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Performance of a Universal Bonding System Associated With 2% Digluconate Chlorhexidine in Carious and Eroded Dentin

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Clinical Relevance

Carious and eroded dentin represent clinical challenges. The use of a universal bonding system, in a self-etching mode, associated with chlorhexidine (CHX) seems to not improve its longevity. This may be attributed to the competition for calcium between the bonding agent functional monomer and CHX.

SUMMARY

Objectives: The purpose of this study was to explore the interaction between two calcium-dependent agents: 10-methacryloyloxydecyl-dihydrogen phosphate (MDP) and 2% chlorhexidine (CHX) digluconate in association with a self-etching universal bonding system.

Methods and Materials: Flat dentin surfaces were obtained from 120 specimens (n=20/group) prepared from extracted sound human third molars and randomly divided into three groups according to the dentin substrate: sound ([S] control), artificial carious ([C] 6 h/demineralization + 18 h/remineralization for 5 days + 48 h/remineralization), and artificial eroded ([E] 3 cycles for 5 min/day for 5 days using orange juice). Before bonding procedures, one-half of the specimens from each group were pretreated with deionized water (W) and the other half with

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2% chlorhexidine (CHX), forming six groups: S-W, S-CHX, C-W, C-CHX, E-W, and E-CHX. All specimens were restored with Adper Single Bond Universal (self-etching mode) and two increments of composite resin (Filtek Z-250), following the manufacturer's instructions. Slices (0.8 mm) were obtained from the specimens and subjected to scanning electron microscopy (SEM) analysis and sticks (0.64 mm²) were obtained and subjected to a microtensile bond strength test (μTBS) in a universal testing machine (0.5 mm/min) after 24 hours and 6 months of storage. Failure modes were classified using optical microscopy (40×). Data was statistically analyzed by three-way ANOVA and Tukey tests (p<0.05).

Results: Substrate type was a statistically significant factor (p<0.0001), whereas neither the pretreatment (p=0.189) nor time (p=0.337) were significant. No interaction considering all the factors was significant (p=0.453). Conclusions: Carious and eroded dentin substrates negatively interfered with the bonding potential of an MDP-based universal bonding system, regardless of the use of CHX. Likely, the reduction of available calcium impaired the effectiveness of the bonding system.

INTRODUCTION

The complexity of the dentin substrate has resulted in a large number of adhesive related studies.[1-4] This is due to the characteristics of a dynamic biological substrate that results in limitations to creating a long-term stable interaction between resin monomers and dentin to achieve an ideal adhesive interface.[5] In clinical practice, dentin substrates are frequently altered by caries and erosion, which creates a vulnerable substrate for adhesion.[1,3,4,6]

Dental caries is still the most common and challenging clinical dental disease.[7] Robust scientific studies and technological advances have pushed for more conservative interventional procedures such as the selective removal of the carious tissue.[8,9] Simultaneously, lifestyle changes have caused an aged, clinical appearance of teeth due to premature enamel loss. This scenario corresponds to an increase in the prevalence of noncarious lesions, such as erosion.[10-12] In this case, the dental surface is demineralized by acids (extrinsic or intrinsic), without bacterial involvement, which provokes the softening of the enamel, followed by its mechanical removal with progressive tissue loss, especially by toothbrushing.[10-12] The erosion process involves two phases. The first phase involves softening of the surface with mineral loss but without structural loss. The second phase involves the removal of this softened tissue, which occurs due to continuing erosion and/or mechanical processes.

