

Effects of Omega-6, Omega-9, Grape Seed Extract, and Chlorhexidine on Dentin Bond Strength

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ABSTRACT

Aim: Biodegradation of collagen matrix is one of the main factors in reducing the strength of dentin–resin bond over time. The present study aimed to evaluate the effect of four natural agents on the prevention of collagen degradation which increases bond strength.

Materials and methods: Twenty-five sound human premolars were selected. The middle one-third of the dentin was exposed by removing occlusal part of the crown. In order to increase the bond strength of the dentin–resin interface, four collagen cross-linking agents and matrix metalloproteinase (MMP) inhibitors were used in this study. The teeth were randomly divided into five groups according to the dentin treatment; control group (no treatment), pretreatment with chlorhexidine (CHX), grape seed extract (GSE), omega-9, and omega-6, respectively. Teeth were restored with Tetric N-Bond adhesive system and resin composite. After thermocycling, all the specimens were mounted on the slicing machine and then sectioned to produce a 1.0 mm² cross-sectional surface area that tested for tensile bond strength. Data were analyzed using one-way analysis of variance (ANOVA) and *post hoc* Tukey test.

Results: The CHX pretreatment group (36.45 ± 1.85 MPa) had a significantly higher bond strength, compared to the other groups ($p < 0.05$).

Conclusion: The application of natural collagen cross-linkers and MMP inhibitors in this study (CHX 2%, GSE 6.5%, omega-9, and omega-6) were capable of significantly increasing resin–dentin bond strength.

Keywords: Chlorhexidine, Dentin, Grape seed extract matrix, Metalloproteinases, Unsaturated fatty acid.

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INTRODUCTION

Dental composite resins have been used in esthetic dentistry for many years. Considering the structure and mineral content of the tooth enamel, adhesive resin can successfully bond to this tissue.^{1,2} However, establishment of a stable bond between the dentin and the adhesive resin remains a challenge in restorative dentistry.³ Despite significant advances in adhesive-bonding systems, the resin–dentin interface is still the weakest area in tooth restoration.⁴ Recent studies have shown that the baseline bond strength is not always associated with the long-term bond strength, as the bonded interface is quickly degraded (even after 6 months).^{4,5}

The endogenous enzyme activity of matrix metalloproteinases (MMPs) in the host is one of the major challenges in resin–dentin bonding, associated with a reduction in bond strength during degradation of demineralized dentin collagen.^{6,7} Matrix metalloproteinases are a group of calcium- and zinc-dependent endopeptidases, which can degrade all the components of the extracellular matrix (ECM). Evidence shows that exposure of collagen fibrils to endogenous MMPs, followed by acid etching, makes them susceptible to degradation.⁸

Several MMPs have been identified in the dentin–pulp complex. During tooth development, MMPs participate in the organization of ECM components. In addition, mature human odontoblasts can synthesize gelatinase A and B (MMP-2, MMP-9), collagenase (MMP-8, MMP-13), and enamelysin (MMP-20).^{9–12} Pretreatment of the dentin–resin interface using inhibitors against MMP activity can improve the bonding stability and strength.¹³ Chlorhexidine (CHX), as a cross-linking agent, can strongly inhibit the proteolytic activity of MMP-2, MMP-8, and MMP-9.³ So far, the effects of inhibitors, such as CHX, zinc, and galardin, have been investigated on the activity of MMPs; nevertheless, further research is needed to reach a definite conclusion.^{14–17}

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Several chemical substances, both natural and synthetic, which have the ability to enhance the collagen bond and prevent the activity of MMPs, are used to prevent the degradation of the hybrid layer.³ Proanthocyanidins (PAs) are oligomeric flavonoids, which can strengthen the dentin collagen and improve the composition of collagen fibers through cross-linking. They can be naturally found in a variety of foods and plant sources, such as grape seeds, pine bark, melon, lemon peel, leaf of hazelnut tree, and many other plants.¹⁸ They are also recognized as exceptional flavonoids with strong antioxidant capabilities.¹⁹

A recent study showed that use of grape seed extract (GSE), which mainly consists of PA, could significantly improve the

mechanical properties of demineralized dentin.^{20,21} Four major mechanisms have been associated with the cross-linking effects between PAs and proteins: Covalent bond, ion bond, hydrogen bond, and hydrophilic bond. Which in general shows the high tendency for PAs to cross-link with collagen.²⁰

According to recent studies, unsaturated fatty acids, such as omega-3, omega-6 (linoleic acid), and omega-9 (oleic acid), can inhibit the proteolytic activity of MMP-2 and MMP-9 in the dentin.²² However, no study has yet examined the effects of these substances on enhancing the resin–dentin bond strength. The aim of the present study was to evaluate the effect of dentin pretreatment with CHX, GSE, omega-6, and omega-9 on the microtensile bond strength (μ TBS) of dentin-bonded resin composite, using the fifth generation of bonding agents after thermocycling.

