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In Vitro Microleakage of Fissure Sealant Following Enamel Surface Treatment with Sodium Hypochlorite and Application of a Universal Adhesive

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ABSTRACT

Objectives: This study aimed to assess the microleakage of fissure sealant following enamel surface treatment with 5.25% sodium hypochlorite (NaOCl) and application of a universal adhesive.

Materials and Methods: In this in vitro study, 75 sound extracted premolars were randomly assigned to 5 groups (n=15) of (I) phosphoric acid etching + sealant (control), (II) NaOCl + etching + sealant, (III) etching + G-Premio Bond application, (IV) NaOCl + etching + G-Premio Bond, and (V) G-Premio Bond + sealant. All groups were then incubated for 24 hours, and underwent 3000 thermal cycles. They were subsequently immersed in 50% silver nitrate and sectioned buccolingually. The dye penetration depth was measured under a light microscope to determine the microleakage score. Data were analyzed by the Kruskal-Wallis and Dunn tests (alpha=0.05).

Results: The microleakage was significantly different among the five groups ($P<0.05$). Application of universal adhesive (groups III, IV and V) significantly increased the microleakage. Application of NaOCl for enamel preparation decreased the microleakage compared with the groups without NaOCl application. The lowest microleakage was recorded in group II (NaOCl + etching + sealant).

Conclusion: Although the difference in microleakage was not significant between the NaOCl and the control groups, lower microleakage in NaOCl groups suggests that enamel surface treatment with NaOCl may decrease microleakage under certain circumstances. Application of G-Premio Bond under sealant increased the microleakage, and is therefore not recommended for microleakage reduction.

Keywords: *Pit and Fissure Sealants; Sodium Hypochlorite; Dental Leakage*

1. INTRODUCTION

Dental caries is an infectious disease with a high prevalence among children [1]. Carious lesions more commonly involve the pits and fissures [2]. These areas are ideal for bacterial retention due to their anatomical structure. Preventive measures such as plaque control and topical application of fluoride have minimal effect on pits and fissures [3]. Therefore, sealing of pits and fissures with fissure sealants especially in areas highly susceptible to caries is an effective caries prevention strategy

[4]. Primary and permanent teeth at risk of caries can benefit from the advantages of fissure sealant therapy [5]

Despite its advantages, fissure sealant therapy has limitations as well. The penetration depth of sealant and its retention gradually decrease with time, leading to treatment failure. Also, leakage of saliva and microorganisms through the tooth-sealant interface is highly common [6]. Thus, absence of microleakage, sufficient retention, and sealant integrity are among the most important factors in assessment of the success of fissure sealant therapy [7].

The preventive function of pit and fissure sealants is mainly achieved through optimal adhesion of sealant to the enamel surface [8]. Currently, a wide range of sealants are available in the market which have differences in terms of basic ingredients, polymerization, fluoride content, and bioactive ingredients [9]. Acid etching is the first step for application of resin-based sealants, because it increases enamel wettability and micromechanical retention of sealant [10]. In a study by Unverdi et al, [10] using different generations of dental adhesives (5^{th} and 6^{th} generations) had a positive effect on increasing the success of pit and fissure sealant therapy. The etch-and-rinse adhesive systems have three steps of etching, rinsing and drying, and bonding application, which prolong the treatment time. Also, they are associated with a higher risk of contamination especially in children if a rubber dam is not used [9]. However, self-etch adhesives have omitted the etching step. In this regard, the use of self-etching adhesives can be useful, especially in children.

One-bottle or universal adhesives are the latest generation of bonding agents with advantages such as simplified and easy application, less technical sensitivity, and decreased working time, which are highly important in pediatric dentistry [11]. These bonding agents contain adhesive monomers that enhance their chemical adhesion to hydroxyapatite [12]. Some other non-invasive techniques have also been proposed to enhance the bond strength and decrease the microleakage of fissure sealants. Enamel surface treatment with sodium hypochlorite (NaOCl) is among the suggested techniques for this purpose, which can increase the penetration depth of adhesive and sealant into the enamel. Freshly erupted teeth have a high organic content, and elimination of this organic content can increase retention [3]. An in vivo study showed that elimination of organic content from the enamel surface by the application of 5.25% NaOCl as a deproteinizing agent for 60 seconds prior to etching doubled the etched enamel surface retention and improved etching of types I and II enamel, resulting in formation of long adhesive tags and improved micromechanical interlocking [3].