Due to changes in these substrates, such as in mineral composition and the biological dynamic involving the organic matrix, the residual demineralized tissue can cause difficulties with bonding.[4,6,13] In this scenario, the bonding substrate can impair the long-term success of adhesion, regardless of the type of adhesive system.[6,13]

To restore these altered substrates, different types of dentin bonding systems are available and can be indicated specifically for each clinical situation. The most recently marketed category of bonding system was classified as universal adhesives.[14,15] Universal systems allow professionals to choose between two bonding methodologies: etch-and-rinse or self-etch. The ease of use of universal bonding systems combined with a promising bonding performance is clinically attractive.[15,16] Since one of the main ingredients in universal bonding systems is an acidic functional monomer, 10-methacryloyloxydecyl-dihydrogen phosphate (MDP), these systems enable the advantages of a chemically stable interaction with dentin and a reduction of sensitivity.[14,15] The deposition of stable MDP-calcium salts with the remaining mineral content seems to be responsible for this stability, due to the formation of a nanolayer that is less susceptible to hydrolysis.[17] Studies have shown increased bond strengths to dentin, attributed to this stable chemical salt formation.[14,16] Oliveira and others[3] demonstrated this optimized bonding performance using sclerotic dentin, which is particularly calcium-enriched.

Enzymatic activity also plays a relevant role in bond degradation and longevity.[18-23] The host matrix metalloproteinases (MMPs) can be activated with a low pH, such as found in carious or erosion processes. In the bonding procedures, MMPs are activated with mineral loss and the consequent exposure and nonprotection of the collagen fibrils due to incomplete monomer infiltration.[24-28] This fact may explain the progressive degradation of the hybrid layer over time.[25,26] As the action of MMPs depends on zinc and calcium, strategies that deprive these ions are being evaluated, such as the application of chlorhexidine (CHX) which presents a satisfactory antiproteolytic potential even at low concentrations.[27,29-31] Often, CHX has been used as a cleaning antimicrobial agent.[27] Its mechanism of action occurs by calcium chelation through the addition of sodium chloride that reverses or prevents the action of MMPs, especially MMP-2 and -9, both of which are present in the dentin substrate.[31] Among the types of

CHX, an aqueous solution of 2% CHX digluconate is the most accessible due to its low cost and can be used in the adhesive process after etching but before the adhesive application.[1,24,28,30,32-34] This methodology presents a high substantivity of the CHX in the organic matrix.[26] Some studies have shown that CHX provides a temporary effect, with 18-month substantivity.[35,36]

The combination of MDP and CHX could improve adhesion and increase the longevity of restorative treatments by minimizing the effects of degradation. However, current studies have demonstrated a possible interaction between these two components by observing precipitate formation near the adhesive interface, which may result negatively impact the interaction between monomers and dentin.[2,28,34,37] Therefore, considering the clinical challenges and possible interaction between these two beneficial strategies (MDP and CHX), more studies are needed to clarify their performance when used in combination on an altered dentin substrate.

The aim of this study was to investigate the microtensile bond strength of an MDP-containing universal bonding system used in self-etching mode combined with CHX in different clinical situations: sound, carious, and eroded dentin substrates. The null hypotheses tested were (1) there is no difference in bond strength to normal, carious, and eroded dentin substrate; (2) there is no difference in bond strength between pretreatment with deionized water or CHX; and (3) there is no difference in bond strength over 6 months, regardless of the substrate and pretreatment.

METHODS AND MATERIALS

Experimental Design

This laboratory study involved the analysis of three factors: substrate condition (three levels – sound [S, control], artificial carious [C], and artificial eroded [E] dentin), dentin pretreatment (two levels – deionized water [W] and CHX digluconate) and storage time (two levels - 24 hours and 6 months). The main response variable was the bond strength measured using a microtensile bond strength test. The failure mode was assessed using optical microscopy (40×), while SEM was used for additional qualitative analyses.

The sample size (120 specimens; n=20/group) was determined based on a pilot study. The current authors considered six groups, a power of 80%, and an $\alpha = 5\%$. Based on the effect size of 10 and an estimated standard deviation of 8, the sample size was calculated as 18. Also, 10% more was added, as losses could occur, resulting in n=20.

