Original Research

The Effect of Expiration Date on Microleakage of Fissure Sealants: An In Vitro Study

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Abstract:

Background: Although the prevalence of dental caries has decreased in recent decades, caries is still a major problem. The idea of preventing caries on masticatory surface of teeth has led scientists to use substances as fissure sealants to prevent pit caries and dental fissures. No study has been performed on potential changes in properties of expired fissure sealants. Therefore, considering the current conditions of society and potential coercion to use some expired substances, particularly in some deprived areas, we decided to evaluate the microleakage of these substances.

Method: In the present study, healthy human premolars extracted for orthodontics were used. First, enameloplasty was performed for premolar fissures by round bur; acid etching and fissure sealant were used to provide similar conditions in different groups and to remove the bonding effect. Then the samples were subjected to thermocycles; dye penetration was used to check the amount of microleakage.

Results: The results showed that, on average, the amount of microleakage increases after expiration date; in this way, the amount of microleakage 1 year after expiration date is about 13% higher than the non-expired group and the amount of microleakage 6 months after expiration date is about 7% higher than the non-expired group; however, this difference is not statistically significant.

Conclusion: Based on this study and previous studies, it seems that properties of dental substances will be less affected after about 1 year from the expiration date if they are stored properly and there is no prohibition to use them.

Keywords: Dental caries, Fissure sealant, Microleakage, Dye penetration.

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Introduction

Although the prevalence of dental caries has decreased in recent decades, caries is still a major problem.1 Tooth decay occurs when there is an imbalance between normal flora and cariogenic organisms. Cariogenic bacteria such as Streptococcus mutans, Actinomycetes species and Lactobacillus produce various acids by metabolizing carbohydrates, which in turn damage the surface of the enamel. This acidic environment causes the pH to drop below 5.5; as a result, it leads to tooth decay with demineralization of hydroxyapatite crystals.2,3 Despite the significant reduction in decay of smooth surfaces due to fluoridation of drinking water, pit and fissure decay is a major problem in advanced and developing societies.1 At present, grooved surface caries account for 80 to 90% of all caries in children and adolescents.4 Caries occurs 5 times more often in occlusal pit and fissures than on smooth surfaces; special anatomy of pit and fissures is an ideal place for trapping bacteria and food debris and difficult to access for mechanical washing. Enamel of these areas also takes less advantage of the effect of fluoride in preventing decay than enamel of smooth surfaces.5

The idea of preventing caries on masticatory surface of teeth has prompted scientists for many years to use substances as fissure sealants to prevent pit caries and tooth fissures.6 Inhibitory properties of fissure sealants are due to physical blockage of pit and fissures. In this way, it prevents the colonization of new bacteria in pit and fissures and the arrival of fermentable carbohydrates to any remaining bacteria so that the remaining bacteria cannot produce acid in cariogenic concentrations.7 Various factors such as microleakage on the sealant-tooth surface, loss of fissure sealant bandage, presence of caries in the depth of fissures, spread of caries after sealing and dentist skills contribute to success or failure of sealant therapy.8,9

The most worrying and important issue about fissure sealants is microleakage. Microleakage is important because it opens the way for bacteria, food and chemicals to enter the fissures and dents of the teeth and eventually causes decay,6 so that microleakage on the sealant-tooth surface is responsible for failure of sealant therapy.8,9 Expiration date of consumables in dentistry is an important factor that should be considered. Theoretically, if materials are used after the specified expiration date, their properties may change. Clinically, this may lead to complications such as fractures, excessive wear, discoloration, and ultimately treatment failure. In practice, dentists may use some dental substances, including fissure sealants, after the expiration date announced by the manufacturer. Do they have to throw away these substances or can they use them in a short time? Various studies have examined the properties of expired dental substances.10-18 However, no study has been conducted on possible change in properties of expired fissure sealants. Therefore, considering the current conditions of the society and possible coercion to use some expired substances, particularly in some deprived areas, we decided to evaluate the microleakage of these substances.

Methods

2.1. Study Type, Population and Sampling

This is an experimental-laboratory study in which data was collected by observation. Microleakage of fissure sealant (dye penetration) was calculated by stereo microscopy with magnification of 10 and 50. Forty-five maxillary first premolars extracted for orthodontic treatment were collected. The teeth did not have any caries, cracks or any restorations. Based on previous studies,[1,15,19] 15 samples were selected for each group (45 samples totally).