Nonetheless, information about the effect of enamel surface treatment with NaOCl on fissure sealant microleakage in use of universal adhesives is limited. Thus, this study aimed to assess the microleakage of fissure sealant following enamel surface treatment with 5.25% NaOCl and application of a universal adhesive. The null hypothesis of the study was that enamel surface treatment with NaOCl, application of G-Premio Bond, or their combination would have no significant effect on microleakage of fissure sealant.

2. MATERIALS AND METHODS

The protocol of this in vitro study was approved by the ethics committee of Qazvin University of Medical Sciences (IR.QUMS.REC.1400.413). The patients consented to the use of their extracted teeth for research purposes.

Sample size:

The sample size was calculated to be a minimum of 15 in each group according to a previous study [13] using G*Power software and one-way ANOVA assuming alpha=0.05, study power of 805, and effect size of 0.42.

Specimen preparation:

This in vitro experimental study was conducted on sound premolars with no caries, restoration, crack, or defect, which had been extracted as part of orthodontic treatment. The collected teeth were inspected under a light microscope at x10 magnification to ensure their soundness. The teeth were immersed in 0.5% chloramine T solution for one week for disinfection, and were then stored in distilled water at 4°C.

Application of sealant:

The occlusal pits and fissures of the teeth were cleaned with a dental explorer, and a rubber cup and pumice paste (without additives) and the teeth were then randomly assigned to 5 groups (n=15) as follows:

Group I (control): The teeth were etched with 37% phosphoric acid (Morvabon, Iran) for 15 seconds, rinsed for 15 seconds, and dried with oil-free spray. Next, Clinpro™ resin-based pit and fissure sealant (3M ESPE, St. Paul, MN, USA) was applied into the pits and fissures, gently dispersed with a dental explorer, and light-cured for 20 seconds using an i-LED light-curing unit (Woodpecker, Guiliin, China) with 385-515 nm wavelength and 1000-1700 mW/cm^2 light intensity.

Group II (NaOCl + etching): In this group, 5.25% NaOCl (Nikdarman, Tehran, Iran) was applied into the pits and fissures with back-and-forth movements for 1 minute [14]. It was then rinsed with sterile distilled water for 10 seconds, and dried with oil-free air spray. Next, acid etching was performed and fissure sealant was applied [15,16].

Group III (etching + bonding): The enamel surface was conditioned by etching with phosphoric acid for 15 seconds as instructed by the manufacturer, rinsed and gently dried with air spray. G-Premio Bond (GC Corp., Tokyo, Japan) was applied into the pits and fissures by a microbrush and after 10 seconds, dried with maximum pressure of air spray for 5 seconds. It was then light-cured for 20 seconds. Sealant was then applied and light-cured as explained earlier.

Group IV (NaOCl + etching + bonding): The pits and fissures were first etched with 5.25% NaOCl which was rubbed on the surface with back-and-forth movements for 1 minute. Etching and bonding were then performed as explained for group III.

Group V (self-etch bonding): Adhesive was applied into the pits and fissures by a microbrush as explained earlier, and cured for 20 seconds. Sealant was then applied and cured.

Table 1 presents the composition and characteristics of the materials used in this study.

