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Özgün Araştırma Makalesi

Efficacy of Pit and Fissure Sealant Containing S-PRG Filler on Inhibition of Enamel Demineralization

S-Prg Doldurucu İçeren Pit ve Fissür Örtücünün Minede Demineralizasyonu Engelleme Üzerine Etkisi

Yasemin Akın[1], Ayşegül Ölmez[2]

ABSTRACT

Aim: The aim of this in-vitro study was evaluating the effectiveness of an S-PRG filler containing fissure sealant (Beautisealant) to inhibit enamel demineralization and to compare it with two different fluoride-containing (Helioseal-F) and non-fluoride-based (Helioseal) conventional fissure sealants, using enamel microhardness tester.

Material and Methods: In this study 30 caries-free 3rd molar teeth were used. Helioseal, Helioseal-F or Beautisealant fissure sealant was applied to the buccal surfaces of the teeth and subjected to a 14-day pH cycle. Lingual surfaces were the control group. Sections were taken in the bucco-lingual direction and cross-sectional microhardness values were measured at different depths. The significance of the mean difference between the groups was examined by One Way Analysis of Variance, the significance of the difference between buccal and lingual surfaces at different depths (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 µm) within the groups by Dependent t-test (p<0.05).

Results: It was determined that BeautiSealant fissure sealant gave statistically significantly higher values than other fissure sealants at 25, 50, 75 µm depths (p<0.001). When Helioseal, Helioseal-F and Beautisealant were evaluated individually, no significant difference was found between the depths in any material at different depths (p=0.784, p=0.568 and p=0.039, respectively).

Conclusion: S-PRG filler containing fissure sealant is found to be the most effective in preventing demineralization.

Keywords: Inhibition of demineralization; Fissure sealant; Microharness; S-PRG filler

ÖZET

Amaç: Bu çalışmada fluorid içermeyen (Helioseal), fluorid içeren (Helioseal-F) ve S-PRG doldurucu içeren (BeautiSealant) rezin esaslı fissür örtücülerin demineralizasyonu engelleme özelliklerinin kesitsel mikrosertlik cihazı kullanılarak değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada çürüksüz 30 adet 3. Molar diş kullanılmıştır. Dişler bukkal yüzeylerine Helioseal, Helioseal-F veya Beautisealant fissür örtücü uygulanıp 14 gün pH siklusuna tabi tutulmuştur. Lingual yüzeyleri kontrol grubu olarak kullanılmıştır. Bukko-lingual yönde kesit alınıp, farklı derinliklerde (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 µm) kesitsel mikrosertlik değerleri ölçülmüştür. Gruplar arasında ortalamalar yönünden farkın önemliliği Tek Yönlü Varyans Analizi, gruplar içinde farklı derinliklerdeki bukkal ve lingual yüzeyler arasındaki farkın önemliliği Bağımlı t-testiyle incelenmiştir (p<0.05).

Bulgular: BeautiSealant fissür örtücünün 25, 50, 75 µm derinliklerde diğer fissür örtücülere göre istatistiksel olarak anlamlı düzeyde daha yüksek değerler verdiği tespit edilmiştir (p<0.001). Helioseal, Helioseal-F ve Beautisealant kendi içlerinde değerlendirildiklerinde farklı derinliklerde, hiçbir materyalde derinlikler arasında önemli bir fark bulunmamıştır (sırasıyla p=0.784, p=0.568 ve p=0.039).

Sonuç: Demineralizasyonu engellemede en etkin fissür örtücü materyalin S-PRG doldurucu içeren Beautisealant fissür örtücü olduğu gözlenmiştir.

Anahtar Kelimeler: Demineralizasyonu engelleme; Fissür örtücü; Mikrosertlik; S-PRG doldurucu

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İletişim: Yasemin Akın
Emek Mah, Bişkek Cd. 1. Sokak No:4, 06490 Çankaya/Ankara
E-mail: yasemin.akdemir@gazi.edu.tr
[1] Araştırma Görevlisi, Gazi Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği, Ankara, Türkiye
[2] Prof. Dr., Gazi Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği, Ankara, Türkiye

INTRODUCTION

Occlusal surface pits and fissures of posterior teeth are highly prone to caries development because of their anatomical structure that favors plaque stagnation, accumulation of food residues and bacteria.[1] Initial caries in these areas can be stopped and even reversible in the early stages with non-invasive and/or minimally invasive and preventive approach.[2] The role of fissure sealant applications, which is one of the most basic preventive approaches, in the prevention of dental caries has been proven by many studies.[3-7]

