# Evaluation of microleakage of three different types of pit and fissure sealants using invasive and non-invasive techniques (An in-vitro study)

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***Backgrounds:*** The aims of this study were to; Evaluate the amount of in-vitro microleakage of three different types of pit and fissure sealants (Vertise Flow, Kerr), (Helioseal-F, IvoclarVivadent), (GC Fuji TRIAGE, GC corporation) and the effect of occlusal preparation on the leakage value.

***Methods:*** Sixty extracted human premolars randomly divided into 6 groups (n=10/group). Teeth fissures of three non-invasive groups (1, 3, 5) left intact, fissures of other three invasive groups (2, 4, 6) were opened up with ¼ round bur. Teeth fissures in group (1, 2) sealed with self-adhesive Vertise Flow, group (3, 4) Helioseal-F, while group (5, 6) fissures sealed with Glass Ionomer GC Fuji TRIAGE. The teeththermocycled between 5±2°C and 55±2°C for 500 cycles with a dwell time of 30 seconds; All teeth sealed apically and coated within 1.5 mm of the sealant margin with two layers of nail varnish, then immersed in 1% Methylene blue solution. Subsequently, two buccolingual sections were made parallel to the long axis of tooth yielding 3 sections and 4 surfaces per tooth for microleakage analysis. The surfaces were scored 0 to 3 for extent of microleakage using a binocular microscope at 25X magnification. Microleakage was analyzed by using paired t-test and ANOVA.

***Results:*** Invasive technique produced significantly less microleakagethan Non-Invasive groups ($P<0.05$). In all six groups Helioseal-F in Invasive technique showed significantly the least degree of microleakage ($P<0.05$).

***Conclusion:*** Helioseal-F was the best material in terms of reducedmicroleakage. Invasive techniquecompared to non- invasive technique had produced less degree of microleakage.

***Keywords:*** Fissure Sealant, Helioseal-F,Invasive Technique, Microleakage, Non-Invasive Technique.

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## Introduction

The term fissure caries was earlier used to describe the caries lesions found in pits and fissures. This definition was based on the assumption that the high incidence of caries lesions in molar pits and fissures was directly related to poor cleaning accessibility to these surfaces.[1] A fissure sealant is defined as a material, which is placed in the pits and fissures of teeth in order to prevent or to arrest the development of dental caries.[2] Pit and fissure sealants are one of the best methods of preventing caries. It occludes the fissures and the pits from the accumulation of plaque and the cariogenic microflora. But caries still occurs in pits and fissures with sealant loss, and in adjacent pits and fissures or along cuspal inclines which were not initially sealed.[3] Several types of resin, both filled and unfilled, have been employed as Pit and Fissure Sealants. The main component of the fissure sealant is Bis-GMA resin. The success of the sealant technique is highly dependent on obtaining and maintaining an intimate adaptation of the sealant to the tooth surface.[4] The marginal sealing ability of a sealing material is extremely important for success of sealants, which can be assessed by evaluating microleakage. Weak sealing can lead to marginal leakage, resulting in bacterial invasion, caries initiation and progression underneath the restoration. In vitro microleakage studies can predict the marginal integrity of restorative materials.[5]integrity of restorative materials.5

Microleakage assessment may be qualitative or quantitative with different systems, including both simple and computer based methods. Dye penetration has been used in several studies, to assess the presence of marginal leakage at the sealant/enamel interface.6

In invasive technique the preparation of fissures with burs has been suggested to provide better access to the deeper areas of the fissures, thus enabling debris removal and deeper sealant penetration.7

## Materials and methods

Sixty sound human maxillary and mandibular premolar teeth were collected which were extracted for orthodontic purpose. The selected teeth were free from obvious carious lesions, morphological defects, restorations and sealants. The selected (60) teeth were kept in (0.1%) thymol solution (Figure 1).

**Figure 1: Storage medium for extracted premolars.**

The occlusal surface debridement was done with hand scaling instruments. After cleaning of all teeth, construction of the plaster blocks was done by pouring mixed plaster of Paris onto the mold withlength, width and height dimensions (38 x 28 x 20) mm. before initial set of plaster every tooth was embedded perpendicular (90°) into the plaster by using two Triangle rulers as seen in figure2.