Table 1. Composition and characteristics of the materials used in this study

Material type	Commercial name	Manufacturer	LOT number	Composition
Sealant	Clinpro Sealant	3M ESPE, St. Paul, MN, USA	NA86659	Triethlene glycol, bis-GMA, silane-treated silica tetrabutylammonium tetrafluoroborate, diphenylidonium, hexafluorophospate, ethyl 4-dimethyl aminobenzoate, titanium dioxide, hydroquinone [39]
NaOCl deproteinizing agent	Hyponic Beta	Nikdarman, Tehran, Iran	93215826	5.25% NaOCl
Dye	Silver nitrate	Merck & Co Inc., Darmstdt HE, Germany	K52188912008	$AgNO_3$
Universal adhesive	G-Premio BOND	GC Corp., Tokyo, Japan	1912021	methacryloyloxydecyl dihydrogen phosphate (MDP), 4-methacryloxyethyl trimellitic acid (4-MET), MDTP (methacryloxydecyl dihydrogen thiophosphate), dimethacrylate monomer, distilled water, acetone, photo initiators, fine silica powder pH=1.5
Etchant	Morva Etch	Morvabon, Tehran, Iran	101204203	37% phosphoric acid

Aging:

The teeth were incubated (Azmateb, Iran) in distilled water at 37°C for 24 hours [17]. They were then thermocycled (Dorsa, Iran) for 3000 thermal cycles between 5-55°C with a dwell time of 20 seconds and a transfer time of 20 seconds [15,18].

Microleakage assessment:

After complete drying of the specimens, the apical part of the root was sealed with auto-polymerizing acrylic resin. Also, the entire surface of the specimens to 1 mm of the area sealed with sealant was coated with 2 layers of water-resistant nail varnish [17]. The second layer was applied after drying of the first layer. Next, the specimens were immersed in 50wt% silver nitrate dye (Sigma Aldrich, Germany) at room temperature for 6 hours [19]. The teeth were then rinsed under running water for 5 minutes, immersed in photochemical developer solution (RRD10; World Chemical Industries, Iran), and irradiated with a 150-W lamp for 6 hours [17].

The specimens were coded and mounted in auto-polymerizing acrylic resin to the level of their cementoenamel junction. They were then sectioned buccolingually by a Mecatome (T201A; Presi, France) with a diamond disc (Drux, Germany) under water coolant [15]. The microleakage was then quantified by measuring the dye penetration depth under a stereomicroscope (Zostrad, Iran) at 40x magnification, and scored as follows (Figure 1) [20]:

Score 0: No dye penetration depth through the tooth-sealant interface

Score 1: Dye penetration to less than one-third of the tooth-sealant interface

Score 2: Dye penetration by one-third to two-thirds of the tooth-sealant interface

