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Impact of Pretreatment of Dentin with Omega-9 on Microtensile Bond Strength of Fifth-Generation Dentine Adhesive at Different Times

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ABSTRACT

Background and Objective: Matrix metalloproteinases (MMPs) reduces the resin-dentin bond and bond strength over time. One of the substances introduced as MMps inhibitors is omega-9. This study aims to investigate the effect of pretreatment of dentin with omega-9 and chlorhexidine (CHX) on the durability of bond strength in certain time intervals and modes of failure.

Methods: In this laboratory study, 45 permanent third molar teeth without caries were cut with a disc to expose the dentin of the middle part of the crown. Then, they were randomly divided into three groups: without pretreatment, pretreatment with CHX, and with Omega 9. This experiment was done after etching and before placing Single bond 2. Each group was divided into three subgroups, one day, 3 months and 6 months. A 4mm composite block was placed on the tooth and then cut into dimensions of 1×1 mm and microtensile bond strength test was performed. The failure mode was examined by stereo microscope using X40 magnification.

Findings: In the comparison of pretreatment groups with CHX and Omega-9, there was no significant increase in bond strength after 24 hours. After three months, the bond strength in the pretreatment group with CHX (13.40±2.64 MPa) was significantly higher compared to the control group (10.46±3.5 MPa) (p=0.014). Moreover, in the omega-9 pretreatment group, the bond strength (13.16±2.7 MPa) was significantly higher compared to the control group (10.46±3.5 MPa) (p=0.028). Average microtensile bond strength in the Omega 9 and CHX groups decreased after six months compared to three-month period but increased compared to the one-day period, which was not significant.

Conclusion: Pretreatment with omega-9 increases the strength of the resin-dentin bond, and this increase was present for up to 3 months.

Keywords: *Matrix Metalloproteinase, Tensile Strength, Adhesive Factors, Omega-9, Chlorhexidine.*

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Introduction

Dental composite resins are the most common materials for restoring tooth color in a direct way. However, the durability and integrity of composite restorations depend on various factors such as shrinkage polymerization, and hydrolysis of the hybrid layer, which may lead to postoperative sensitivity, secondary caries, and as a result, failure of the restoration (1-3). Recently, many researchers have focused on the durability of the bond between tooth and dentin adhesive systems. Hussein et al. have shown that pretreatment with ethanol reduces the hydrolysis of the hybrid layer and the destruction of collagen fibers even in the presence of bacteria and their toxins (4). Matrix metalloproteinases (MMPs) are multi-molecules that play an important role in various types of biological processes, including tissue repair and angiogenesis, as well as diseases such as cancer, arthritis, and neurological diseases (5).

Human dentine matrix contains MMP-2, MMP-9 (with gelatinolytic activity) and MMP-8 (with collagenolytic activity), and tooth enamel contains MMP-20 (6). Nearly 90% of dentine proteins are collagen, and the rest are non-collagenous. With the activation of MMPs, MMP8 breaks down collagen proteins, and these proteins undergo further degradation by MMP9 and MMP2. MMPs can be present in the pulp, saliva, and dentin and are released during caries (1). Studies suggest that MMP-2 may be the most common MMP in dentin. Incomplete penetration of collagen fibers by dental adhesive leads to exposed fibers and the collagenolytic activity of MMPs, and low pH values may activate during dentin etching (7).

Collagen in biological tissues strengthens itself by forming internal bonds, which increases the resistance of fibrils to enzymatic degradation and also provides more tensile properties. There are several synthetic and natural chemicals that can increase the number of internal collagen junctions (7, 8). Strengthening collagen fibers using adhesive agents in order to increase mechanical properties and reduce enzymatic degradation can be an important application in restorative dentistry (6). Chlorhexidine (CHX) is widely recognized as an antimicrobial agent in oral health. In addition, it has been reported that CHX is capable of inhibiting the collagenolytic activity of MMPs and improving the durability of the bond between adhesive and dentin (4, 6).

In recent studies, it has been shown that omega-3, omega-6, and omega-9 fatty acids inhibit the proteolytic activity of MMP2 and MMP9, two enzymes found in dentin (5). Gelatinase inhibition depends on the length of the fatty acid chain, and it has been shown that in the presence of unsaturation capacity, the inhibitory effect on both types of gelatinases increases (9), and the carbon chain length between 18 and 20 carbons has the greatest effect on the inhibition of MMPs (5).