MATERIALS AND METHODS

Materials Used in the Study

The current study was approved by ethics committee of Babol University of Medical Science (IR.MUBABOL.REC.1397.027). In this experimental study, omega-9, omega-6, and 6.5% grape seed oligomeric PA that all of them were from Sigma-Aldrich (St. Louis, Missouri, USA) were used to increase the resin–dentin bond strength. Also, 2% CHX digluconate from Maquira (Maquira Dental Products – Maringá, PR, Brazil) was used as the positive control.

Specimen Preparation

In this study, 25 permanent premolar teeth with no caries were selected. The teeth were extracted for orthodontic reasons. The extracted teeth were then immediately placed in 0.1% thymol solution at room temperature for 24 hours. Afterward, they were added to normal saline until further analysis. No extracted tooth was kept for >3 months before the analyses. Also, normal saline used for storing the samples was replaced once a week (Fig. 1).

The occlusal one-third of crown was removed with a disk (D and Z, Darmstadt, Germany) attached to a slow-speed dental handpiece in cooling water. The middle one-third of the dentin was exposed by removing occlusal one-third of the crown. Then, the dentin surface was polished with silicon carbide papers (600 grit) to create a standard smear layer. The teeth were etched with 37% phosphoric acid gel (Etch-Rite; Pulpdent, Watertown, Massachusetts, USA) for 30 seconds, washed for 30 seconds, and dried under gentle air pressure using a cotton ball to maintain the dentin moisture. The teeth were randomly divided into five groups, and five teeth were randomly selected for each group. The teeth were prepared for restoration by etching, rinsing, drying, and using five different preparation methods. The preparation methods in each group are presented in Table 1.

In the next stage, the Tetric N Bond (Ivoclar-Vivadent, Schaan, Liechtenstein) with microbrush was scrubbed on the dentin surface and dried under moderate air pressure for 10 seconds; this process was repeated in duplicate. Then cured for 20 seconds using a Light-Cure device (Valo, Ultradent, Salt Lake City, USA) at 800 mW/cm². A1-shade composite (Filtek Z250, 3M ESPE Dental Products, St. Paul, Minnesota, USA) layers were placed incrementally (1 mm for each layer), and each layer was cured for 40 seconds until at least a 6 mm composite block was formed. Thickness of 6 mm was considered for the samples, which were placed in the Universal Testing Machine for μ TBS testing after slicing (Fig. 2).



Fig. 1: Twenty-five permanent premolar teeth with no caries were selected

Thermocycling Test

All the samples were exposed to the aging process by thermocycling device (Willytech Thermocycler V.2.8, Haake, Munich, Germany) at $55 \pm 2^\circ\text{C}$ and $5 \pm 2^\circ\text{C}$, respectively (dwell time, 30 seconds) at 5000 rpm.

μ TBS Test

Teeth were mounted on the slicing machine (Delta precision sectioning machine, Mashhad, Iran) and the samples were sliced into about 1×1 mm beam shaped sections under constant water coolant (Fig. 3).

From each premolar, five beams were selected (25 beams per group) and placed on the Universal Testing Machine (BISCO, Schaumburg, USA) with a cyanoacrylate glue (Zapit; Dental Ventures of America, Corona, California, USA). Tensile force was then applied at a crosshead speed of 0.5 mm/minute to produce the maximum fracture force (μ TBS) in Newton (9.8 kg). Afterward, using a digital caliper (Mitutoyo Corp., Kawasaki, Japan), the cross-sectional area at the site of fracture was determined for each slice (mm²), and the value was expressed in MPa relative to the cross-sectional area (F/A).

Statistical Analysis

Data were analyzed in SPSS version 17 (SPSS Inc., USA). To analyze the differences in μ TBS, one-way analysis of variance (ANOVA) was performed at a 95% confidence interval, followed by Tukey's *post hoc* test.

Table 1: The materials and pretreatment methods used for each group

Group	The substance used for pretreatment	Application time
I	Dentin surface without any pretreatment (control group)	—
II	Pretreatment with CHX with microbrush and its additions with sterile cotton were removed	60 seconds
III	Pretreatment with GSE then rinse with distilled water and dry with cotton wool	5 minutes
IV	Pretreatment with oleic acid (omega-9) then rinse with distilled water and dry with cotton wool	5 minutes
V	Pretreatment with linoleic acid (omega-6) then rinse with distilled water and dry with cotton wool	5 minutes

