RESEARCH ARTICLE

Recevied/ Geliş Tarihi: 21.12.2024
Accepted/ Kabul Tarihi: 08.04.2025
Published / Yayın Tarihi: 30.04.2025

Aydın Dental Journal - Volume **11** Issue **1** - April **2025** (57-68)

Aydın Dental Journal

Journal homepage: http://dergipark.ulakbim.gov.tr/adj

DOI: 10.17932/IAU.DENTAL.2015.009/dental_v011i1005

Evaluating In Vitro Performances of Various Pit and Fissure Sealing Materials

Farklı Pit ve Fissür Örtücülerin In Vitro Performansının Değerlendirilmesi

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ABSTRACT

Objectives: The aim of the study was to evaluate the penetration depth and microleakage of a resin infiltrant (Icon, DMG), and two fissure sealants (Glass Carbomer, GCP Dental; Teethmate F-1, Kuraray) applied to fissures and pits on the enamel surface in vitro.

Materials and Methods: For the penetration test, 90 human molars were kept in 0.1% ethanolic tetramethyl rhodamine isothiocyanate 24 hours to label all accessible pores. Prior to application, materials were labeled with a 0.1% rhodamine isothiocyanate. A confocal microscope was used to analyze slices of teeth that were perpendicular (n=20). The maximum percent penetration (maxPDY) was computed by measuring the maximum depth of penetration (maxPD) and the maximum depth of lesion (maxLD). For the microleakage test, 30 human molars were preserved in basic fuchsin dye. Teeth were divided in the mesiodistal direction, pieces were examined with a stereomicroscope and scored (n=10). Kruskal-Wallis and Mann-Whitney U tests were used for the statistical analysis (p=0.05).

Results: The maxPD values of the Icon group was statistically higher than the Glass Carbomer and the Teethmate F-1 groups ($p<0.05$). The maxPD values of Glass Carbomer and the Teethmate F-1 groups were similar ($p>0.05$). Glass Carbomer group showed severe microleakage values and internal cracks. There was no difference between Icon and Teethmate F-1 groups statistically ($p>0.05$).

Conclusion: Icon and Teethmate F-1 groups displayed favorable performance. Although the penetration depth of the Glass Carbomer is similar to Teethmate F-1 group, further research on the clinical performance of this material is needed due to its excess microleakage.

Keywords: *Dental resins, Fissure sealants, Minimally invasive surgical procedures*

ÖZET

Amaç: Bu çalışmanın amacı, mine yüzeyindeki pitlere ve fissürlere uygulanan bir infiltrantın (Icon, DMG) ve iki fissür örtücünün (Glass Carbomer, GCP Dental; F1-Teethmate, Kuraray) penetrasyon derinliğini ve mikrosızıntısını in vitro olarak değerlendirmektir.

Gereç ve Yöntemler: Penetrasyon testi için, 90 adet insan azı dişi kullanıldı. Erişilebilir tüm gözenekleri işaretlemek için dişler 24 saat boyunca %0,1 etanolik tetrametil rodamin isotiyosiyanat'da bekletildi. Materyaller uygulanmadan önce %0,1 rodamin isotiyosiyanat ile işaretlendi. Dik diş kesitleri konfokal mikroskopta incelendi (n=20). Maksimum penetrasyon derinliği (maxPD) ve lezyon derinliği (maxLD) ölçüldü ve maksimum penetrasyon yüzdesi hesaplandı (maxPDY).

Mikrosızıntı testi için, 30 adet insan molar dişi kullanıldı (n=10). Dişler 24 saat bazik fuksin boyasında bekletildi. Dişlerden meziodistal yönde kesitler alınarak stereomikroskopta incelendi ve skorlandı (n=10). İstatistiksel analizde Kruskal-Wallis ve Mann-Whitney U testleri kullanıldı (p=0.05).

Bulgular: Icon grubunun maxPD değerleri Glass Carbomer ve Teethmate F-1 gruplarına göre istatistiksel olarak daha yüksekti($p<0.05$). Glass Carbomer ve Teethmate F-1 gruplarının maxPD değerleri benzerdi ($p>0.05$). Glass Carbomer grubu ciddi mikrosızıntı değerleri ve içsel çatlaklar gösterdi. Icon ve Teethmate F-1 grupları arasında istatistiksel olarak anlamlı fark yoktu ($p>0.05$).