Specimen Preparation

The specimens with a flat dentin surface were randomized using Excel software. Extracted sound (caries and restoration-free) human third molars, obtained under the approval of the Local Institutional Ethics Committee (protocol CAAE 79124217.0.0000), were stored in a 0.1% salt solution of thymol at nearly 8°C until use. The occlusal enamel and roots were removed (perpendicular to the long axis of the tooth) using a water-cooled diamond disc (Isomet, Buelher Ltd. Lake Bluff, IL, USA). A 600-grit SiC abrasive paper was used under running water for 30 seconds (Politriz APL-4 AROTEC, Cotia, São Paulo, Brazil) to standardize the smear layer. The specimens were divided according to dentin substrate (S, C or E) and pretreatment (W or CHX) to constitute the groups: S-W, S-CHX, C-W, C-CHX, E-W, and E-CHX.

The control group (S) was maintained in artificial saliva (1.5 mM $Ca[NO_3]_2$-$4H_2O$, 0.9 mM $NaH_2PO_4.2H_2O$, 150 mM KCL, 0.1 mol/L Tris, 0.05 ppm F, pH 7.0). The C lesions were produced by 6-hour cycles in demineralization solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50.0 mM lactic acid buffer, pH 5.0),[38] followed by 18 hours in remineralization solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130.0 mM KCl, 20 mM HEPES buffer, 5.0 mM NaN_3, pH 7.0), which simulated the clinical situation of the carious process with an imbalance of demineralization and remineralization.[39] Each specimen was immersed in 30 mL of solution for each cycle. The solutions were renewed each day for 5 days, followed by 48 hours of incubation in a remineralizing solution, which was also renewed daily, for a total of 7 days of immersion. To create the E lesions, the specimens were immersed in industrialized orange juice at a pH of 4.0 (Suco Del Valle do Brasil, Leão Alimentos e Bebibas Ltda, Linhares, Espírito Santo, Brazil) that was composed of reconstituted orange juice, dietary fiber (acacia gum), vitamin C, and natural aroma. The specimens were immersed for 5 minutes, 3 times a day, for 5 days and stored in artificial saliva at all other times. Orange juice was selected for this step since it is a popular beverage in the population and is easy to use.[2] Both altered substrates were assessed by transverse microradiography after the challenge was completed to validate the formation of caries and erosion in dentin. In the C specimens (Figure 1), a thin demineralized subsurface was found with preservation of the outer surface, while eroded dentin revealed a superficial loss (Figure 2).

For the restorative treatment, as per the manufacturer's directions, enamel-selective acid-etching with 37% phosphoric acid gel (Dentscare LTDA, Joinville, Santa

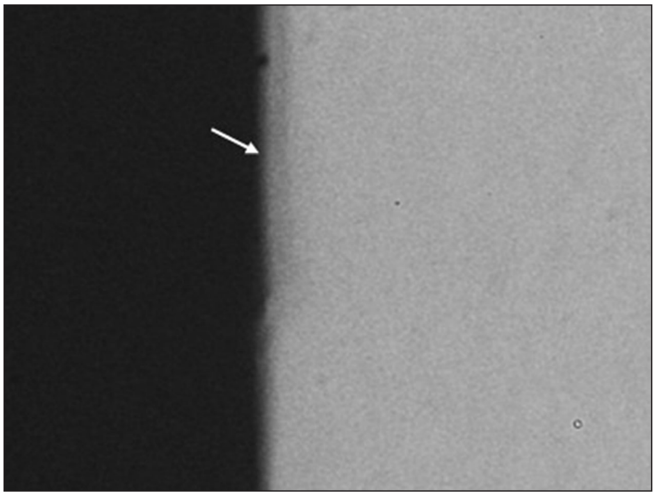

Figure 1. *Transverse microradiography representative image of artificially carious dentin substrate. A subsurface lesion is observed.*

Catarina, Brazil) was performed for all specimens for 30 seconds, followed by abundant washing with water for 30 seconds, and drying with absorbent paper (wet technique). Dentin was not etched, since the self-etching method was selected for this study. The specimens from the two dentin substrate pretreatment groups received an application of W or CHX aqueous solution at pH 5.8 (Sigma-Aldrich, Saint Louis, MN, USA). After 30 seconds of passive application, the excess was removed with absorbent paper. In sequence, the universal bonding system (Adper Single Bond Universal, 3M ESPE, St Paul, MN, USA; see Table 1) was applied following the self-etching protocol:

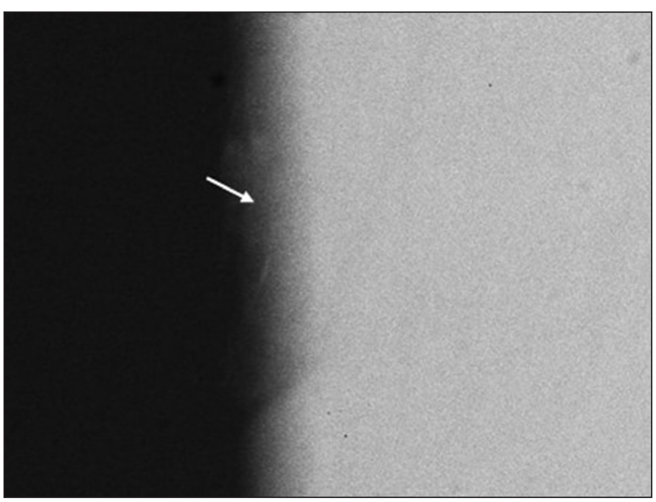

Figure 2. *Transverse microradiography representative image of artificially eroded dentin substrate. Different from Figure 1, the external surface is not intact.*

Table 1. *Composition of Adhesive System – Adper Single Bond Universal*

Adhesive system	Composition
Adper Scothbond Universal (3M ESPE, St. Paul, MN, EUA)	Methacryloyloxydecyl dihydrogen phosphate, dimethacrylates, 2-Hydroxyethylmethacrylate, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane.

rubbing for 20 seconds and solvent evaporation for 5 seconds. The bonding agent was light cured using a 1000 mW/cm^2 LED unit (Radii-Call, SDI, Bayswater, Victoria, Australia) for 10 seconds. Irradiance was checked after every 5 activations. The composite resin was placed in two increments of 2 mm layers (Filtek Z350 Universal Restorative, 3M ESPE, St Paul, MN, USA), which were both light cured for 20 seconds. The specimens were stored in artificial saliva for 24 hours at 37°C. All procedures were performed according to the manufacturer's instructions and by the same operator.

Scanning Electron Microscopy

After 24 hours, the specimens were longitudinally sectioned, perpendicular to the bonding interface, using an Isomet 1000 digital saw (Buehler, Lake Bluff, IL, USA) to obtain slices of approximately 0.8 mm thickness. One slice from each subgroup was randomly selected for initial scanning electron microscopy (SEM) (24 hours) and 6 month analysis. The slices were stored in artificial saliva until time of observation. Then, they were immersed in an 18% hydrochloric acid solution for 30 seconds to remove the superficial smear layer, washed for 30 seconds in water, followed by immersion in a 5% sodium hypochlorite solution for 15 minutes to remove all noninfiltrating collagen by the dentin bonding system and subsequently washed for 30 seconds. The specimens were then dried for 12 hours at room temperature and then mounted on aluminum stubs to be sputter coated with palladium gold (DentronVaccum, Desk IV Moorestonn, NJ, USA). The adhesive surface of all specimens was analyzed using SEM (JSM – T22OA, JOEL LTDA, Tokyo, Japan) at a magnification of 1,500×.[40,41]

Microtensile Bond Strength Test

The test followed the current guidance.[42,43] The remaining slices of the restored specimens were again longitudinally sectioned to prepare resin–dentin sticks of approximately 0.64 mm^2 area (0.8x0.8 mm) verified using a digital caliper (Mitutoyo Americana LTDA, Aurora, IL, USA). The sticks were then fixed to a device (JIG 1 – Plus, Odeme Dental Research, Luzerna, Santa Catarina, Brazil) using cyanoacrylate resin (Super Bonder Power Flex Gel – Loctite, Henckel LTDA, Itapevi, São Paulo, Brazil) and tested in tension with a universal testing machine (Instron 3342, Instron Co., Canton, MA, USA) at a 0.5 mm/minute crosshead speed and with a 500 N load cell. The µTBS was expressed in MPa by dividing the maximum load (kgf) by the specimen cross-sectional area (mm^2). For this test, the operator was blinded regarding the stick group. During aging, the sticks were stored in weekly-renewed artificial saliva at 37°C.

