Microleakage and penetration depth of different fissure sealant materials after cyclic thermo-mechanic and brushing simulation

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The aim of the study was to evaluate microleakage and the penetration-depths of different fissure-sealant materials applied with/without enameloplasty after cyclic aging. One-hundred-sixty mandibular molars were divided into non-invasive and enameloplasty preparation groups and eight material subgroups, including: flowable composites (microhybrid, nanohybrid, and nanofilled), three resin-based (unfilled, filled, and highly-filled), a giomer-based, and a glass-ionomer-based fissure sealant. Specimens were subjected to two-year cyclic chewing and brushing simulation. After 5% basic-fuchsin dye penetration, specimens were sectioned and scored under stereomicroscope. Kruskal-Wallis statistical data showed that preparation type significantly affected the penetration of all tested materials ($p < 0.05$), but not significantly affected microleakage ($p > 0.05$). Flowable composites showed the best and the glass-ionomer-based sealant showed the worst penetration and microleakage. Slight preparation of fissures is not important in microleakage. However, enameloplasty significantly enhanced the depth of penetration of the sealants. Flowable composites offer promising results at the fissure sealing.

Keywords: Enameloplasty, Fissure sealant, Cyclic thermo-mechanic and brushing simulation microleakage, Penetration depth

INTRODUCTION

Dental caries is one of the most affecting bacterial diseases known to the humankind; however, the levels of dental caries in children and adolescents have declined because, in recent decades, preventive measures have been taken in developed countries. While smooth surfaces, especially proximal surfaces, have benefited from caries-prevention protocols, the high prevalence of occlusal caries is still a problem¹. The main reason for this problem is complex pit and fissure morphology on the occlusal and buccal or palatal surfaces of molar teeth, which are the most susceptible sites to developing caries. Foods and dental plaque easily accumulate in these areas and cannot be removed effectively by the patient. Moreover, lack of saliva flow to the fissures and insufficient intake of remineralization agents do not compensate for a high incidence of occlusal caries².

Fissure sealants isolate pits and fissures from the bacteria and their by-products, provide a mechanical barrier, and avoid accumulation of dental plaque. Therefore, fissure sealant application is one of the most reliable and effective methods for preventing occlusal caries. The advantage of the sealant application is significant caries risk reduction compared to non-sealed controls and lower cost compared to restoration placement³.

Many methods have been defined for applying fissure sealant and many materials used as sealant have been developed. However, there is no clear consensus regarding which application technique is superior or

which type of sealant material is the most durable under oral conditions⁴.

Air abrasion and enameloplasty or fissurotomy with bur preparation are some of the techniques used before fissure sealant application. It is expected that the sealants be applied without any intervention; however, fissure morphology and depth of fissures may vary from teeth to teeth. The deepest part of the fissures usually contains organic debris, including bacteria¹. Fissure enameloplasty with rotary instruments makes deeper fissures accessible; therefore, penetration and adaptation of the sealant material can be enhanced, and removal of the debris becomes possible clinically. Moreover, a layer of prismless enamel, which may hinder the effect of the etching application, can be removed with mechanical enamel preparation⁵. Conversely, it was reported that the enameloplasty technique injures the sound enamel layer, possibly increasing the caries susceptibility of those fissures⁶.

Glass ionomer, resin, recently giomer-based fissure sealants, and flowable composites are the main material groups that can be used as fissure sealant. *In vivo* or *in vitro* performance of most of these materials has been investigated intensively; however, there is no one material suggested as an ideal pit and fissure sealant⁷. Generally, resin-based materials are recommended with the advantage of better retention and glass-ionomer-based materials are recommended with the advantages of fluoride release and lower moisture sensitivity⁸. Recently developed giomer-based fissure sealant material represents advantages of resin-based and glass-ionomer-based sealants. The use of flowable composites as fissure-sealant material was also

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suggested⁹⁾.

The preventive effect of pit and fissure sealing is mainly based on the ability of sealant materials to flow through pits and fissures and completely fill them without any gaps or air entrapments. As long as the sealant material remains bonded to enamel, effective protection will continue. Microleakage is the most affecting factor on adhesion failure between sealant and tooth structure and can be determined by many techniques *in vitro*. With the advantages of reliability, simplicity, and ease of application, the dye penetration test is a well-established and commonly used method for the determination of microleakage *in vitro*¹⁰⁾.

