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Bond Durability of Self-etch Adhesive to Ethanol-Based Chlorhexidine Pretreated Dentin after Storage in Artificial Saliva and under Intrapulpal Pressure Simulation

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Clinical Relevance

Ethanol-based chlorhexidine pretreatment is not recommended as a generalized approach to enhance bonding durability.

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SUMMARY

Objective: **To evaluate the bond strength durability of a single-step self-etch adhesive to dentin pretreated with either ethanol-based chlorhexidine (ECHX) or water-based chlorhexidine (WCHX) after storage in artificial saliva and under intrapulpal pressure simulation (IPPS).**

Methods: **The occlusal enamel of 30 freshly extracted premolars was trimmed to expose midcoronal dentin. Roots were sectioned to expose the pulp chamber. Specimens were distributed over three groups (n=10) according to the dentin pretreatment used. In the first group, Adper Easy One (3M ESPE) was**

applied to the dentin surfaces according to the manufacturer's instructions (control group). In the second group, dentin was pretreated before bonding with 1 mL of 2% CHX diacetate dissolved in 100% ethanol (ECHX). The third group received the same pretreatment; however, CHX was dissolved in distilled water (WCHX). Pretreatment and bonding were carried out while the specimens were subjected to IPPS. Resin composite (Valux Plus, 3MESPE) buildups were made. After curing, specimens were stored in artificial saliva and under IPPS at 37°C in a specially constructed incubator (n=5/group) either for 24 hours or six months prior to testing. Thereafter, bonded specimens were sectioned into sticks with a cross section of 0.9 ± 0.01 mm^2 and subjected to microtensile bond strength (μTBS) testing (n=25/subgroup) using a universal testing machine. Data were statistically analyzed using two-way analysis of variance (ANOVA) with repeated measures, one-way ANOVA , and Bonferroni post hoc tests ($p \leq 0.05$). Failure modes were determined using a scanning electron microscope.

Results: **After 24 hours of storage, control and WCHX groups revealed significantly higher μTBS than the ECHX group. After six-month storage in artificial saliva and IPPS, only the WCHX group maintained its μTBS value. The predominant mode of failure was the mixed type, except for the ECHX group, which was mostly adhesive.**

Conclusion: **Pretreatment of the dentin with ECHX had a negative effect on bonding of the tested single-step self-etch adhesive; however, WCHX showed bond stability under IPPS.**

INTRODUCTION

Based on both *in vitro* and *in vivo* work done over the past decade, considerable evidence has been accumulated concerning the lack of bond durability of resin adhesives to dentin.[1] The tooth-restoration interface, which consists of dentin organic matrix, remaining hydroxyapatite, adhesive resin, and solvents, is still the weakest part for any adhesive restoration.[2] Hydrolytic degradation of denuded dentin collagen has been suggested as a possible mechanism responsible for adhesive bond degradation. Hence, some approaches were encountered to enhance bond durability.[3]

The wet-bonding technique introduced by Kanca[4] has been adopted as a standard protocol for the latest generation of dentin adhesive systems. It made use of the continued presence of water in demineralized dentin, which is critical to prevent collapse of the dentin matrix. However, this technique was found to cause phase separation of hydrophobic BISGMA monomer in the hybrid layer.[5] Furthermore, there is no general consensus on how wet the dentin should be. Recently, a new philosophical approach has been developed called ethanol wet bonding.[6] This philosophy embraces the concept of water replacement from interfibrillar and intrafibrillar collagen spaces by ethanol to create a comparatively hydrophobic, ethanol-suspended demineralized collagen matrix for infiltration by hydrophobic resin monomers.[7-9] This allows for more and deeper resin penetration and better collagen fiber encapsulation. Replacement of water by ethanol from the collagen intrafibrillar compartments also removes the hydrolytic medium necessary for the function of the collagen-bound matrix metalloproteinases (MMPs). MMPs are responsible for the degradation of resin-sparse collagen fibrils within the hybrid layer.[10,11]

Ethanol wet bonding differs conceptually from the use of chlorhexidine (CHX), a potent MMP inhibitor in preventing hybrid layer degradation.[11-13] Substantivity of CHX, or its ability to be retained in dentin matrices, could be the reason why CHX-treated acid-etched dentin may form hybrid layers that are more stable over time.[11-15] Once bound, CHX is relatively resistant to the displacing effects of ethanol but is more easily removed by water.[16] Thus, if CHX-treated acid-etched dentin is not rinsed with water, most of the CHX applied to the matrix will remain bound during the application of a solvated etch-and-rinse adhesive.[16]

