**Original Article**

# Evaluation of Microleakage in Fissure Sealants following Contamination with Artificial Saliva at Different Curing Times with or without using bonding agent

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**Abstract**

**Background and Aim:** One of the important factors in microleakage of fissure sealants is saliva contamination. Researchers believe that applying bonding agents can reduce the microleakage. The present study compared the microleakage of a fissure sealant contaminated with artificial saliva at different curing times with or without using a bonding agent.

**Materials and Methods:** In this in-vitro study, 96 extracted premolars were randomly divided into eight groups. Fissure sealant was applied to all groups; in 4 groups after putting on a bonding agent and in remaining four without a bonding agent. Specimens of each group were contaminated with saliva at different times (5, 10, 15 and 20 seconds) during curing. Then the microleakage of samples was measured. Mann-Whitney and Kruskal-Wallis tests were used to statistically analyze the collected data.

**Results:** No-bonding groups had significantly higher microleakage values than bonding groups. Among bonding groups, the microleakage values were significantly lower when contamination occurred after 15 and 20 seconds of light curing. Amounts of microleakage were similar in no-bonding groups ($P=0.39$) when contamination occurred at 5, 10 and 15 seconds of curing; however, contamination after 20 seconds led to significantly lower microleakage ($P<0.001$).

**Conclusion:** The findings revealed that when using the bonding agent, retreatment is not required if contamination occurs after 10$^{th}$ second of curing; however, retreatment is required when no bonding has been applied, and contamination happens before 20$^{th}$ s of curing. Thus the isolation is more critical when pits and fissure sealants are applying without using a bonding agent.

**Key Words:** Dental Leakage, Pit and Fissure Sealants, Dental Bonding

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## Introduction

Sealing pits and fissures is one of the most effective methods in preventing occlusal caries. However, achieving this goal depends directly on the complete sealing of the pits and grooves with adhesive resins with proper longevity [1]. Proper isolation and prevention of oral fluids contamination are essential factors for successful pit and fissure sealants which is more difficult to carry out in children [2,3]. According to the

Borsatto et al [4] study, contamination of the sealant with saliva remarkably increases microleakage. Moreover, it has been reported that microleakage is one of the causes of treatment failure [4]. The etched enamel is very sensitive, and even one-second contact with saliva would be enough to fill the enamel microporosities with pellicle and preventing proper attachment of sealant. Controlling saliva in uncooperative, young, and mentally challenged children are difficult and almost impossible [5,6]. Using a rubber dam is one of the isolation techniques that cannot be used easily in pediatrics as in adults. Moreover, using cotton rolls requires enough proficiency and there is a possibility of saliva contamination due to sudden and unwanted movements of the tongue. Another important factor is using a suitable adhesive bonding agent, which could lead to more successful fissure sealant. However, using bonding agent is optional in this kind of treatments, it improves bonding strength particularly when isolation is questionable [7-11]. Poor marginal integrity in pit and fissure sealants results in microleakage and consequently influx of microorganisms which subsequently induce recurrent caries. If the isolation is not correctly carried out and the destructive factors (i.e., salivary contamination) are not well controlled, the treatment fails, and adverse effects accure [12].

The ideal time for fissure sealant placement is right after the eruption of permanent molars in children who might not be fully cooperative; therefore, saliva contamination could happen anytime during application and curing of the sealants. Since the time and duration of saliva contamination may affect the rate of microleakage, the present study was carried out to evaluate the effect of artificial saliva contamination on fissure sealant microleakage at different times during light curing with and without the use of bonding .

## Materials and Methods

The present in vitro study was performed on 96 healthy premolars with deep occlusal pits and grooves, which were extracted for orthodontic purposes up to six months before the start of the study. Teeth were washed with tap water, and the remaining tissues and debris on the teeth were removed. Then teeth were disinfected by storing in 0.5% chloramine-T for one week. Then they were washed and kept in normal saline at 4°C. Afterward, they were randomly assigned to eight groups (n=12).

