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Original Article

Sodium fluoride addition to a two-step etch-and-rinse adhesive system: effect on dentin microtensile bond strength and durability

Yasaman Samani [1], Homayoun Alaghehmand[2]✉, Zahra Jafari[3], Soraya Khafri [4], Hamed Tashakkorian[5]

1. Postgraduate Student, Student Research Committee, Babol University of Medical Sciences, Babol, IR Iran. **ORCID** (0000-0002-2381-212X)
2. Associate Professor, Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
3. Assistant Professor, Department of Operative Dentistry, Faculty of Dentistry, Mazandaran University of Medical Sciences, Sari, IR Iran.
4. Assistant Professor, Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
5. Assistant Professor, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.

✉**Corresponding Author:** Homayoun Alaghehmand, Faculty of Dentistry, Babol University of Medical Sciences, Babol, IR Iran.
Email: halagheh@yahoo.com **Tel:** +98 1132202035 **ORCID** (0000-0002-5319-0309)

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Abstract

Introduction: Studies have suggested that sodium fluoride (NaF) has an inhibitory effect on the activity of endogenous matrix metalloproteinase enzymes. This study assessed the effect of a fluoride-containing adhesive on microtensile bond strength (μTBS) over time.

Material & Methods: In this experimental study, 36 extracted third molars were used to evaluate the μTBS of a 5th generation adhesive modified by NaF. The exposed dentin surfaces were abraded and built up using composite resin. Then, the specimens were randomly divided into three groups of 12 teeth based on the type of adhesive used: Solobond M with no inhibitor (control); Solobond M with 5,000 ppm NaF; and Solobond M with 10,000 ppm NaF. The μTBS and failure mode of specimens were evaluated after 24-hour and 3-month storage in distilled water. Data were analyzed using ANOVA and Tukey's test. $P<0.05$ was considered as significant level.

Results: The control group demonstrated a lower μTBS than the experimental groups after 24 hours and 3 months ($p<0.05$). The μTBS was higher in adhesive with 5,000 ppm NaF than in control group after 3 months ($p<0.05$). The group with 10,000 ppm NaF had the highest μTBS after 24 hours and 3 months ($p<0.05$).

Conclusion: The fluoride-containing adhesives showed significantly higher bond strength values than the original adhesive without fluoride after 24-hour and 3-month storage in distilled water, leading to the improvement of resin-dentin bonds.

Keywords: Dental bonding, Dentin, Sodium fluoride, Matrix metalloproteinase inhibitors

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بررسی تاثیر افزودن سدیم فلوراید به ادهزیو نسل پنجم بر دوام باند ریزکششی عاجی

یاسمن سامانی[۱]، همایون علاقه مند[۲*]، زهرا جعفری[۳]،ثریا خفری[۴] ، حامد تشکریان[۵]

۱. دانشجوی دستیاری، کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی بابل، بابل،ایران.
۲. دانشیار، مرکز تحقیقات مواد دندانی،پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.
۳. استادیار، گروه دندانپزشکی ترمیمی، دانشکده دندانپزشکی، دانشگاه علوم پزشکی مازندران، ساری، ایران.
۴. استادیار، مرکز تحقیقات بهداشت باروری و ناباروری، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.
۵. استادیار، مرکز تحقیقات بیولوژی سلولی و مولکولی، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

***نویسنده مسئول:** همایون علاقه مند، دانشکده دندانپزشکی، دانشگاه علوم پزشکی بابل، بابل، ایران.
پست الکترونیکی:halagheh@yahoo.com تلفن: ۱۱۳۲۲۰۲۰۳۵ ۹۸+

چکیده

مقدمه: مطالعات نشان داده اند که سدیم فلوراید دارای اثر مهاری بر روی فعالیت آنزیم های ماتریکس متالوپروتئینازهای اندوژن می باشد. در این مطالعه تاثیر ادهزیو عاجی حاوی فلوراید بر دوام باند ریزکششی عاجی در طی زمان ارزیابی گردید.