Very few studies[17-19] examined the benefits of the adjunctive use of CHX and ethanol as an efficient simplification step on the durability of resin-dentin bonds. However, the authors tested this adjunct with an etch-and-rinse adhesive system at normal laboratory conditions. This study compared the effectiveness of using CHX dissolved in either water or ethanol on the prevention of bond degradation of recently simplified single-step self-etch adhesive under some clinical simulating conditions (artificial saliva immersion, intrapulpal pressure simulation at 37°C). The null hypothesis tested was that water-based CHX as well as ethanol-based CHX do not influence the microtensile bond strength of single-step self-etch adhesive after storage in artificial saliva at 37°C and under intrapulpal pressure simulation for 24 hours and six months.

MATERIALS AND METHODS

Specimen Preparation

Sound single-rooted premolars, extracted from an age group of 18 to 20 years, were stored in phosphate buffer solution containing 0.2% sodium azide at 4°C. The teeth were collected from patients after the protocol had been approved by the Faculty of Dentistry's Ethics Committee at Cairo University, Egypt. All teeth were used within 1 month after extraction. Each tooth was trimmed perpendicular to its long axis, exposing the dentin using a slow-speed diamond saw sectioning machine (Buehler Isomet Low Speed Saw, Lake Bluff, IL, USA) under water coolant. Another cut was made parallel to the occlusal surface, 2 mm below the cementoenamel junction, exposing the pulp chamber. Remnants of pulp tissue in the pulp chamber were removed using a discoid excavator (Carl Martin GmbH, Solingen, Germany) without touching the walls of the pulp chamber.[20] Dentin surfaces were then wet polished with 600-grit SiC paper to create a standard surface roughness and smear layer. The specimens (n=30) were connected to the intrapulpal pressure assembly during bonding and storage following the same procedures described by Mobarak.[21]

Restorative Procedures

Prepared specimens were divided into three groups (n=10) according to the dentin pretreatment used: in the first group (control group), Adper Easy One (single-step self-etch adhesive system; 3M ESPE dental products, St Paul, MN, USA) was applied to the prepared dentin surface according to the manufacturer's instructions. It was applied using a disposable brush tip, left undisturbed for 20 seconds, dried with oil-free mild air flow for 5 seconds, then light cured for 20 seconds. In the second group, dentin was pretreated before bonding with 1 mL of 2% CHX diacetate (Sigma-Aldrich, St Louis, MO, USA) dissolved in 100% ethanol (ECHX). The third group received the same pretreatment; however, CHX was dissolved in distilled water (WCHX). CHX was left undisturbed for 60 seconds and dried with oil-free mild air flow for 30 seconds at a distance of 10 mm.[21]

Materials specifications, manufacturers, compositions, and batch numbers are listed in Table 1. Resin composite (Valux Plus, 3M ESPE dental products) of shade A1 was applied in two increments of 2 mm each. Each increment was polymerized for 40 seconds using Bluephase C5 (Ivoclar Vivadent, Schaan, Liechtenstein) with an intensity ≥500 mW/cm^2. Light intensity was checked using an LED radiometer (Kerr Dental Specialties, West Collins, Orange, CA, USA). The specimens were then immersed in artificial saliva[11] at 37°C in a specially constructed large incubator, to accommodate the intrapulpal pressure assembly. Specimens of each group (n=10) were divided into two subgroups (n=5) according to the storage duration, which was 24 hours or six months.