Sonuç: Icon ve Teethmate F-1 grupları istenen performansı göstermiştir. Glass Carbomer'in penetrasyon derinliği Teethmate F-1 grubuna benzer olsa da mikrosızıntısının fazla olması nedeniyle bu materyalin klinik performansı konusunda daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: *Dental rezinler, Fissür örtücüler, Minimal invaziv cerrahi işlemler*

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Introduction

Minimal intervention concept is relied on all the factors that included progression of disease and thus completes concepts of prevention, control and treatment. Detection of lesions early as possible, risk assessment and practice of preventive strategies is the most important stages for the patient. Therefore, patient education is also important. If the disease are present, other therapeutic strategies is required. For example demineralization, therapeutic sealants and restorative care such that minimal loss of tissue.[1] Pits and fissures of occlusal surfaces are the most important locations for dental caries risk, because of its unfavorable morphology. Even in individuals with good oral hygiene, the presence of caries in pits and fissures makes the situation more remarkable and important.[2] Although fissure sealant application is an effective method for preventing fissure caries, the cariostatic effects of fissure sealants depend on the physical closure of pits and grooves.[3] Fissure sealants isolate the lesion from the oral environment and can prevent the progression of the caries process by inhibiting the glycolytic activity of bacteria.[4] The protective effect of fissure sealants varies from 87% in the first year to approximately 60% after 4-5 years depending on the permanence of the restoration.[5,6] Nevertheless, they penetrate only to the upper layers of caries lesions.[7] Although authors allegation that sealing caries provides the occasion arrest the decay process but, caries progression can be prevented by covering the enamel initial lesions before they become cavitated. [2,8,9]

Caries infiltration is a minimally invasive dentistry approach used to treat incipient caries lesions on the proximal and flat surfaces of teeth. Resin infiltration aims to seal the demineralized enamel lesion body in incipient caries with light-cured low-viscosity resins.[10]

Various fissure sealants have been used from past to present. These; cyanoacrylates, polyurethanes, polycarboxylate cements, composite resins, polyacid modified composite resins, resin modified glass ionomer cements, glass ionomer cements, ormocers (organic modified ceramics), glass carbomer and resin infiltration systems. Resin-based and glass ionomer-based materials are the most commonly used fissure sealants. [11,12] The aim of the study was to evaluate the penetration depth and microleakage of a resin infiltrant (Icon, DMG), and two fissure sealants (Glass Carbomer, GCP Dental; Teethmate F-1, Kuraray) applied to fissures and pits on the enamel surface in vitro. The tested null hypothesis is that there were no statistically significant differences in the penetration depth and microleakage of different fissure sealing materials.

Materials and Methods

The Selçuk University Faculty of Dentistry Non-Interventional Clinical Research Evaluation Committee ethically approved the study (2014/03).

Penetration test and image analysis

According to the results of the Power analysis (G*Power software v3.1.10), a requirement of at least 18 samples in each group was determined with 95% confidence (0.05-α) and f=0.5 effect size for penetration test; a requirement of at least 10 samples in each group was determined with 95% confidence (0.05-α), 90% test power (1-β) and f=0.3 effect size for microlekage test. The number of samples was considered to be 20 for the penetration test (n=20) and 10 for the microleakage test (n=10).

Icon etch resin infiltrant (DMG, Hamburg, Germany), Glass Carbomer sealant (GCP Dental, Germany) and Teethmate F-1 sealant (Kuraray Medical, Okoyama, Japan) fissure sealing materials are used in this study.

Ninety non-carious third human molars extracted in the last 6 months were included. Sixty samples used for penetration test. The samples were randomly divided into three groups (n=20) then stored in 0.1% thymol solution. Fissures scored as according to ICCMS[13] (International Caries Classification and Management System) codes 0 and 1 independently.

Teeth were cleaned and stored in 0.1% ethanolic tetramethyl rhodamine isothiocyanate (TRITC, Sigma-Aldrich, Steinheim, Germany) for 24 hours to label all reachable pores in pre-staining for penetration test. Fissure sealing materials were labeled with 0.1% rhodamine isothiocyanate

(RITC, Sigma-Aldrich, Steinheim, Germany) before applying fissures.[14] HCl-gel was applied to teeth where Icon infiltrant would be applied for 120 seconds, and 37% phosphoric acid was applied to teeth where Glass Carbomer and Teethmate F-1 sealants would be applied for 40 s.