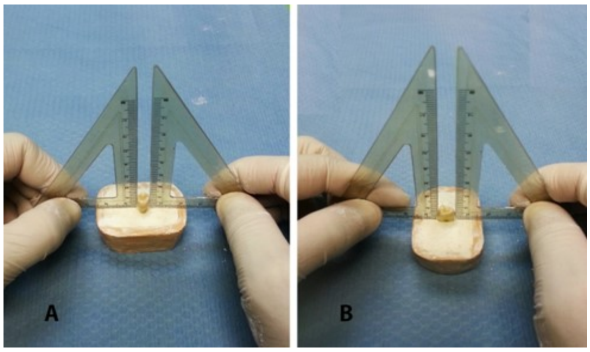


**Figure 2: Angulation of tooth on plaster block, A. perpendicular in mesiodistal direction, B. Perpendicular in buccolingual direction.**

The occlusal surfaces of teeth were cleaned with a disposable prophylaxis brush with tapered end by using a low speed contra angle hand piece for ten seconds. No pumice was used (Figure 3). The 60 premolars were randomly divided into six groups of 10 teeth (Figure 4).

**Figure 3: Tapered end prophylaxis brush.**

In group 1, 3, and 5 the occlusal surfaces of the teeth were left intact (Non–Invasive technique).In group 2, 4, and 6 the fissures of the teeth were opened up by using of ¼ round carbide bur in a high speed handpiece (Turbine). The high speed handpiece was fixed on a removable surveyor by a special

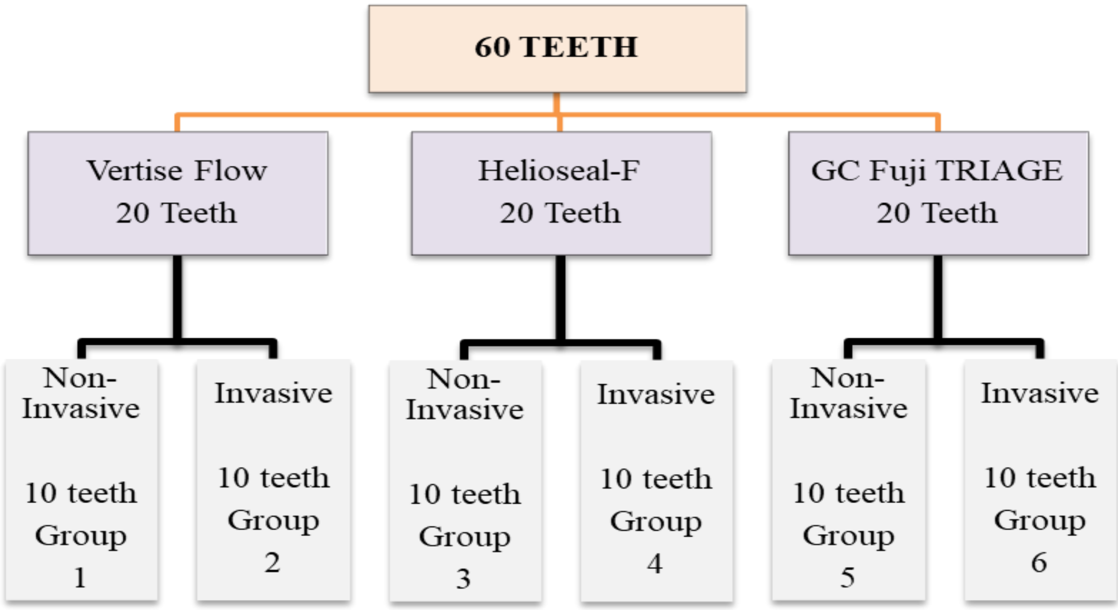


**Figure 4: Scheme of sample size and distribution.**

part which was specially designed for this purpose. Plaster blocks were fixed on the base of surveyor.The bur on turbine was applied parallel to the long axis of the tooth, and placed on the deepest point of fissure with forward and backward motion by hand with no pressure to open central fissures. Cutting Dimension was equivalent to the diameter of the round carbide bur 0.5mm (Figure 5).