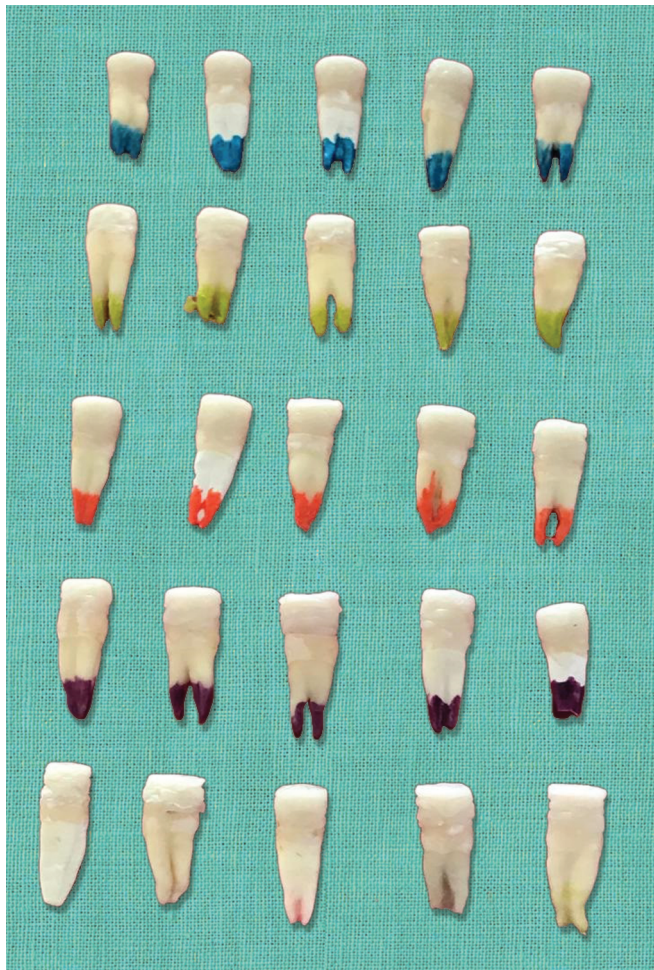


Fig. 2: Specimens after restoring with composite block

RESULTS

The μ TBS (MPa) is presented in Table 2. The μ TBS in all experimental groups was significantly higher than control group according to *post hoc* Tukey's test ($p < 0.001$). Based on the results of one-way ANOVA, the CHX pretreatment group (mean bond strength: 36.45 ± 1.85) showed significantly higher bond strength, compared to the other groups ($p < 0.001$). As shown in Table 2, the difference in bond strength between the three pretreatment groups with GSE, omega-9, and omega-6 was not significant ($p > 0.05$).

DISCUSSION

Use of collagen cross-linking and enhancing agents can increase the mechanical properties, stability, and resistance to collagen biodegradation in tissues, such as dentin.^{21,23–25} Also, cross-linking agents can disable the activity of MMPs by cross-linking peptide chains after demineralization by acids.²⁶ In fact, the stability of collagen fibers depends on the formation of these internal and external cross-links.^{27,28}

Various natural cross-linking agents, such as PA, have been examined to investigate their effects on improving the mechanical properties of resin–dentin bonds.^{20,21,23,24} In this study, 6.5% GSE, rich in PA, was used in one of the groups, and the mean microtensile strength was found to be significantly higher than that of the control group; this finding is consistent with studies by Al-Ammar et al.,²⁹ Castellan et al.,³⁰ Gajjela et al.,³ Bedran-Russo et al.,²⁰ Pinto et al.,³¹ Kaur et al.,³² and Srinivasulu et al.³³

The findings of most studies in this area indicated the increasing of bond strength or mechanical properties of the dentin–resin bond area. Although the rate of bond strength increase varies in different studies, we have better understanding when the efficacy of these compounds is compared with other substances, such as CHX, which is a recognized standard agent for improving the bond strength.

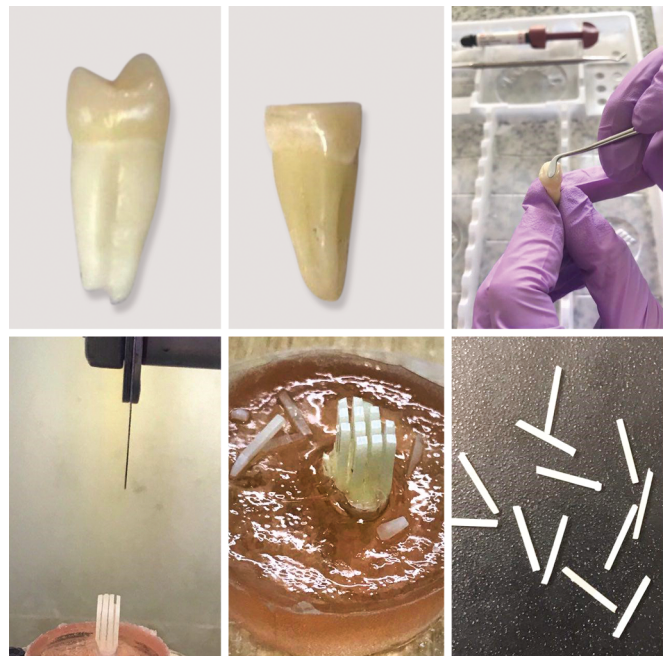


Fig. 3: Illustration of specimens preparation for microtensile bond strength testing

