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Original Article

Effect of sodium fluoride in fifth-generation adhesive (Solobond M) on microleakage and dentin type I collagen content

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Abstract

Introduction: The purpose of this study was to evaluate the addition of sodium fluoride to fifth-generation adhesive (Solobond M) on the degree of microleakage and type I collagen content of dentin.

Material & Methods: The present in vitro study was conducted on 120 orthodontically extracted human premolars devoid of decay and anatomical defects. Two series of 20 premolars were selected to test immunohistochemistry (IHC) and two series of 40 premolars to evaluate microleakage in two time points of 24 hours and 3 months. In both tests and at both time points, the tests were performed on the samples divided into four groups: 1-control (only Solobond M), 2-bonding group (Solobond M) containing fluoride, 3-bonding group (Solobond M) containing Chlorhexidine (CHX) after acid etching, and 4-bonding group (Solobond M) containing fluoride+CHX after acid etching.

Results: The IHC score at 24 hours and 3 months was significantly higher in the CHX, Fluoride, and Fluoride+CHX groups compared to the control group. The IHC score in the CHX+fluoride group was higher than that in the CHX group (p=0.04). The degree of microleakage at 24 hours and 3 months was significantly lower in the Fluoride+CHX and Fluoride groups compared to the control group. The degree of microleakage in the Fluoride group was lower than in the CHX group. The IHC score and the microleakage degree had no significant difference in 24 hours and 3 months between the Fluoride+CHX, Fluoride and CHX groups.

Conclusion: It seems that the effect of fluoride on non-degradation of collagen is greater than that of the CHX.

Keywords: Sodium fluoride, Adhesives, Collagen type I

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بررسی تاثیر افزودن سدیم فلوراید به ادهزیو نسل پنجم (Solobond M) بر میزان ریزنشت و محتوای کلاژن نوع I عاج

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چکیده

مقدمه: مطالعه حاضر با هدف ارزیابی افزودن سدیم فلوراید به ادهزیو نسل پنجم (Solobond M) بر میزان ریزنشت و محتوای کلاژن نوع I عاج انجام گرفت.

مواد و روش ها: این مطالعه آزمایشگاهی بر روی ۱۲۰ دندان پرمولر انسانی بدون پوسیدگی و نقص آناتومیک که به دلیل ارتودنسی خارج شده بودند، انجام گرفت. به منظور آزمون ایمنوهیستوشیمی ۲ سری ۲۰ تایی از دندان پرمولر و برای ارزیابی ریزنشت ۲ سری ۴۰ تایی از دندانهای پرمولر در دو بازه ی زمانی ۲۴ ساعت و ۳ ماه انتخاب شدند. در هر دو تست و در هر دو بازه ی زمانی نمونه ها به ۴ گروه: ۱-کنترل (فقط شامل Solobond M)، ۲-گروه باندینگ (Solobond M) حاوی فلوراید، ۳-گروه باندینگ (Solobond M) و اعمال کلرهگزیدین بعد از اسید اچ، ۴-گروه باندینگ (Solobond M) حاوی فلوراید به همراه اعمال کلرهگزیدین بعد از اسید اچ تقسیم شده و آزمون ها انجام پذیرفت.

یافته ها: توزیع ایمنوهیستوشیمی در زمان‌های ۲۴ ساعت و ۳ ماه، در گروه های کلرهگزیدین، فلوراید، فلوراید و کلرهگزیدین بطور معناداری بالاتر از گروه کنترل بود. میزان نمره ایمنوهیستوشیمی در گروه کلرهگزیدین-فلوراید بیشتر از کلرهگزیدین بود ($p=0.04$). میزان میکرولیکیج در زمان‌های ۲۴ ساعت و ۳ ماه، در گروه های فلوراید-کلرهگزیدین و فلوراید بطورمعناداری پایین تر از گروه کنترل بود. میزان میکرولیکیج در گروه فلوراید کمتر از کلرهگزیدین بود. میزان ایمنوهیستوشیمی و میکرولیکیج در زمان ۲۴ ساعت با ۳ ماه در گروه های فلوراید-کلرهگزیدین، فلوراید، کلرهگزیدین تفاوت معناداری نداشت.