Today, with the development of restorative materials, many different types of fissure sealants have been produced. According to the most preferred classification, fissure sealants are divided into 2 groups as glass ionomer-based fissure sealants and resin-based fissure sealants. Although glass ionomer-based fissure sealants are known to have anti-cariogenic effect around the enamel and in adjacent teeth due to their fluoride release capability, their retention was found to be lower compared to resin-based fissure sealants.[1] Since resin-based fissure sealants have time-consuming application steps, they increase the risk of contamination with saliva. If this occurs after etching it can have a detrimental effect on bonding, causing microleakage and gap formation resulting in secondary caries formation.[8] As with many restorative materials, the integrity of the bond between the tooth surface and the material for fissure sealants is a key factor in preventing the leakage of bacteria and oral fluids. The caries prevention abilities and retention of fissure sealants are directly related to the bond strength between the material and the enamel surface.[4] Since the retention of fissure sealing materials is not continuous, microleakage may occur over time and marginal discoloration, secondary caries, postoperative sensitivity and ultimately pulp destruction may be observed as a result.[8] In this case, the fact that the material used has caries prevention properties such as preventing demineralization/promoting remineralization emerges as an advantage, especially in societies with high caries risk. Fissure sealants containing fluoride play a caries preventive role both by forming a barrier between teeth and bacteria, and by the ability of fluoride to inhibit demineralization and promote remineralization.[9,10] The clinical importance of fluoride release of fissure sealants has been proven by many studies.[11-13]

In recent years, a new resin-based fissure sealant that contains S-PRG filler has been introduced. In this technology, fluoro alumina silicate glass is added to the silica-filled urethane resin after reacting with polyalkenoic acid in water. As it bonds with its own self-etch primer it does not require enamel etching, which increases clinical time. So, application of fissure sealant that contains S-PRG filler simplify the technique and provide acceptable cooperation from children. These materials have fluoride release and recharge abilities like glass ionomer cements.[5,9,14] In vitro studies have concluded that they also have high buffering capacity and antibacterial properties as they can release a large number of bioactive ions in neutral and lactic acid conditions.[15,16] In this way, it is expected that these materials will have high caries prevention activities.

Based on this information, the aim of this in-vitro study was to evaluate the effectiveness of an S-PRG filler containing fissure sealant bonded by self-etching primer to inhibit enamel demineralization and to compare it with two different fluoride-containing and non-fluoride-based conventional fissure sealants bonded by acid etching, using enamel microhardness tester. The null hypothese tested was that the inhibition of the enamel surface's demineralization properties does not vary with respect to the sealant types.

MATERIAL AND METHODS

Sample Size Calculation

Before starting the study, sample size calculaion was performed in order to determine the sampling volume. Considering the technical limitations that may be encountered in the study; It was planned to carry out the study with a significance level of 0.05, a sensitivity of 0.40, a reliability of 95%, and a theoretical power of 80%, with a total of 30 samples.

Preparation of samples

Ethical approval was granted by the Ankara University Faculty of Dentistry Clinical Research Ethics Committee (25.05.2016, No:10/1). A total of 30 third molar teeth extracted for therapeutic indications were obtained under patient's informed constent.

The enamel surfaces of the extracted teeth were examined with a magnifying glass. The teeth were free of caries, had no hypomineralization on the enamel surface and had no defects due to extraction. After extraction the teeth were stored in 0.5% chloramine-T trihydrate solution for 1 week; teeth were cleaned with deionised water and stored in distilled water at +4 °C and used within 3 months. In this study, fissure sealants from each group were applied to the buccal surface in accordance with respect to the manufacturer's instructions as implemented by Tantbirojn *et al.*[17] The lingual surface of the same tooth was chosen as the control group in this study because it is thought that the enamel structure and organic-inorganic content will have higher differences in different teeth and this may affect the microhardness. Three experimental groups were formed. The names, composition, brand and the codes of the fissure sealant materials used in the study were given in Table-1; and the images were also given in Figure-1.

Group Hel: A fluoride-free resin-based fissure sealant Helioseal (Ivoclar-Vivadent, Schaan, Liechtenstein) was applied to the enamel surfaces after 30 seconds of 37% orthophosphoric acid (Eco Etch, Ivoclar-Vivadent, Schaan, Liechtenstein) application, 20 seconds of washing with air water spray and 10 seconds of drying. It was polymerized with LED light device for 20 seconds (Woodpecker LED-G, Guilin Woodpecker medical Instrument, China).