Each fractured surface was analyzed with a handheld digital microscope (Dino-Liteplus digital microscope, AnMo Electronics Corp, Hsinchu, China) at approximately 40× magnification and failure classified as adhesive, cohesive in dentin, cohesive in composite resin, or mixed.

The experimental unit considered was the tooth, so the sticks of each tooth were divided into two groups for the initial and six month evaluations. The average µTBS value for each tooth and time based on all the sticks was calculated and the premature failures were considered as zero for calculating the mean values. For the statistics analysis, the data satisfied the assumptions of a normal distribution and the equality of variance was tested for all the variables using Statistica software (Statsoft, Tulsa, OK, USA). Data was analyzed using three-way ANOVA and multiple comparison tests (Tukey test) with $\alpha = 0.05$.

RESULTS

Bond strength mean values and standard deviations are shown in Table 2. The type of substrate was the only significant factor ($p<0.0001$). Pretreatment ($p=0.189$), time ($p=0.337$), and the interaction between all the factors ($p=0.453$) were not statistically significant.

Overall, the results suggest that the sound dentin substrate consistently provided the highest bond strength values, which were statistically different from the carious and eroded dentin substrates. The bond strength was compromised related to altered dentin substrates, presenting lower values in artificial carious and eroded conditions, regardless of pretreatment or storage time in this laboratory evaluation. Regarding the demineralized substrates, they presented similar performance, with no statistical differences between them.

Table 2. *Mean (MPa) and standard deviation values of micro-tensile bond strength of a universal bonding system to dentin substrates treated or not with chlorhexidine.*

Groups	Initial	6 months
S-W	39.27 (10.16) Aa*	39.23 (9.88) Aa*
S-CHX	40.55 (15.75) Aa*	33.39 (13.64) Aa*
C-W	27.67 (13.09) Ba*	26.17 (10.69) Ba*
C-CHX	24.09 (7.21) Ba*	24.44 (7.70) Ba*
E-W	25.73 (12.64) Ba*	26.63 (12.75) Ba*
E-CHX	25.83 (10.71) Ba*	24.87 (8.94) Ba*

N=20. Different capital letters mean statistical significance between substrates (S x C x E) (p<0.05). Equal lowercase letter means no statistical significance between pretreatments (W x CHX) (p<0.05). Asterisk symbol means no statistical significance between time (initial x 6 months) (p<0.05).

When considering time, no significant difference was noted for any condition. The same performance was attributed to CHX, which did not provide any differences with regards to substrate or time.

Figure 3 shows the distribution of the failure modes, revealing that adhesive failure was observed mostly in the initial groups and mixed failure was predominant in the six-month storage groups. Cohesive failures were not absent, although they were present at only a small percentage.

Representative SEM images (1500×) of sound, carious, and eroded dentin are presented, respectively, in Figure 4 and combined with their subgroups (W and CHX, initial and six months). The images of the sound group showed a homogeneous distribution of adhesive agent (self-etching mode) constituting a thin hybrid layer with the presence of some resinous tags, which are notable on the groups treated with CHX. When altered substrates were observed, a discontinuous structure was visible, even for the carious and eroded dentin.