Considering the limited number of articles on the effect of omega-9 on bond strength and the lack of a study on determining the effect of this substance on bond strength over time, in this study, we decided to measure the effect of pretreatment of dentin by omega 9 on the microtensile bond strength during the intervals of one day, 3 and 6 months and compare the results with chlorhexidine and to determine the modes of failure in the studied groups.

Methods

This study has been approved by the ethics committee of Babol University of Medical Sciences with the code R.MUBABOL.REC.1400.121. In this experimental study, 45 third-molar teeth without caries and cracks were selected (10). The extracted teeth were immediately placed in 0.1% thymol solution for 24 hours at room temperature. Then, they were placed in normal saline until the time of the test, and the normal saline was changed once a week (11). The maximum storage time of the teeth was three months.

The coronal third of the teeth was cut with a disk (Ivoclavar, Vivadent, Liechtenstein) at low speed and water coolant so that a smooth surface was created. Then the dentin surface was polished with 699 grit silicon carbide paper. The teeth were etched with 37% phosphoric acid (FGM-Condac37 Brazil) for 30 seconds, washed for 30 seconds, and dried with very gentle air pressure and cotton balls so that the dentin retains a little moisture (11).

The teeth were randomly divided into three groups of 15:

Group 1: Dentin surface without any pretreatment

Group 2: Dentin pretreatment with chlorhexidine digluconate solution 2% (CHX) (ADS-China) by micro brush for 30 seconds and removal of excesses with sterile cotton

Group 3: pretreatment of dentin with Omega 9 (Merk-Germany) by micro brush for 5 minutes and removal of excesses with sterile cotton.

In all three groups, two layers of dentin bonding (Single bond2, 3M-ESPE, USA) were placed on the dentin surface with a micro brush and dried with gentle air pressure. Then, for 20 seconds, it was cured with a light cure device (LED VALO Ultradent, USA) with an intensity of 1000 Mw/ cm^2. Composite restoration (3M ESPE Z350 - USA) with color A2 was placed as a layer with a thickness of 2 mm for each layer, and each layer was cured for 40 seconds until a composite block of at least 4mm was created on the cut surface of the samples (12). The samples were stored in distilled water at 100% humidity and at a temperature of 37 degrees for further tests (13).

Microtensile bond strength test: In order to check the strength of the microtensile bond, the teeth of each group were selected separately and mounted on the pad of the cutting machine with cyanoacrylate glue and then under the cutting machine (Delta Precision Sectioning Machine, Nemo - USA), samples were created with a cross-sectional area of about 1×1 mm. Two appropriate slices were selected from each molar tooth (10 slices in each subgroup), then the cross-sectional area of each slice was accurately calculated by a digital caliper (Mitutoyo, Japan). The slices were placed on the microtensile testing machine (Microtensile tester Bosco, USA), and then the tensile force was applied at a speed of 0.5 mm per minute to obtain the maximum breaking force (μTBS) in Newton (9.8×kg). Then, according to the amount of cross-sectional area (F/A), this number was calculated in megapascals (MPa). Next, each slice was observed in a stereo microscope (Dewinter, Italy) with 40X magnification, and the location of the fracture was examined (14).

Finally, the statistical data were analyzed using SPSS software version 20, as well as the Post hoc Tukey Test and One-Way ANOVA statistical tests. P-value less than 0.05 was considered significant.

Results

On day one, none of the three groups were significantly different in terms of microtensile bond strength ($p<0.05$). In the period of 3 months, the control group had significantly lower microtensile bond strength than the chlorhexidine ($p=0.014$) and omega 9 ($p=0.028$) groups. After the period of six months, all three groups showed an increase in the strength of the microtensile bond in the Omega 9 and CHX groups, but this amount was not significant ($p<0.05$) (Table 1, Diagram 1). In this study, the type of failure was also investigated, the results of which are shown in Table 2.

