

Evaluating the Effect of Pretreatment with Matrix Metalloproteinase Inhibitors on the Shear Bond Strength of Composite Resin to Primary Teeth Dentin: A 6-Month *In vitro* Study

Abstract

Objective: The purpose of this study was to evaluate the effect of pretreatment with of matrix metalloproteinase inhibitors on the shear bond strength (SBS) of Adper Single Bond 2 total etch adhesive to the primary teeth dentin following 6 months of storage in artificial saliva. **Materials and Methods:** One hundred and twenty primary anterior teeth extracted for orthodontic reasons were selected. After etching, dentin blocks from each tooth were pretreated for 60 s with: (i) phosphate-buffered saline (0.01 M, pH 7.2) as the control group, (ii) 17% ethylenediaminetetraacetic acid (EDTA), (iii) 2% doxycycline (DO) solution, and (iv) 2% chlorhexidine (CHX) solution, with subsequent application of an etch-and-rinse adhesive system (Adper Single Bond 2). The composite was placed in clear Teflon cylinders. The SBS values were determined immediately and following 6 months of aging with a universal testing machine. Failure mode was evaluated using the stereomicroscope and scanning electron microscope. Data were analyzed by the SPSS software using the one-way analysis of variance and *post hoc* tests ($P = 0.05$). **Results:** At baseline, no significant difference was observed between control, EDTA, CHX, and DO groups ($P = 0.554$). Following 6 months of aging, the SBS of the CHX group was significantly higher than the control group ($P = 0.013$). However, the SBS of the control, EDTA, and DO groups was not statistically different ($P < 0.05$). **Conclusions:** Following 6 months of aging, among different groups of the study, only CHX significantly preserved the SBS of composite resin to primary teeth dentin using Adper Single Bond 2 adhesive.

Keywords: Composite, matrix metalloproteinases inhibitor, primary teeth, shear bond strength

Introduction

Long-term studies have shown a decrease in the bond strength of resin-bonded dentin over time.^[1] Deterioration of the hybrid layer is considered as the primary reason for compromising the resin-dentin bond durability.^[1,2] Apart from the extrinsic factors, such as water or oral fluid sorption and polymer swelling, some intrinsic host-derived enzymes like matrix metalloproteinases (MMPs) are also responsible for disintegration of the hybrid layer.^[3]

MMPs are a group of zinc and calcium-dependent enzymes, which are trapped within the mineralized dentin matrix during the tooth development process. Simplified etch and rinse adhesives and less destructive versions of self-etch adhesives used during dental procedures, and the

caries process itself can contribute to the release and activation of these endogenous MMPs and lead to dentin-adhesive bond failure.^[3,4]

Moreover, lower grades of resin monomer infiltration within the acid-etched dentin using etch-and-rinse adhesives may result in the formation of incompletely infiltrated zones and denuded collagen fibrils at the bottom of the hybrid layer.^[5] Dentin MMPs can degrade these unprotected collagen fibrils.^[6] From a clinical standpoint, MMP inhibitors such as chlorhexidine (CHX) can play an imperative role in the longevity of the resin bond to dentin.^[2]

CHX chelates and sequesters cations such as calcium and zinc which are required for the activation of the MMPs, thus inhibiting collagenase and gelatinase activity in dentin matrices.^[7] Recent *in vivo* and *in vitro* studies have demonstrated that the application of CHX has a broad-spectrum

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MMP-inhibitory effect and considerably preserves the unity of the hybrid layer formed by etch-and-rinse adhesives.^[3]

Moreover, tetracyclines (TCs) and their semisynthetic forms (minocycline and doxycycline [DO]) that are commonly used in the treatment of periodontitis, show inhibitory effects on the collagenase and gelatinase enzymes like MMPs.^[4] Based on the findings of a recent research, pretreatment of acid-etched dentin with aqueous solutions of semisynthetic TCs (minocycline and DO) improves immediate bonding performance.^[8]

Another extrinsic agent with MMP inhibitory capacity is ethylenediaminetetraacetic acid (EDTA). The application of EDTA as a chelating compound has been shown to inactivate the endogenous MMP action in human dentin.^[4] Singh *et al.* showed that EDTA had an MMP inhibitory effect, which could enhance the durability of the resin-dentin bond.^[9]

The beneficial effects of dentin surface pretreatment with MMP inhibitors would possibly become apparent over time, as the dentin bond strength is not immediately impaired.^[10]

To date, the efficacy of these MMP inhibitors to prevent the loss of dentin bond strength over time has not been determined in the primary dentition. Thus, the present study intended to evaluate the effect of primary teeth dentin pretreatment with inhibitors of MMP enzymes on the shear bond strength (SBS) of Adper Single Bond 2 adhesive, immediately and following 6 months of aging, with microscopic evaluation of the bond failure mode.