All steps were performed according to the manufacturer's instructions. All specimens were etched with 37% phosphoric acid (Ultradent, USA) for 15 seconds, followed by irrigating with water and air spray for 15 seconds. The samples were then dried with compressed air until the chalky white appearance has appeared on the teeth surfaces. After this point, the procedures were followed differently and is shown in Figure 1. In four groups bonding (Single Bond 3M ESPE, U.S.A) was used before the fissure sealant (3M, ESPE, U.S.A) placement while in the other four groups no bonding agent was used.

Light curing was performed in all samples with an LED light-curing device (Radii, SDI, Australia) with an output of 1000 $mW/cm^2$ at the 2 mm distance. A radiometer measured the light intensity of the light-curing device before each use. It should also be mentioned that light was irradiated horizontally and vertically in order to reach all the surfaces equally. To simulate the contamination, artificial saliva was applied to all specimens for 5 seconds then air dried for 10 seconds. Artificial saliva was prepared at the chemistry laboratory of School of dentistry, Tehran University of Medical Sciences [13].

All the samples were subjected to 1000 cycles of thermocycling between 5°C and 55°C. Next, the apical third of the roots were sealed with sticky wax and then, all tooth surfaces were painted with two coats of nail varnish except a 1 mm wide zone around the margins of the sealant. Afterward, teeth were immersed in a 1mol silver nitrate solution (prepared at the chemistry laboratory of School of dentistry, Tehran University of Medical Sciences) and kept in the dark place for 24 hours. After thoroughly rinsing with distilled water and drying, the specimens were immersed in a processing solution (Iran Chemical, World Co, Iran) under fluorescent light for 4 hours. Next, the samples were mounted in translucent self-hardening acrylic resin and sectioned buccolingually using a high-speed sectioning device (DORSA Teb, Iran) with a 0.3 mm thick double-edged diamond disc under water coolant with the 8000 rpm rotational speed and 10 mm/min forward speed. In order to measure the microleaakage, each section was evaluated under LEICA EZ4D stereomicroscope (Tokyo, Japan) at 40× magnification and digital photographs were taken. In the present study the percentage of microleakage was calculated using the following formula:

Microleakage (%) = (A+B)/(C+D) × 100 [14]

A= The extent of dye penetration in the buccal wall along the enamel-sealant interface (μm)

B= The extent of dye penetration in the lingual wall along the enamel-sealant interface (μm)

C= Length of buccal enamel-sealant interfaces (μm)

D= Lengths of lingual enamel-sealant interfaces (μm) [14] (Figure 2).

After the microleakage value of each tooth was calculated, the SPSS-20 software was used to statistically analyze the collected data using Kruskal-Wallis and Mann-Whitney tests and $P<0.05$ was considered significant.