DISCUSSION

In clinical practice, the most common substrates are morphological and structurally modified, as with carious and eroded dentin, which frequently require restorative treatment.[4,6,8] As most studies often use sound dentin, this study evaluated the influence of a demineralized substrate in different interactions, resembling the actual clinical condition.[14,15,16,25]

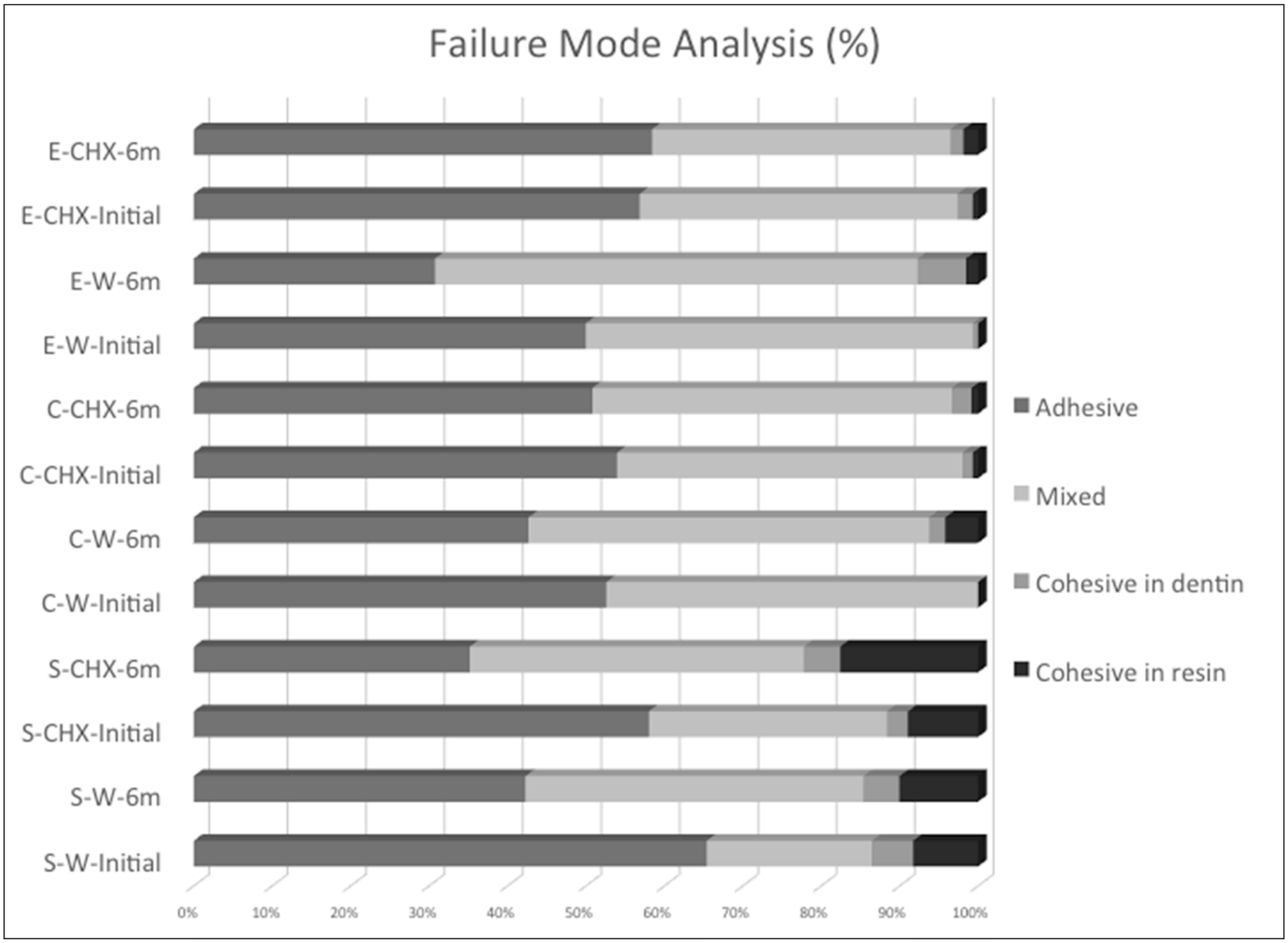

Figure 3. *Classification of the failure mode distribution (%) for all substrates, pretreatment, and time evaluation (initial and 6 months). Predominance of adhesive and mixed failure pattern in all groups.*

According to the present results, when sound dentin was used, bond strength reached the highest values, regardless of time and pretreatment. Both artificially carious and eroded dentin impaired the bond strength of the self-etching mode of a universal bonding system to dentin (Table 2), which supports rejecting the first null hypothesis tested. Likely, this performance suggests that a lower mineral content negatively affects the chemical interaction of these remaining minerals with MDP-based bonding agents, even immediately after bonding. Therefore, a reduced calcium content in dentin might reduce the formation of stable calcium-based salts and impair the quality of bonding.

