyX ARC failure modes. Dentin sides of fractured sticks are shown. (A): Adhesive failure along the cement/dentin interface (1). (B): Adhesive failure along the cement/composite interface (2). (C): Cohesive failure within resin cement (3). (D): Mixed failure of 1 and 3 (4). (E): Mixed failure of 2 and 3 (5). (F): Higher magnification showing a mixed failure at the base of the hybrid layer and cohesively along resin cement, with fractured resin tags tightly occluding the enlarged dentinal tubule entrances after H_3PO_4-etching.*

DISCUSSION

Since 2% CHX application produced no negative effects on the dentin microtensile bond strength of either the conventional or the self-adhesive resin cement, the first hypothesis is accepted. Conventional resin cement bonded to phosphoric acid-etched dentin exhibited higher microtensile values than self-adhesive resin cement, irrespective of CHX application, which can be explained by the differences in the bonding mechanism of both cements. Self-adhesive resin cements are composed of acidic monomers that

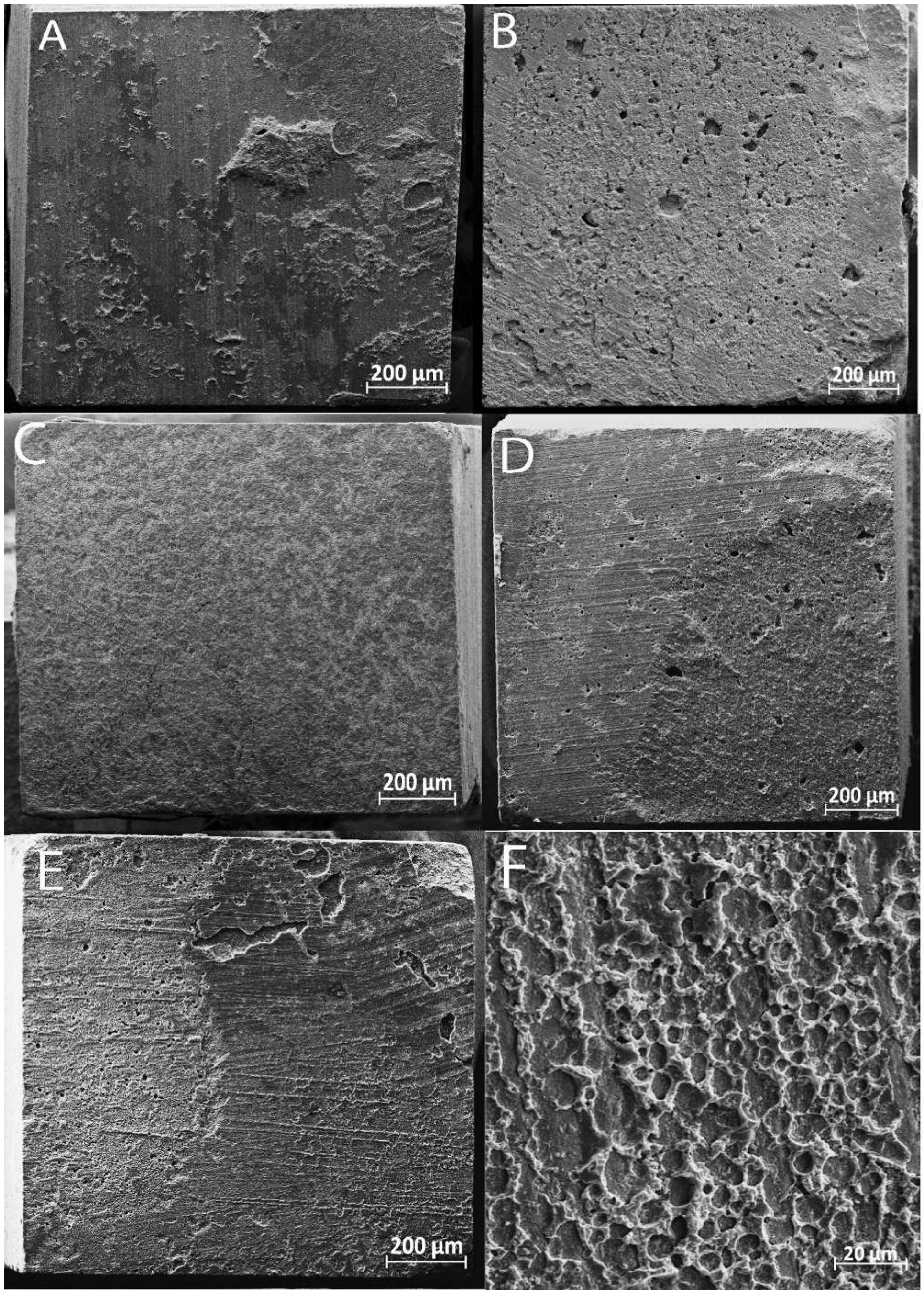

Figure 2. *Representative scanning electron micrographs for RelyX U100 failure modes. Dentin sides of fractured sticks are shown. (A): Adhesive failure along the cement/dentin interface (1). (B): Adhesive failure along the cement/composite interface (2). (C): Cohesive failure within resin cement (3). (D): Mixed failure of 1 and 3 (4). (E): Mixed failure of 2 and 3 (5). (F): Higher magnification of adhesive failures along dentin/cement interface when dentin was pretreated with chlorhexidine with formation of a high number of bubbles. Notice the absence of open dentinal tubules, which was observed in all specimens luted with RelyX U100 irrespective of dentin pretreatment.*

