Article published online: 2021-11-01

Original Article

Effect of Different Matrix Metalloproteinase Inhibitors on Shear Bond Strength of Composite Attached to Primary Teeth Dentin

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Abstract

Aim: This study is aimed to evaluate the effect of exogenous matrix metalloproteinases (MMPs) inhibitors on immediate shear bond strength (SBS) of resin composite attached to primary teeth dentin. **Methods:** Sixty extracted anterior primary teeth were selected. A dentin block (6.0 mm × 6.0 mm × 2.0 mm) was obtained from each tooth. All dentin blocks (n = 60) were pretreated for 60 s with either 0.01 M phosphate-buffered saline (pH 7.2) in the control group (Group I), chlorhexidine (CHX) 2% (Group II), doxycycline (DOX) solution 2% (Group III), and ethylenediaminetetraacetic acid (EDTA) 17% (Group IV) before applying etch-and-rinse adhesive system (Adper Single Bond 2). After adhesive application, composite resin (Filtek 3M, USA) was applied, and then, SBS values were determined with a universal testing machine. **Results:** There were no significant differences between SBS values of DOX, EDTA, and the control group ($P < 0.05$). SBS values reduced when CHX was applied, although the reduction was not statistically significant ($P = 0.961$). The DOX group showed the highest bond strength values (8.82 ± 3.29), which was significantly greater than the CHX ($P = 0.026$). **Conclusion:** Based on the results, pretreatment with MMPs inhibitors was not advantageous with regard to improving the immediate SBS of composite attached to primary teeth dentin.

Keywords: Composite, laboratory research, matrix metalloproteinase inhibitors, primary dentin, shear bond strength

Introduction

Adhesion between the resin-based materials and the tooth substrate is the foundation of restorative dentistry. Despite all the improvements in adhesive systems, the hybrid layer, which is the interface of the bonding between the tooth substrate and adhesive materials, remains the weakest area in adhesive dentistry.[1]

In general, it is accepted that the resin-dentin bond created by adhesives deteriorates over time. Degradation of the hybrid layer is the main cause of this phenomenon. Decreased resin monomer diffusion within the acid-etched creates incompletely infiltrated zones along the bottom of the hybrid layer that contains denuded collagen fibrils. These denuded collagen fibrils are vulnerable to degradation by endogenous matrix metalloproteinases (MMPs).[2] MMPs are a group of calcium- and zinc-dependent host-derived enzymes that are trapped within the mineralized dentin matrix during tooth development. They can hydrolyze components of the extracellular matrix.

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Quick Response Code:

Website: www.ejgd.org

DOI: 10.4103/ejgd.ejgd_167_20

Dentinogenesis is a complicated developmental phenomenon that requires the active extra-cellular enzymatic function of several different proteinases, mainly of the MMP family. Recent studies revealed the contributions of host-derived proteinases to the breakdown of the collagen matrices in the pathogenesis of dentin and periodontal disease.[3] The existence of an acidic environment due to caries leads to the activation of different MMPs and degradation of the collagen fibrils, hence weakening the bond between the adhesive and dentin interface occurs.[4] During bonding procedures with etch-and-rinse or self-etch systems, demineralization of dentin activates proteolytic enzymes (MMPs), which degrades unprotected

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How to cite this article: Mohammadi N, Parsaie Z, Jafarpour D, Bizolm F. Effect of different matrix metalloproteinase inhibitors on shear bond strength of composite attached to primary teeth dentin. Eur J Gen Dent 2020;9:147-51.

Submitted: 12-May-2020 **Revised:** 30-Jun-2020
Accepted: 15-Jul-2020 **Published:** 15-Sep-2020

collagen fibrils. Therefore, at the bonding interface we need to counteract the adverse effects of enzymes such as MMP.