Group Hel-F : Helioseal-F (Ivoclar-Vivadent, Schaan, Liechtenstein), a resin-based fissure sealant that contains fluoride, was applied to the enamel surfaces after 30 seconds of 37% orthophosphoric acid application, 20 seconds of washing with air water spray, and 10 seconds of drying. It was polymerized with LED light device for 20 seconds.

Table 1. Brand, composition and code of the materials used in this study

Materials	Types/ Geneation	Composition	Manufacturer	Code
Helioseal	Resin sealant/ 3rd generation	Bis-GMA, TEDGMA, titanium, dioxide, initiators	Ivoclar Vivadent, Schaan, Liechenstein	Hel
Helioseal-F	Resin sealant/ 3rd generation	Bis-GMA, UDMA,TEGDMA, Fluorosilicate glass, silicon dioxide, initiators	Ivoclar Vivadent, Schaan, Liechenstein	Hel-F
Beautisealant	S-PRG containing resin sealant/ 3rd generation	S-PRG fillers, fluoroboroaluminosilicate glass, UDMA, TEGDMA, micro fumed silica and others, distilled water, carboxylic acid monomer, phosphonic acid monomer and others Primer: Acetone	Shofu Inc., Kyoto, Japan	Bs

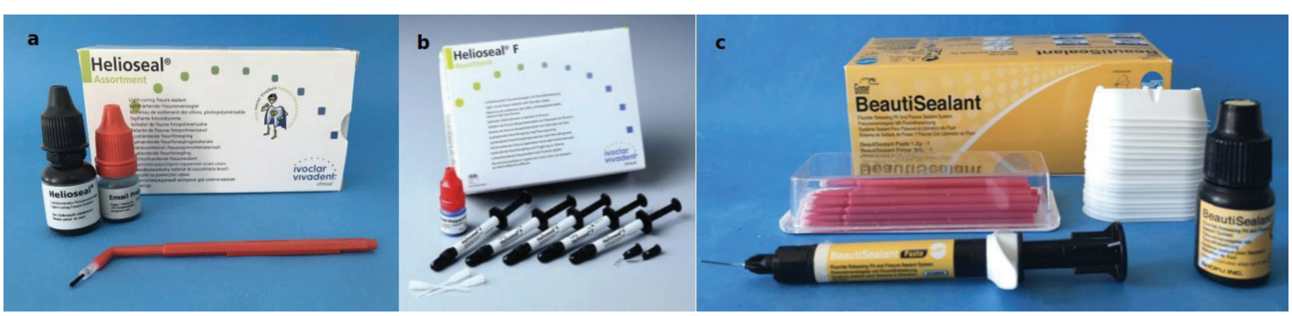

Figure 1. Images of fissure sealant materials
a) Helioseal (Ivoclar Vivadent, Schaan, Liechenstein),
b) Helioseal-F (Ivoclar Vivadent, Schaan, Liechenstein), c) Beautisealant (Shofu Inc., Kyoto, Japan)

Group BS: Beautisealant (Shofu Inc., Kyoto, Japon), a resin based sealant that contains S-PRG (Surface pre-reacted glass ionomer) filler, was applied to the enamel surface after 5 seconds of waiting and 5 seconds of air drying after its own primer was applied and it was polymerized with LED light device for 20 seconds.

The demineralization inhibition properties of fissure sealants were evaluated using the cross-sectional microhardness measurement technique with Shimadzu HMV (Shimadzu, Kyoto, Japan) microhardness device. After the surfaces of the teeth were cleaned with fluoride-free pumice and a polishing brush, all surfaces were coated with two coats of acid-resistant nail polish (Colorama Maybelline, São Paulo, Brazil) except an area of 3x3 mm² on the vestibule surfaces and all of their lingual surfaces. Fissure sealants from each group were applied to the exposed area on the buccal surface with respect to the manufacturer's instructions. The lingual surfaces of the teeth that were not coated were used as the control group.