Firstly, for Group 1, RITC labeled Icon infiltrant was applied for 180 s entire deepest surface area of fissures. After, fissures were filled with resin infiltration material from beginning to end, light curing was performed with Monitex Blue Lex GT-1200 (Monitex Industrial Co., Taiwan) for 60 s.

Group 2 RITC labeled Glass Carbomer sealant capsule was activated by mixing for 15 s and applied onto the fissures in 60 s working time. Glass Carbomer surface gloss (GCP Dental, Germany) was applied and polymerized 60 s with Carbo-Led (GCP Dental, Germany).

Group 3 RITC labeled Teethmate F-1 sealant was applied for 60 s onto fissures and were polymerized for 20 s Monitex Blue Lex GT-1200.

The roots of the teeth were cut and the crown parts were embedded in acrylic. Three pieces of approximately 1200 µm thickness were obtained from each tooth perpendicular to the lesion surface (Isomet Buehler, Illinois, USA). Each piece of sample was fixed on a microscope slide. Samples polished with 1200, 2400, 4000 abrasive sandpaper and then were kept in 30% hydrogen peroxide for 12 s to remove free red fluorophore. After washing with water, the samples were kept in 100 µm sodium fluorescein in 50% ethanol solution (NaFI-Aldrich, Steinheim) for 3 minutes to stain the dentin and the porous structure where the fissure sealant was not infiltrated.[14] Finally, the samples were washed with deionized water for 10 s and dried with air-water spray.

Evaluation of samples with microscope was imaged at Selcuk University Advanced Technology Application and Research Center. Samples were examined at 10X magnification in a confocal laser scanning microscope (Nikon A1R-A1Confocal Microscope, Japan). Depth of penetration and depth of lesion were evaluated and compared using a confocal microscope images.

In dual fluorescence mode, images were acquired simultaneously in samples stained with RITC and NaFl. Images were recorded at 1024x1024 pixels and 2606 µm x 2606 µm. It allows the separation of the red (RITC) dye in the infiltrant part, the green (NaFI) dye in the non-infiltrating part and the porous part in dual flourescence mode technique.[14] Sections with the deepest fissure from each tooth sample were selected and used for analysis (n=20).

Microleakage test and image analysis

Thirty non-carious third human molars were used for mikroleakage test (n=10). Nail polish was applied 1 mm outside the fissure borders of the teeth and materials applied onto the fissures. The teeth were kept in 0.5% basic fuchsin (VWR Prolabo Chemicals, USA) solution at 37 ^{o}C for 24 h and then washed with water.

The teeth were cut in the mesio-distal direction using a water-cool bidirectional diamond-coated separator (Isomet Buehler, Illinois, USA). One side of the cut teeth samples were examined with stereomicroscope (Olympus, Tokyo, Japan). The dye penetration of the samples was assessed under top illumination with a stereomicroscope at 40X magnification. Microleakage values were evaluated using with the data obtained in the previous study of Pardi et al.[15]

The ranked scale used to score dye penetration was 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. The flow chart figure of the study is shown below (Fig. 1).