نتیجه گیری: به نظر می رسد که تاثیر فلوراید بر عدم تخریب کلاژن بیشتر از تاثیر کلرهگزیدین بوده است.

واژگان کلیدی: سدیم فلوراید، ادهزیو، کلاژن نوع ۱

Introduction

Dental composite resins are common due to their excellent aesthetics, direct restoration ability and force-bearing capacity. [1-3] After bonding to the tooth structure with the adhesive [4], it is advisable to have a long-lasting restoration-tooth bonding interface. Although most adhesives have shown excellent short-term bond strength, the durability of the bonded interface remains a challenge. [5, 6] Microleakage is introduced as a way of passing bacteria, fluids, molecules and ions between dental cavity-restorative material interfaces. [7] The microleakage has great importance in the bonding restorations, so if the bond does not exist or the strength is low, the consequence will be restoration weakness. [8] Complications caused by microleakage in dentistry include increased penetration of saliva and microorganisms between restoration and tooth, secondary caries, marginal degradation and discoloration, pulp injury [9] and post-treatment sensitivity, which can endanger the clinical durability of treatment. [10] Performing acid etching and bonding are the basis of marginal adaptation and seal against microleakage in all restorations bonded with enamel. [8] Etch-and-rinse adhesive systems are the oldest evolution of several generations of resin-bonding systems. [6] In two-step etch-and-rinse systems, the

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primer and the adhesive are in a bottle, but the separate step of the etching is still needed and the two successive layers are applied on the tooth. [11] The bonding of resin adhesive to dentin involves the penetration of liquid monomers into the surrounding spaces of collagen fibrils in the demineralized matrix. This area contains approximately 50% collagen matrix and 50% resin adhesive, which is called the "hybrid layer", and the maximum depth of the hybrid layer produced by etch-and-rinse adhesives is about 5 micrometers. [12] It seems that the degradation of the hybrid layer in the dentin-adhesive interface is the main cause of the bond failure that occurs by various mechanical and chemical factors, including hydrolytic and enzymatic degradation of exposed collagen and resin adhesive. [13]

The dentin matrix contains endopeptidases called matrix metalloproteinases (MMPs) that form during the formation of the teeth and contribute to dentinal decay. [14] Recent studies have shown that the MMPs activated during etching acid bonding process and due to lactic acid induced by oral pathogenic bacteria can cause gradual degradation of the hybrid layer and weaken the dentin-resin bond, resulting in secondary decay, marginal discoloration and ultimately the failure of restoration. [15-18]

The MMPs are known as a family proteolytic enzyme with 26 members. A range of MMPs have been identified in decay lesions, including MMP2 (gelatinase), MMP8 (collagenase), MMP9 (gelatinase), and MMP20 (enamelysin). The dentin contains 90% of collagenous proteins (mainly type 1) and 10% of non-collagenous proteins. Collagenous proteins are initially fragmented by MMP8 and eventually degraded by MMP9 and MMP2 after demineralization of dentin in carious lesions. These MMPs can be present in saliva and pulp, or located in dentin and released during caries. These enzymes are activated by reducing the acidic pH to ≤4.5; they will remain active if the activation is performed even if pH returns to normal. It has been suggested that organic acids, as well as acids such as phosphoric acid, play a special role in activating these enzymes. [16]

Chlorhexidine (CHX) was introduced as an antiseptic for skin wound in the 1940s, and was widely used in dentistry due to its broad-spectrum antibacterial properties. The CHX is used as the most effective anti-plaque and anti-gingivitis agent in the treatment of periodontal disease and as an effective antiseptic cleanser for endodontic treatment. [19] This material is available in forms of toothpaste (0.4%), solution (0.12%, 0.2%), gel (1%) and varnish (1%, 10%, 20% and 35%). [20] The CHX is a cationic chlorophenyl bisbiguanide antiseptic with a unique inhibitory effect on dental plaque, which is mainly due to its substantivity and antimicrobial properties. The CHX was reported to inhibit some MMPs, which have a significant effect on periodontal disease. The CHX has recently been studied as a protease inhibitor to maintain the hybrid layer by inhibiting MMPs20 and cysteine cathepsins. [21]