مواد و روش ها: در این مطالعه آزمایشگاهی، از ۳۶ دندان مولر سوم سالم کشیده شده انسانی جهت ارزیابی استحکام باند ریزکششی ادهزیو نسل پنجم مدیفاید شده با فلوراید استفاده گردید.. سطوح عاجی اکسپوز ساییده شدند و توسط کامپوزیت رزین بیلدآپ گردید. سپس نمونه ها به صورت تصادفی بر اساس نوع ادهزیو به کار رفته به سه گروه ۱۲ تایی تقسیم شدند. ۱) دارای Solobond M به عنوان عامل ادهزیو (کنترل)، ۲) دارای Solobond M حاوی یون فلوراید با غلظت 5,000 ppm به عنوان ادهزیو، ۳) دارای Solobond M حاوی یون فلوراید با غلظت 10,000 ppm به عنوان ادهزیو، استحکام باند ریزکششی و الگوی شکست نمونه ها پس از ۲۴ ساعت و۳ ماه نگهداری در آب مقطر ارزیابی شدند. آنالیز آماری با استفاده از آزمون دو طرفه ANOVA و Tukey انجام شد. $p<0.05$ معنی دار تلقی گردید.

یافته ها: گروه کنترل استحکام باند ریزکششی پایین تری در مقایسه با گروهای مورد آزمایش پس از ۲۴ ساعت و ۳ ماه نشان داد ($p<0.05$). ادهزیو حاوی یون فلوراید با غلظتppm 5,000 استحکام باند بالاتری از گروه کنترل پس از ۳ ماه داشت ($p<0.05$). بالاترین مقدار استحکام باند در گروه حاوی 10,000 ppm فلوراید پس از ۲۴ ساعت و ۳ ماه بود.($p<0.05$).

نتیجه گیری: ادهزیو های حاوی فلوراید استحکام باند بالاتری در مقایسه با ادهزیو بدون فلوراید پس از ۲۴ ساعت و ۳ ماه نگهداری در آب مقطر نشان دادند که باعث بهبود باند رزین- عاجی گردید.

واژگان کلیدی: باندینگ عاجی، عاج ، سدیم فلوراید، مهارکننده ماتریکس متالوپروتئیناز

Introduction

Dental resin composites as direct-filling restorative materials have become very popular because of their excellent esthetics and improved load-bearing properties.[1-3] Although most adhesives show excellent short-term bonding strength, bond failure at the adhesive-dentin interface still remains a challenge.[4,5] It has been proven that the resin–dentin bonds are less durable than resin–enamel bonds since the dentin bonding relies on organic components.[6] Bond durability affects the longevity of dental restorations because the resin–dentin bonds deteriorate over time, leading to the formation of microgaps between the restorative material and teeth.[7] It has been suggested that the main reason for bond failure is the degradation of the hybrid layer in the adhesive–dentin interface, which may be triggered by mechanical and chemical factors. [8] Armstrong et al. for the first time, represented that the collagen fibrils in hybrid layers disintegrated as bond strengths degraded over five years. [9] Pashley et

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al. have shown that the dentin contains matrix metalloproteinase (MMP) enzymes, which can cleave the collagen.[10] MMPs are a family of zinc-and calcium-dependent host-derived proteases that are present in the saliva, dentinal tubules and bacterial products. One of the physiological roles of MMPs is to degrade the extracellular matrix of connective tissue, such as collagen and gelatin.[11]