The pH cycle model of Hu and Featherstone18 was used to simulate pH changes in the oral cavity. Each sample was kept in 40 ml of demineralization solution with pH 4.4 at 37°C for 6 hours. Then, the specimens were rinsed with distilled water . Subsequently they were placed to 20 ml of remineralization solution with pH 7.0 at 37°C for 17 hours. This pH cycle was repeated for 14 days. Afterward, the teeth were washed with distilled water and embedded in acrylic blocks. The contents of demineralization and remineralization solutions were prepared as stated by Gomez *et al*.[19]

The teeth in the prepared acrylic block were divided into two in the buccolingual direction with a precision sectioning device. The formed blocks were placed in acrylic molds again in a way that the sectioned surface was above for making proper measurements in the microhardness device. Surface polishing of the sections was done under running water using 320, 600 and 1200 grid Al_2O_3 paper discs. At each stage of the study, the samples were stored in a humid environment.

Two lines, oriented perpendicular to the enamel margin, were subjected to measurements. First line was at the junction of the occlusal triad and the middle triad in the area where the fissure sealant was applied. The second line was at the junction of the middle triad and the gingival triad, on the cross-sectioned surfaces of the samples. Measurements were made on these two lines, from the outer surface of the tooth to the inner parts, from 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 µm depths. The measurement points were determined with the digitometer on the table. Measurements were made with Vickers hardness device by applying 100 gr force for 10 seconds.

Statistical analysis

Analysis was conducted with the software package IBM SPSS Statistics 17.0 (SPSS IBM, New York, NY, USA). The normality of the distribution of continuous numerical variables was examined using the Kolmogorov-Smirnov test, while the homogeneity of variances was assessed using the Levene test. Data are represented as means and standart deviations (SD). One-Way Analysis of Variance (One-Way ANOVA) was employed to examine the importance of the mean differences between the groups. In cases where the results were found to be significant, the condition(s) that are responsible for the difference were identified using the post hoc Tukey HSD (Tukey's Honestly Significant Difference Test). The significance of the difference with respected microhardness averages between buccal and lingual at different depths within the groups was examined with the Dependent t-test, and whether there was a statistically important change in the microhardness averages according to the depths with the Analysis of Variance in Repeated Measurements. Results with a p-value < 0.05 were deemed statiscally important, unless otherwise specified. Nevertheless, to account for the possibility of Type I errors in all potential multiple comparisons, the Bonferroni Correction was applied.

RESULTS

Table 2 shows the microhardness values of the tested materials obtained from different buccal depths according to the groups.There was a statistically important difference among the groups in terms of mean microhardness value at buccal 25 µm depth ($p<0.0001$), and the mean microhardness value of the BS group was statistically significantly higher than Group Hel and Hel-F ($p<0.001$ and $p<0.001$).

There wasn't any statistically important difference between Group Hel and Hel-F in terms of mean microhardness values (p=0.984).

There was a statistically important difference among the groups in terms of mean microhardness values at buccal 50 µm depth (p<0.0001), the reason of this difference was that the mean microhardness values of group BS was significantly higher than group Hel-F. (p<0.001). There was no statistically important difference among Group Hel and Gropu Hel-F and between Group Hel and Group BS in terms of mean microhardness levels according to Bonferroni Correction (p=0.1766 and p=0.0025).

The groups demonstrated an important difference with regard to the mean microhardness values at buccal 75 µm depth (p=0.0017), the reason for this difference can be attributed to the noticeably higher mean microhardness value found in Group BS when compared to Group Hel-F (p<0.001). There wasn't any statistically important difference between Group Hel and Group Hel-F and between Group Hel and Group BS in terms of mean microhardness levels according to Bonferroni Correction (p=0.179 and p=0.087).

There was a statistically important difference among the groups with regard to the mean microhardness levels at buccal 100 µm depth (p=0.0009), the reason for this difference can be attributed to the noticeably higher mean microhardness value found in Group BS when compared to Group Hel-F (p<0.001).There wasn't any statistically important difference between Group Hel and Group Hel-F and between Group Hel and Group BS in terms of mean microhardness levels according to Bonferroni Correction (p=0.009 and p=0.689).

The groups didn't demonstrate any important difference with regard to the mean microhardness values at buccal 125, 150, 175, 200, 225, 250, 275 and 300 µm depth according to Bonferroni Correction (p>0.0021).