Score 3: Dye penetration by more than two-thirds of the tooth-sealant interface

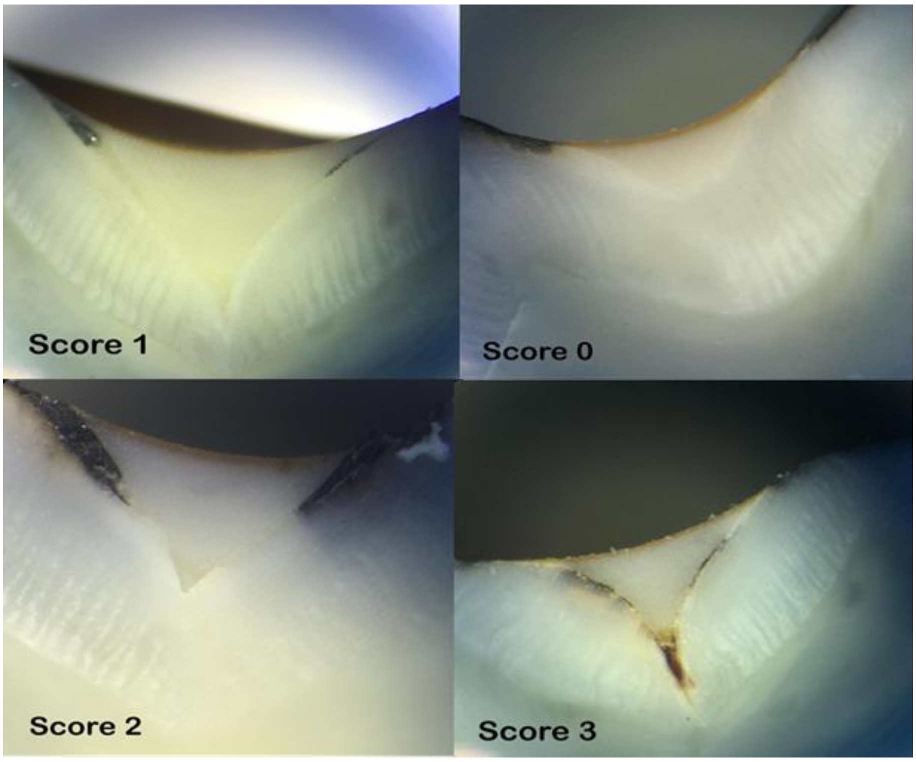

Figure 1. Microleakage scores determined by the dye penetration depth

Statistical analysis:

Data were analyzed using SPSS version 25 (SPSS Inc., IL, USA). Due to non-normal data distribution as shown by the Shapiro-Wilk test ($P<0.05$), the Kruskal-Wallis test was used to compare the microleakage score among the groups. Pairwise comparisons were performed by the Dunn test with Bonferroni adjustment. $P<0.05$ was considered statistically significant.

3. RESULTS

Table 2 and Figure 2 show the frequency of different microleakage scores in the study groups. The Kruskal-Wallis test showed a significant difference in the microleakage score among the five groups ($P<0.05$). For a more accurate comparison, the mean rank was used, which showed the highest microleakage in group V with a mean rank of 58.83 followed by group III with a mean rank of 51.83, group IV with a mean rank of 43.17, group I (control) with a mean rank of 21.50, and group II with a mean rank of 14.67.

Table 2. Frequency distribution of different microleakage scores in the study groups

Score / Group	Score 0		Score 1		Score 2		Score 3		Total		Mean rank	P value
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage		P<.05
Group I (control)	6	35.3%	7	38.9%	2	16.7%	0	0%	15	20%	21.50	

Group II (NaOCl + etching)	11	64.7%	3	16.7%	1	8.3%	0	0%	15	20%	14.67	
Group III (etching + bonding)	0	0%	3	16.7%	2	16.7%	10	35.7%	15	20%	51.83	
Group IV (NaOCl + etching + bonding)	0	0%	5	27.8%	5	41.7%	5	17.9%	15	20%	43.17	
Group V (self-etch bonding	0	0%	0	0%	2	16.7%	13	46.4%	15	20%	58.83	

Pairwise comparisons of the groups by the Dunn test with Bonferroni adjustment showed that groups I (control) and II did not differ significantly (P>0.05). Groups III, IV, and V were not significantly different from each other either (P>0.05), but had significantly greater microleakage than groups I and II (P<0.05).