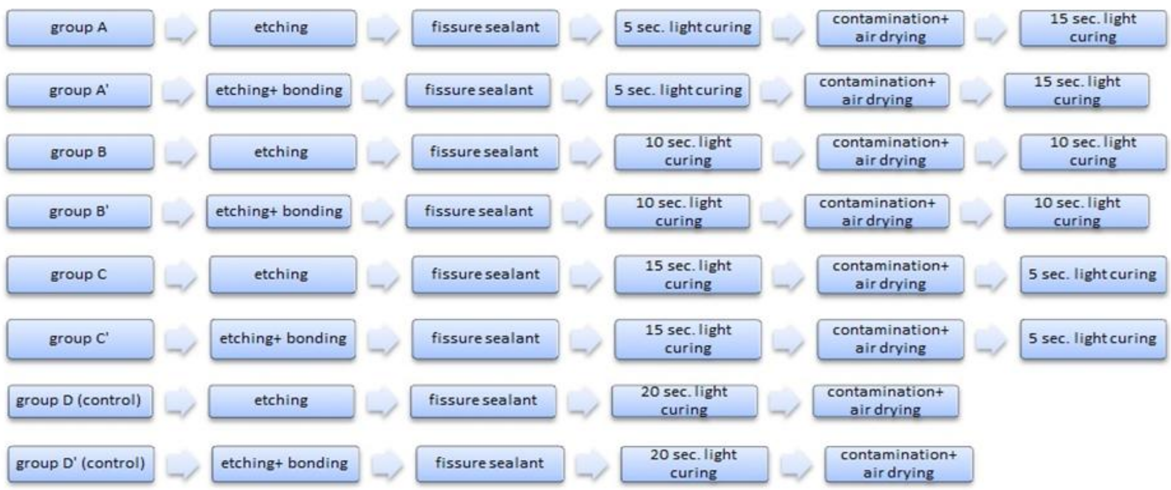


**Figure 1.** The representative chart of the sample preparation in different groups

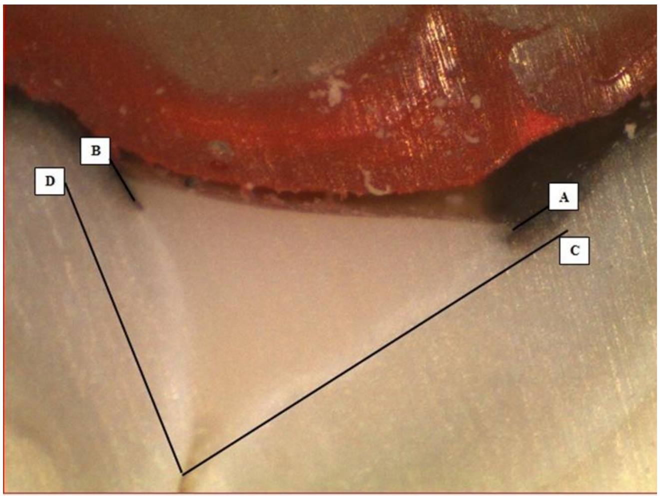


**Figure 2.** A digital photograph (40×) of the tooth section is shown in the figure; A = Extent of dye penetration in the buccal wall along the enamel-sealant interface (μm)

## Results

The mean microleakage values and standard deviations of each group after contamination with artificial saliva at different curing times are presented in Table 1.

**Table1.** Comparison of the mean percentage of microleakage of the bonding and non-bonding groups following saliva contamination at different curing times. Values (%) are presented as Mean±SD

| Radiation and contamination time (second) | With Bonding | Without Bonding | Adjusted P-value |
|---|---|---|---|
| 5 | 15±11 | 31±33 | P<0.001 |
| 10 | 16±8 | 27±22 | P<0.001 |
| 15 | 9±3 | 19±21 | P<0.001 |
| 20 | 9±3 | 10±7 | P<0.001 |

-A significant difference was observed between all groups. Microleakage values in all non-bonding groups were significantly more than their counterpart bonding groups (P<0.001).

-The microleakage value of the bonding groups that the saliva contamination occurred after 15 and 20 seconds of curing was significantly lower than groups of 5 and 10 seconds of curing before contamination (P<0.001).

-The mean percentage of the microleakage was not significantly different among the non-bonding group that contaminated after 5, 10 and 15 seconds of curing times; however, there was a significant difference between them and 20 seconds group (P<0.001).

## Discussion

The present study compared the microleakage values of fissure sealant with and without using a bonding agent following saliva contamination at different curing times.

In our study, non-bonding groups had significantly higher microleakage compared to bonding groups. Among bonding groups, the microleakage percentage was significantly lower when contamination occurred after 15 and 20 seconds of light curing. While in the non-bonding groups, the microleakage measurement indicated no significant difference (P=0.39) between 5, 10, and 15 seconds curing time subgroups. However, contamination after 20 seconds of curing led to significantly lower microleakage (p<0.001). Therefore, the findings revealed that when using a bonding agent, retreatment is not required if contamination occurs after curing for 10s. On the other hand, retreatment is necessary when bonding agents are not used, and saliva contamination occur anytime before the 20s of curing. Thus, the isolation is more important when pits and fissure sealant is applied without using a bonding agent. To our knowledge, no study has been evaluated the effect of the time of contamination on the bonding strength of fissure sealant. Therefore, the comparison between the studies in this field is not possible.