A variety of MMPs have been identified in carious lesions including MMP-2, MMP-9 (gelatinase), MMP-8 (collagenase) and MMP-20 (enamelysin). [12,13] The dentin protein matrix is composed of 90% collagen (primarily type I) and 10% non-collagen proteins. Collagen proteins can be cleaved by MMP-8 and further degraded by MMP-2 and MMP-9 after the demineralization of dentin from acids in the carious lesions. [11-14] A common method for inhibiting the MMPs or decreasing their activity is the application of MMP inhibitors. The MMP inhibitory effects of some agents such as fluoride, chlorhexidine (CHX), galardin and benzalkonium chloride have been evaluated. [15-19] For example, Kato et al. concluded that the addition of sodium fluoride (NaF) gel to demineralized dental matrix reduced the degradation of the dentin by MMPs.[20] Another study illustrated that the addition of NaF to the incubation buffer resulted in the reversible inhibition of MMP-2 and MMP-9. [21] The effect of NaF on the endogenous MMP activity of dentin matrices has also been studied. [22] The specific aim included evaluating the resin–dentin bonds created with a commercially available adhesive modified with different concentrations of NaF (5,000 ppm and 10,000 ppm) by measuring the microtensile bond strength (μTBS) after 24 hours and 3 months. Therefore, the purpose of this study was to investigate the efficacy of NaF as an inhibitor of dentin MMP activity via assessing the changes in bond strength.

Materials & Methods

Adhesive preparation: The used adhesive in the current study was a two-step etch-and-rinse (Solobond M,Voco Co, Cuxhaven, Germany). Its composition based on the manufacturer's description is illustrated in Table1.

Table 1. Commercial name, components, and manufacturers of the materials used in this study

Material	Components	Manufacturer
Solobond M	BIS-GMA,HEMA, phosphate methacrylates, BHT, acetone, CQ, amine accelerator	Voco, Cuxhaven, Germany
Grandio (shade A2)	Nanohybrid ,87s% w/w (71s% volume) inorganic nanohybrid filler, Bis-GMA, UDMA, TEGDMA	Voco GmbH, Germany

BisGMA: bisphenol-A-diglycidylether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; BHT: butylated hydroxy toluene; CQ: camphorquinone; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

NaF powder at amount of 20 mg (5,000 ppm) and 40 mg (10,000 ppm) was individually added to 4 mL of dentin adhesive, which were accurately measured using a Digital Scale (GR200, A&DCo, Tokyo, Japan) with accuracy of 0.0001mg. The NaF-containing adhesives were shaken using a tube agitator for 10 minutes at 2,000 rpm to promote complete dissolution and formation of a homogeneous solution. Unpolymerized NaF-containing adhesives were placed on Teflon molds (4 mm in diameter and 4 mm in thickness), which were covered with a Mylar strip and a glass side. Specimens were light-cured from the top for 20 seconds with an LED light unit (Bluephase C8, Ivoclar Vivadent, Liechtenstein, Austria) with a power density of 800 mW/cm^2. Moreover, the specimens were cured on the surfaces covered by the mold for 20 seconds after removal from the mold. Then, specimens were polished with Sof-lex disks (3M/ESPE St. Paul, MN, USA) to obtain a flat surface with standardized roughness. Energy dispersive X-ray analysis (EDAX) was applied to identify the homogeneity of the mixture and the amount of fluoride ion dispersion in each adhesive (Fig. 1).