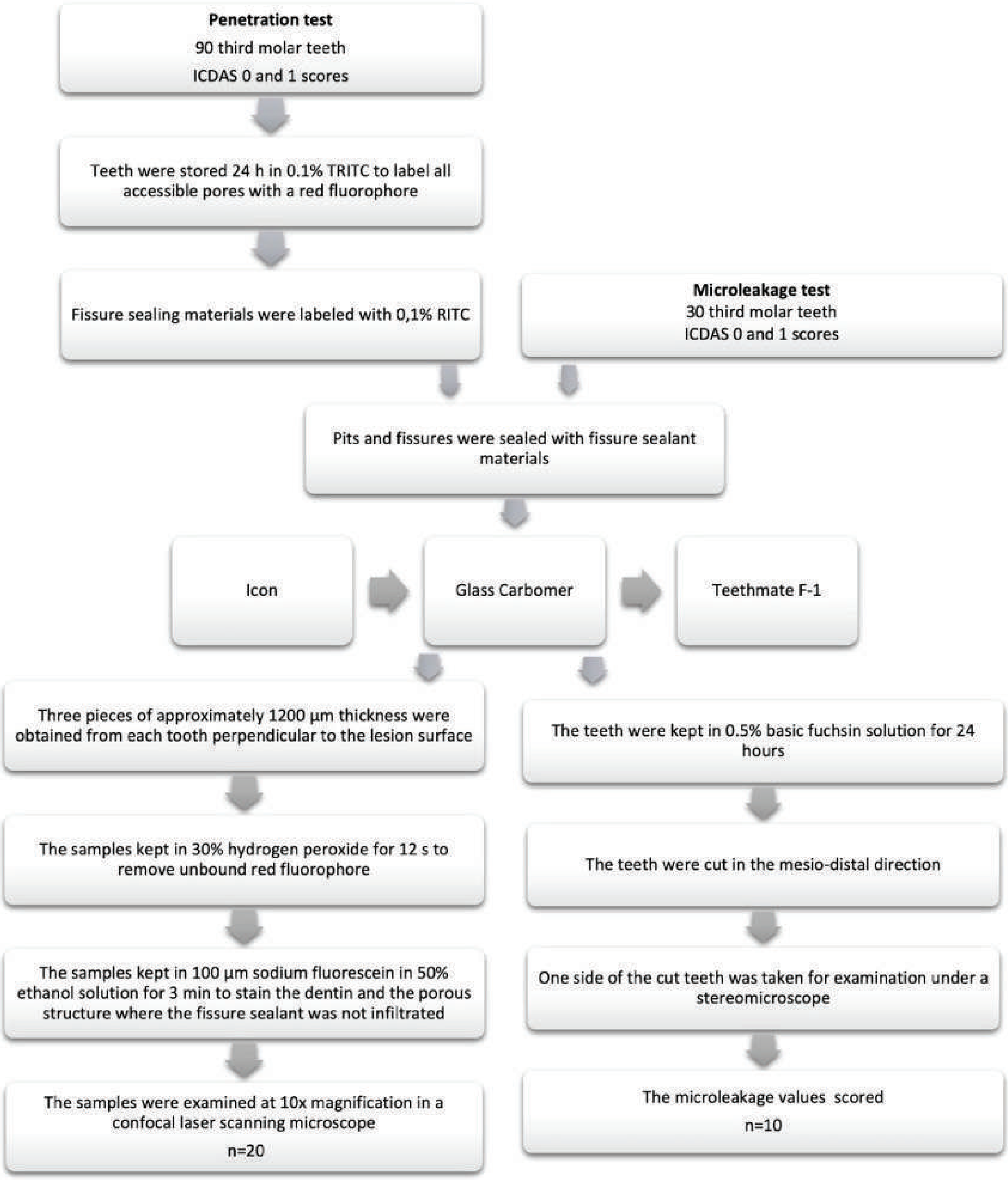

Figure 1. Flowchart of the study.

Statistical analyzes

Statistical analyzes were made using IBM SPSS Statistic 20 and MS Excel 2007 programs. The depth of penetration (maxPD) and the depth of the lesion (maxLD) were analyzed, and the percent penetration was calculated as the outcome variable.

(max)PDY = (max PD)/ (max)LDx100.

The normal distribution of the data was controlled with the Shapiro-Wilk test. Differences between groups were evaluated with the One-way ANOVA test ($p<0.05$).

Differences between microleakage scores were evaluated statistically by Kruskal Wallis and Mann-Whitney U-test ($p<0.05$).

Results

It was observed that the maxPD values different from each other according to the viscosity of the applied materials, the presence of air bubbles, organic deposits at the base of the fissure and the shape of the fissures (Figure 2). There was no statistically significant difference between the group 1 and 3 ($p>0.05$). All materials showed almost full penetration in V-type and U-type fissures (Fig. 2).