2.2. Procedure

In this laboratory study, 45 maxillary first premolars extracted for orthodontic treatment were collected. The teeth did not have any caries, cracks or any restorations. Soft tissue debris was removed from the teeth using periodontal curette. The teeth were first kept in 0.1% thymol solution for 24 hours; then, to prevent dehydration, the samples were exposed to normal saline at 5°C. Then, all teeth were mounted in self-cure acrylic and occlusal fissures of the samples were cleaned using a catheter and enameloplasty was performed with 1.4 diamond round bur (Tizkavan, Iran) with cooler. Surfaces were etched with 37% phosphoric acid gel (pulpdent, US) for 15 seconds and gently dried with oil-free rubber bulb after washing. After etching, the teeth were examined to ensure a white plaster appearance; otherwise, the teeth were etched for another 15 seconds (1). Then the teeth were randomly assigned to 3 groups of 15. Conventional fissure sealant (Clinpro Sealant, 3M ESPE, US) was used in all samples. All fissure sealants used were stored at about 6°C in the refrigerator before use.

In the first group, fissure sealant was placed within the expiration date; in the second group, it was placed 6 months after the expiration date; in the third group, fissure sealant was placed 1 year after the expiration date. The fissure sealant spread slowly by a catheter; polymerization was then performed for 40 seconds. Finally, the presence of bubbles or overhang was checked by a catheter. For aging, the samples were subjected to thermocycling (Dersa thermocycling device, Iran) for 1000 cycles between 5±2 and 2±55 °C with a setting time of 30 seconds (1, 4, 19). To check for microleakage, the entire surface of the samples up to one millimeter of the sealed areas were covered with two layers of nail polish. The samples were then immersed in a 2% aqueous solution of basic fuchsin (Ghatran Shimi, Iran) for 24 hours. In the next step, all samples were cut from the middle of the mesiodistal dimension in the buco-lingual direction using a precision cutting machine with water cooling (Mecatome T201A, Presi, France), 1 mm thick disk with a rotation speed of -100 rpm. After cutting, microleakage was measured with a magnification of 10 and 50 times by stereomicroscope (Russia, 2-MБC) (Figure 1, Figure 2). Microleakage was assessed with the following scale:

Code zero: no color penetration

Code 1: Color penetration between 1/3 to 2/3 of the buccal/lingual wall length

Code 2: Color penetration between 1/3 to 2/3 of the buccal/lingual wall length

Code 3: Color penetration along the entire length of the buccal/lingual wall

The amount of dye penetration between the fissure sealant-tooth interface was calculated and coded by 2 researchers who were not aware of the purpose and method of the study (4, 19) (Figure 3).

Results

3.1. Descriptive Analysis

3.1.1. Hypothesis: microleakage (dye penetration) increases after the expiration date.

Descriptive results related to microleakage of 15 studied samples within the expiration date versus 30 expired samples are shown in Table 1.

The results showed that out of 30 samples for which expired substances were used, 15 (50%) samples had no penetration and 15 (50%) samples had penetration to 1/3 of the wall, while out of 15 samples for which non-expired substances were used, 9 (60%) samples had no penetration and 6 (40%) samples had penetration to 1/3 of the wall. These results show that expired fissure sealants increased dye penetration in the wall by about 10% compared to non-expired fissure sealants. Then, distribution of microleakage (dye penetration) was examined in three groups with non-expired fissure sealant, 6 months after expiration date and 1 year after expiration date. Table 2 lists the descriptive results related to microleakage (dye penetration).

Regarding the amount of microleakage (dye penetration) in the wall of 45 samples studied, regardless of expiration date of fissure sealant, 24 (53%) samples had no penetration and 21 (47%) samples had penetration to 1/3 of the wall length. The results also show that none of the three groups had penetration up to 2/3 of the wall length or full penetration in the wall and had almost the same penetration ratios.

3.1.2. Question 1: How much is the microleakage of non-expired fissure sealant (dye penetration)?

Out of 15 samples studied, 9 (60%) had no dye penetration into the wall and 6 (40%) had dye penetration up to one third of the wall. These results indicate that dye penetrates into the wall even within the expiration date.

3.1.3. Question 2: How much is the microleakage of fissure sealant (dye penetration) 6 months after expiration date?

Out of 15 samples studied, 8 (53%) had no dye penetration into the wall and 7 (47%) had dye penetration up to one third of the wall. These results indicate that that the fissure sealant used 6 months after expiration date increased penetration rate by 7% compared to the non-expired fissure sealant.