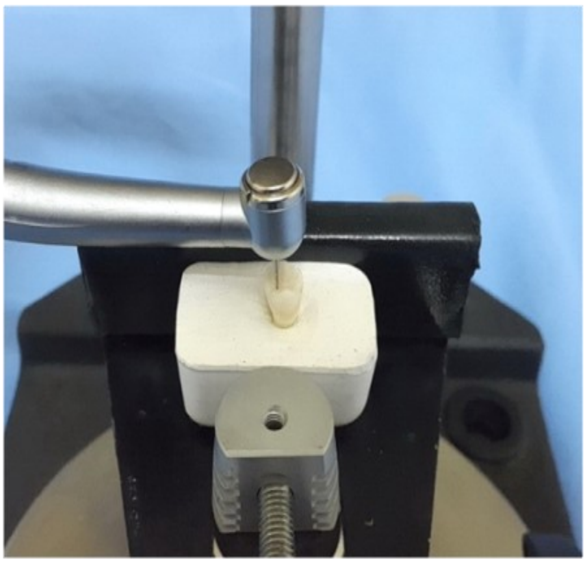

**Figure 5: Dental bur parallel to the long axis of the tooth.**

**Fissure sealants application.** The teeth in Group 1 and 2, Vertise Flow was placed onto the fissures according to the manufacturer's instructionsand light cured for 20 seconds (Figure 6 and 7).

In Group 3 and 4,Helioseal F were used according to its manufacturer instructions the teeth were etched with 37% phosphoric acid gel for 30 seconds. The teeth were rinsed for 20 seconds. The fissures were air -dried completely with oil and moisture free air syringe until mat white appearance occurred, then the Helioseal F was applied on to the fissures and light cured for 20 seconds (Figure 8 and 9).In group 5 and 6, GC Fuji TRIAGE (GC Corporation, Tokyo Japan), The tooth washed for 10 seconds and dried for 20 seconds with an air syringe,the surface of the tooth appeared moist, glistening appearance. Then cement powder and liquid were mixed for 25 seconds and placed in to fissures, when sealant was chemically set GC Fuji Varnish applied to the sealant (Figure 10 and 11).The teeth in blocks were removed and cleaned from excess plaster and stored in distilled water for one week.After, all teeth were thermocycled between 5±2°C and 55±2°C for 500 cycles with a dwell time of 30 secondsby using thermocycling machine, followed by storage in distilled water at room temperature for three days.


**Figure 6: Vertise flow sealant.**

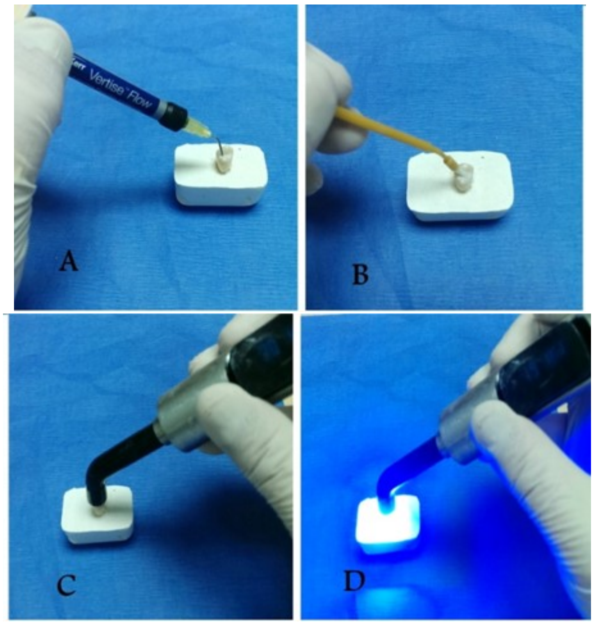


**Figure 7:A. application of Vertise flow, B. excess material removed with brush, C. tip of light cure on the tip of buccal cusp, D. 20 seconds light curing.**