It is well-known that certain substances are promising MMPs inhibitors. The most common MMP inhibitors are chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), tetracycline (TC) compounds (minocycline and doxycycline [DOX]), ammonium compounds, etc. In adhesive dentistry, CHX is now used as a common MMPs inhibitor. CHX is the first candidate to be applied on dentin surface to inhibit collagenolytic enzymes. CHX has been shown to effectively inhibit MMP-2, MMP-9, and MMP-8. The effective concentration varies, yet the best concentration is 2%.[5] EDTA is one of the most common agents for irrigating the root canal system during mechanical instrumentation. EDTA reacts with calcium ions from dentin hydroxyapatite and forms soluble calcium salts. The inhibitory effect on MMP activity is the result of its Zn2 + and Ca2 + chelating efficacy. When applied for 1–5 min, EDTA has an inhibitory effect against human dentin MMP-2 and MMP-9. During longevity tests, it has been shown that it preserves the dentin-adhesive interface.[6] The latest MMPs inhibitors are TCs. They are commonly used as antibiotics in the treatment of periodontitis. Both *in vitro* and *in vivo* studies have shown that TC and its semi-synthetic forms, DOX and minocycline, have the ability to inhibit MMP-1, MMP-2, and MMP-12.[2] Different medical and dental applications have been offered for minocycline and DOX. Recently, the effect of applying minocycline and DOX aqueous solution as a preliminary step after acid-etching on the immediate bonding performance has been investigated. Researchers have proven that this technique increases the bond strength.[1,6] There are limited data on the use of TCs and their analogs in dentin bonding as a way to increase the longevity of the resin-dentin bond, and this subject should be the focus of further investigations.[6]

Even though there are several researches on the effect of MMP inhibitors on adhesion physical properties in permanent dentition, information about their effect on bond strength of composite to primary teeth dentin is limited. Thus, with this in mind, in this study, we evaluated the effect of exogenous MMP inhibitors such as CHX 2%, DOX solution 2% and EDTA 17% on bond strength of resin composite to primary teeth dentin.

Methods

Initial specimen preparation

The local ethics committee of the institution approved the study protocol (Grant # 20,969). Sixty extracted anterior primary teeth were selected from patients who required tooth extraction due to orthodontic reasons, which was done after obtaining written informed consent from their parents/guardian. The selected teeth were inspected visually when wet and confirmed as teeth free of discoloration, carious lesions, and any defects. The enamel was removed to expose the flat dentinal surface and then the root was cut at the cementum-enamel junction. A dentin block (6.0 mm × 6.0 mm × 2.0 mm) was obtained from each tooth. The lack of enamel residue was confirmed with a stereomicroscope (12× SZ51/61, Olympus, Tokyo, Japan). Next, the dentin block was polished with a #600-grit wet silicon carbide abrasive paper on a grinder for 60 s. Then, the sample was rinsed thoroughly with water.

Etching and bonding procedures and treatment groups

37% phosphoric acid (Scotchbond Etchant, 3M ESPE) was used to create the acid conditioning procedure, which was done for a period of 15 s and further washed in water for the same period. Dentin was further exposed to three different MMP inhibitors. All the dentin blocks ($n = 60$) were pretreated for 60 s with either 0.01 M phosphate-buffered saline (PBS, pH 7.2) in the control group, CHX 2%, DOX solution 2% and EDTA 17% after etching with phosphoric acid 37%, and before applying etch-and-rinse adhesive system (adper single bond 2).

The four groups in this study were as follows:

- Group I: Control group ($n = 15$): 0.01M PBS of pH 7.2 was used. The dentinal surface was dried with the help of an absorbent and air stream. After this, the application of the resin adhesive layer was made
- Group II: CHX ($n = 15$): CHX gluconate 2% was applied for 60 s onto the dentin blocks. The application was done with the help of a micro-brush. The dentinal surface was dried with the help of an absorbent and air stream. After this, the application of the resin adhesive layer was done
- Group III: Doxcycycline ($n = 15$): DOX solution 2% was applied for 60 s onto the dentin blocks. The application was done with the help of micro-brush. The dentinal surface was dried with the help of an absorbent and air stream. After this, the application of the resin adhesive layer was done
- Group IV: EDTA ($n = 15$): EDTA 17% was applied for 60 s onto the dentin blocks. The application was done with the help of micro-brush. The dentinal surface was washed and then dried with the help of an absorbent and air stream. After this, the application of the resin adhesive layer was done.