Therefore, the aim of the study was to evaluate microleakage and the penetration depth of different fissure-sealant materials applied with/without enameloplasty after cyclic chewing and brushing simulation. The tested null hypotheses were that there were no statistically significant differences between enameloplasty and non-invasive preparation methods, and among tested materials in terms of microleakage and penetration depth.

MATERIALS AND METHODS

Sample selection

A total of 160 freshly extracted non-carious human mandibular third molar teeth with intermediate deep fissures¹¹⁾ were collected and stored in 0.5% Chloramine-T solution (Merck, Darmstadt, Germany) for 48 h for disinfection, then stored in 4°C of distilled water until use. After scaling and cleaning with water/pumice slurry and a bristle brush at low speed, the buccal-lingual and mesio-distal dimensions were recorded for each specimen using a digital calliper (Mitutoyo, Suzano, Brazil) at the most prominent point of the tooth's surface. Teeth were embedded in self-curing acrylic resin (Leaddent, Izmir, Turkey) up to 1 mm below the cemento–enamel junction (CEJ), using a PVC cylinder (3 cm in height and 2 cm in diameter), which is compatible with the specimen sockets of a chewing and brushing simulator. Occlusal fissures were carefully cleaned by using an air abrasion unit (Gnatus Prophyl Jet, Sao Paulo, Brazil) with sodium bicarbonate powder (Clinpro Prophyl Powder, 3M ESPE, Seefeld, Germany). Fissures were controlled with loupe magnification for the presence of any debris.

The teeth were randomly divided into two main groups according to preparation technique, as non-invasive or invasive ($n=80$), and a further eight subgroups according to materials, including three flowable composites (Filtek Ultimate Flow, GrandioSo Flow, and Majesty Flow), and three resin-based (Clinpro Sealant, Fissurit FX and GrandioSeal), a giomer-based (BeautiSealant), and a glass-ionomer-based fissure sealant (Fuji Triage) ($n=10$). While enameloplasty was standardized by extending fissure entrance to the diameter of half length of micro narrow taper fissure carbide bur (Fissurotomy® Micro NTF, SS White, Lakewood, NJ, USA) throughout the fissures in

invasive groups, no preparation was performed in non-invasive groups.

Pit and fissure sealant application

The pits and fissure sealant materials used in the study are listed in Table 1.

1. Flowable composite application

The occlusal fissures were etched for 30 s with 35% orthophosphoric acid (Scotchbond Universal Etchant Gel, 3M ESPE). During the etchant gel application, ultrasonic vibration was applied to the buccal surface of the teeth to enhance penetration of the gel¹²⁾. Fissures were washed thoroughly with water spray for 20 s, then specimens were dried with oil-free air spray. For optimal dehydration, fissures were dried with 60% ethanol for 5 s.

An adhesive system (Singlebond Universal, 3M ESPE) was applied with a brush applicator for 20 s, dried with a mild air flow for 5 s, and light cured with an LED curing unit (Valo Cordless, Ultradent, South Jordan, UT, USA) in standard power mode for 10 s. Flowable composites were applied from the distal to the mesial part of the fissures by using a probe, and ultrasonic vibration was applied to the buccal surface of the specimens for 30 s to enhance penetration of the flowable composites. Following light curing in the distal and mesial half of teeth for 20 s each, restoration surfaces were covered with glycerin gel and light cured for 60 s. The glycerin gel was removed with water spray, and the specimens were stored in distilled water for 24 h at 37°C for post-cure polymerization.

2. Resin-based pit and fissure sealant application

The procedures were the same after etching, except that adhesive system application in the flowable composite groups was performed.

3. Giomer-based pit and fissure sealant application

Self-etch primer (Beautiprimer, Shofu, Kyoto, Japan) was applied to fissures with a brush applicator for 5 s, then dried with a mild air flow according to manufacturer instructions. Giomer-based sealant material (BeautiSealant, Shofu) was applied from the distal to the mesial part of the fissures by using a probe. Ultrasonic vibration was applied to the buccal surface of the specimens for 30 s. Similarly, light curing and storage procedures were performed in the flowable composite groups.