The inhibitory effects of CHX, zinc, benzalkonium chloride and galardin on MMPs have been studied so far. [22] Carilho et al. in 2007 reported that the collagen scaffold in the case group treated by CHX showed no alteration or degradation. [23] Hebling et al. in 2005 indicated that the hybrid layer integrity was successfully maintained following the application of CHX after acid etching with phosphoric acid. [24]

As a well-known antimicrobial anion, fluoride improves the demineralization resistance of enamel and dentin by reducing the solubility of hydroxyapatite. Studies have reported that fluoride prevents the loss of relatively demineralized dentine tissue when exposed to acid under erosive conditions. This protective effect may depend on several factors, including fluoride composition, fluoride concentration, exposure time, and exposure device. Although the protective effect of dentin reinforcement is thought to be due to the formation of fluoride-containing hydroxyapatite, protection of the collagen matrix against enzymatic degradation may also contribute to the overall efficiency of fluoride ions, which has recently been shown to be an inhibitor of the catalytic activity of recombinant MMP. The mechanism of the inhibitory effect has been attributed to the high electronegativity of fluoride ions that can bind to Zn^{2+} and Ca^{2+} (required for the catalytic function of MMPs). Furthermore, the fluoride has been shown to be effective in inhibiting recombinant human cathepsin K and B.[25] A study also reported that 1.23% sodium fluoride (NaF) gel significantly reduced the degradation of demineralized organic matrix.[26] Samani et al. in 2018 concluded that 10000 ppm of sodium fluoride addition to fifth-generation adhesive (Solobond M) increased significantly the dentin microtensile bond strength after 24 hours and 3 months. [27] Kato et al. in 2012 concluded that adding sodium fluoride gel to demineralized dentin matrix reduced the dentin degradation by MMPs. [26] Brackett et al. in 2015

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showed that the addition of sodium fluoride to the incubation solution reduced dentin matrix degradation by dentin MMPs. [28]

Therefore, the aim of this in vitro study was to evaluate the effect of sodium fluoride addition in the fifth-generation adhesive and its Comparative effect with CHX on the degree of microleakage and type I collagen content of dentin.

Materials & Methods

The present in vitro study was conducted on 120 orthodontically extracted human premolars devoid of decay and anatomical defects. Ethical approval was given by Babol University of Medical Sciences (IR.MuBABOL.HIR.REC1397.194).

1- The process of preparing the adhesive solution: To prepare a fifth-generation adhesive with a concentration of 10000 ppm, 40 mg of sodium fluoride powder (Merck Co, Germany) was weighed by digital scales (Bel Engineering, MG314Ai, Itlay) with an accuracy of 0.0001, and added to Solobond M (Voco Co, Germany)-containing 4-ml bottle and vibrated for one minute. To determine the homogeneity of the mixture and the dispersion rate of the fluoride ion, a bonding with new concentration (10000 ppm of fluoride) was used to fabricate a sample at a dimension of 4 × 4 × 1 mm in a Teflon mold, followed by polishing using Sof-Lex (3M, ESPE, USA) polishing discs from rough to soft, respectively, and tested for EDAX (Figure 1).

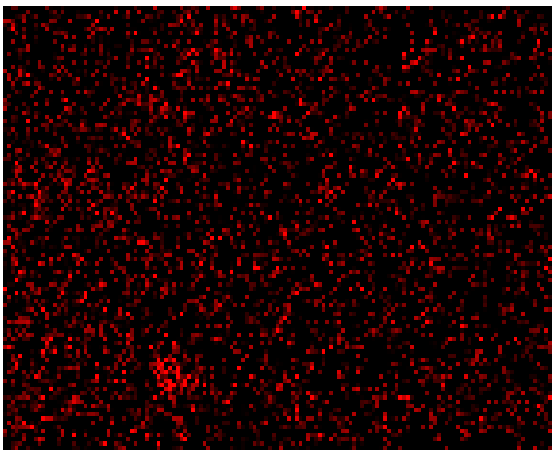


Figure 1- EDAX map of cured adhesive containing 10000 ppm of sodium fluoride

2- The process of immunohistochemistry (IHC)

A) The preparation of teeth: Two series of 20 human extracted premolars devoid of decay and anatomical defects were selected (one series for studying in 24 hours and another after 3 months). The extracted teeth were placed in 0.2% Thymol solution for 24 hours and then stored in normal saline solution until testing. Class V cavities with dimensions of 2 × 2 × 2 were applied to each tooth at the buccal surface 1 mm above the CEJ.