Microtensile Bond Strength Testing

After storage of the bonded teeth, each tooth was longitudinally sectioned in both mesiodistal and buccal-lingual directions across the bonded interface to obtain multiple sticks of approximately 0.9 ± 0.01 mm^2 for the microtensile bond strength (μTBS) test. From each tooth, the central sticks were collected. A digital caliber was used to check the cross-sectional area and length of the sticks. Sticks of similar length and remaining dentin thickness (average of five sticks from each tooth) were tested. This resulted in a total of 25 sticks for each subgroup to be tested. Each stick was fixed to the attachment with a cyanoacrylate adhesive (Rocket Heavy, Corona, City, CA, USA) and stressed in tension using a universal testing machine (Lloyd Instruments Ltd, Ametek Company, West Sussex, UK) at a cross-head speed of 0.5 mm/min until failure. The tensile force at failure was recorded and converted to tensile stress in MPa units using computer software (Nexygen-MT Lloyd Instruments). Sticks that failed before testing were counted as 0 MPa.[22,23] Cohesively failed specimens in the resin composite or the dentin were discarded and not included in the calculations.[24] Two-way analysis of variance (ANOVA) with repeated measures was used to compare the effect of dentin pretreatment, storage time, and their interaction. One-way ANOVA was used to test the effect of the difference in dentin pretreatment on the bond strength values for each storage time. This was followed by the Bonferroni post-hoc test for pairwise comparison. A *t*-test was used to compare between 24-hour and six-month μTBS mean values for each adhesive system. The significance level was set at $p \leq 0.05$. Data were analyzed using the SPSS program for windows (Statistical Package for the Social Sciences, release 15 for MS Windows, 2006, SPSS Inc., Chicago, IL, USA).

Fractured sides of all specimens were inspected under a scanning electron microscope (SEM; 515;

Table 1: *Materials Specifications, Manufacturers, Compositions, and Batch Numbers*

Material (Specification; Manufacturer)	Composition	Batch No.
Adper Easy One (single-step self-etch adhesive system; 3M ESPE dental products, St Paul, MN, USA)	HEMA, Bis-GMA, methacrylated phosphoric esters, 1.6 hexanediol dimethacrylate, methacrylate functionalized polyalkenoic acid (vitre bond copolymer), finely dispersed bonded silica filler with 7 nm primary particle size, ethanol, water, initiator based on campharquinone, stabilizers	D-82229
Valux Plus $A_{3.5}$ (light-cured microhybrid resin composite; 3M ESPE dental products, St Paul, MN, USA)	Monomer matrix: Bis-GMA and TEGDMA resins	$5540A_{3.5}$
	Inorganic fillers: zirconia/silica of a particle size range 3.5 to 0.01 μm (66% by volume)	
	Additional contents: catalysts, stabilizers, and pigments (0.8% by weight)	

Abbreviations: Bis-GMA, bisphenol-A-diglycidyl-dimethacrylate; HEMA, hydroxyethyl-methacrylate; TEGDMA, tri-ethylene-glycol dimethacrylate.

Philips, Einhoven, the Netherlands) at different magnifications. Failure modes were evaluated at 100× and classified into four types: type 1, adhesive failure at the dentin side; type 2, cohesive failure in the adhesive layer; type 3, mixed failure (adhesive failure at the dentin side and cohesive in the adhesive layer); type 4, mixed all (adhesive failure at the dentin side, cohesive in adhesive, and cohesive in resin composite). The frequency of each mode of failure was calculated for each subgroup.[25]

RESULTS

Two-way ANOVA with repeated measures revealed that there were statistically significant differences for dentin pretreatment ($p<0.0001$) and storage time ($p=0.04$) as well as for their interaction ($p<0.05$). The means and standard deviations (SD) of the μTBS values of all tested groups are presented in Table 2. One-way ANOVA indicated that there was a significant difference among the dentin pretreatments when tested after 24 hours ($p<0.001$) and after six-month storage ($p<0.0001$). After 24 hours of storage, the Bonferroni post hoc test revealed no statistically significant difference between the mean microtensile values of the control group and the WCHX group, which were significantly higher than ECHX (Table 2). After six months of storage, there was a statistically significant difference among all tested groups. The WCHX-pretreated group maintained its higher values. However, the control group and the ECHX group decreased significantly (Table 2). For the control group, after 24 hours of storage, the predominant mode of failure was the mixed type

Table 2: *Microtensile Bond Strength Values (in MPa) of Tested Adhesive Systems*[a]

Storage Period	Dentin Pretreatment						
	Control		Water-Based Chlorhxidine Pretreatment		Ethanol-Based Chlorhxidine Pretreatment		*p*-value
	Mean (SD)	ptf/tnt	Mean (SD)	ptf/tnt	Mean (SD)	ptf/tnt	
24 h	28.5 (1.4)^{Aa}	0/25	28.3 (1.8) ^{aA}	0/25	16.2 (2.2) ^{bA}	4/25	0.001
6 mo	18.1 (1.8) ^{aB}	0/25	32.2 (4.8) ^{bA}	0/25	5.8 (0.9)^{cB}	8/25	0.001
p value	0.001		1.02		0.00001		