**Figure 8: Helioseal-F.**

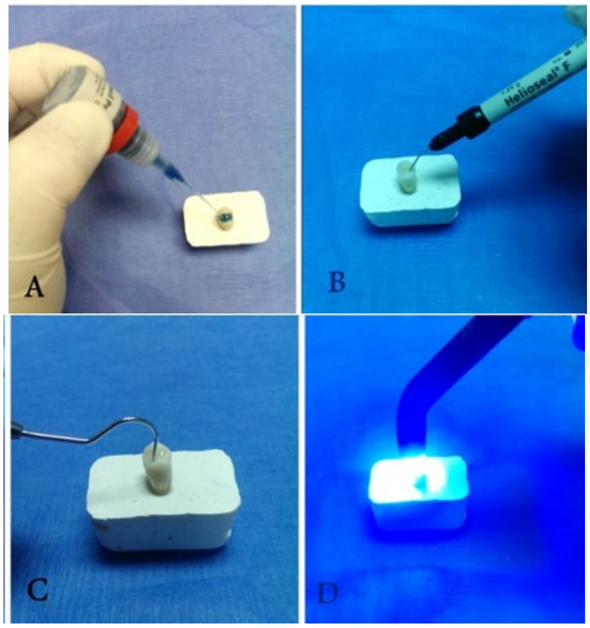


**Figure 9:A. acid etching, B. Helioseal-F application, C. using tip of probe for adaptation. D. light curing.**


**Figure 10: GC Fuji TRIAGE- White shade.**

**Microleakage Assessment.** The apices of teeth were sealed with sticky wax. All tooth surfaces were painted except 1.5mm around the sealant margin with two layer of nail varnish. The teeth were immersed in a 1% Methylene blue solution for 48 hours at room temperature (Figure 12).

Upon removal from the dye, the teeth were rinsed with distilled water, and the excess sticky wax from root apices removed and then each tooth was placed on a plaster block and each block positioned, and

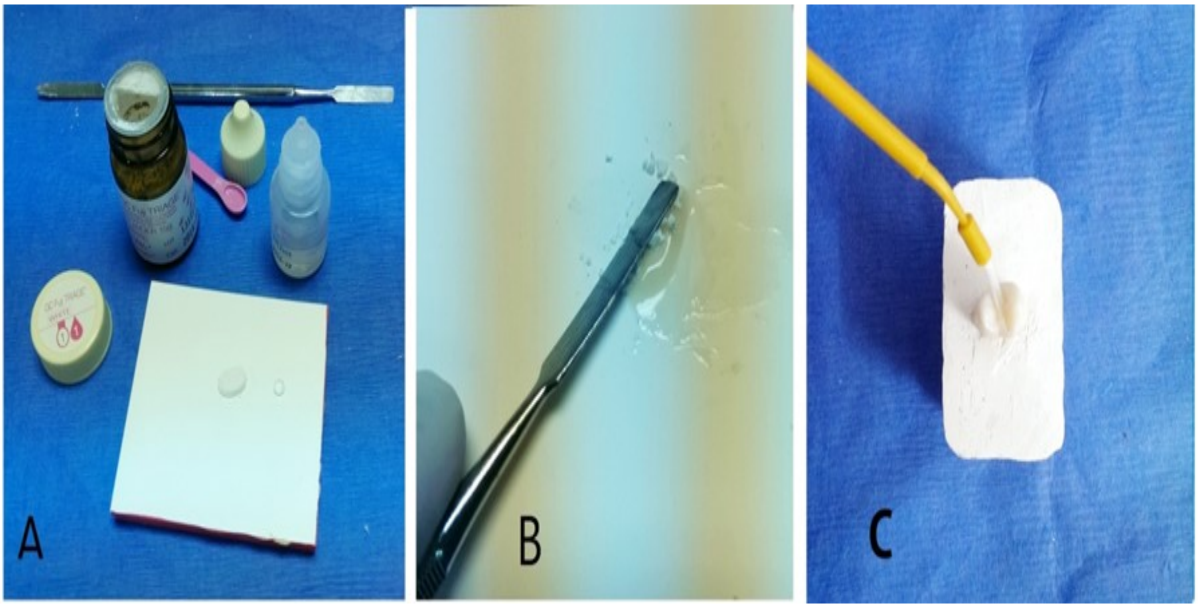


**Figure 11:A. one drop of liquid and one spoon of powder, B. mixing by spatula, C. application of GC with small brush.**