simultaneously demineralize and infiltrate the tooth substrate, resulting in micromechanical retention. This single step resin cement complies with the demand for simplification of luting procedures. Although this bonding strategy makes clinical practice easier, the absence of a dentin conditioning step creates a limited decalcified substrate and decreases resin monomer diffusion into dentin.[21] Even with a low initial pH of 2.1,[22] nearly no demineralization of the dentin surface below the smear layer is noticed after cement setting.[23,24] The relatively high viscosity of resin cement[25] associated with a low demineralizing capacity[24] contributes to low monomer infiltration into dentin, reducing micromechanical retention. Nevertheless, RelyX U100 has a dual-set polymerization reaction, a dual-cured redox-reaction for polymerization of the resinous phase, and an acid-base reaction. In this bonding mechanism, the calcium atoms present in the dentin hydroxyapatite act as electron acceptors promoting chemical union between the acidic resin monomers and the hard dental tissues,[26] resulting in the formation of calcium phosphates.[24] Such bonds do not exhibit a high bonding energy. As a consequence, low dentin microtensile values were obtained for the groups luted with RelyX U100. The use of CHX along with total etch adhesive resins does not impair their ability to bond to dentin.[14,16,27-31] This finding is in agreement with the present study where a bisphenol A-glycidyl methylmethacrylate (Bis-GMA)/hydroxyethyl methacrylate (HEMA) three-step etch and rinse adhesive system was used along with a conventional dual resin cement.

The priming step was performed with a HEMA-based primer that does not debond CHX molecules from the dentin substrate[32] allowing the CHX molecules to remain trapped within the base of the hybrid layer. In addition, a total-etch hybridization process involving twostep adhesive resins forms a superficial hydrophobic layer previous to the application of the resin cement. This layer acts as a barrier decreasing the possibility of CHX molecules located in the dentin substrate to chemically interact with methacrylate monomers present in the resin cement; any interference in the bonding process of conventional resin cements would be minimized. As a consequence, chlorhexidine application did not alter failure modes when dentin was luted with RelyX ARC. Similarly, CHX had no influence on the immediate dentin bond strength of tested self-adhesive resin cement. This finding is not in agreement with a previous study[19] where moisture removal was not properly performed and lower dentin bond strength values were obtained when CHX was used prior to the application of a self-adhesive resin cement with the same composition as RelyX U100. Water has a critical role in the effectiveness of self-adhesive resin cement bonding. It is generated during neutralization of functional groups modified by phosphoric acid and reused to react with acidic functional groups and ion-releasing basic filling bodies. However, when excess water is present, the polymerization reaction might be affected because the accumulation of oversaturated water droplets in the microvoids within the polymer network might decrease the cohesive strength of the self-adhesive resin cement. Since adequate moisture removal was performed, dentin pretreatment with CHX did not affect RelyX U100 bond strength. The prevalent mode of fracture for RelyX U100 occurred cohesively within the resin cement, which is in agreement with other studies.[19,20] When chlorhexidine was used, an increased number of mixed failures involving adhesive failure in the cement/dentin interface and cohesive failure along the resin cement occurred. In addition, resin cement remnants on the dentin side of fractured sticks presented a consistent bubbly pattern (Figure 2), which might suggest a possible interaction between RelyX U100 and CHX. Regardless, such interaction did not impair the immediate dentin bond strength of the resin cement. Further studies are needed to support the use of CHX as a widespread dentin pretreatment along with self-adhesive resin cements.