This poor bond strength performance for carious lesions is supported by Isolan and others.[6] In this systematic review, a significantly higher bond strength to sound dentin was found when compared to carious substrates, regardless of the cycling protocols. Also, the lower values found for eroded dentin are in accordance with the literature, which indicates a reduction of adhesion to these substrates.[1,2,32,33]

Controversially, Giacomini and others[2] did not present differences between sound and eroded substrates, with only artificially carious dentin diminishing bond strength to dentin. According to the authors, this difference may be attributed to structural and chemical changes of carious dentin,[44,45] as the denuded collagen fibrils of the organic matrix are degraded.[45] In eroded dentin, the main modification relies on mineral loss, without affecting the organic matrix. For the artificial

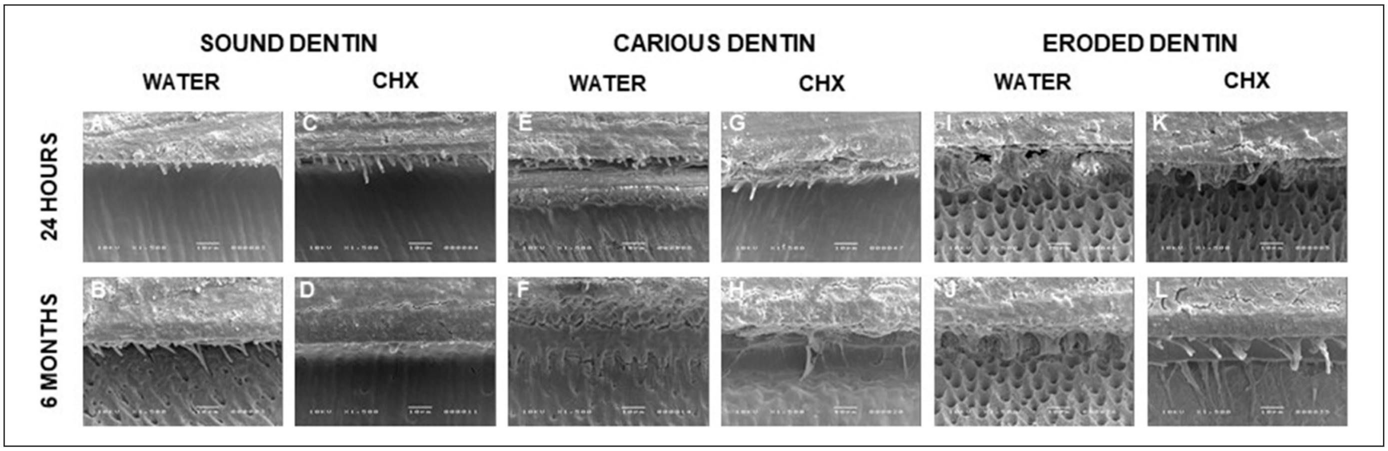

Figure 4. *Representative scanning electron microscope images (1500×) of the sound groups (A–D), carious groups (E–H), and eroded groups (I–L), with water or chlorhexidine (CHX) at initial and 6-month evaluation. A specific and particular pattern of the self-etching adhesive system was observed in the sound groups, with eventual formation of short resin tags with a sparse and homogeneous distribution. A pattern similar to that found in the sound group, but with lower homogeneity, was observed in the carious and eroded groups due to the complexity of the demineralized substrates. Additionally, a standard feature of eroded dentin substrate was observed with the exposure of dentin tubules.*

carious substrate, a greater effect to the adhesive quality is likely due to the degradation of collagen.[45] Muñoz and others[16] did not observe any difference between application modes (etch-and-rinse or self-etch) in the use of Adper Single Bond, although it should be noted that those authors used sound dentin. Based on the present study, this substrate overestimates the bonding performance and is probably not realistic enough to simulate clinical conditions.