After etching, pretreatment and adhesive application in accordance with the manufacturer instructions [Table 1], the composite was applied. In all four groups, a clear Teflon trade cylinder 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 for 20 s according to the manufacturer instructions from three different sides (one from the top and two from the sides). The specimens were stored in distilled water for 48 h at 37°C and then shear bond strength (SBS) values were determined 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.

Statistical analysis

The collected data were analyzed using SPSS Ver. 20 (SPSS Inc., IL, USA). Data were analyzed using one-way ANOVA and

Table 1: Etching, pretreatment, and adhesive application procedure

	Acid etching	Adhesive application
Material	Acid etch (Scotchbond Etchant, 3M ESPE)	Adper Single Bond 2 (3M ESPE, USA)
Composition	Acid phosphoric 35%	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator system, methacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5 nm-diameter spherical silica nanopaticles
Application technique	Apply etchant for 15 s Rinse for 15 s Gently air-dry (10 s at 20 cm)	Apply one coat of adhesive for 10 s with gentle agitation Gently air-dry (10 s at 20 cm) Apply one coat of adhesive for 10 s with gentle agitation Gently air-dry (10 s at 20 cm) Light curing for 10 s

Bis-GMA – Bisphenol A-glycidyl methacrylate, HEMA – 2-hydroxyethyl methacrylate

post hoc Tukey's tests. $P < 0.05$ were considered statistically significant.

Results

The mean SBS values and their respective standard deviations for different groups are presented in Table 2. The lowest SBS values were observed when CHX was applied (5.60 ± 2.69). DOX group showed the highest bond strength values (8.8 ± 3.29).

Data analysis with one-way ANOVA showed statistically significant differences between the groups ($P = 0.023$). Therefore, a *post hoc* Tukey test was used for pairwise comparison. The results revealed a significant difference just between the CHX group and DOX group ($P = 0.026$). There were no significant differences between SBS values of DOX, EDTA, and the control group ($P < 0.05$). SBS values reduced when CHX was applied, although the reduction was not statistically significant ($P = 0.961$).

Discussion

In the present study, the effect of three different MMP inhibitors on the SBS of resin composite to primary teeth dentin was assessed. MMP enzymes play a critical role in many biological processes, such as embryogenesis, normal tissue remodeling, wound healing, angiogenesis, and also in diseases such as atheroma, arthritis, cancer, and tissue ulceration.[7] Preventing the detrimental effects of MMPs on incomplete resin-infiltrated collagen fibrils is an important issue to be investigated. This leads to increased durability of restorations that involve bonding to the dentin substrate.[8]

CHX is a widely used antimicrobial agent that possesses a broad activity against oral bacteria and has low toxicity.[8,9] In this study, CHX did not increase the bond strength of the specimens immediately subjected to the shear test. This result corroborates the findings of some other studies that evaluated the pretreatment of dentin with 2% CHX after acid-etch conditioning. In the mentioned researches, using CHX before acid etching showed negative effects on the bonding of the etch-and-rinse adhesive system.[9,10] Memarpour *et al.* also found that 2% CHX pretreatment after acid etching and before the application of adhesive (adper single bond 2) increased the microleakage of the composite restoration.[11]

One reason for this undesirable effect might be the fact that CHX can bind to phosphate groups of apatite in the smear layer or dentin surface owing to its cationic properties. This might have an adverse effect on the resin infiltrations.[12] In addition, the most important ingredient of CHX solution is water. Its uncontrolled application on the dentin surface after acid-etch might make an over wetted surface.[13] On the contrary, there are other studies (mostly on permanent teeth) which concluded that by applying CHX after phosphoric-acid-etching, the integrity of the hybrid layer improves and complete inhibition of the proteolytic enzymes is achieved.[5,14-16]