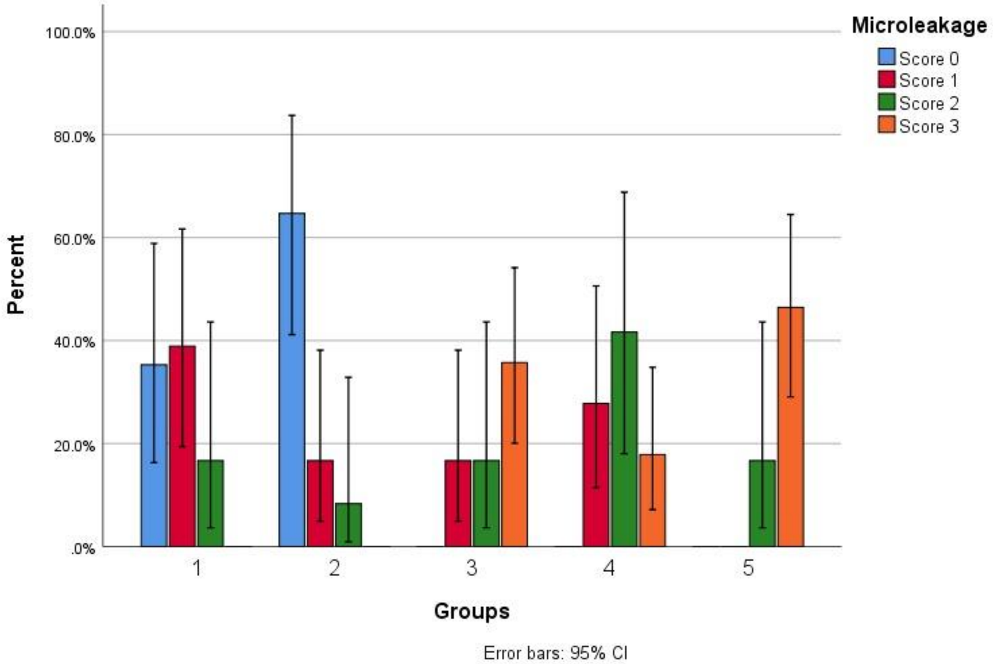

Figure 2. Comparative frequency of microleakage scores in the five study groups (1: etching, 2: NaOCl + etching, 3: etching + bonding, 4: NaOCl + etching + bonding, 5: self-etch bonding)

4. DISCUSSION

This study assessed the microleakage of fissure sealant following enamel surface treatment with 5.25% NaOCl and application of a universal adhesive. In the preset study, thermocycling was performed to simulate thermal stresses present in the oral environment. Thus, the specimens were subjected to 3000 thermal cycles between 5-55°C, which is close to the range of thermal alterations in the oral environment [21].

Silver nitrate is a valuable dye for use in dye penetration test due to small size of its particles (0.059 nm), enabling their easy leakage through the tooth-restoration interface. However, due to small size, this dye penetrates easier through the interface than the bacteria which normally range in size from 0.5 to 1 µm. Thus, although this technique may appear to be strict, it may be assumed that a restoration that prevents the penetration of silver ions can also prevent bacterial leakage, and may show a better performance compared with the clinical setting [22].

The present results showed a significant difference among the study groups in microleakage such that application of NaOCl significantly decreased the microleakage. Also, application of G-Premio Bond significantly increased the microleakage.

Thus, the null hypothesis of the study was rejected.

Acid-etching is performed aiming to create a retentive surface with high energy. Although phosphoric acid has been successful in this regard, the etching quality may not be homogenous in all parts of the enamel surface. The enamel contains 96% minerals and less than 1% organic content, half of which, is protein. Phosphoric acid etching affects the enamel minerals, but cannot eliminate its organic content. Thus, elimination of enamel organic content may improve the quality of etching; this idea led to development of the concept of enamel deproteinization [23]. Therefore, 5.25% NaOCl was used for 1 minute for enamel surface deproteinization in the present study [14]. NaOCl denatures the proteins non-invasively without affecting the tooth structure. It has optimal antibacterial activity and decreases the surface tension through saponification of fatty acids. Application of NaOCl for deproteinization may serve as a suitable strategy to enhance bonding by elimination of organic content of the enamel and acquired pellicle formed by enamel etching with phosphoric acid, and improves the etching pattern as such [24].

Microleakage in group II that the specimens were deproteinized with NaOCl before etching was lower than that in the control group with only etched specimens; however, this difference did not reach statistical significance. Also, microleakage in group IV (NaOCl + etching + bonding) was lower than that in group IIII (etching + bonding). Abdelmegid [16] showed that enamel surface roughness increased by the application of NaOCl before or after etching. Improvement of enamel surface properties such as surface roughness increased retention and bond strength of adhesives [16]. Valencia et al. [23] demonstrated that application of 5.25% NaOCl is an effective strategy to increase surfaces with types I and II etching patterns. Application of NaOCl as a deproteinizing agent prior to etching significantly increased the retentive enamel surface by up to 94.47%, and increased types I and II etched enamel [24]. Justus et al. [25] demonstrated that application of NaOCl for 1 minute prior to enamel etching enhanced the penetration of acid etchant through elimination of enamel organic content, and improved the shear bond strength by increasing types I and II patterns. Occlusal enamel surface preparation with NaOCl exposes a higher number of enamel crystals to acid etchant and clinically results in a better etched surface [23]. Valencia et al. [23] discussed that in absence of deproteinization, the etchant easily attacks the center of enamel prisms since these areas have a less organic content; while after deproteinization, etching attacks inter-prismatic areas that have a greater organic content. Thus, they concluded that types I, II, and III etching patterns are determined by the level of elimination of enamel organic content (biofilm, enamel pellicle, and inter-prismatic proteins) [23]. Nonetheless, Ahuja et al. [26] reported that enamel deproteinization with 5.25% NaOCl prior to etching did not efficiently affect the enamel surface topography, and Roopa et al. [3] showed that application of 5.25% NaOCl before or after etching compared with etching alone had no significant effect on shear bond strength or microleakage.