Table 1. The average microtensile bond strength of the groups (in MPa)

Groups	1 Day Mean±SD	3 Months Mean±SD	6 Months Mean±SD
Control	10.18±2.3^{Aa}	10.46±3.5^{Aa}	10.28±3.51^{Aa}
Omega 9	11.29±2.8^{Aa}	13.16±2.7^{Ba}	12.56±3.01^{Aa}
CHX	11.41±2.79^{Aa}	13.4±2.64^{Ba}	12.89±3.73^{Aa}

Lowercase letters indicate statistically significant differences in each row (p<0.05)

Capital letters indicate statistically significant differences in each column (p<0.05)

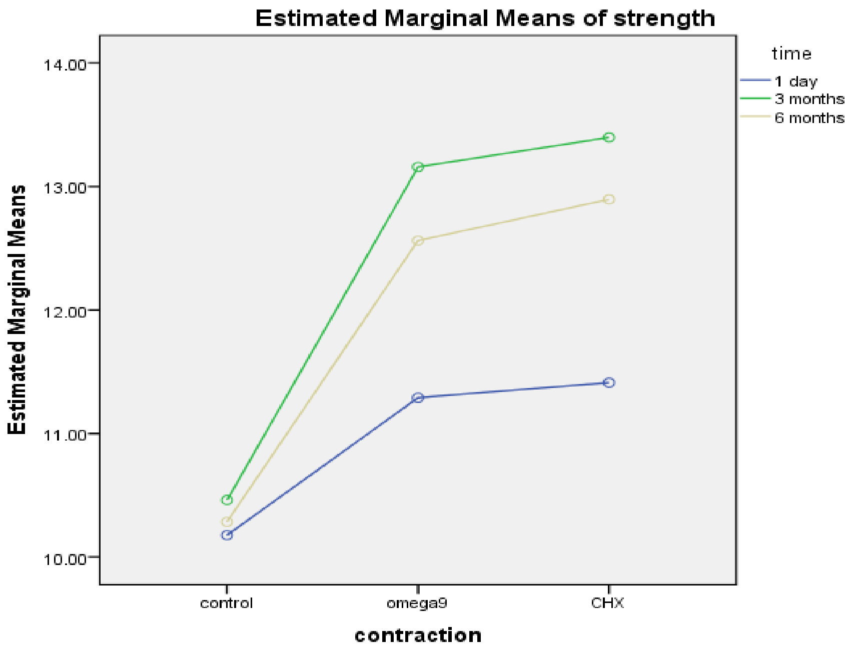

Diagram 1. Microtensile bond strength of the study groups

Table 2. Percentage of failure types in the study groups

	1 Day			3 Months			6 Months		
Role of failure	Control	CHX	Omega 9	Control	CHX	Omega 9	Control	CHX	Omega 9
Cohesive	30%	40%	40%	30%	20%	20%	40%	30%	20%
Adhesive	50%	40%	30%	40%	30%	20%	30%	30%	30%
Mix	20%	20%	30%	30%	50%	60%	30%	40%	50%

Discussion

The results of the present study showed that after a period of three months, the teeth that were pretreated with chlorhexidine and omega-9 showed a significantly higher tensile bond strength than the control group. Considering the role of omega-9 and chlorhexidine in inhibiting dentine matrix metalloproteinases, this finding is expected. However, there was no difference in bond strength between chlorhexidine and omega-9.

Collagen cross-linking and strengthening agents can increase mechanical properties, structure stability and resistance to collagen biodegradation in tissues such as dentin (15, 16). Moreover, crosslinkers can deactivate the activity of matrix metalloproteinases by creating crosslinks between peptide chains after demineralization by acid (17). In fact, the relative hardness of collagen fibrils depends on the formation of these internal and external crosslinks (18). Various natural cross-linking agents such as proanthocyanidin, omega-3, omega-6, and omega-9 have been studied in order to investigate their effect on improving the mechanical properties of the resin-dentin bond (15, 17-19). The reason for the increase in bond strength following the use of chlorhexidine and omega-9 is that they act as matrix metalloproteinases inhibitors and prevent collagen degradation. By preventing the activity of host endopeptidases (which are responsible for the self-degradation of collagen in this area), chlorhexidine maintains the morphological properties of the hybrid layer. In addition to increasing the strength of the resin-dentin bond, chlorhexidine also prevents the weakening of the bond strength over time in this area (13, 20-22). In the present study, omega-9 was used as a substance for dentin pretreatment to increase the bond strength. In a study, Nicolai et al. found the inhibitory role of unsaturated fatty acids in preventing the activity of matrix metalloproteinases in dentin. In their investigation, which was carried out by both fluorescence and electron microscopy, it was found that omega-9 inhibits the activity of MMP-2 and MMP-9 in dentin (5).