4. Glass-ionomer-based pit and fissure sealant application

GC Cavity Conditioner was applied to fissures with a brush applicator for 10 s, then dried with a mild air flow. A glass-ionomer-based sealant material (Fuji Triage, GC, Tokyo, Japan) capsule was activated, triturated for 10 s according to manufacturer instructions, and subsequently loaded to the capsule applicator. A thin film of the material was applied into the occlusal fissures and light cured for 30 s. Fuji Coat LC was applied immediately after curing. The specimens were stored in

Table 1 Materials used in the study

Material		Composition and filler % (wt/vol)*	Manufacturer	Batch no
Filtek Ultimate Flow	Nanofilled flowable composite	Bis-GMA, UDMA, TEGDMA, prokrlat resin, ytterbium trifluoride, silica nano-fillers, zirconia nano-fillers (78.5–63.3)	3M ESPE, St Paul, MN, USA	N577695
GrandioSO Flow	Nanohybrid flowable composite	HEDMA, Bis-GMA, TEGDMA, glass ceramics, silicon dioxide (80.2–65.7)	Voco, Cuxhaven, Germany	1339440
Majesty Flow	Microhybrid flowable composite	TEGDMA, Hydrophobic aromatic dimethacrylate, silanized colloidal silica and barium glass (80–62)	Kuraray, Tokyo, Japan	6D0003
ClinproSealant	Resin-based unfilled fissure sealant	Bis-GMA, TEGDMA, EDMAB, BHT, TBATFB diphenyliodoniohexafluorofosfat (NA)	3M ESPE	N581514
Fissurit FX	Resin-based filled fissure sealant	Bis-GMA, TEGDMA, UDMA, BHT, benzotriazole derivatives, inorganic glass ionomer filler, NaF (NA)	Voco	1341127
GrandioSeal	Resin-based highly-filled fissure sealant	Bis-GMA, TEGDMA, nano fillers (70-NA)	Voco	1340270
BeautiSealant	Giomer-based fissure sealant	TEGDMA, UDMA, S-PRG fillers (NA)	Shofu, Kyoto, Japan	031309
Fuji Triage	Glass ionomer-based fissure sealant	Powder: alumina-fluoro-silicate glass (amorphous) Liquid: polyacrylic acid, Specific component	GC, Tokyo, Japan	1311121

Bis-GMA: bisphenol-A-diglycidyl methacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethyleneglycol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; EDMAB: Ethyl P-dimethylamino benzoate BHT: Butylhydroxytoluene TBATFB: Tetrabutyl dimethacrylate S-PRG; surface-pre reacted glass.

distilled water for 24 h at 37°C.

Artificial aging

For the purpose of artificial aging, specimens were subjected to two-year cyclic thermo-mechanic and brushing simulations. A one-year artificial aging cycle was the combination of one-year thermo-mechanic and one-year brushing simulation. This cycle was repeated twice to create two-year artificial aging.

Specimens were positioned and mounted to holders of the chewing simulator (Chewing Simulator CS-4, SD Mechatronik, Westerham, Germany). Buccal cusps of extracted upper premolar teeth were sectioned and used as an antagonist in a chewing simulation. For one-year, thermo-mechanic simulation, 240,000 load cycles with a load of 49 N and a frequency of 1.7 Hz, were applied through 1.5 mm horizontal and 3 mm vertical movement of the antagonist on the center of the sealed occlusal surfaces¹³. Specimens were also subjected to thermocycling (1,000 cycles of 5 and 55°C; 100 s per cycle, and a 10-s interval to remove water from the chambers) at the same time with the chewing simulation.

Simulated toothbrushing (Toothbrush Simulation ZM-3.4, SD Mechatronik) was performed using toothbrushes with medium-hard bristles under a 200

g load. By mixing a 2:1 ratio by volume of deionized water and a daily used dentifrice (Colgate Total, Colgate-Palmolive, England), the slurry, which was used as a third medium in the brushing simulation, was prepared. For one year, 10,000 strokes were performed at a frequency of 1 Hz; a double pass of the toothbrush head on the specimen was considered a stroke^{14,15}. The toothbrushes were changed after every 10,000 strokes. After a brushing simulation, the specimens were cleaned thoroughly with running water.