The first series of teeth (n=20) were divided into four groups of five:

In the first group, Solobond M (Voco Co, Germany) bonding was used without fluoride and without CHX solution (control group). In the second group, CHX solution (Extra, cercamed, Poland) and solobond M containing 10000 ppm of fluoride was applied. In the third group, solobond M containing 10000 ppm of fluoride was used without CHX solution application. In the fourth group, CHX solution and Solobond M bonding was utilized without fluoride.

In the groups where CHX solution (after acid etching) was used, the cavities were prepared with CHX 2% solution for 30 seconds and then the residues were removed with cotton. [24]

In the etching process, all teeth were respectively etched for 30 seconds and 15 seconds for enamel and dentin with 35% phosphoric acid (Vocoacid, Voco, Germany), washed and dried. [29] The used bondings were applied by a microbrush to the walls of the cavities and after 10 seconds of drying gently by an air blower were cured with an intensity of 600 mw/cm^2 (LEDVAIO, ULTRADENT, USA) for 20 seconds. The second series of samples (including 20 premolars) was prepared in the same manner and stored in normal saline/Penstrep2x with weekly exchange of solution at 37 ° C for 3 months [30] for decalcification process.

B) Decalcification and cutting process: After the desired time points (24 hours and 3 months) for chelating the mineralization phase for 8 weeks of preparation for cutting, all teeth were immersed in an EDTA solution at pH of 7.4 (neutral) (1750 ml of distilled water+disodium salt (Merck, Germany)+250 g of EDTA) (22). Then, from each tooth in each group, two samples with a thickness of 4 micrometers were obtained by microtome. (leitz 1600, Ernst leitz, German)

C) IHC test: The sections were deparaffined, dehydrated by ethanol, and placed in an autoclave with

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EDTA for 15 seconds. When the buffer solution reached room temperature, the samples were washed and the peroxidase activity was suppressed with 0.3% hydrogen peroxide in 100% methanol in a dark room for 10 minutes. The control group was coated with tris-buffered saline and other sectioned samples with Type 1 collagen antibodies (NB600-408, Novus Biologicals) for 30 minutes and then tissue surfaces were placed in peroixidase-labeled polymer (Envision Code K 800-DAKO LTD) at 37 ° C for 30 minutes. After washing them in a buffer solution, the tissue surfaces were coated with Diaminobenzidine (DAB-Code-K 8004-DAKO LTD) at room temperature for 10 minutes, and all samples were stained with hematoxylin and mounted. Next, the slides were explored via an observer with a microscope (BX51, Olympus optical, and Tokyo, Japan). The staining intensity was classified according to the degree of "DAB" penetration into weakly positive (+), relatively positive (++) and strongly positive (+++) grades. [22]

3- The microleakage test

Two series of 40 human extracted premolars devoid of decay and anatomical defects were selected. The extracted teeth were kept in 0.2% Thymol solution for 24 hours and then in normal saline solution until testing. The Class V cavities with dimensions of 2 × 2 × 2 were applied to each tooth at the buccal surface 1 mm above the CEJ. The first series of teeth (n=40) were divided into four groups of ten similar to IHC group.