Table 2: The microtensile bond strength (MPa)

Group	The microtensile bond strength MPa (mean \pm SD)
Control	24.68 \pm 3.33 ^a
CHX	36.45 \pm 1.85 ^b
GSE	31.43 \pm 2.23 ^c
Omega-9	29.89 \pm 1.66 ^c
Omega-6	30.11 \pm 1.77 ^c

The lowercase letters represent a significant difference between the groups ($p < 0.05$).

Meanwhile, in a study by Feiz et al.,³⁴ the control group showed a higher bond strength compared to the pretreatment group with GSE. In their study, differences in factors, such as type of GSE, tooth, adhesive, and composite, were described as the reasons for the discrepancy between their results and other studies.

Moreover, Paulose and Fawzy³⁵ concluded that the mean bond strength in the GSE pretreatment group was not significantly higher than that of the control group. It should be noted that in studies by Feiz et al.³⁴ and Paulose and Fawzy,³⁵ the duration of GSE pretreatment was 30–60 seconds, whereas in studies reporting an improvement in the mechanical properties of resin–dentin bond with GSE pretreatment, a longer pretreatment duration was documented. For instance, in studies by Al-Ammar et al.²⁹ and Castellan et al.,³⁰ the duration of pretreatment with GSE was 1 hour and 10 minutes, respectively. Since these pretreatment durations are not clinically feasible²³ in our study, the duration of GSE pretreatment was set at 5 minutes, which is more acceptable for clinical purposes.

Chlorhexidine digluconate is another compound used in the present study for comparisons with other substances to increase the dentin bond strength. It is well established that CHX can maintain the morphological properties of the hybrid layer by preventing the host endopeptidases (which are responsible for collagen self-degradation), increase the strength of the resin–dentin bond, and prevent the reduction of bond strength in this area over time.^{17,36–39} In this study, we used CHX as the positive control to compare its effects with other new compounds evaluated in this study.

The findings of this study showed that the teeth which were pretreated with CHX had the highest μ TBS of all groups. These findings are in consistence with the results reported by Gajjela et al.³ In their study, the mean bond strength in the CHX pretreatment group was significantly higher than that of the other groups, including GSE and chitosan.

Multiple studies have used different concentrations of CHX for pretreatment. In this study, 2% CHX digluconate was used as pretreatment for 60 seconds, as suggested in a study by Loguercio et al.⁴⁰ In their study, they used different CHX concentrations for 15 and 60 seconds of dentin pretreatment. Their findings showed that use of 2% CHX for 60 seconds led to the highest μ TBS in the resin–dentin bonding area.

In the present study, omega-6 and omega-9 were also used as dentin pretreatment materials to increase the bond strength. So far, no studies have examined the effects of unsaturated fatty acids on the mechanical properties of dentin bonding. Our hypothesis regarding the effect of these substances on the dentin bond strength is based on a study by Nicolai et al.²² In a microscopic study, they concluded that unsaturated fatty acids, such as omega-3, omega-6, and omega-9, could inhibit the proteolytic activity of

MMP-2 and MMP-9 in the dentin. Since the endogenous proteolytic activity of these MMPs in the host is one of the main causes of degradation of resin–dentin interface over time, we aimed to examine the effect of pretreatment with these materials on the dentin bond strength.

The findings of this study showed that omega-6 and omega-9 could significantly increase the resin–dentin bond strength, compared to the control group. Although the mean bond strength in the omega-6 and the omega-9 groups was significantly lower than that of the CHX group, the difference with the GSE pretreatment group was not significant.

So far, no study has evaluated the efficacy of omega-6 and omega-9 in increasing the resin–dentin bond strength. In this study, pretreatment duration in the omega groups was similar to that of the GSE pretreatment group, providing a better comparison between these compounds; the results indicated an almost similar increase in the dentin–resin bond strength. Further studies are suggested on the effects of these compounds on the resin–dentin bond strength. The factor of time also needs to be considered, and histological tests are recommended for the accurate analysis of the hybrid layer.

CONCLUSION

The application of natural collagen cross-linkers and MMP inhibitors in this study (CHX 2%, GSE 6.5%, omega-9, and omega-6) were capable of significantly increasing resin–dentin bond strength.

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