Evaluation of microleakage and depth of penetration

Apices of the specimens were sealed with sticky wax and the coronal surfaces of the specimens (except the occlusal surfaces) were covered with a double layer of nail varnish and allowed to dry. The teeth were immersed in 0.5% basic fuchsin solution for 24 h at 37°C to allow dye penetration into gaps between the enamel and the sealant, then rinsed under running water to remove excess solution. All specimens were buccolingually sectioned into three fragments with a high-speed precision saw (Isomet 1001, Buehler, Evanston, IL, USA). Four surfaces per tooth were analyzed by two calibrated examiner who were blinded to the methods and materials under a stereomicroscope (Stemi 2000

C, Zeiss, Oberkochen, Germany) at 40× magnification. Microleakage and the penetration depth of sealant materials were graded according to the following criteria.

1. The microleakage of the materials^{16,17)}

- 0=no dye penetration
- 1=dye penetration limited to the outer half of the sealant
- 2=dye penetration extending to the inner half of the sealant
- 3=dye penetration extending to the underlying fissure

2. The penetration ability of the materials¹⁸⁾

- 1) Sealant penetrated to 1/3 the total length of the fissures
- 2) Sealant penetrated to 1/2 the total length of the fissures
- 3) Sealant penetrated to total length of the fissures

The data were analyzed with Kruskal-Wallis and Mann-Whitney U *post-hoc* tests at a significance level of 0.05.

RESULTS

Microleakage test results

Microleakage test results revealed that tested materials with different application methods affect the degree of dye penetration. Figure 1 shows the microleakage score distribution of the tested materials according to preparation methods and Fig. 2 shows the examples of the microleakage results according to material brands and preparation methods. In the non-invasive group, 14 specimens (17.5%) did not show dye penetration. In the enameloplasty group, 8 specimens (10%) did not show dye penetration. There were no statistically significant differences between enameloplasty and non-invasive application groups ($p>0.05$).

Table 2 shows mean, minimum and maximum microleakage scores of the groups according to material type and preparation. In non-invasive groups, flowable composite group showed the least microleakage, and the difference was statistically significant ($p<0.05$). In addition, there were no statistical differences among flowable composites, nanofilled, nanohybrid, or microhybrid ($p>0.05$) (Table 3). Maximum microleakage was observed in glass-ionomer-based (FJT) fissure sealant material. However there were no differences among the glass-ionomer, resin (unfilled, filled or highly-filled), and giomer-based material groups statistically ($p>0.05$) (Table 3).

In the enameloplasty group, flowable composites showed the least and glass-ionomer-based fissure sealant showed the highest microleakage scores. While the difference between flowable composites and glass-ionomer-based fissure sealant was statistically significant ($p<0.05$), there were no significant differences among other pairwise comparisons ($p>0.05$).

Depth of penetration test results

Depth of penetration test results revealed that tested materials with different application methods affect the degree of penetration depth. Figure 3 shows the penetration depth score distribution of the tested materials according to preparation methods and Fig. 2 shows the examples of the penetration depth results according to material brands and preparation methods. In the non-invasive group, 30 specimens (37.5%), and in the enameloplasty group, 64 specimens (80%), showed penetration to the total length of the fissures. There were statistically significant differences between enameloplasty and the non-invasive application groups ($p<0.05$).

Table 4 shows mean, minimum and maximum penetration depth scores of the groups according to material type and preparation. In the non-invasive group, whereas flowable composites showed the highest

Fig. 1 Microleakage score distribution of the tested materials according to preparation methods.

Fig. 2 Examples of microleakage and penetration depth results. Black arrows indicate limit of dye leakage and red arrows indicate penetration failures. a) Microleakage score 0 and penetration depth score 3 in non-invasive Filtek Ultimate Flow group. b) Microleakage score 1 and penetration depth score 3 in invasive GrandioSo Flow group. c) Microleakage score 1 and penetration depth score 3 in non-invasive Majesty Flow group. d) Microleakage score 2 and penetration depth score 3 in invasive Clinpro Sealant group. e) Microleakage score 0 and penetration depth score 2 in non-invasive Fissurit FX group. f) Microleakage score 1 and penetration depth score 1 in non-invasive GrandioSeal group. g) Microleakage score 2 and penetration depth score 3 in invasive BeautiSealant group. h) Microleakage score 3, and penetration depth score 3 in invasive Fuji Triage group.