EDTA is used in endodontics to facilitate instrumentation of constricted canals and to dissolve the inorganic portion of the smear layer created during the shaping of the canal space.[17] To compare different MMP inhibitors, our study showed that pretreatment of primary teeth dentin with EDTA increased the bond strength of the composite resin. This is in agreement with other studies that reported higher SBS values.[18-21] Studies have shown that EDTA removes the hydroxyapatite of dental hard tissue selectively without destroying the collagen matrix structure. Youm *et al*. reported the pretreatment of dentin surface with 18% EDTA to eliminate the smear layer appeared to be a desirable procedure for improving the μTBS of the self-adhesive resin cement. Their study showed that 17% EDTA significantly inhibits endogenous MMP activity of human dentin within 1–2 min and degradation of hybrid layer following resin bonding procedures in the root canal space might be minimized.[18] Furthermore, EDTA contains carboxylic acid groups and can chelate calcium. It has been used to dissolve the mineral phase of dentin without altering dentin proteins by causing major alterations to the fibrillar structure of the dentin collagen network; hence, the resin infiltration is probably facilitated.[19]

Multiple steps involved in MMP transcription, protein synthesis, and enzyme activation can theoretically be inhibited by doxycycline through binding with the MMP active site.[2] In the present study, primary teeth dentin pretreatment with DOX showed the highest values of SBS among all groups. Zheng and Chen also concluded that DOX could be used

Table 2: The mean shear bond strength values and their respective standard deviations for the different groups

	Group	*n*	Mean±SD	Minimum-maximum
I	Control	15	$6.20^{A,B}$±2.11	2.79-10.10
II	CHX	15	5.60^{B}±2.69	2.26-10.30
III	DOX	15	8.82^{A}±3.29	3.09-15.60
IV	EDTA	15	$7.50^{A,B}$±3.94	3.66-16.50

Different upper case letters show significant difference between groups. CHX – Chlorhexidine, DO – Doxycycline, EDTA – Ethylenediaminetetraacetic acid, SD – Standard deviation

to increase the dentin resin adhesive bond strength. MMP inhibitor application for 1 min after the etching procedures significantly improved the bond strength.[1] This finding is in contrast with Stanislawczuk *et al.* and Elkassas *et al.* study, who found that DOX jeopardizes the bond strength and quality of the hybrid layer of the etch-and-rinse adhesive systems.[2,9]

The diversity of the results could be attributed to the different methods and materials used. Elkassas *et al*., in their study, used MTAD, which is an acidic solution that contains both DOX and citric acid. The use of MTAD negatively affected the bonding of the etch-and-rinse adhesive. The mild etching effect followed by the phosphoric acid etching might have led to an increased depth of demineralization, which was not compatible with the depth of resin monomer infiltration.[9]

On the other hand, in a study by Stanislawczuk *et al*., minocycline and doxycycline (2%) were dissolved in water, acetone, and ethanol and then transferred to a small glass and mixed with the adhesive system before application. Signs of phase separation, which were observed for doxycycline resulted in lower SBS values observed in this study.[2]

Despite promising results of DOX in the present study, it should be mentioned that due to its ability to stain permanent teeth, applying a TC derivative on primary teeth should be done with caution and in situations where isolation is achieved by a rubber dam technique.

Another limitation of the present study is the scarcity of studies evaluating the effect of pretreatment with MMP inhibitors on primary teeth. Therefore, in some parts of this article, the results of studies on permanent teeth have been mentioned and compared with the results of the present study on primary teeth. The study did not investigate the effect of MMP inhibitors for aged specimens. However, our data can be used as baseline data for further studies.

Although primary teeth are expected to work in the mouth for a limited duration (6–8 years), this time seems enough to have the degradation of the bonding interface as a real clinical problem. Consequently, the promising results from some of the groups should be further evaluated clinically.

Conclusion

Based on the results, it can be concluded that pretreatment with different MMP inhibitors did not show a significant effect on the SBS of composite attached to primary dentition using etch and rinse adhesive system.

Clinical significance

Although MMP inhibitors have been proven effective in improving bond strength of composite to permanent teeth, they might not be efficient in primary teeth.

Acknowledgment

The authors would like to thank the Vice-Chancellery of the Shiraz University of Medical Science for supporting this research. This manuscript is based on thesis #18127 by Fatemeh Bizolm with the advisory of Najmeh Mohammadi. The authors also thank Dr. Mehrdad Vosughi of the Center for Research Improvement of the School of Dentistry for the statistical analysis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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