The hypothesis for conducting this study, the effect of these materials on the strength of the dentine bond, originates from the study of Ahangari et al. (15). They showed that pretreatment of the dentin surface with omega 6 and omega 9, as well as grape seed extract, significantly increases the bond strength of the resin-bond contact area. In this comparison between materials, the effect of pretreatment with chlorhexidine was higher than other compared materials.

The findings of this study showed that after 24 hours, although the microtensile bond strength increased in the chlorhexidine and omega-9 groups, these increases were not significant compared to the control group. This finding is consistent with the findings of de Castro et al. (22). In their study, the use of chlorhexidine did not have a significant effect on the microtensile bond strength of different dental adhesives within 24 hours. In the study of Mobarak et al. (23), after using 5% and 2% chlorhexidine within 24 hours, no significant effect was seen on the microtensile bond strength of caries-affected dentin. The study of Kazemi-Yazdi et al. (24) in relation to pretreatment with chlorhexidine also confirmed the same results. According to the study of Soares et al. (25), the use of different doses of chlorhexidine in the period before and after etching, after 24 hours, had no difference in bond strength, but according to the findings of Gajjela et al., the microtensile bond strength of the group pretreated with chlorhexidine was significantly higher than the control group (26).

In this study, there was no difference in bond strength between the control group and the chlorhexidine and omega 9 groups after six months. However, the findings of Campos et al. (27) showed that pretreatment with chlorhexidine 2% over a period of 6 months reduces the loss of microtensile bond strength. Also, Francisconi-dos-Rios et al. (13) showed that the strength of the tensile bond could be higher than the control group during a period of six months, but this ratio is not sustainable during a period of 12 months. Also, Parsaei et al. (28) compared pretreatment with several matrix metalloproteinase inhibitors (EDTA, doxycycline solution) during six months with chlorhexidine on primary teeth and concluded that the highest compressive strength in pretreatment with chlorhexidine 2% was observed. The study of Jidi Shen et al. showed that although the use of chlorhexidine increases the microtensile bond strength after six months, it may interfere with the formation of MDP-Ca salts (29). Unfortunately, there are limited articles related to the role of omega-9 on tensile bond strength, which makes it difficult to compare. Regarding the role of chlorhexidine over time, different percentages of chlorhexidine (20, 25, 27, 30, 31), different bonding systems (19, 21, 23, 26, 31), and different research methods (19, 20, 22, 24, 26, 30, 32) have been performed, which have led to different results.

Regarding the results of failure type, one day after pretreatment with omega 9 and chlorhexidine, the most common failure type was cohesive. After three months, the failure in the Omega 9 group was mostly mixed and cohesive, and in the chlorhexidine group, it was mostly mixed and adhesive. This can indicate an increase in the bond strength that is higher than the microtensile strength of composite resin or dentin, therefore these types of failure have increased. After six months, the failure in both groups was mostly mixed and adhesive, which can confirm that the strength of the microtensile bond has started to decrease. Unfortunately, no article could be found in this field about omega 9. The research of Rayar et al. (33) showed that after pretreatment with chlorhexidine 2%, the failures were mostly adhesive and mixed, both in dentin and in the composite.

In order to ensure the decreasing trend in bond strength, we suggest increasing the pretreatment time of omega 9 in the future. Also, it is necessary to conduct more studies on the effect of unsaturated fatty acids on the bond strength of the resin-dentin contact area in order to investigate the effectiveness of these materials in terms of clinical use.

Dentin pre-treatment with chlorhexidine and omega-9 did not increase the bond strength in the 24-hour group, while in the 3 months group, it increased compared to the control group, but in the six months group, there was no change compared to 24 hours group.

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