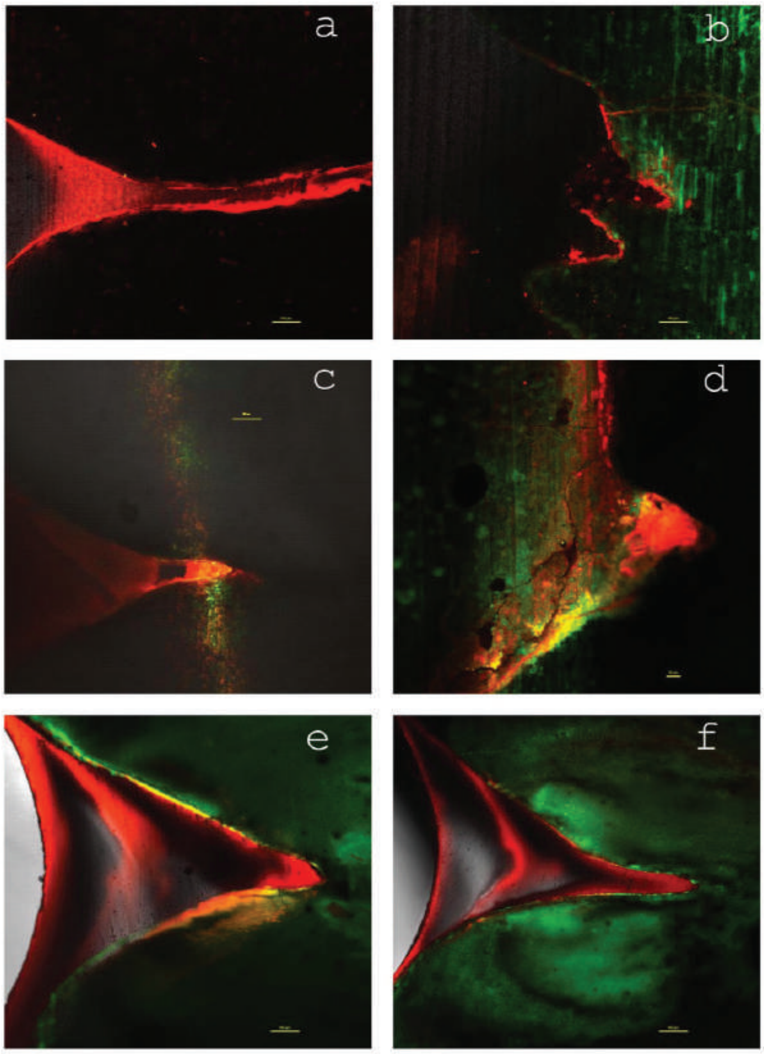

Figure 2. Penetration depth image of Group 1(a,b); Group 2(c,d) and Group 3(e,f).

The number of samples with 100% maxPD value were determined as 13 in the Group 1, 6 in the Group 2 and 10 in the Group 3. The maxPD values of the group 1 was statistically higher than the group 2 and the group 3 ($p<0.05$). The maxPD values of group 2 and 3 were similar ($p>0.05$) (Table 1).

Table 1. Mikroleakage scores of materials.

SCORES OF MICROLEAKAGE	Group 1 (Icon)	Group 2 (Glass Carbomer)	Group 3 (Teethmate-F1)
0	7	-	8
1	3	1	2
2	-	2	-
3	-	7	-

Group 2 showed significantly higher microleakage values than group 1 and 3 ($p < 0.05$). Group 2 showed severe microleakage values and internal cracks. There was no statistically significant difference between group 1 and 3 ($p>0.05$). Low microleakage values were observed in group 1 and 3 ($p > 0.05$). Microleakage values was determined as group 2>1>3 respectively (Table 2).

Table 2. Penetration depth of materials.

MATERIALS	PD_{mean} ± SD	PD_{min}-PD_{max}
Group 1 (Icon)	90,54 ± 14,30[a]	61,64 -100
Group 2 (Glass Carbomer)	77.30 ± 16,01[b]	44,74 - 100
Group 3 (Teethmate-F1)	85,11 ± 21,07[ab]	45,32 -100

*PD_{mean}: Penatration depth mean, SD: Standart deviation. **Small letters show the statistical differences. There is no statistical difference between the average values with the same letter according to the One-Way Anova test.