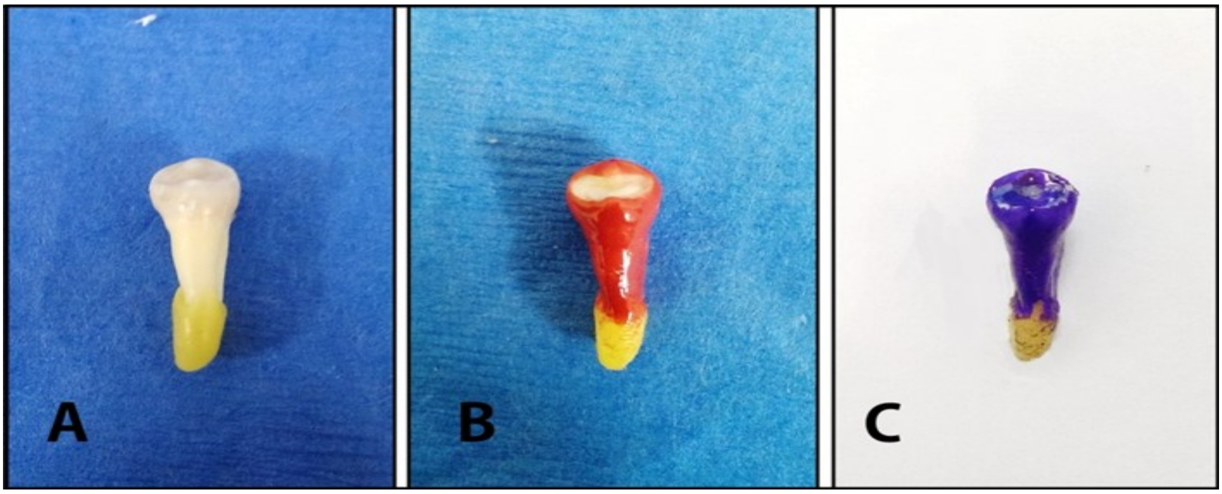


**Figure 12: A, Sticky wax on root apex, B, Nail Varnish applied, C, tooth immersed in Methylene blue solution.**

secured on the base of manual surveyor for sectioning. Based on deepest part of the fissures two buccolingual sectioning cuts parallel to the long axis of each tooth were made, yielding 3 sections and 4 surfaces per tooth for analysis. For each tooth mesial and distal sections were secured on glass slides by wax and the middle section placed on slide glass. All sections were examined under stereo microscope (x25) for microleakage level according to the method described by Övrebö and Raadal.[8]
As shown in the figure 13, the scoring method was:
Score 0 = no dye penetration
Score 1= dye penetration restricted to the outer half of the sealant
Score 2 = dye penetration to the inner half of the sealant
Score 3 = dye penetration into underlying fissure.
Each surface score was determined by the greatest dye penetration detected on the buccal occlusal and/or lingual occlusal fissure wall (Figure 14). The overall score for each tooth equaled the highest score of the 4 surfaces. Mean microleakage scores and standard errors were calculated for each treatment group.