Table 2 Mean ($\pm$ SD), minimum and maximum microleakage scores of the groups according to material type and preparation

Material	<i>n</i>	Non-invasive			Enameloplasty		
		Mean ($\pm$ SD)	Median	Min–Max	Mean ($\pm$ SD)	Median	Min–Max
Flowable composite	30	0.6 ($\pm$ 0.6) ^a	1	0–2	1.1 ($\pm$ 0.66) ^a	1.12	0–2
Resin-based sealant	30	1.53 ($\pm$ 0.9) ^b	1	0–3	1.4 ($\pm$ 0.77) ^a	1	0–3
Giomer-based Sealant	10	2.10 ($\pm$ 0.73) ^b	2	1–3	1.9 ($\pm$ 0.87) ^{a,c}	2	1–3
Glass ionomer-based sealant	10	2.5 ($\pm$ 0.52) ^b	2.5	2–3	2.6 ($\pm$ 0.51) ^{b,c}	3	2–3

*Different lowercase letters in the same column indicate significant differences between the material groups.

depth of penetration, the lowest penetration was observed in the glass-ionomer-based fissure sealant. The difference between these materials was statistically significant ($p < 0.05$). However, there were no significant differences among other pairwise comparisons ($p > 0.05$). Despite flowable composites showing the highest penetration depth among that type of materials, resin-based unfilled sealant material showed the highest scores among all tested materials. Conversely, resin-based, highly filled fissure sealant showed the lowest scores among all tested materials, and the difference

between unfilled resin and highly-filled resin was statistically significant ($p = 0.014$) (Table 5).

In enameloplasty groups, flowable composites received the highest and glass-ionomer-based fissure sealants showed the least penetration depth scores. While the difference between flowable composites and glass-ionomer-based fissure sealant was statistically significant ($p < 0.05$), there were no significant differences among other pairwise comparisons ($p > 0.05$).

It was observed that the application method significantly affected resin-based fissure sealant

Table 3 Mean ($\pm$ SD), minimum and maximum microleakage scores of the groups according to brand or content and preparation

Material	<i>n</i>	Non-invasive			Enameloplasty		
		Mean ($\pm$ SD)	Median	Min–Max	Mean ($\pm$ SD)	Median	Min–Max
Filtek Ultimate Flow Nanofilled flowable composite	10	0.6 ($\pm$ 0.51) ^a	0.5	0–1	0.6 ($\pm$ 0.69) ^a	0.5	0–2
GrandioSo Flow Nanohybrid flowable composite	10	1 ($\pm$ 0.66) ^{a,c}	1	0–2	1.5 ($\pm$ 0.52) ^{a,c}	1.5	1–2
Majesty Flow Microhybrid flowable composite	10	0.4 ($\pm$ 0.51) ^a	0	0–1	1.2 ($\pm$ 0.42) ^{a,c}	1	1–2
Clinpro Sealant Resin-based unfilled fissure sealant	10	1.6 ($\pm$ 0.96) ^{a,d}	1.5	0–3	1.5 ($\pm$ 0.85) ^{a,c}	1.5	0–3
Fissurit FX Resin-based filled fissure sealant	10	1.3 ($\pm$ 0.94) ^{a,d}	1	0–3	1 ($\pm$ 0.67) ^a	1	0–2
Grandio Seal Resin-based highly-filled fissure sealant	10	1.7 ($\pm$ 0.82) ^{a,d}	1.5	1–3	1.7 ($\pm$ 0.67) ^{a,c}	2	1–3
BeautiSealant Giommer-based fissure sealant	10	2.1 ($\pm$ 0.73) ^{b,c,d}	2	1–3	1.9 ($\pm$ 0.87) ^{a,c}	2	1–3
Fuji Triage Glass ionomer-based fissure sealant	10	2.5 ($\pm$ 0.52) ^{b,d}	2.5	2–3	2.6 ($\pm$ 0.51) ^{b,c}	3	2–3

*Different lowercase letters in the same column indicate significant differences between the material brand groups.

Fig. 3 Penetration depth score distribution of the tested materials according to preparation methods.

($p < 0.05$), but not flowable composite, giomer, and glass-ionomer-based sealants ($p > 0.05$).