Discussion

Minimally invasive approaches aimed at protecting healthy tooth tissues as much as possible have become remarkable in dental applications in recent years. As a result of this approach, the use of adhesive restorative materials has increased. The performance of a restoration is closely related to the material selection. For this reason, penetration depth and microleakage characteristics of different pit and fissure sealant materials were investigated in this study. Considering the results obtained from the study, the null hypothesis was rejected. Both the penetration depth and the microleakage of the Glass Carbomer sealant group were found to be different from the other groups.

Clinical studies ensure that all properties of the materials are determined in the most realistic conditions.[16] However, in vivo studies, it cannot be determined which properties of the materials cause success and which properties cause failure. In vivo conditions, it is impossible to determine the effect of physical and mechanical stresses that occur during function in the oral environment on the failure of the material. In vitro tests have advantages such as being easy to apply, fast and cheaper.[17] Therefore, penetration and microleakage properties, which are important in the clinical success of the materials, were evaluated in our study.[2]

From past to present, the most preferred fissure sealants have been glass ionomer-based and resin-based materials. However, resin-based fissure sealants are considered to be the most successful materials in terms of their high retention rates and effectiveness. Viscosity is another factor for the ideal penetration, sealing and ideal marginal adaptation of the fissure sealant material.[18,19] Irinoda et al.[20] evaluating the effect of viscosity reported that low viscosity fissure sealant penetrates better into enamel than high viscosity ones. On the other hand, Barnes et al.[21] stated that viscosity does not affect the sealing properties of fissure sealants, so clinical success can be affected by changing the surface energy of enamel rather than making modifications in fissure sealants. In this study, the confocal laser microscope analysis method, which is used by many researchers was used to evaluate the penetration depth.[7,22]

Simultaneous images were obtained by staining the healthy enamel surface, porous tissue and fissure sealant with fluorescent dyes. Using more than one fluorescent dye separates images of different materials in studies. The fluorescent dye should be easily soluble and a good stimulant in the emission wavelength range.[23] Rodamin and fluorescein derivatives display these characteristics. Therefore, fluorophores are most commonly used materials in dental research. Fluorophores usually dissolve in non-polimerized monomers when marked with fluorescent resin dye. Chemical combination of fluorescent dyes with TEGDMA, HEMA or BIS-GMA-based resin bonding groups is not possible. For this reason, the dye is thrown out of the resin matrix and penetrates into the surrounding structures .[17,23]

Researchers have shown that fissure sealants penetrate the V and U shaped fissures the most; and penetrates the I and IK shaped fissures the least.[24-28] Although the fissure sealant has low viscosity, it cannot penetrate well and the fissure sealant becomes more difficult as the viscosity increases in I and IK shaped deep fissures. Our study supports previous studies. In our study, the penetration of Glass Carbomer sealant group which is the highest viscosity was found to be lower than other materials. The penetration depth of Icon Infiltrant and Teethmate F-1 sealant, both with low viscosity, was not statistically different from each other ($p>0.05$). These results show that viscosity of the sealant influences the penetration ability. Unlike other studies found that there was no difference in penetration between materials of different viscosity.[25,29]

Icon infiltrant contains TEGDMA that is a methacrylate-based resin matrix.[30]Also, before the Icon infiltrant is implemented, 99% ethanol is applied to the enamel surface to evaporate the remaining water. This application reduces viscosity and contact angle of the material, thus increasing the penetration of the material to the surface.[30,31] In some studies, TEGDMA containing infiltrants in two different forms with and without solvent was applied to the enamel lesions to compare the penetration ability and the effect of stopping progress of lesion, so infiltrants containing TEGDMA and ethanol solvent were found to be more effective.[32]

Paris et al.[7] compared infiltration abilities of fissure sealants and resin infiltrants in non-cavitated enamel fissure caries. The penetration of fissure caries treated with resin infiltrants was found to be significantly better compared to fissure sealants or soft etch infiltrants. However, no significant difference was observed in the penetration of resin sealants and resin infiltration in shallow lesions (ICDAS-code 0 and 1). Again, to this study, the hydrochloric acid used for infiltration provides deeper penetration in the enamel than phosphoric acid used in fissure sealants. In recent study, fissure morphology is another parameter that affects penetration depth. The effectiveness of the penetration depends on the wettability of the infiltrant and this is related to the complete accession of the acid to the fissures. Paris et al.[33] revealed that the most important step before caries infiltration was a complete drying. Clean surfaces with high free energy increase wettability compared to lower free energy surfaces coated with organic biofilm. Furthermore, thanks to the lower contact angle between the liquid and the solids, liquids penetrate easier to the porous solid structure. Presence of biofilm on the surface also changes the surface features, increases the contact angle between solids and liquids and possibly reduces the wettability of infiltrant.[34] In addition, Paris et al.[33] revealed that air bubbles remained in the lesion when infiltrant was penetrating and air gap obstructs the infiltrant flow, especially on the fissure base. In our study, air bubbles were also observed into the fissure.