In the groups where CHX solution (after acid etching) was used, the cavities were prepared with CHX 2% solution for 30 seconds and then the residues were removed with cotton. [24]

In the etching process, all teeth were respectively etched for enamel and dentin with 35% phosphoric acid (Vocoacid, Voco, Germany) for 30 seconds and 15 seconds, washed and dried. [28] The used bondings (SolobondM, Voco, Germany) were applied by a microbrush to the walls of the cavities and after 10 seconds of drying gently by an air blower were cured (LEDVAIO, ULTRADENT, USA) with an intensity of 600 mw/cm^2 for 20 seconds. After that, the cavities were restored by incremental method with 1-mm layer of composite (Grandio, Shade A2, Voco, Germany), and cured for 20 seconds. The surface of the restored cavities was finished and polished by Sof-Lex (3M, ESPE, USA) polishing discs. Two layers of varnish were applied to coronal and radicular surfaces of restoration with the exception of restoration and 1 mm around it, as well as four layers of varnish to protect the apex to prevent dye penetration into the teeth. Subsequently, the samples were immersed in 0.2% fuchsine solution for 24 hours, and then the teeth were removed from the dye, rinsed with water and placed in transparent acrylic resin.

The teeth were cut buccolingually by cutting machine (Nomov Industrial Group, Mashhad, Iran) and disc, and one sample was obtained from each tooth. Therefore, there were 10 samples in each group (n=10). The sectioned samples were prepared in each group for observational evaluation and record of microleakage degree in the cavities under Stereo microscope (MEIJI, JAPAN). For the long-term evaluation, the second series of 40 premolars was selected, prepared and grouped with the above method and stored in distilled water (with weekly renewal) for 3 months, followed by the microleakage test.

The samples were classified by the following grading system:

Gingival margins

0: no dye penetration

1: dye penetration up to 1/2 of the beginning of the gingival margins

2: dye penetration beyond 1/2 of the beginning of the dentinal walls without reaching the axiogingival line angle

3: dye penetration to axiocervical line angle and axial wall [22]

4- The process of statistical analysis

Data were analyzed using SPSS 17 software. T-test was used to compare the two time points. To measure the staining intensity in the groups, Kruskal-Wallis test was carried out at each time, and multiple comparisons were used for pairwise comparisons. Wilcoxon and Chi-square tests were performed to compare the two time points. The significance level was considered to be $p<0.05$.

Results

1- The IHC scores

According to Chi-square test, the IHC score was significantly correlated with the groups at 24 hours and 3 months ($p<0.0001$) (Table 1.). Based on Kruskal-Wallis test, the IHC scores at 24 hours and 3 months were not the same for the studied groups, and a significant difference was observed ($p<0.0001$). Based on multiple comparisons, the IHC scores at 24 hours

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was lower in the control group than in the CHX (p=0.04), Fluoride (p<0.0001), Fluoride-CHX (p<0.0001) groups. The IHC score in the Fluoride and Fluoride+CHX group was greater than in the CHX (p=0.04). Based on multiple comparisons, the IHC score at 3 months was lower in the control group than in the CHX group (p=0.004), Fluoride (p<0.0001) and Fluoride+CHX (p<0.0001) groups.

There was no significant correlation in the IHC scores at 24 hours and 3 months between the control, CHX, Fluoride and Fluoride-CHX groups. Based on the Wilcoxon test, the IHC score in the control group at 24 hours was more than at 3 months (p=0.02). In the Fluoride+ CHX (p=0.414), Fluoride (p=0.414), CHX (p=0.414) groups, the IHC scores were not significant between 24 hours and 3 months.

2- The degree of microleakage

Based on the Chi-square test, the degree of microleakage was significantly associated with the groups at 24 hours and 3 months (p<0.0001) (Table 2.).

Table 1. IHC score at 24 hours and 3 months

IHC	24 hours Groups				3 months Groups			
	Control	Fluoride+CHX	Fluoride	CHX	Control	Fluoride+CHX	Fluoride	CHX
+	5(83.3%)	0(0.0%)	0(0.0%)	1(16.7%)	10(90.9%)	0(0.0%)	0(0.0%)	1(9.1%)
++	5(31.2%)	2(12.5%)	3(18.8%)	6(37.5%)	0(0.0%)	4(23.5%)	5(29.4%)	8(47.1%)
+++	0(0.0%)	8(44.4%)	7(38.9%)	3(16.7%)	0(0.0%)	6(50.0%)	5(41.7%)	1(8.3%)