DISCUSSION

Occlusal surfaces constitute only 12% of the total tooth surface in the mouth; however, they are eight times

more vulnerable to caries than the smooth surfaces¹⁹. Fissure sealants have been proven to be effective in the prevention of pit and fissure caries^{20,21}. To enhance clinical service and effectiveness, several sealant materials with different contents have been developed, and many improvements have been made to fissure sealant application. Current advances in this area

Table 4 Mean ($\pm$ SD), minimum and maximum penetration depth scores of the groups according to material type and preparation

Material	<i>n</i>	Non-invasive			Enameloplasty		
		Mean ($\pm$ SD)	Median	Min–Max	Mean ($\pm$ SD)	Median	Min–Max
Flowable composite	30	2.53 ($\pm$ 0.57) ^a	3	1–3	2.93 ($\pm$ 0.25) ^a	3	2–3
Resin-based sealant	30	2.13 ($\pm$ 0.73) ^{a,c}	2	1–3	2.7 ($\pm$ 0.53) ^a	3	1–3
Giomer-based Sealant	10	2.2 ($\pm$ 0.63) ^{a,c}	2	1–3	3 ($\pm$ 0) ^a	3	3
Glass ionomer-based sealant	10	1 ($\pm$ 0.42) ^{b,c}	2	1–2	2.4 ($\pm$ 0.51) ^a	2	2–3

*Different lowercase letters in the same column indicate significant differences between the material groups.

Table 5 Mean ($\pm$ SD), minimum and maximum penetration depth scores of the groups according to brand or content and preparation

Material	<i>n</i>	Non-invasive			Enameloplasty		
		Mean ($\pm$ SD)	Median	Min–Max	Mean ($\pm$ SD)	Median	Min–Max
Filtek Ultimate Flow Nanofilled flowable composite	10	2.6 ($\pm$ 0.51) ^{a,c}	3	2–3	2.9 ($\pm$ 0.31) ^a	3	2–3
GrandioSo Flow Nanohybrid flowable composite	10	2.5 ($\pm$ 0.7) ^{a,c}	3	1–3	3 ($\pm$ 0) ^a	3	3
Majesty Flow Microhybrid flowable composite	10	2.5 ($\pm$ 0.52) ^{a,c}	2.5	2–3	2.9 ($\pm$ 0.31) ^a	3	2–3
Clinpro Sealant Resin-based unfilled fissure sealant	10	2.8 ($\pm$ 0.42) ^a	3	2–3	2.8 ($\pm$ 0.42) ^a	3	2–3
Fissurit FX Resin-based filled fissure sealant	10	1.9 ($\pm$ 0.73) ^{a,c}	2	1–3	2.7 ($\pm$ 0.48) ^a	3	2–3
Grandio Seal Resin-based highly-filled fissure sealant	10	1.7 ($\pm$ 0.48) ^{b,c}	2	1–2	2.6 ($\pm$ 0.69) ^a	3	1–3
BeautiSealant Giomer-based fissure sealant	10	2.2 ($\pm$ 0.63) ^{a,c}	2	1–3	3 ($\pm$ 0) ^a	3	3
Fuji Triage Glass ionomer-based fissure sealant	10	1.8 ($\pm$ 0.42) ^{b,c}	2	1–2	2.4 ($\pm$ 0.51) ^a	2	2–3

*Different lowercase letters in the same column indicate significant differences between the material brand groups.

include the development of giomer-based fissure sealant material and the use of flowable composites as fissure sealant.

The microleakage test is an evaluation method that has been used for assessing the marginal and internal adaptation of restorative materials²²⁾. Dye penetration is the most used and most widely accepted method for this purpose. While there are many studies regarding microleakage of fissure sealants after thermal aging, no studies are available in dental literature regarding microleakage of sealants after aging with thermo-mechanic loading and brushing simulation. A two-

year cyclic thermo-mechanical chewing and brushing simulation was performed in this study to evaluate the effect of intraoral conditions on the fissure sealants. This predicts the possible clinical success of sealants.

Dental literature demonstrates the importance of preparing the enamel surface of pits and fissures, to reduce the risk of microleakage, by improving the sealant penetration²³⁾. This preparation consists of a slight abrasion on the enamel surface with a diamond bur to improve the sealant retention and, consequently, reduce the risk of its partial or total loss. It was reported that enameloplasty increases surface area for bonding and

helps to clean debris and pellicle within the fissures²⁴. However, the material volume used for sealing fissures and polymerization shrinkage increase in light-cured materials after enameloplasty, and it has been reported that enameloplasty injures the sound enamel layer, and this leads to higher caries susceptibility of that fissure in the future⁶. In the present study, the effect of non-invasive or enameloplasty preparation methods and various materials on microleakage were evaluated. The null hypothesis was partially rejected because the difference between preparation techniques was not significant, but there were significant differences among tested materials.