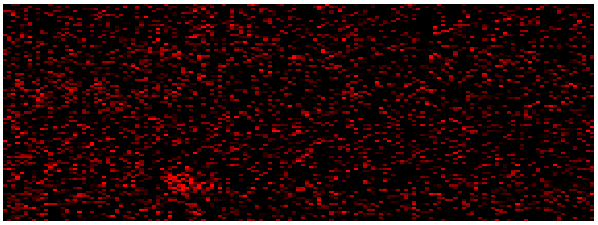


Fig 1. EDAX map of Solobond M containing 5,000 ppm NaF

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Specimen preparation: In this experimental study, 36 extracted and non-carious human third molars were used. Before using, the teeth were stored in physiological saline containing 0.1% thymol. A flat dentin surface was obtained using a water-cooled low-speed diamond disk (D&Z, Darmstadt, Germany). The smear layer of the dentin surfaces was created using 600-grit silicon carbide paper (SiC paper, Buehler). The dentin surfaces were etched with a 35% phosphoric acid gel for 15 seconds (Vococid Acid Etching Gel, Voco Co, Cuxhaven, Germany), rinsed and then, the excess moisture was removed using absorbent paper (DiaDent, Korea). The teeth were equally and randomly assigned into three groups with 12 teeth in each group as follows: treatment with Solobond M (group 1 or the control group); treatment with Solobond M containing 5,000 ppm NaF (group 2); treatment with Solobond M containing 10,000 ppm NaF (group 3). The adhesive was used for the moist dentin surfaces according to the polymerization and wet-bonding technique based on manufacturer's instructions using an LED curing device (Bluephase C8) with a power density of 800 mW/cm^2. Composite build-ups (Grandio, Voco Co, Cuxhaven, Germany) in shade A2 were placed 4-mm height on the bonded surfaces in 2 increments. Each 2-mm increment was polymerized for 20 seconds. The bonded teeth were stored in distilled water and placed in an incubator at 37ºC for 24 hours to ensure the polymerization.

Microtensile bond strength: After 24 hours, all teeth were embedded in an acrylic resin. The specimens were longitudinally sectioned in both mesiodistal and buccolingual directions across the adhesive interface using a diamond disk under copious irrigation (Delta precision sectioning machine, Mashhad, Iran) to obtain 5 resin-dentin sticks. Each stick was further sectioned to produce beams with an adhesive area of approximately 1 mm^2. The cross-sectional area of each stick was measured with a digital caliper (Shinwa digital caliper, Niigata, Japan). The sticks were divided into two groups for μTBS evaluation after 24h and 3 months of storage at 37ºC in distilled water. The storage media were changed weekly. Each stick was attached to a jig for microtensile testing with a cyanocrylate adhesive and subjected to a tensile force in a universal testing machine (Zwick/Z250, Zwick Roell Group, Ulm, Germany) at a crosshead speed of 1mm/min.

The failure mode was analyzed by a single examiner using a stereomicroscope (Nikon SMZ-U, Melville, NY, USA) at ×40 magnification. Failure modes were classified as adhesive, cohesive in composite, cohesive in dentin or mixed failure. Data were analyzed using SPSS 22 (SPSS inc., Chicago, Illinois, USA), ANOVA and Tukey's post-hoc test. The significant level was considered $p \leq 0.05$. Chi-square test was used for evaluation of failure modes.

Results

Table 2 demonstrates the mean μTBS for all study groups after 24 hours and 3 months of storage. Kolmogorov-Smirnov test exhibited normal distribution of data ($p>0.05$). The Two-way ANOVA indicated that the variables of NaF treatment and storage time as well as their interactions had a significant effect on the bond strength. After 24 hours, the experimental groups containing 10,000ppm NaF presented significantly higher bond strength than the other groups ($p<0.05$); there was no significant difference between the control group and the group treated with 5,000ppm NaF. When bond strength was evaluated after 3 months, there was a significant difference in bond strength between the control group and experimental groups.

The groups treated with NaF were not significantly different from each other. After 24 hours, the most frequently observed failure modes were cohesive in dentin in all experimental groups. After 3 months, the most prevalent failure modes were cohesive in composite (Table 3).

Table 2. Microtensile bond strength mean(±SD) values (MPa) for the study groups after 24 hours and 3 months

Time	NaF 10000 ppm	NaF 5000 ppm	Control	p-value ANOVA
24 hr	15.78± 6.90^{A,a*}	13.88± 6.75^{A,ab}	10.21± 6.01^{A,b}	0.005
3 mon	16.77± 5.72^{A,a}	14.21± 5.47^{A,a}	9.67± 4.16^{A,b}	< 0.001

NaF, sodium fluoride; SD, standard deviation

* The same superscript capital letters in the same column represent that there is no statistically significant difference between measuring times. Different superscript lower case letters in the same row represent that there are statistically significant differences among groups.