Fissure sealants that are the low viscous materials easily penetrate the pores formed by acids on the enamel surface. However, despite the improvement of their physical properties, polymerization shrinkage has not been prevented. The fissure sealants shrink by volume 1,5-4% during their polymerization with visible light which causes marginal gap formation. Marginal gaps are habitat for bacteria, causing microleakage and thus cause failure of restoration.[35,36] For this reason, another important factor affecting the efficacy and success of the fissure sealants is the adaptation performance to enamel. The ability to reduce the microleakage of the fissure sealent is achieved by a good marginal adaptation.[37,38] In our study, Icon infiltrant and Teethmate F-1 sealant revealed successful results with favorable adaptation and microleakage values.

Paris et al.[10] investigated the penetration potential of caries infiltration system in proximal lesions of different ICDAS (2,3,4,5) codes. Resin-based materials penetrate into the micropores formed by acidification, thus resin tags varying from 25 to 100 microns in length are formed, thus providing a mechanical lock between the resin and the enamel surface.[2,39] Resin tags not only contribute to the mechanical retention of the sealer but also surround the enamel crystals, reducing the effect of microorganism-derived acids.[40] In the resin infiltrant, these extensions are generated up to 800 microns thanks to hydrochloric acid. Contrary to this, glass ionomer-based fissure sealants are chemically bonded to enamel and dentine. Using relatively high molecular weight, acidic, polycarboxylic-based polymers in its own structure, glass ionomers roughened the tooth structure due to its low pH (self-etch). However, this structure is weaker.[41] For this reason, the microleakage of Glass Carbomer sealant that is the glass ionomer-based fissure sealant was found to be higher than other materials in the present study. Glass Carbomer sealant could not penetrate up to the fissure depths. In the confocal microscope analysis, we observed adaptation losses with wide and long intervals in the material and enamel interface which support our detections.
Possible causes of differences in microleakage values are thought to be the content and percentage of fillers, the concentration of light-sensitive agents and the intensity of the polymerization light.[42] Microleakage for fissure sealants is an important indicator of adaptation and hiding power of the material to the dental tissue.[43]

Microleakage becomes more important issue because there is no cavity preparation in fissure sealants. Long-term adaptation of fissure sealants are weaker than composite restorations.[42] Today, it is reported that the most important reasons for replacing or repairing of restorative materials are marginal leakage and related complications. Therefore, it is important firstly to determine the marginal leakage and adaptation of temporary materials.[17]

The advantages of using glass ionomer-based materials can be determined in long-term use. For this reason, these materials tested in vitro must be tested in oral applications with clinical trials. More in vivo and in vitro studies will be useful to assess the efficacy of novel fissure sealant materials resulted from rapid developments and increased demand for minimal intervention.

Conclusion

Resin-based fissure sealants have been found to be more successful. In vitro penetration depths and internal fractures were observed in glass ionomer-based material. Although the penetration depth of glass ionomer-containing materials is deemed insufficient according to the results of this study, there will be indications for the use of different materials in different clinical situations due to the need to use them. Regular restoration checks will overcome this problem.

Acknowledgements
The authors thank to Emine Yavuz for laboratory phase of the study.

Ethical Approval
The necessary ethical approval for this study was obtained from The Selçuk University Faculty of Dentistry Non-Interventional Clinical Research Evaluation Committee (2014/03).

Conflict of interest
None of the authors of this article has any relationship, connection or financial interest in the subject matter or material discussed in the article.

Sources of Funding
Selcuk University Scientific Research Projects Governing Unit supported this study was a thesis of Nuray Günaydın (Project No: 14102031)

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