Studies reveal a conflict in the effects of preparation techniques on microleakage of sealants. Whereas a number of authors revealed no difference between non-invasive and enameloplasty groups similarly to the present study^{23,25}, some of the authors revealed less or increased microleakage for enameloplasty groups^{4,16,20}. The difference between tested materials, application methods, and aging procedures is the main factor in the controversial results of the studies.

The application of a bonding agent gives the possibility of obtaining a better adhesion of a biomaterial to the tooth surface²⁶. It was reported that retention and microleakage have shown improvement when a bonding agent is used, especially in a moist environment, because of the advantage of the hydrophilic head of the adhesive system^{6,26}. However, when isolation is under control, there is no significant difference in bond strengths, whether or not a bonding agent is used under the sealant²⁶.

When considered the glass-ionomer sealants link predominantly *via* chemical bond to tooth and the resin-based sealants encompass some of the benefits inherent in the latest bonding systems, such as penetration or wetting, a bonding agent was only used under the flowable composites in the current study²⁶. As a result the least dye penetration was observed in flowable composites. Resin, giomer, and glass-ionomer-based fissure sealants showed more microleakage, respectively. These findings were consistent with the result of recently published review, which concluded that applying a bonding agent under fissure sealants enhanced the adhesion of the material and increased the retention of the sealant²⁷.

Penetration depth is an important parameter that may increase the longevity of sealant²⁶ and affect the retention and adaptation of the sealant²¹. The present study revealed a significantly better penetration depth in the enameloplasty groups than in the non-invasive groups, so that the null hypothesis was rejected. By the enlargement of the fissure entrance with enameloplasty, sealant material easily penetrates into the fissures. The penetration-depth results of the our study, in terms of application method, are consistent with previous studies^{23,28,29}.

Material properties and the fissure morphology have a significant influence on the penetration ability of the sealants^{28,30}. Glass-ionomer sealants are more viscous than resin-based sealants, making the penetration into

the fissure depth more difficult. Insufficient penetration ability also makes them less retentive mechanically when compared with the opponents. The study results revealed that glass-ionomer-based sealants showed the least penetration depth with more microleakage, in harmony with previous studies^{31,32}.

The chemistry of resin-based sealants is not different from the composites used for dental restorations. The resin-based sealants should be less viscous to allow better penetration onto conditioned enamel surfaces³³. Changing ratios of monomers and fillers in the composition affect viscosity and mechanical properties of the sealants. In certain circumstances, different size and proportion fillers, such as silica microparticles or vaporized inorganic glass, can be added to provide rigidity and enhance wear resistance, changing the structure of unfilled sealant to filled sealant or flowable composites³³. In the present study, an unfilled, a filled, and a highly filled resin-based sealant, and a microhybrid, a nanohybrid, and a nanofilled flowable composite, were also evaluated. Theoretically, penetration is inversely proportional to viscosity. Thus, an unfilled resin would be penetrated more deeply into the fissure system and, therefore, perhaps be better retained according to the results of previous studies^{21,34}. In this regard, the unfilled, resin-based sealant Clinpro Sealant showed the highest and the highly-filled resin-based sealant Grandio Seal showed the least penetration depth scores in non-invasive groups in this study. However, enameloplasty made up the difference significantly between resin-based sealants. In addition, there were no differences among used flowable composites in both preparation groups in terms of penetration, which could be caused by the positive effect of the applied bonding agent.

This *in vitro* study evaluated the microleakage and penetration depth of different commercially used fissure sealant materials after two-year cyclic thermo-mechanic and brushing simulations. This kind of aging affects not only microleakage of sealant materials, intensively affects marginal adaptation or wear properties of the sealant materials, as well. Further studies should be focused on the aforementioned issues.

CONCLUSIONS

Within the limitations of this study, the following conclusions can be drawn:

1. Whereas there were no significant differences in terms of microleakage between enameloplasty and non-invasive preparation, enameloplasty significantly enhanced the penetration depth of the sealant materials.
2. Flowable composites applied with bonding agents showed better results than other tested fissure sealant materials in both preparation groups in terms of microleakage and penetration depth.
3. Glass-ionomer-based sealant exhibited the worst microleakage and penetration depth scores in both preparation groups.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests in writing this article.

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