Table 2. The degree of microleakage at 24 hours and 3 months

Microleakage	24 hours Groups				3 months Groups			
	Control	Fluoride+CHX	Fluoride	CHX	Control	Fluoride+CHX	Fluoride	CHX
0	0(0.0%)	3(75.0%)	1(25.0%)	0(0.0%)	0(0.0%)	2(66.7%)	1(33.3%)	0(0.0%)
1	2(9.5%)	7(33.3%)	9(42.9%)	3(14.3%)	0(0.0%)	8(47.1%)	8(47.1%)	1(5.9%)
2	2(22.2%)	0(0.0%)	0(0.0%)	7(77.8%)	0(0.0%)	0(0.0%)	1(12.5%)	7(87.5%)
3	6(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	10(83.3%)	0(0.0%)	0(0.0%)	2(16.7%)

Based on Kruskal-Wallis test, the degree of microleakage was not similar in the studied groups at 24 hours and there was a significant difference (p<0.0001). Based on multiple comparisons, the degree of microleakage was significantly higher at 24 hours in the control group than in the Fluoride+CHX (p=0.003), fluoride (p=0.01) and CHX (p=0.01) groups. The degree of microleakage in the CHX group was higher than in the Fluoride+CHX group (p=0.018). Based on Kruskal-Wallis test, the degree of microleakage was not the same in the studied groups at 3 months and there was a significant difference (p<0.0001). Based on multiple comparisons, the degree of microleakage at 3 months was significantly higher in the control group than in the Fluoride+CHX (p<0.0001), Fluoride (p=0.008) and CHX groups. The degree of microleakage in the CHX group was higher than in the fluoride (p=0.018) group.

Based on Chi-square test, there was no significant correlation between the degree of microleakage at 24 hours and 3 months in the control, CHX, Fluoride, Fluoride and CHX groups. Based on Wilcoxon test, in the control (p=0.6), Fluoride+CHX (p=0.564), Fluoride (p=0.664) and CHX (p=0.17) groups, the degree of microleakage had no significant difference at 24 hours and 3 months.

Discussion

The results of this study showed that using fluoride-containing bonding, applying CHX solution after acid etching and using bonding containing Fluoride+CHX after acid etching increased significantly the IHC score at 24 hours and 3 months compared to the control group. Also, in the group with bonding containing Fluoride+CHX after acid etching, the IHC score was higher than that of CHX after acid etching alone (p=0.04). Using fluoride-containing bonding and bonding with Fluoride+CHX after acid etching significantly reduced the degree of microleakage at 24 hours and 3 months compared to the control group. The use of fluoride-containing bonding significantly reduced the degree of microleakage compared to the use of CHX

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after acid etching. There was no significant difference in IHC and microleakage between time points of 24 hours and 3 months in Fluoride+CHX, fluoride and CHX groups.

One of the most important inherent challenges of dental composite is marginal leakage, which leads to a gap between restoration and tooth structure and the subsequent problems due to this gap. The major cause of this leakage is polymerization shrinkage of a dental composite that has a different thermal linear expansion than the thermal linear expansion of enamel and dentin. One of the rules to prevent marginal leakage is the use of dentin adhesive to apply sealing capacity to prevent leakage and ensure restoration durability. [31] The present study was conducted to evaluate adding of sodium fluoride to fifth-generation adhesive (Solobond M) on the degree of microleakage and type I collagen content of dentin. According to our information, no studies have ever evaluated the effect of fluoride adding to adhesives on the microleakage and type I collagen content of the dentin.

The concentration of CHX used in most studies to inhibit MMP is 2%. [32] However, Breschi et al. (2010) [33] and Mazzoni et al. (2011) found that the CHX was able to control MMPs even at a concentration of 0.2%. [34] Gendron et al. (1999) found that 0.002% is the minimum concentration of CHX to completely inhibit the MMP-9 activity, while 0.0001% is the minimum concentration to inhibit the MMP-2 activity, which is probably due to chelation of Zn^{2+} cation. [35] The CHX effectively inhibits MMP-2, MMP-8, and MMP-9 and Cysteine cathepsins. [32] Therefore, CHX 2% was used in the present study.