The comparison and distribution of microhardness values obtained from different depths between the buccal and lingual surfaces of Group Hel in Table 3, Group Hel-F in Table 4 and Group BS in Table 5 are shown. When Group Hel, Group Hel-F and Group BS were evaluated at different depths, none of the materials showed an important difference betwe-

Table 2. The mean ± standard deviation of the microhardness values obtained from different buccal depths according to the groups

	Grup Hel	Grup Hel-F	Grup BS	p-value †
25 µm	335.90±39.56[a]	332.70±52.92[b]	423.05±32.04[a,b]	<0.0001
50 µm	348.20±23.52	320.30±44.82[b]	404.85±29.91[b]	<0.0001
75 µm	345.40±36.99	311.90±49.22[b]	385.85±35.05[b]	0.0017
100 µm	364.25±29.74	315.00±41.58[b]	376.90±29.73[b]	0.0009
125 µm	348.15±41.00	313.00±42.71	367.30±32.03	0.0140
150 µm	341.30±35.35	314.90±44.43	352.70±26.01	0.0728
175 µm	349.15±27.05	313.10±48.61	339.55±29.90	0.0911
200 µm	331.20±38.66	311.70±42.69	337.40±21.89	0.2589
225 µm	327.15±40.79	316.40±37.40	336.75±27.57	0.4539
250 µm	326.80±21.51	313.35±30.04	322.60±20.27	0.4598
275 µm	337.85±34.19	309.40±25.63	308.00±21.08	0.0365
300 µm	324.40±19.78	288.30±29.53	296.80±21.53	0.0061
p-value ‡	0.784	0.568	0.039	

† Comparisons between groups at each depth, results for p<0.0021 according to One-way analysis of variance, Bonferroni Correction were considered statistically significant, same superscript letter represents significiant difference in each row (p<0.001)

‡ Comparisons between depths within groups, Analysis of variance in repeated measurements, according to Bonferroni Correction, the results were considered statistically significant for p<0.0083

Buccal : fissure sealant applied surface

Lingual: Surface without fissure sealant (control) en the depths. (respectively p=0.784, p=0.568 and p=0,039). When the buccal and lingual surfaces of Group Hel and Group Hel-F were evaluated within themselves, there wasn't any statistically important difference at any depth (p>0.0014). In the lingual surface of Group BS, there was a statistically important decrease in the mean microhardness value compared to the buccal surface at 25, 50, 75, 100, 150, 200, 225 and 250 µm depths (p<0.0014).There wasn't any statistically important difference according to Bonferroni Correction in terms of mean microhardness levels between buccal and lingual at 125, 175, 275 and 300 µm depths (p>0.0014).

Table 3. The mean ± standard deviation of microhardness values obtained from different depths between buccal and lingual in Group Hel

	Buccal	Lingual	p-value†
25 µm	335.90±39.56	328.15±50.85	0.7371
50 µm	348.20±23.52	322.15±32.22	0.1356
75 µm	345.40±36.99	319.15±38.68	0.0841
100 µm	364.25±29.74	323.35±32.93	0.0067
125 µm	348.15±41.00	355.10±47.54	0.6018
150 µm	341.30±35.35	324.30±36.45	0.3007
175 µm	349.15±27.05	327.55±40.71	0.1958
200 µm	331.20±38.66	321.90±43.41	0.5440
225 µm	327.15±40.79	334.25±55.72	0.7282
250 µm	326.80±21.51	320.65±45.75	0.6885
275 µm	337.85±34.19	316.25±43.72	0.1776
300 µm	324.40±19.78	309.80±39.19	0.1919

† Dependent t-test, results were considered statistically significant for p<0.0014 according to Bonferroni Correction.
Buccal : fissure sealant applied surface
Lingual: Surface without fissure sealant (control)

Table 4. The mean ± standard deviation of microhardness values obtained from different depths between buccal and lingual in Group Hel-F

	Buccal	Lingual	p-value†
25 µm	332.70±52.92	330.50±35.35	0.8933
50 µm	320.30±44.82	324.20±35.48	0.7770
75 µm	311.90±49.22	324.30±39.78	0.4955
100 µm	315.00±41.58	317.05±32.39	0.8793
125 µm	313.00±42.71	309.55±28.18	0.7944
150 µm	314.90±44.43	302.80±25.28	0.4232
175 µm	313.10±48.61	293.90±26.11	0.2388
200 µm	311.70±42.69	294.90±24.37	0.2222
225 µm	316.40±37.40	296.05±24.98	0.0857
250 µm	313.35±30.04	299.05±27.55	0.1342
275 µm	309.40±25.63	303.45±28.86	0.5733
300 µm	288.30±29.53	291.35±26.09	0.8145