Abbreviation: ptf/tnt, pretest failure/total number of tested sticks.
[a] *Within rows, means with different small letters are statistically significantly different (p≤0.05, Bonferroni test). Within columns, means with different capital letters are statistically significantly different (p≤0.05, t-test).*

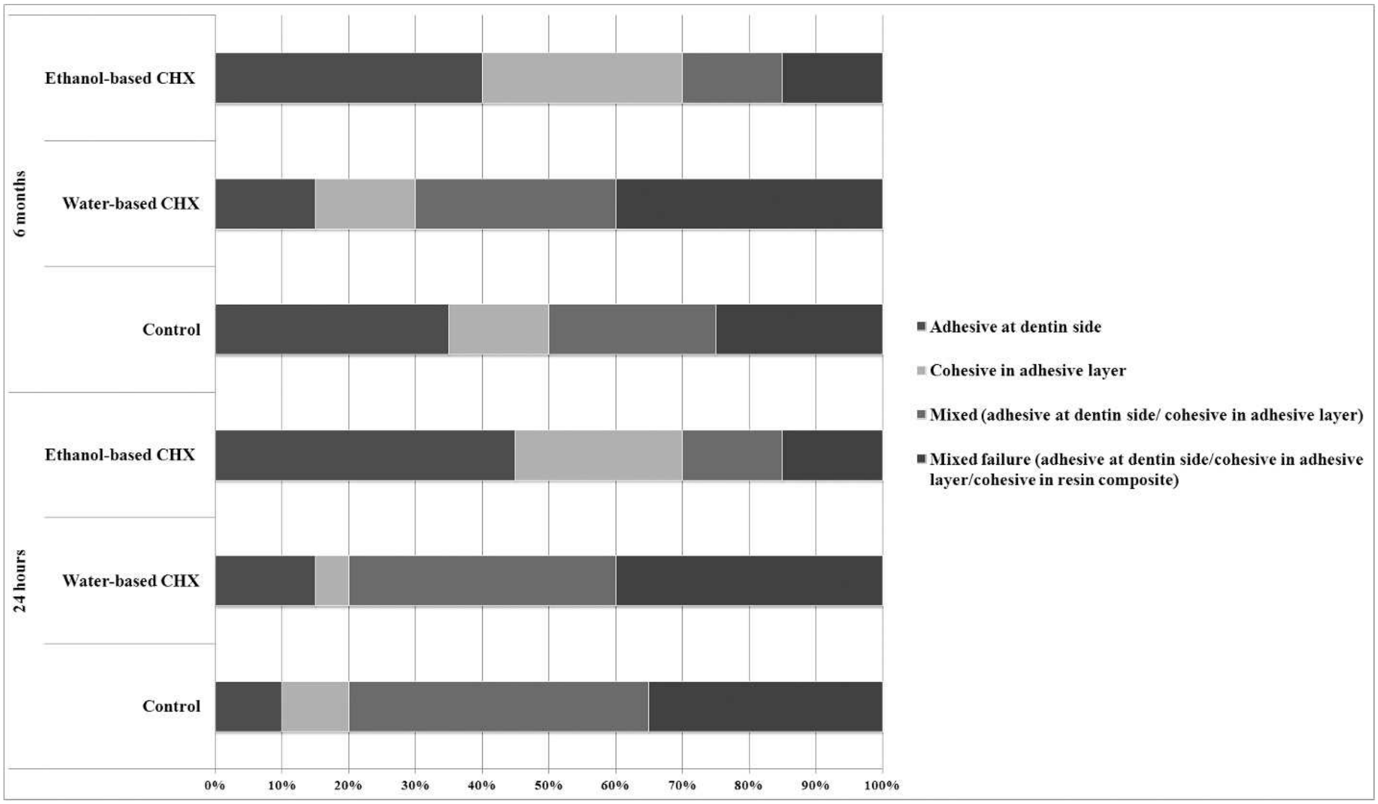

Figure 1. *Failure mode percentages of all tested groups.*

(type 3), which changed to adhesive failure at the dentin side after six months of storage. For the WCHX group, the major modes of failure were mixed types (types 3 and 4) after both aging periods. For the ECHX group, adhesive failure at the dentin side (type 1) was the predominant type after 24 hours and six months of storage (Figure 1). Representative SEM micrographs for some modes of failure are presented in Figure 2.