Table 3. Failure mode distribution in numbers and percentages for the study groups after 24 hours and 3 months

Group	Time	Mixed failure	Adhesive failure	Cohesive in composite	Cohesive in dentin	*p*-value Fisher's Exact Test
Control	24h	1 (3.3%)	11 (36.7%)	5 (16.7%)	13 (43.3%)	0.270
	3mo	2 (6.7%)	7 (23.3%)	11 (36.7%)	10 (33.3%)	
NaF	24h	0 (0.0%)	8 (26.7%)	9 (30.0%)	13 (43.3%)	0.005
5000 ppm	3mo	0 (0.0%)	1 (3.3%)	12 (40.0%)	17 (56.7%)	
NaF	24h	1 (3.3%)	6 (20.0%)	8 (26.7%)	15 (50.0%)	0.002
10000ppm	3mo	1 (3.3%)	2 (6.7%)	22 (73.3%)	5 (16.7%)	

NaF: sodium fluoride.

The numbers in the parentheses are the percentages of the failure mode in the same row

Discussion

The present study evaluated the effects of NaF as an inhibitor of dentin MMP activity on bond degradation. The null hypothesis was rejected because adding NaF displayed higher bond strengths of the adhesive after both 24 hours and 3 months. The results of the current study confirmed the previously showed effectiveness of NaF as an MMP inhibitor .[21]

The release of fluoride from restorative materials has been widely studied. The importance of fluoride is well understood in facilitating the mineralization or preventing the demineralization of dentin matrices. Different studies have suggested that the fluoride-containing dentin adhesives probably release the fluoride into the marginal gap, which has a beneficial effect on the demineralized enamel and dentin.[22,23]

NaF is one of the most widely used compounds in dentistry.[24] Other fluoride-containing compounds such as stannous fluoride, titanium tetra-fluoride and silver diamine fluoride have additional ions which may exhibit the relevant activity against caries and/or erosion.[21] However, it is hard to compare the different salts and dissociate the effects of fluoride from those of the other ions because their modes of action are various.[25] For this reason, the NaF was evaluated in the current study as it is unknown that the sodium ion has effect on either the demineralization and remineralization processes or MMP activity in dentin.[21] Moreover, there is little evidence available on the effect of fluoride- containing adhesives on bond strength. The bond strength after 3 months and 24 hours in all groups had no significant differences and even increased in groups with NaF. In the present study, the groups treated with fluoride compared to the control group represented an increased bond strength. The effect of fluoride was dose-dependent; the mean bond strength values after 24 hours were 13.8 MPa and 15.7 MPa for 5,000ppm NaF and 10,000ppm NaF, respectively, while after 3 months, the values were 14.2 MPa and 16.7 MPa for 5,000ppm NaF and 10,000ppm NaF, respectively. After 3-month water storage, the specimens of the fluoride-containing adhesives illustrated a significant increase in bond strength values. These findings corroborate those of Nakajima et al. [25] who concluded that a decrease in bond strength after 6-month water storage could be prevented by using fluoride-containing adhesives. They reported that the degradation of dentin was somehow prevented by fluoride so that the long-term stability of the dentin interface was improved. Although the results of the current study have shown the stability of resin-dentin bonds after 3 months with the use of fluoride-containing adhesive, it is possible that this positive effect may have been only for a storage time of 3 months and longer storage time may achieve different results.

Therefore, it can be suggested that fluoride-containing adhesive has a positive effect on resin-dentin interface. In the present study, it was surmised that the fluoride could react with the dental components in hybrid layer, leading to the remineralization of the dentin substrates. Similar results were obtained by Shinohara et al. in 2009, [26] who reported that fluoride-containing adhesive indicated a significant increase of bond strength values after 3-month water storage and a significant increase of conversion degree after 1 month. These findings illustrated that fluoride can be safely combined with the resin monomers present in Solobond M without compromising its initial bond strength and polymerization. In addition, El-Deeb et al. assessed the dentin bond strength durability of adhesives containing modified-monomer with/without-fluoride after 24-hour and 6-month storage times in artificial saliva and under intrapulpal pressure simulation and reported that fluoride addition had no effect on dentin bond