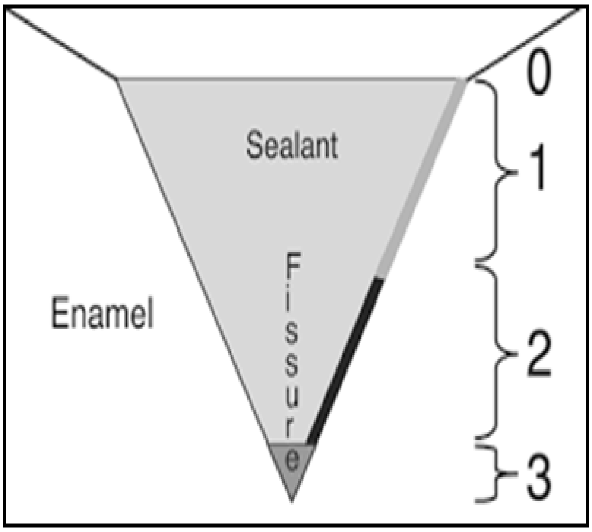


**Figure 13: Schematic diagram for dye penetration scoring[9]**

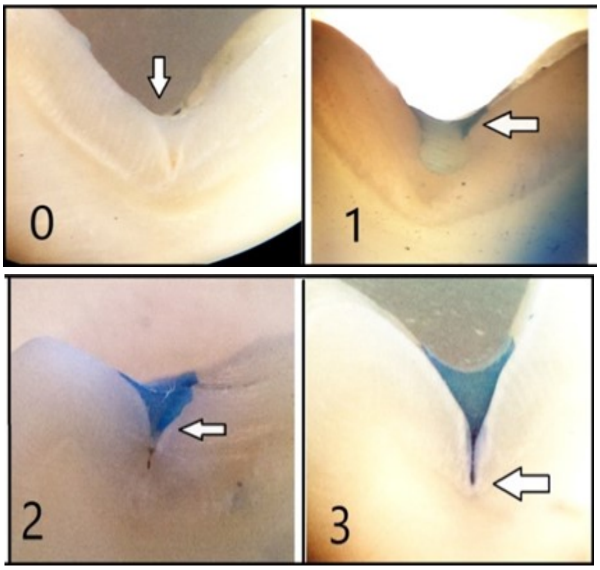


**Figure 14: Score 0, Score1, Score2, Score3.**

## Statistical Analysis:

Data were analyzed using SPSS software version 19. The data were summarized using means and standard deviations. Statistical analysis with one-way analysis of variance (ANOVA) was performed to compare the differences in the means among groups and paired t-test was used to compare between two groups with P>0.05.

## Results

Table 1 shows the dye penetration scores according to highest tooth level analysis with 4 surfaces measurement per tooth totaling 60 scores. No leakage (score 0) was noted in 10 of 60 teeth (16.6%). Dye penetration restricted to the outer half of the sealant (score 1) was noted in 12 of 60 teeth (20%). Dye penetration to the inner half of the sealant (score 2) was noted in 16 of 60 teeth (26.6%). Dye penetration into underlying fissure (score 3) was noted in 22 of 60 views (36.6%).

The highest level of microleakagewas noted in group (5) GC Fuji TRIAGE non-invasive technique with (mean 2.6) per 10 teeth. While the lowest level
was noted in group (4) Helioseal-F invasive technique with mean (0.8). Mean and standard deviation of each group was conducted in Table (2).

**Table 1: Dye penetration scores of samples**

| | | | Dye penetration | | | | |
|---|---|---|---|---|---|---|---|
| **Fissure Sealant** | **Technique** | **Group** | **0** | **1** | **2** | **3** | **Total** |
| **Vertise Flow** | **Non- Invasive** | **1** | 0 | 1 | 4 | 5 | 10 |
| | **Invasive** | **2** | 0 | 5 | 4 | 1 | 10 |
| **Helioseal-F** | **Non- Invasive** | **3** | 3 | 4 | 0 | 3 | 10 |
| | **Invasive** | **4** | **6** | 1 | 2 | 1 | 10 |
| **GC Fuji TRIAGE** | **Non- Invasive** | **5** | 0 | 1 | 2 | **7** | 10 |
| | **Invasive** | **6** | 1 | 0 | 4 | 5 | 10 |
| **Total** | | | 10 | 12 | 16 | 22 | 60 |
| **Percentage** | | | 16.6% | 20% | 26.6% | 36.6% | 100% |

**Table 2: Mean and standard deviation of each group.**

| Group | No. | Mean | Std. Deviation |
|---|---|---|---|
| **1** | 10 | 2.4 | 0.69 |
| **2** | 10 | 1.6 | 0.69 |
| **3** | 10 | 1.3 | 1.25 |
| **4** | 10 | 0.8 | 1.13 |
| **5** | 10 | 2.6 | 0.69 |
| **6** | 10 | 2.3 | 0.94 |