EDTA is a MMP synthetic inhibitor[18] that has been widely studied as a dentin-etching agent.[29,33-37] At neutral pH,[38] it is considered a mild chelating agent that produces different effects on dentin depending on its concentration and time of exposure.[39] EDTA dissolves dentinal mineral phase without shifting dentin proteins,[40] which avoids major alterations of the collagen fibrillar structure, conferring stability to the organic-matrix.[33,38,41] The extent of dentin demineralization is reduced when compared with phosphoric acid etching; hydroxyapatite is selectively removed, promoting partial removal of the smear layer. Maintenance of about 30% of the smear plugs and no morphologic alteration of the dentin surface are observed following application of 17% EDTA for 60 seconds.[37] The self-limited chelation reaction between EDTA and calcium present on the dentin substrate occurs in a ratio of 1:1, creating a stable EDTA-Ca compound responsible for dentin demineralization.[42] When dentin is etched with 30%-40% phosphoric acid, the smear layer is completely removed forming a layer of mineral-depleted collagen fibrils. Inadequate monomer infiltration of such compact collagen mesh impairs the dentin bond strength of self-adhesive resin cement to phosphoric acid-etched dentin.[43] Therefore, partial removal of the smear layer with EDTA solution was tested in a previous study, but no improvement in the self-adhesive resin cement-dentin bond occurred.[20] In the present study, an even higher EDTA concentration was tested to combine MMP synthetic inhibition properties with more pronounced smear layer removal[44] in an attempt to promote better interaction between resin cement and underlying dentin without compromising bond strength. As a consequence, dentin pretreatment with 24% EDTA gel significantly increased the self-adhesive resin cement bond to dentin, so the second hypothesis must be accepted. Statistically similar bond strengths were obtained between RelyX ARC and RelyX U100 when EDTA gel was used. The 24% EDTA gel improved the RelyX U100 dentin bond, while it decreased RelyX ARC bond strength. Nevertheless, RelyX ARC conventionally bonded to dentin still exhibited superior dentin bond strengths. In the ARC/EDTA group, an increase in mixed failures along the cement/dentin interface and resin cement occurred when compared to the ARC group. In this manner, the EDTA-treated dentin surface acted as a weak link to the bonded restoration. Sparse enlarged dentinal tubule entrances were evident when adhesive failures occurred at the dentin/cement interface, suggesting that adhesive system penetration into dentin was hampered. The adhesive system inability to properly infiltrate dentin certainly contributed to bond strength reduction when dentin was treated with EDTA. No differences in failure modes occurred between EDTA-treated and nontreated dentin when RelyX U100 was used. In specimens with adhesive failures at the dentin/cement interface, incomplete removal of the smear layer was observed when EDTA was used. Despite the use of a demineralizing agent, no open dentinal tubules and resin tag occlusion of dentinal tubule entrances were evident demonstrating low capacity of RelyX U100 to demineralize and infiltrate dentin.

From a clinical perspective, it remains to be proved if EDTA pretreatment will be able to effectively prevent resin-dentin bond degradation once it does not impair immediate self-adhesive dentin bonding. Consequently, longer term studies would be advisable. Dentin demineralization by acidic monomers may release some sequestered growth factors, which could, along with unpolymerized acidic monomers, diffuse through dentinal tubules stimulating the expression of MMP by odontoblasts,[7] possibly contributing to bond degradation. For these reasons, dentin pretreatment with MMP inhibitors might play an important role preserving the self-adhesive resin cement dentin bond. Regarding EDTA pretreatment and conventional resin cements, the third hypothesis was rejected because replacement of phosphoric acid etching for 15 seconds with 24% EDTA gel for 60 seconds reduced dentin bond strength of the conventional resin cement tested. Because one of the most important factors determining the bonding effectiveness of conventional resin cements is the adhesive system,[1] impairment of its capability to diffuse into substrate affects dentin bond quality. While self-etching adhesives seem to benefit from additional dentin pretreatment with mild chelating etchants,[34] conflicting results are found in the literature regarding total-etch adhesive systems when EDTA is used in place of phosphoric acid etching. Even though different application times (which may vary from 30 to 240 seconds) of EDTA 24% gel do not affect dentin shear bond strengths,[38] EDTA can improve,[45] impair,[34] or produce similar dentin bond strength values[29] to phosphoric acid etching depending on EDTA concentration and on the adhesive system used.[45] Lower dentin microtensile bond strength values were obtained when 24% EDTA gel replaced phosphoric acid etching prior to RelyX ARC luting. Since EDTA is a mild etchant, it may not sufficiently demineralize dentin as happens when lower pH etching agents such as phosphoric acid are used In addition, the chemical composition of adhesive systems is a determining factor influencing the ability to bond to EDTA etched dentin.[46] When acidic monomers capable of dentin demineralization, such as PENTA, which has a functional phosphoric acid group, are bonded to EDTA etched dentin, higher dentin bond strengths can be obtained.[45] However, the water-based adhesive system composed by HEMA, Bis-GMA, and polyalkenoic acid copolymers was not able to produce comparable bond strength values when dentin was etched conventionally with phosphoric acid or 24% EDTA gel for 60 seconds. Another relevant aspect that must be considered is EDTA delivery form. Even at a higher concentration, a 24% EDTA gel might not be able to etch dentin in the same manner as EDTA in aqueous solution due to its lower wetting capacity. This might explain why a lower concentration of EDTA produced equivalent microtensile bond strength to phosphoric acid etching when a one-step total etch adhesive with similar monomer composition was bonded to EDTA etched dentin.[29]

CONCLUSIONS

Based on the results and taking into consideration the limitations of this *in vitro* study, the following conclusions were drawn:

1. Application of 2% CHX does not compromise immediate microtensile dentin bond strength of self-adhesive resin cements.

2. Conventional resin cements bonded to phosphoric acid etched dentin produce higher bond strength compared to self-adhesive resin cements.
3. A 24% EDTA gel is a useful alternative to increase dentin bond strength of self-adhesive resin cements.
4. Dentin pretreatment with 24% EDTA gel for 60 seconds does not substitute for conventional phosphoric acid etching when resin cements are used along with HEMA/Bis-GMA adhesive systems.

Conflict of Interest Declaration

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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