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durability,[27] which opposes that of ours and previous researchers .[25, 26] This might be due to the adhesive used in the present study contained NaF fillers rather than potassium fluoride fillers, and sodium (41.89 g/mol) had lower molecular weight compared to potassium (58.0967 g/mol). Particularly, the bond stability was found in some studies which applied an adhesive system with NaF fillers, characterized by their easy solubility. [26]

Hence, it might be explained that the fluoride might be released using the adhesive system containing fillers with lower molecular weight, which stabilizes the bond strength over the tested period. It is worth mentioning that the fluoride-released adhesive is time-dependent. Therefore, more studies are needed to investigate the possible positive effect of fluoride-containing adhesives on the bond durability over a longer duration compared to that tested in the current study.

After 3-month water storage, the specimens of the fluoride-containing adhesives represented immediate increase in bond strength. The increase in initial bond strength could be elucidated by different mechanisms. The increase in immediate bond strengths of the additive systems in the present study might be due to the preservation of collagen from MMP activity alone. Another possible reason can be associated with the fluoride, which effectively interacts chemically with hydroxylapatite.[23] This interaction generates the fluorapatite which is insoluble. Further research is required to clarify the mechanisms which inhibit the collagen matrix degradation and preserve it over time.

Currently, the etch-and-rinse system is the gold standard for bonding to enamel. Shinohara et al. in 2006, [28] used a self-etch system in their study. It seems that adding an MMP inhibitor such as fluoride to an etch-and-rinse system is more effective than adding it to a self-etch system. It was indicated that bond degradation was decreased when CHX and MMP-2/MMP-9 specific inhibitors were used with etch-and-rinse adhesive system.

However, these incorporated MMP inhibitors did not improve the self-etch adhesive systems.[28] Ferracane et al. found that the adhesives containing fluoride could release it into the microspaces of a restored cavity and finally, they offered some level of protection from demineralization and secondary caries.[29] They also expressed that fluoride-containing adhesives could release fluoride into the water for more than 4 months.[29] These results were similar to ours since after 3-month water storage, the μTBS values significantly increased in the specimens treated with fluoride-containing adhesives. Resin-dentin bond stability has been achieved with the non-specific synthetic protease inhibitor, CHX. CHX can inhibit MMP-2, -8 and -9 and the activity of cysteine cathepsins.[27-30] It has been suggested that CHX inhibits MMPs and cathepsins by a cation-chelating process and electrostatic binding.[31] Kato et al. indicated that the inhibition of MMP-2 and MMP-9 at high concentrations of NaF such as 5,000ppm was irreversible but reversible at low concentrations.[21] It seems that the fluoride has no effect on the direct inhibition of proteolysis via dentin matrix-bound MMPs. [32] The possible mechanism involved in the inhibition of MMP by NaF still remains unclear. Regarding that MMPs are Zinc-and-Calcium-dependent enzymes, maybe, the excess fluoride causes these cations not to be accessible for participation in catalytic process because of the electronegative property of fluoride. Therefore, it was decided to use the high concentrations of NaF in the present study.

This is the first study to describe the ability of NaF added to a two-step etch-and-rinse system in order to improve the stability of resin-dentin bonds after 3 months. Fluoride is the most common compound in oral hygiene products. Due to the limitations of this study, only a filled acetone-base adhesive was evaluated so the results of this study might not attribute to all filled adhesives. Moreover, further in vivo and in vitro studies are recommended to find the causes of the increase in immediate bond strengths, to optimize the MMP inhibitory effect (*e.g.*, concentration of NaF, period of storage) and to illuminate the NaF mechanisms which inhibit the collagen matrix degradation and preserve it over time.

Conclusion

Despite the limitation of this in vitro study, it can be concluded that the Solobond M containing fluoride promotes the highest value of bond strength. This may describe that the durability of resin-dentin bonds is increased by using fluoride-containing adhesive.

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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. Yasaman Samani carried out the in vitro procedures, collected the data and wrote the manuscript. Zahra Jafari and Hamed Tashakkorian supervised the procedure and edited the manuscript. Soraya Khafri developed the interpretation of data and statistical analysis.

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