Peng et al. [15] studied Marginal microleakage of triage sealant under various moisture contamination. The results of their study showed that Fuji Triage sealant had the least marginal leakage under a moisture-controlled environment. Nevertheless, when saliva was introduced during the application of the material, microleakage significantly increased, and 1-second air-thinning of the saliva, 10-second air-drying of the saliva, or reconditioning before sealant application did not significantly decrease the microleakage. Memarpour et al. [16] also have evaluated the effect of saliva contamination on sealing properties of fissure sealant bonded with universal adhesive systems. They revealed that saliva contamination after etching the tooth had an adverse effect on the adhesion of the sealant to the treated enamel. The increase of microleakage after saliva contamination, which observed in these two studies, is similar to the results of the present study. However, the effect of the time of contamination during light curing was not considered in the Peng et al. [15] and Memarpour et al. [16] studies.

Basir et al. [17] carried out a study on the effect of the different adhesive system on fissure sealant microleakage with or without saliva contamination. They have reported that microleakage was reduced by using bonding systems, although it was not eliminated and sealants without any bonding agent could not tolerate slightest saliva contamination.

Hence, bonding systems were recommended to be used before sealant to reduce the effects of saliva contamination.

Mesquita-Guimarães et al. [18] evaluated the effect of saliva contamination on bond strength of a Bisphenol-A free fissure sealant with and without an adhesive layer. They concluded that an adhesive system layer should be used prior to the sealant placement in either dry and saliva-contaminated enamel.

Similar to these studies, the results of the present study also showed a significant difference between bonding and non-bonding groups, and a higher percentage of microleakage in non-bonding groups. These findings are in line with the results of the studies carried out by Bahrololumi et al. [12] and Mesquita-Guimarães et al. [18].

Cehreli and Gungor [14] evaluated the microleakage of Helioseal F fissure sealant with and without different bonding agents. The results showed using etch-and-rinse adhesives led to lower microleakage compared to self-etching adhesives or acid etch alone without bonding. Moreover, using sealant with no bonding revealed the highest level of microleakage after 48 hours. Although the particulars are different in Cehreli and Gungor [14] research and the present study, results are comparable, even though, Cehreli and Gungor [14] didn't consider saliva contamination.

Bahrololumi et al. [12] compared the microleakage of two types of fissure sealants (Helioseal F; and Tetric flow) with and without using a bonding agent. Their findings indicated that Helioseal F was better sealant than Tetric flow, and the use of bonding was recommended in all groups especially with the Tetric flow. They also have reported that the use of a bonding agent reduce microleakage. In general, using flowable composite along with bonding agent, especially in specific clinical conditions (e.g., uncooperative children and saliva contamination), has been recommended [19].

In the present study, the enamel surfaces were not cleaned or changed by pumice prophylaxis or enameloplasty respectively. One study has considered these procedures as a factor to increase fissure sealant bonding strength [20]. However, other study pointed out that pumice paste may remain at the bottom of the grooves and prevent complete penetration of fissure sealant [21]. In the present study, artificial saliva was used at different curing times to simulate the different clinical situation in which saliva contamination might occur. Also, silver nitrate was used to reveal the extent of the microleakage because of its high penetration capability. In a study by Hebling and Feigal [5], a one-bottle adhesive was used as the bonding agent to reduce microleakage in the enamel with saliva contamination. In their study, when applied saliva to the tooth was dried with compressed air, the microleakage was reduced from 94% to 42% compared to the group that saliva contamination was not dried. Furthermore, in the groups to which a bonding agent was applied, the microleakage level was decreased to 6%. This could be a confirmation of the efficiency of bondings, especially single bond, in situations that saliva contamination occurs. It also indicates that drying the saliva at the time of contamination could reduce microleakage by half, although it is still considered a high percentage [5]. Therefore, the specimens in the present study were dried after saliva contamination in order to obtain more acceptable and applicable results.

The results of the present study could be considered as a starting point for further investigations since no other studies have been conducted in this regard. Particularly because the results obtained from in vitro studies cannot adequately reflect and be indicative of the clinical conditions, further studies are needed.

## Conclusion

In conclusion, when fissure sealant is applied with a bonding agent, retreatment is not required if contamination occurred after 10s curing. However, without using bonding agent retreatment is required if the contamination happens anytime before the 20s of curing. Thus the isolation is more critical when pits and fissure sealant is applying without using a bonding agent.

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