DISCUSSION

The results of the present study indicate the rejection of the null hypothesis, where there was a significant difference among tested groups at 24 hours and six months of storage in artificial saliva at 37°C and under simulated intrapulpal pressure.

After 24 hours of storage, the μTBS values of the control group and WCHX-pretreated group showed no statistically significant difference. Previous studies[13,15,21,26-28] revealed that 2% WCHX dentin pretreatment did not show a negative effect on the bond strength after 24 hours of storage. Surprisingly, the ECHX group recorded the least readings after 24 hours and six months of storage. The reason behind this finding is not yet clear. Previous studies[29,30] on etch-and-rinse adhesives revealed that the use of absolute ethanol (100%) for water replacement is a very sensitive technique compared with the use of ascending ethanol concentrations (50%, 70%, 80%, 95%, then 100% three times for 30 seconds each). The collapse of the collagen matrix caused by water evaporation during transition from the water to ethanol phase could result in stiffening and stabilization of the matrix in its collapsed state. Thus, reducing the application time by using a simplified absolute ethanol replacement protocol was not recommended.[29]

For this study, the concentration of CHX used with the ECHX group could be crucial. Nor and others, in 2011,[17] observed that when 2% or 3% CHX diacetate that was dissolved in absolute ethanol was used as dentin pretreatment before bonding, reduction in the initial μTBS was recorded. Nevertheless, this did not occur when 1% CHX was used. However, it should be noted that those concentrations were used with the etch-and-rinse technique. Further study is needed to investigate the most suitable CHX concentration to be dissolved in ethanol when used with single-step self-etch adhesives. Another possible reason for the loss of the μTBS values with ECHX groups is that, after its evaporation, the dentin was left dry to ionize