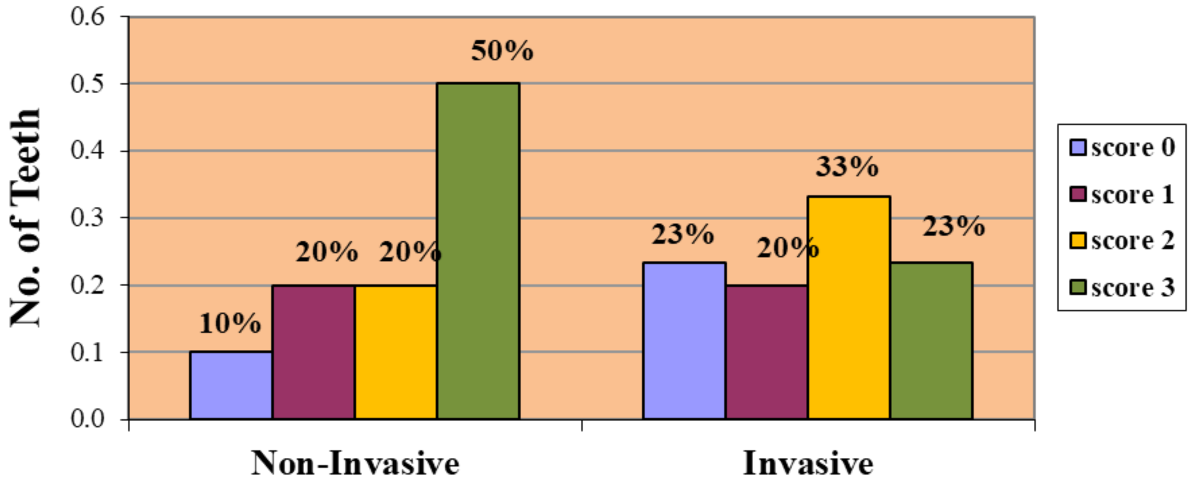


**Figure 15: Bar chart illustrating number and percentage of teeth with two different techniques for the three sealants.**

**Table 3: Comparison between Non-invasive and Invasive technique for the three sealants.**

| Method | Mean±SD | Std.Error mean | df | P |
|---|---|---|---|---|
| **Non-Invasive** | 2.1±1.06 | 0.19 | 29 | *0.043 |
| **Invasive** | 1.56±1.1 | 0.20 | | |

*Significant P<0.05

**Table 4: ANOVA Test comparing all Study Groups**

| Groups | Sum of Squares | df | Mean Square | P |
|---|---|---|---|---|
| **1** | 11.60 | 9 | 0.90 | 0.55 |
| **2** | 4.40 | 9 | 0.55 | 0.61 |
| **3** | 14.10 | 9 | 0.95 | 0.60 |
| **4** | 4.40 | 9 | 1.82 | *0.02 |
| **5** | 4.40 | 9 | 1.20 | 0.06 |
| **6** | 8.10 | 9 | 1.07 | 0.34 |

*Significant P<0.05

**Quality of two different techniques for the three sealant materials.** Results showed that the invasive technique for sealants reveals better microleakage reduction than non-invasive technique as shown in Figure (15) with significance difference between two techniques for all sealants together (P<0.05) as shown in Table (3).

**Quality ofsealant material and technique.** Better results were seen in Helioseal-F with Invasive technique (group 4), with significant difference between (group 4 and other five groups) (p<0.05) shown in Table (4)

## Discussion

Pit and fissure sealants recently have been considered outstanding with oral health promotion and care as preventive strategies in the decrease of occlusal caries.[10]

Marginal microleakage following sealant placement allows bacterial and bacterial byproducts to penetrate beneath the sealant, potentially initiating and continuing the caries formation process.[11]