In the Giacomini and others[2] study, the etch-and-rinse strategy used on artificial carious and eroded substrates exacerbated the lack of mineral content. This scenario may impair the ability of the acidic functional monomer MDP to bond to dentin, with a reduced formation of MDP-calcium salt.

The 10-MDP monomer is often present in the composition of universal bonding systems and allows for chemical bonding to the dental structure by a calcium-dependent mechanism, forming a stable nanolayer with various MDP-calcium salts in the adhesive interface.[46,47] Substrates with a lower calcium ion concentration associated with MDP-based bonding systems could result in greater adhesion, especially on modified substrates if they undergo further demineralization by etching during the adhesive process. Therefore, the self-etching mode could allow for more effective interaction of MDP with the calcium in the substrate due to its greater availability, which would be less harmful for adhesion. The same rationale could be applied to any other phosphate-based functional monomer.

In terms of pretreatment with CHX at the initial and six-month evaluations, no statistical difference was observed, regardless of substrate type and time. Therefore, the second null hypothesis was accepted. This association was anticipated, as CHX is robustly supported as an antiproteolytic agent.[1,28] However, as no influence was observed, it is supposed that the available calcium concentration, even in demineralized dentin, was sufficient to allow complete action for both agents (MDP and CHX) when the self-etching mode was employed, as no adverse additional demineralization was provoked by phosphoric acid.[31,46,47] In Giacomini and others[2] the difference between treatments (W and CHX) on all substrates (sound, carious and eroded) when using the etch-and-rinse mode would support this observation. Over time, this perspective may change, as substantivity of CHX is approximately 18 months, according to other studies.[35,36] When considering the time of evaluation, no statistical difference was observed among the initial and six-month groups; therefore, the third null hypothesis was not rejected.

Failure mode analysis (Figure 3) indicated a predominance of adhesive and mixed failure patterns, which validate the bond strength test and was compatible with the current literature. These analyses guarantee that the interface was being tested while avoiding a material cohesive test. The increase in the percentage of cohesive fractures in the sound groups confirmed the fact that the adhesive resistance in the sound substrate was higher than the observed bond strength for the carious and eroded demineralized substrates. The representative SEM image analyses are shown in Figure 4, which supports the quantitative data and is in accordance with the literature.[48] The overall performance of the bonding agent shows a classical particular pattern of self-etching adhesive systems. The hybrid layer was not distinct in the SEM images and the eventual formation of short resin tags of sparsely and homogeneous distribution was detected.

The characteristics presented in the images also support the stable values of bond strength detected for the different groups in the time comparison, regardless of the pretreatment used. However, poorly homogeneous images are observed in the demineralized groups, which suggests a greater complexity of the substrates when affected by caries or erosion. For the eroded group, it is possible to note the characteristic pattern of eroded substrates with great exposure of dentinal tubules throughout the dentin surface.[4] Possible fractures can be seen in areas corresponding to the composite resin, especially in the six-month sound groups, demonstrating a probable degradation of the resin, which may correspond to the increase of cohesive fractures in resin over time.

Therefore, the supposed competition in this study between MDP and CHX for the calcium ions present in the substrate would not cause an interference in bonding effectiveness when testing initially and at six months in sound, carious, and eroded substrates with an MDP-based bonding system. Within the limitations of this study, this strategy is feasible and reliable for clinical use, even though demineralized substrates always impair the bonding potential.

CONCLUSION

Carious and eroded dentin substrates negatively interfered with the bond strength of an MDP-based universal bonding system when using the self-etch mode, regardless of its use with CHX.

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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