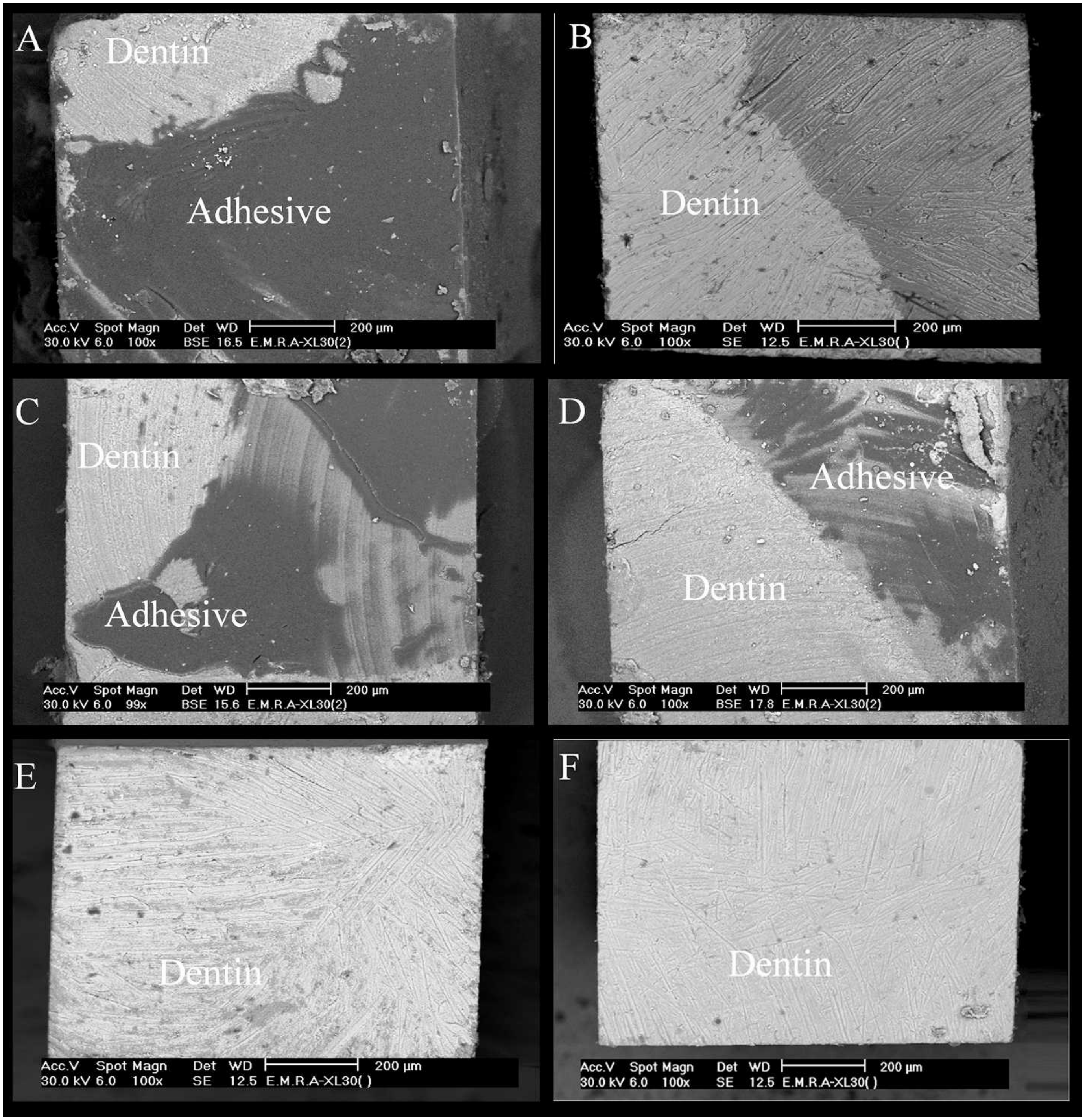

Figure 2. *Representative SEM photomicrographs of fractured surfaces of the control group showed (A) mixed failure (adhesive failure at dentin side and cohesive in adhesive layer) after 24 hours of storage, while (B) showed adhesive failure of the control group after six months. The fractured surface of the WCHX group showed a mixed type of failure (adhesive failure at the dentin side and cohesive in the adhesive layer) after 24 hours (C) and after six months (D) of storage. (E, F): Adhesive failure at the dentin side of the ECHX group after 24 hours and six months of storage.*

this single-step adhesive. For the self-etch adhesives, the water is required for the decalcification process and for preservation of the hydrated state of demineralized collagen and underlying dentin integrity.[31] However, we still need further investigation and chemical analysis to confirm this suggestion.

The mode of failure of the tested groups confirmed the μTBS results, as the control and WCHX groups showed almost similar results, with an increased percentage of mixed type of failures. The results of the control group corroborated with the Mine and others[32] study, which showed high rates of mixed failure with Adper Easy One. This was explained by the micromechanical as well as chemical interaction of the functional monomer included in this adhesive with the hydroxyapatite crystals that remain at the surface. For the ECHX group, the most common mode of failure was adhesive failure at the dentin side (type 1), denoting that the defect was located at the dentin/adhesive interface.

After six months of storage under simulated intrapulpal pressure and in artificial saliva at 37°C, the WCHX pretreated group had a statistically higher value than the control group. Clearly, there was a drop in the bond strength of the control group, and this loss of bond strength can be due to the action of water (hydrolytic degradation) and dentin enzymes (enzymatic degradation). Regarding the enzymatic degradation, it is known that mild acids have a potential to activate MMPs[33]; pH values ranging from 2.3 to 5 are effective in activating gelatinases. The self-etch adhesive used in this study has a pH value of 2.4 and so is capable of enhancing dentin proteolytic activity without denaturation of the enzymes.

After 6-month storage under simulated pulpal pressure and in artificial saliva at 37°C, the μTBS of the groups pretreated with WCHX was significantly higher than that of the control group. This result was in accordance with the findings of other researchers.[13,15,26,27] The maintained mode of action of CHX over the six-month study period could be due to its substantivity. CHX is characterized as a strong base with cationic properties. The cationic part of the CHX molecule binds to the negative site of the substrate, which results in a cationic-anionic reaction. This involves an electrostatic attraction between the protonated amine groups of the CHX and the mineral phosphates and the carboxylic groups of collagen.[34] In the present study, the mode of failure confirmed the six-month μTBS results in which the WCHX-pretreated group maintained a higher percentage of mixed failures. On the other side, the control group showed a higher percentage of adhesive failures at the dentin side.

It seems that dentin pretreatment using CHX, as an antibacterial and antiproteolytic agent, is a beneficial way to enhance adhesive/dentin bond strength durability. However, the effect of ethanol as a solvent still needs further investigation to elucidate its advantages and its precautions for use.

CONCLUSIONS

Pretreatment of the dentin with ECHX had a negative effect on bonding of the tested single-step self-etch adhesive; however, WCHX showed bond stability under intrapulpal pressure simulation.

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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