Materials and Methods

Initial specimen preparation

The study was approved by the local ethics committee. One hundred and twenty primary anterior teeth (from boys and girls, with ages between 8 and 10 years) extracted for orthodontic reasons (from June/2019 to August/2019) were selected for the study (September/2019). Written informed consent was obtained from the parents or guardians at the time of tooth extraction. The parents were informed about the purpose of the study, privacy preservation, and data anonymity. After visual inspection, the selected teeth were confirmed to be devoid of discoloration, carious lesions, or any other defect. The enamel was removed to create a flat dentinal surface, and the root was cut at the cemento-enamel junction. A dentin block (6.0 mm × 6.0 mm × 2.0 mm) was obtained from each tooth. Lack of enamel residue was confirmed using a stereomicroscope (12 × SZ51/61, Olympus, Tokyo, Japan). The dentin blocks were polished with a #600-grit (Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany) wet silicon carbide abrasive paper. Subsequently, the samples were rinsed thoroughly with water.

Etching and bonding procedures and treatment groups

The samples were conditioned with 37% phosphoric acid gel (Scotchbond Etchant, 3M ESPE) for 20 s and washed with water for 15 s. Then, the dentin samples were pretreated with three MMP inhibitors as the following groups, with subsequent application of an etch-and-rinse adhesive system (Adper Single Bond 2).

- Group I: control group ($n = 30$): 0.01M phosphate-buffered saline, pH 7.2 (Sigma-Aldrich, USA) was used. Dentinal surfaces were dried with an absorbent and a stream of air. Subsequently, the resin adhesive layer was applied
- Group II: EDTA (Coltene/Whaledent AG, Altstätten, Switzerland) was applied for 60 s^[8,10] on the dentin blocks with a micro-brush. Dentinal surfaces were washed and then dried with an absorbent and a stream of air. Subsequently, the resin adhesive layer was applied
- Group III-DO ($n = 30$): 2% DO solution (Sigma-Aldrich, USA) was applied for 60 s on the dentin blocks with a micro-brush. Dentinal surfaces were dried with an absorbent and a stream of air. Subsequently, the resin adhesive layer was applied
- Group IV-CHX ($n = 30$): 2% CHX gluconate solution (Consepsis, Ultradent, USA) was applied for 60 s on the dentin blocks using a micro-brush. Dentinal surfaces were dried with an absorbent and a stream of air. Subsequently, the resin adhesive layer was applied.

Acid-etching, pretreatment, and adhesive application was completed according to the manufacturer's instructions [Table 1]. Subsequently, the composite resin was placed. In all four groups, a clear Teflon trade cylinder (Tygon tubes, ET, Shandong China) measuring 2.65 mm in diameter and 3 mm in length was secured to the lapped tooth surface and served as a mold into which the composite (Filtek 3M, USA) was inserted. The composite was cured (Woodpecker, China) for total time of 40 s from three different sides (20 s from the top and two 10 s from the sides). The specimens were stored in artificial saliva at 37°C. The SBS values were determined immediately for half of the samples in each group ($n = 15$) and also following 6 months of aging in artificial saliva for another half ($n = 15$) with a universal testing machine (Zwick/Roll Z020, Zwick GmbH and Co, Germany). The test was performed by securing the specimens in a mounting jig, and a sharp straight-edge chisel attached to the cross-head was used to apply a shearing force of 0.5 mm/min until failure.