Causes for microleakage is related to several factors, such as dimensional changes of materials due to polymerization shrinkage, thermal contraction, absorption of water, mechanical stress and dimensional changes in tooth structure.[9,12]The results of this study are valid for *in vitro* conditions. Depending on the environment, all pit and fissure sealants may act differently due to other variables like type of fissures, preparation of fissures, enamel etching and conditioning, application of bonding agent and contamination of prepared surfaces of fissures. Appropriate method of application of sealants and viscosity of the sealant, are also a factors influencing the microleakage, and if a proper application method is followed, it can increase the length of resin tag and thus improve the efficiency of the sealant in preventing caries.[13]

In this study the highest level of microleakage (score 3) among 6 groups was noted in group 5 (GC Fuji TRIAGE,Non-Invasive) with (mean 2.6) per 10 teeth. While the least level of microleakage (score 0) among 6 groups was noted in group 4 (Helioseal-F, Invasive) with mean (0.8) (Table 1)

**Quality of two different preparation techniques for the three sealant materials.** Results showed that the invasive technique for sealants reveals better score readings than non-invasive technique.Enameloplasty technique is specially indicated for deep narrow discolored fissures, suspected of being carious. Opening of the fissure promotes mechanical retention, reduces microleakage and most important, it permits diagnosis of the presence or extent of the carious lesion.[14]

This result agree with the study was done by Singla *et al*[15], because invasive technique widens and deepens the pits and fissures eliminates organic material and plaque and exposes a more reactive tooth enamel, therefore, enabling a thicker layer of sealant, which would be more wear resistant, superior sealant adaptation can be obtained.

In the study done by Chan *et al.*[16] mechanical preparation of fissures with burs is believed to provide certain advantages, such as removal of surface demineralization, creating a higher retention rate, and reducing the risk of microleakage. This could be the possible explanation for less microleakage in the invasive group of their study.

**Quality of Sealant Material and Technique.** Helioseal F in invasive technique produced significantly the least microleakage among all six groups. The superior results of Helioseal F seem to be related to itis higher flowability rate which contain triethylene glycol dimethacrylate (TEGDM) make sealant less viscous and high flowable,On the other hand, the monomer matrix of Helioseal-F consists of Bis-GMA which provide lower polymerization shrinkage due to long chain polymer, rigid monomer, ring aromatic bond not flexible. This result is in consistent with the findings of previous studies. [17,18,19] Vertise Flow showed higher of microleakagerate than Helioseal-F, this is due to that Vertis Flow contain 70wt% fillers while Helioseal-F contain 40.5wt% filler, on the other hand Vertise Flow contain Hydroxy Ethyl MethAcrylate (HEMA) which is hydrophilic material and absorb water that affect shrinkage of sealant. this result agrees with Eliades *et al*[20].By including the bonding in its formulation, Vertise Flow eliminates the additional steps of etching/ priming/bonding otherwise necessary to bond a resin composite to dentin and enamel.[21]In the present study, Vertise Flow fissure sealants used without acid etch. In light of the findings of Horiuch *et al*.[22] and Iijima *et al*.[23] they found that the low bond strength of Vertise Flow was due to its minimal effects on tooth enamel.

Moreover, it is important to consider that the enamel surface has an aprismatic configuration in the zone of occlusal fissures. Therefore, treatment with self-etching agents does not eliminate a significant amount of the surface layer of enamel without prisms, since it is not washed after applying to the tissue. The aprismatic structure of the enamel might prevent penetration of self-etching adhesives, leaving some zones partly unetched and with inadequately sealed fissures.[24,25]

According to some studies higher extent of microleakage was observed under glass ionomer sealant, which is attributed to the solubility of the material.[26,27]These results were in agreement withastudyby (Alonso *et al*.)[28] that indicated poor retention rates of glass ionomer-based materials placed as occlusal sealants.

## Conclusion

Within the limitation of this invitro study the following conclusions was drawn: Helioseal-F in comparison with Vertise Flow and GC Fuji TRIAGE provided the least microleakage while GC Fuji TRIAGE produced the highest amount of microleakage as a fissure sealant material. This study revealed that invasive technique for fissure treatment compared to non- invasive technique had lesser degrees of microleakage.

### Conflict of interest

The authors reported no conflict of interests.

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