3.1.4. Question 2: How much is the microleakage of fissure sealant (dye penetration) 1 year after expiration date?

Out of 15 samples studied, 7 (47%) had no dye penetration into the wall and 8 (53%) had dye penetration up to one third of the wall. These results indicate that the fissure sealant used 1 year after expiration date increased penetration rate by 6% compared to the fissure sealant expired for 6 months. Comparison of the fissure sealant expired for 1 year with non-expired fissure sealant showed that although both have dye penetration in the wall, but its permeability ratio is about 13 percent higher.

3.2. Inferential Analysis

In order to perform inferential analysis and test the main hypothesis (the amount of microleakage or dye penetration increases after the expiration date), non-parametric analysis and Mann-Whitney-U test were used at a significant level of 5%, the results of which are shown in Table 3.

Based on Table 3 and considering Mann-Whitney-U test value and sig = 0.51> 0.05, the results show that microleakage of the expired fissure sealant is on average slightly higher than the non-expired fissure sealant, but there is no significant difference between the two groups. Since microleakage (dye penetration) increases as expiration date passes, a comparison was made between non-expired group, expired for 6 months and expired for 1 year in terms of microleakage (dye penetration). To compare the average amount of microleakage (dye penetration) of these three groups, non-parametric analysis and Kruskal-Wallis test were used at a significant level of 5%, which is used to compare three independent groups; the results are shown in Table 4.

According to Table 4 (sig=0.77>0.05), although microleakage (dye penetration) increases as expiration date passes and it is higher than that of non-expired fissure sealant, this difference is not significant and there is no difference between three groups in terms of microleakage (dye penetration). This means that fissure sealant microleakage (dye penetration) is not related to its expiration date and there is no significant difference between them. Based on available evidence, therefore, the hypothesis that microleakage of fissure sealant (dye penetration) increases after the expiration date is rejected.

Discussion

Dental caries is a multifactorial disease that, with changes in composition of bacterial biofilms, upsets the balance between remineralization and demineralization stages and can occur in deciduous and permanent teeth.[20] Throughout the 21st century, dental caries remains a major global problem that has changed the health and quality of life of people. Certainly, regular oral hygiene using fluoride toothbrushes and toothpaste, reducing the consumption of cariogenic foods, and using topical and systemic fluorides are effective ways to prevent caries, but there must be another way for anatomically sensitive areas such as pits and fissures.[21] The idea of sealing and covering pits and fissures was formed in the 1960s.[22] Covering these surfaces by creating a physical barrier prevents the biofilm from feeding and thus prevents its growth.[23] There are various substances for covering pits and fissures; the most suitable substance for this purpose is still in question. But resin-based sealants and glass ionomer-based sealants are the most commonly used materials.[24] Sealant trap is very important in order to maintain the caries prevention effect. Improper sealing causes microleakage in the margin area (between the substance and the tooth) which causes bacteria, liquids, molecules and ions to pass through this area, resulting in onset or progression of decay beneath the substance used. Therefore, correct bondage of the substance with tooth enamel without any microleakage is a key point in survival of fissure sealants.[25,26]

Most dental substances have a limited half-life, which is the length of time that the substance has retained the physical and mechanical properties required for the purpose used. Although manufacturers encourage dentists to use the substances within the dates stated on the package, sometimes the dentist may use some substances on or near the expiration date. Theoretically, it seems that properties of substances change after the expiration date. Clinically, this may lead to difficult mixing, fracture, bondage loss or leakage. In everyday use, dentists may have expired substances and the question arises as to whether it is possible to use these substances shortly after the expiration date.[10] This study tended to investigate this question. In this study, non-expired fissure sealant, expired for 6 months and expired for 1 year were used to measure the amount of microleakage, which is a key factor in success of fissure sealant.

In the present study, healthy human premolars extracted for orthodontics were used. The reason for choosing this tooth was because the most common cause for extracting other teeth is caries; moreover, this tooth was not used because the morphology of pit and fissures of the third molars is very diverse and may affect the test results. Many studies have used premolars in their experiments.[1,6,15] First, enameloplasty was performed for fissures of premolars by round bur.[19,27] In order to provide similar conditions in different groups and eliminate the bonding effect, acid etching and fissure sealant were used. The samples were then thermocycled.[2,19] Dye penetration was used to investigate the amount of microleakage, which, as Arastoo claims, is a cheap and non-toxic method and has been widely used in studies.[1,2,19] The results showed that, on average, the amount of microleakage increases as expiration date passes; in this way, the amount of microleakage 1 year after expiration date is about 13% higher than the non-expired group and the amount of microleakage 6 months after expiration date is about 7% higher than the non-expired group; however, this difference is not statistically significant.