Mode of fracture failure

All specimens were examined under a stereomicroscope (BS-3060C, BestScope, China) to determine modes of failure, which were categorized as follows:

Table 1: Acid etching and adhesive application procedures

	Acid etching	Adhesive application
Material	Acid etch (Scotchbond Etchant, 3M ESPE)	Adper Single Bond 2 (3M ESPE, USA)
Composition	Acid phosphoric 37%	Bis-GMA, HEMA, water, dimethacrylates, ethanol, photoinitiator system, methacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5 nm-diameter spherical silica nanoparticles
Application technique	Apply etchant for 20 s Rinse for 15 s Gently air-dry (10 s at 20 cm)	Apply one coat of adhesive with gentle agitation Gently air-dry (10 s at 20 cm) Apply second coat of adhesive Gently air-dry (10 s at 20 cm) Light curing for 20 s

Bis-GMA: Bisphenol A glycerolate dimethacrylate; HEMA: Hydroxyethyl methacrylate

- Type I: adhesive failure in the tooth-composite interface
- Type II: cohesive failure in the composite or dentin structure
- Type III: mixed adhesive and cohesive failure.

Preparation for visualization using field-emission scanning microscope

Two cut sections of sheared dentinal surfaces from each group were examined using magnifications up to ×1130 for analysis, with emphasis on areas of adhesive or cohesive failure. The specimens were mounted on aluminum stubs with conductive silver liquid, sputter-coated with gold, and examined under a field-emission scanning electron microscope (SEM) (TE-SCAN, VEGA3, USA) for verification of the type of failure.

Statistical analysis

SPSS version 20 (SPSS Inc., IL, USA) was used to assess the collected data. Data were analyzed using the one-way analysis of variance (ANOVA) analysis and least significant difference (LSD) *Post hoc* test. $P < 0.05$ was considered statistically significant. The mean results of baseline SBS tests and aging tests were compared by the paired *t*-test.

Results

The mean SBS values and their respective standard deviations of different groups of the study for immediate tests (0 month) and following 6 months of aging tests are presented in Table 2. The highest numerical SBS values were observed when CHX was applied in both periods of times. DO group recorded the second highest bond strength values.

At baseline, one-way ANOVA analysis did not show any significant difference between control, EDTA, CHX, and DO groups ($P = 0.554$) [Table 1].

The results of one-way ANOVA following 6 months of intervention in four groups showed that there was not a significant difference between control, EDTA, CHX, and DO groups ($P = 0.070$). However, LSD *Post hoc* test was used to compare the differences between these groups.

Table 2: Comparison of mean shear bond strength between 0 and 6 month in groups of the control, chlorhexidine, ethylenediaminetetraacetic acid, doxycycline and comparison of 4 group (the control, chlorhexidine, ethylenediaminetetraacetic acid, doxycycline) in baseline and 6 months

Variable	SBS base (0 month)		SBS after 6 months		Paired <i>t</i>	<i>P</i>
	Mean	SD	Mean	SD		
Control	19.20	2.55	16.44	2.48	89.28	≤0.001*
EDTA	19.50	3.44	17.45	3.48	78.40	≤0.001*
DO	19.80	3.89	18.30	3.80	13.84	≤0.001*
CHX	21.60	5.73	21.18	5.72	-1.33	0.214
<i>F</i>	0.708		2.56			
<i>P</i>	0.554†		0.070†			

*Paired sample *t*-test; †One-way ANOVA test ≤0.05 is significant.
*EDTA: Ethylenediaminetetraacetic acid; DO: Doxycycline;
CHX: Chlorhexidine; SBS: Shear bond strength; SD: Standard deviation

The results showed that the mean of postintervention test in the CHX group was significantly higher than the control group ($P = 0.013$). According to the findings, there were no statistically significant differences between the mean SBS values of the control, EDTA, and DO groups ($P > 0.05$) [Table 3].

Finally, comparing the mean results of baseline SBS tests and aging tests by paired *t*-test showed statistically significant differences ($P \leq 0.001$) except for the CHX group. Bond strength values were preserved when CHX was used ($P = 0.214$) [Table 2].

Microscopic analysis

The prevalence of the failure modes confirmed by stereomicroscope is shown in Table 4. The most commonly occurring failure modes were mixed failures. Figure 1 shows the images related to the stereomicroscope evaluation for all groups of the study after 6 months of aging. The results of SEM analysis were also consistent with the mentioned data analysis. Figure 2 illustrates representative images from visualization of cut sections of sheared dentinal surfaces by SEM In images I (CHX group) and II (control group), which are related to the groups with

highest and lowest SBS values (mixed failure and adhesive failure are evident, respectively.)

Discussion

Numerous factors affect the bond strength of adhesive agents. Mechanical stresses induced by chewing forces,

variations in intraoral pH and temperature, water sorption, resin shrinkage, and enzymatic action of MMPs affect the bond integrity to varying extents.^[11]

Despite the development of simplified adhesive systems in recent years, etch-and-rinse adhesives are still considered as the gold standard in terms of durability and bond strength.^[8] The acidic monomers in etch-and-rinse adhesives expose the collagen fibrils of the organic matrix during bonding procedures.