There are several methods for evaluating microleakage, including the use of air pressure, bacterial penetration, radioisotope application, electrochemical studies, chemical detectors, dye penetration and SEM. [36] The penetration dye is the most original, oldest and common method to evaluate the microleakage. For this reason, the dye penetration method was used in this study. Fuchsine was chosen as the main dye because it is easy to use and inexpensive and does not require complicated laboratory equipment; it is also easy to determine the degree of microleakage by Stereomicroscope due to the fine contrast of the Fuchsine with dental structures. [37] Many studies have been conducted on the release of fluoride from restorative materials. The importance of fluoride in facilitating the mineralization or preventing the demineralization of dentin matrices is not well understood. Various studies have shown that the fluoride-containing dentin adhesives may release fluoride to the marginal gap. [27] The sodium fluoride is one of the MMP inhibitors. The mechanism of inhibiting MMPs by sodium fluoride is not fully understood. [32] Kato et al. [38] showed the inhibition of MMP-2 and MMP-9 by sodium fluoride; high electronegativity of the fluorine and heavy concentrations of this mineral could release the free cations to participate in the catalytic process.

The sodium fluoride is the most widely used fluoride-containing compound in dental products. Some F compounds have additional ions that may indicate activities related to decay or erosion, such as stannous fluoride (SnF2), titanium tetrafluoride (TiF4) and silver diamine fluoride (SDF). It is difficult to compare different salts and to decompose fluoride effects with these ions, because their mechanism of action is different. Hence, the sodium fluoride was used in this study. Moreover, the Na^{+} effect on MMP activity is not known or does not work in the de-remineralization process in dentin. [38]

In the IHC test, the median of staining intensity of type I collagen fibrils was strongly positive (+++) in the group of bonding containing Fluoride+CHX after acid etching, relatively positive (++) in the group of applying CHX after acid etching and relatively positive (++) in the group of using bonding containing fluoride. The inhibitory effects of sodium fluoride [28, 38] and CHX [16, 22, 23] have been investigated in previous studies, but concurrent comparison of inhibitory effect of sodium fluoride and CHX using the two microleakage and IHC tests has not been done so far.

Similar to the present study, Gholam et al. assessed the degree of microleakage of adhesives containing 5% calcium fluoride and found that fluoride reduced the degree of microleakage. [31] Also, Brackett et al. reported that the sodium fluoride inhibits matrix-bound MMP and thus can slow down dentin matrix degradation by endogenous MMP activity of dentin matrices. [28] The results of both studies are in line with the results of this study.

Alaghemand et al. (2014) carried out a study to investigate the effect of CHX and zinc oxide nanoparticles on the degree of microleakage and dentin collagen content using the IHC test, and reported that preparation with zinc oxide nanoparticles can protect the hybrid layer due to the prevention of collagen

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degradation. [22] In another study, Alaghemand et al. (2018) reported that the CHX had no effect on microshear bond strength of dentin. [29] Samani et al. (2018) reported a positive role of fluoride in improving the quality of the bonding, which could be due to the successful inhibition of dentin MMP activity in line with our conclusion. [27]

Contrary to the present study, Altinci et al. (2016) stated that the sodium fluoride does not directly inhibit proteinase, and only if administered with high concentrations can slowly inhibit the activity of dentin matrix due to inhibition of cathepsin K. [25] The difference may be due to the use of different concentrations of sodium fluoride and different methods of testing. It is suggested to evaluate the effects of different concentrations of fluoride in future studies. It appears that the fluoride concentration in the present study is sufficient to inhibit the activity of MMPs. The limitations of this study were: in vivo study was not possible, there were financial problems with the plan and impossibility of immunohistochemical tests at Babol University of Medical Sciences

Conclusion

It seems that the effect of fluoride on non-degradation of collagen is greater than that of the CHX.

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Authors' Contributions

Homayoun Alaghehmand developed the original concept and design as well as supervised the in vitro procedure and preparation of the manuscript. Madeh Zarei carried out the in vitro procedures, collected the data and wrote the manuscript. Soraya Khafri developed the interpretation of data and statistical analysis.

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