In this study, 40% of the non-expired fissure sealant samples had some microleakage, which is consistent with Talreja and Arastoo.[15,19] No completely similar study on microleakage of expired fissure sealants was found. Many studies have investigated mechanical properties such as microhardness, coloration, coefficient of elasticity, flexural strength, water solubility, bond strength of these two substances. Most studies[11-17] have concluded that physical properties of composites will be less affected 1 year after expiration date. Talerja also examined the standard and expired composite microleakage and reported that an acceptable microleakage can be achieved even by using an expired agent.[15] In contrast, Zava reported that dental substances undergo physical and chemical changes after expiration data, which can affect its clinical application. It is noteworthy that this study was performed on glass ionomer cements and the substances used were stored at a temperature of 20-35 °C; the difference in results could be due to differences in the substance used and storage temperature.[10] Garcia et al. also reported that expiration can affect the function and properties of composites; in this study, the type of substance used and mechanical properties evaluated were also different.[18]

Conclusion

Based on this study and previous studies, it seems that properties of dental substances will be less affected after about 1 year from the expiration date if they are stored properly and there is no prohibition to use them. However, potential toxicity of their constituents after expiration should not be ruled out, and certainly further investigation is required in this regard.

Authors' Contributions

RM, MRA, and NM contributed in the design of the study. NM and NG contributed to Writing, review, and editing. All the authors participated in manuscript preparation and revision

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Not applicable

Ethics approval

The study protocol was approved by the Research Ethics Committee of Jundishapur University of Medical Sciences Ethical Code: IR.AJUMS.REC.1399.330

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Competing Interests

The authors declare that they have no conflict of interest

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Table & Figure:

Figure 1. Dye penetration in the tooth/sealant interface under an optic microscope with a magnification of 10

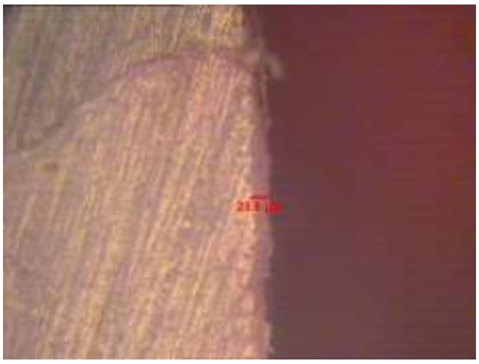

Figure 2. Dye penetration in the tooth/sealant interface under an optic microscope with a magnification of 50

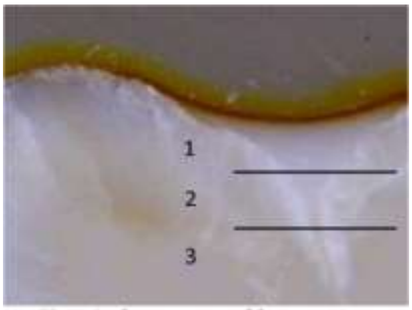

Figure 3. Guide for coding color penetration in the sealant-tooth interface

Table 1. Descriptive data of microleakage (dye penetration) of expired and non-expired fissure sealants

	expired (group 1)	non-expired (group 2)	sum
n	30 (6.66%)	15 (3.33%)	45 (100%)
no penetration	15 (50%)	9 (60%)	24 (53%)
Penetration up to 1/3 of the buccolingual wall	15 (50%)	6 (40%)	21 (47 %)
Penetration to 1/3 to 2/3 of the buccolingual wall	0 (0 %)	0 (0 %)	0 (0 %)
Penetration to the entire buccolingual wall	0 (0 %)	0 (0 %)	0 (0 %)

Table 2. Descriptive data related to microleakage (dye penetration) in three groups based on expiration date

	expired for 1 year (group 1)	expired for 6 months (group 2)	non-expired (group 3)	sum
n	15 (3.33 %)	15 (3.33 %)	15 (3.33 %)	45 (100%)
no penetration	7 (47%)	8 (53%)	9 (60%)	24 (53%)
Penetration up to 1/3 of buccolingual wall	8 (53%)	7 (47%)	6 (40%)	21 (47 %)
Penetration to 1/3 to 2/3 of buccolingual wall	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Penetration to entire buccolingual wall	0(0 %)	0(0 %)	0(0 %)	0(0 %)

Table 3. Comparison of microleakage (dye penetration) between non-expired and expired groups

Group	n	mean	Mann-Whitney-U	sig
expired fissure sealant	30	75.23	5.202	0.531
non-expired fissure sealant	15	50.21		