Hybrid layer formation requires the infiltration of adhesive resin monomers to the exposed collagen network. However, a layer of exposed collagen resulting from incomplete resin infiltration may remain at the bottom of the hybrid layer. These collagen fibrils are susceptible to degradation by MMP host-derived enzymes activated through acid contact and water uptake.^[12]

Pretreatment with MMP inhibitors is considered as a valid alternative to prolong the resin-dentin bond stability by overcoming this self-degradation process.^[9]

On the other hand, deciduous teeth have less mineral and more organic matrix than permanent teeth. The advantages of using MMP inhibitors would be evident in deciduous teeth as their hybrid layer is more susceptible to degradation over time.^[12]

In the present study, the effect of three different MMP inhibitors on the SBS of resin composite to primary teeth dentin was assessed at baseline and following 6 months of storage of samples in the artificial saliva.

The results of the present study showed improved dentin bond strength with the application of CHX on etched dentin at baseline which was preserved following 6 months of aging. No significant reductions in bond strength value were observed when CHX was used ($P = 0.214$). Other studies also observed that applying a 2% CHX digluconate solution before composite placement successfully preserved the bond strength for 6 months when etch-and-rinse adhesive systems were used.^[13-15] Studies by Manfro *et al.* and Breschi *et al.* reported that bond strengths in CHX-treated specimens were significantly higher than the

Table 3: Least significant difference *Post hoc* analysis results

Group	Mean difference	P
Control versus CHX	-4.74	0.013*
Control versus EDTA	-1.01	0.571
Control versus DO	-1.86	0.323
CHX versus EDTA	3.73	0.416
CHX versus DO	2.88	0.130
EDTA versus DO	-0.85	0.643

$P \leq 0.05$ is significant. EDTA: Ethylenediaminetetraacetic acid; DO: Doxycycline; CHX: Chlorhexidine

Table 4: The prevalence of the failure modes

Group	n	Immediate failure mode			Aging failure mode		
		I	II	III	I	II	III
I Control	15	11	0	4	10	0	5
II EDTA	15	5	5	5	4	6	5
III DO	15	4	5	6	4	3	8
IV CHX	15	3	2	10	3	3	9

EDTA: Ethylenediaminetetraacetic acid; DO: Doxycycline; CHX: Chlorhexidine

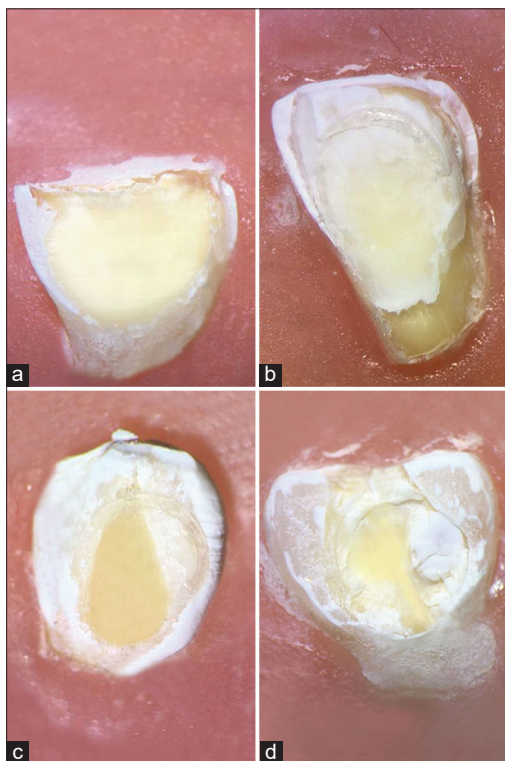


Figure 1: Stereomicroscope evaluation for all study groups. (a) Control group, (b) Ethylenediaminetetraacetic acid group, (c) doxycycline group, (d) chlorhexidine group