It is assumed that application of bonding agents increases the bond strength especially in pits and fissures that have remained slightly moist [27]. Nonetheless controversy exists regarding the effect of application of bonding agents on the microleakage and bond strength of sealants [28,29].

The idea of application of a bonding agent underneath the sealant was first suggested by Feigal [30] in 1993. They used hydrophilic bonding agents to ensure sealant stability in wet environments (where complete isolation was not achievable). The American Dental Association and the American Academy of Pediatric Dentistry supported the application of an adhesive system prior to sealant application to maximize retention [28]. Also, Askarizadeh et al. [31] showed that application of a bonding agent decreased the microleakage in saliva-contaminated teeth. Nonetheless, Marks et al. [29] reported the best results in etched-only specimens and concluded that additional application of adhesive did not decrease microleakage. Moreover, Mehrabkhani et al. [7] concluded that application of bonding agent had no significant effect on microleakage and recommended using a low-viscosity sealant. Similarly, Nirwan et al. [32] reported that application of a bonding agent under the sealant did not improve the retention and did not decrease the microleakage of conventional sealants. Almahdy et al. [33] stated that application of a bonding agent prior to sealant application decreased its penetration depth into the fissures. Furthermore, Almahdy et al. [33] showed that aging decreased the bond strength in groups with a bonding agent probably due to water sorption and dissolution of bonding layer while aging had no significant effect on the bond strength in groups without a bonding agent.

G-Premio Bond is a HEMA-free adhesive that contains acetone. HEMA functional comonomer serves as a solvent to prevent separation of the hydrophilic and hydrophobic phases. Nonetheless HEMA enhances water sorption and hydrolytic degradation [34]. Thus, some manufacturers eliminated HEMA from their adhesive formulations. Elimination of HEMA necessitates a higher concentration of solvent. Thus, G-Premio Bond contains 40% acetone instead [35]. Although acetone is highly volatile, higher concentration of acetone in HEMA-free adhesives may prevent complete water/solvent evaporation [35]. G-Premio Bond also contains 4-META monomer. It is believed that presence of an adhesive monomer other than 10-MDP may compete with it for binding to hydroxyapatite and limit the formation of 10-MDP-Ca nano-layer [36]. Since G-Premio Bond is HEMA-free, it may compromise phase separation at the interface and subsequently the bonding durability [35,37].

The etching strategy of universal adhesives depends on their pH and tooth structure [38]. Omidi et al. [37] reported greater microleakage reduction by the selective etching technique than the self-etch technique in using G-Premio Bond. Even universal adhesives that can chemically bond to enamel require phosphoric acid etching for micromechanical bonding. Thus, selective etching of enamel is recommended in self-etch use of adhesives [37]. Significantly higher microleakage in group V (self-etch bonding) compared with other groups in the current study may be due to not using the selective etch technique.

This study had an in vitro design, which limits the generalizability of the findings to the clinical setting due to the absence of masticatory forces and other intraoral conditions that decrease the retention of sealants. Also, only premolars were evaluated in this study. Furthermore, a stereomicroscope was used for assessment of microleakage. Using an electron microscope may provide more details. Future studies are required on primary teeth using more advanced techniques for evaluation of microleakage. Finally, clinical studies with a long-term follow-up are required to obtain more reliable results.

5. CONCLUSION

Although the difference in microleakage was not significant between the NaOCl and the control groups, lower microleakage in NaOCl groups suggests that enamel surface treatment with NaOCl may decrease microleakage under certain circumstances. Application of G-Premio Bond under sealant increased the microleakage, and is therefore not recommended for microleakage reduction.

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