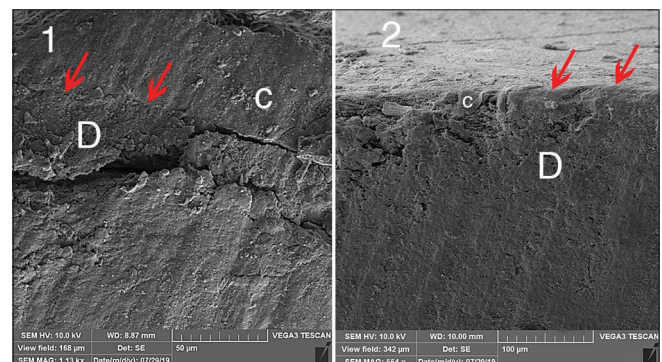


Figure 2: Representative scanning electron microscope images of the cut sections of sheared dentinal surfaces. 1: Chlorhexidine group and 2: Control group (C: Composite, D: Dentin)

nontreated groups after 12 months of aging.^[16,17] The results of the present study corroborated these findings.

The use of CHX preserved the bond interface, possibly due to its inhibitory effect on MMPs found in etched dentin.^[6] This observation was in agreement with the results of the studies by Carrilho *et al.* and Zheng *et al.*^[18,19]

CHX maintains a strong affinity to the dental tissue by binding to the phosphate groups of mineralized dentin crystallites and negative carboxyl groups of the collagen matrix. The long-term effectiveness of CHX can be explained by its high substantivity, regardless of the concentration. CHX can oversaturate protease binding sites and remain bound to collagen fibrils for later release in higher concentrations.^[7]

Furthermore, CHX water-based solutions act as rehydrating agents by maintaining the humidity needed to keep the collagen network of dried dentin in an expanded condition.^[2]

Ricci *et al.* confirmed that the use of CHX reduced the rate of resin-dentin bond deterioration within the first few months of restoration.^[20] An *in vivo* study on primary teeth showed extensive nanoleakage and degradation of the hybrid layer even in teeth with clinically intact cavosurface margins after only 6 months of clinical service. Conversely, when CHX was applied to etched dentin, the hybrid layer deterioration was significantly reduced.^[21]

Contradictory results have been reported in a few studies on the primary dentition. Findings of a study by Lenzi *et al.* showed that CHX did not influence the immediate bond strength to sound or caries-affected dentin of primary and permanent teeth.^[22] The results of the study by Viera *et al.* in this context showed the adverse effect of CHX on bond strength of single bond adhesive to the primary teeth dentin.^[23] The differences observed could be attributed to the different materials and techniques used or the pretreatment of caries-affected dentin.

The study by Thompson *et al.* asserted that 17% EDTA significantly inhibits endogenous MMP activity of human dentin within 1–2 min.^[24]

However, in the present study, the application of EDTA did not show significant differences in SBS in comparison with the control group. Furthermore, EDTA is water soluble, and it might be rinsed off the EDTA-treated dentin. Hence, it may have been unable to sustain MMP inhibition for as long as 6 months.^[7]

Based on our findings, the highest numerical SBS values of the composite to primary teeth dentin were observed for the CHX followed by DO groups after 6 months, as was reported in the study by Li *et al.*^[8] Loguercio *et al.* showed that the use of 2% minocycline and 2% CHX as pretreatment of acid-etched dentin retarded the degradation of the resin-dentin interface over 24 months.^[25]

However, in some studies, DO negatively affected the bonding of etch-and-rinse adhesives.^[26,27] The increased depth of demineralization was not compatible with the extent of resin monomer infiltration in the study by Elkassas and El Zohairy.^[26] In the study by Stanislawczuk *et al.*,^[27] the phase separation observed for DO after mixing with bonding agents resulted in lower SBS values observed.

Evaluating the sheared surfaces by stereomicroscope and SEM, the present study revealed that the failure modes were mostly adhesive for the control group. An increased percentage of mixed failures with MMP inhibitors was observed, which could be attributed to the increased SBS values.

Although the performance of CHX was superior in our study, it should be mentioned that the molecules of this inhibitor are water-soluble and may gradually leach out from the adhesive interface, especially when it comes in contact with an external environment (for example, through marginal gaps). Therefore, further long-term laboratory and clinical studies (longer than 6 months) are suggested to confirm our findings.

Conclusions

Following 6 months of aging, among different groups of the study, only CHX significantly preserved the SBS of composite resin to primary teeth dentin using Adper Single Bond 2 adhesive. Future clinical trials are recommended to verify the effect of CHX and other MMP inhibitors on long-term bonding durability in primary dentition.

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Conflicts of interest

There are no conflicts of interest.

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