†Dependent t-test, results were considered statistically significant for p<0.0014 according to Bonferroni Correction.
Buccal : fissure sealant applied surface
Lingual: Surface without fissure sealant (control)

Table 5. The mean ± standard deviation of microhardness values obtained from different depths between buccal and lingual in Group BS

	Buccal	Lingual	p-value†
25 µm	423.05±32.04	312.45±32.99	<0.0001
50 µm	404.85±29.91	312.85±20.80	<0.0001
75 µm	385.85±35.05	306.65±18.25	0.0002
100 µm	376.90±29.73	300.45±19.13	<0.0001
125 µm	367.30±32.03	298.80±33.26	0.0019
150 µm	352.70±26.01	293.40±25.69	0.0006
175 µm	339.55±29.90	287.00±25.91	0.0033
200 µm	337.40±21.89	286.60±28.27	0.0009
225 µm	336.75±27.57	283.70±32.45	0.0011
250 µm	322.60±20.27	277.00±25.97	0.0003
275 µm	308.00±21.08	277.70±34.32	0.0109
300 µm	296.80±21.53	270.05±29.30	0.0119

† Dependent t-testi, results were considered statistically significant for p<0.0014 according to Bonferroni Correction.
Buccal : fissure sealant applied surface
Lingual: Surface without fissure sealant (control)

DISCUSSION

In this study, the demineralization inhibition properties of 3 different resin-based fissure sealants were evaluated by the cross-sectional microhardness method. The effectiveness of a S-PRG filler containing fissure sealant, Beautisealant, in inhibiting enamel demineralization has been demonstrated especially in 25, 50, 75 and 100 µm depths, so the null hypotese tested was rejected. This finding aligns with previous in-vitro studies.[20,21]

Mineral loss acknowledged as an essential stage in the development of enamel demineralization.[22] Studies indicates that bioactive materials have the potential to counteract the demineralization impact of bacterial acids on the enamel surface by promoting mineral gain.[19,20] For the assessment of this effect, various models of demineralization and remineralization have been applied *in vitro* or research laboraory set-ups. These models can be categorized into two main goups: microbial models and chemical models. In this study a chemical model was utilized due to its benefits of simplicity, cost-effectiveness, efficiency, and the stability it provides for the experiment.[23] In this study, sealants were administered to sound enamel surfaces, and subsequently, the samples underwent a 14-day cycle of exposure to demineralization and remineralization solutions. This process aimed to simulate the dynamic pH changes observed in the oral environment. Following the pH-cycling, microhardness of the samples were tested. The results of this study showed that S-PRG filler containing fissure sealant, Beautisealant, applied enamel surfaces showed higher microhardness scores after 14 days of pH-cycling compared to the other resin-based fissure sealants.

In a study conducted to evaluate the remineralization abilities of different bioactive and non-bioactive resin pit and fissure sealants, enamel surface microhardness changes were evaluated in samples subjected to 7-day pH cycling. When the results were examined, Beautisealant specifically promoted enamel remineralization and showed surface hardness gain, which was significantly different when compared to other 2 resin based fissure sealant groups.[6]

Shimazu *et al*.[20] conducted a study for evaluating the caries preventive effect of Beautisealant, versus those of 2 different fluoride containing resin sealants in terms of it's effect on enamel remineralization and demineralization, using polarized light photomicrographs and micro-radiographs. In the Beautisealant group the enamel surface was maintened and demineralization did not proceed. Conversely, in fluoride containing resin sealant groups, notable defects were detected on the enamel surface. Important differences in lesion depth were evident between the Beautisealant group and the resin sealant groups. They concluded that the S-PRG filler-containing fissure sealant exhibited a superior preventive impact on demineralization compared to the conventional resin sealants.

Kaga *et al*.[21] demonstrated *in vitro* that various ions releaesed from S-PRG filler containing fissure sealant swiftly buffered the lactic acid solution and prevented enamel demineralization. Ushimura *et al*.[24] investigated the effects of fissure sealants in protecting against demineralization of primary teeth utilizing an automatic pH-cycling system. They found that mineral loss and lesion depth was the lowest in glass ionomer sealant, followed by S-PRG filler containing sealant, Beautisealant, and fluorid containing resin-based sealant; thus, inhibition of demineralization was higher in the Beautisealant group than resin-based sealant group. Ogawa *et al*.[25] conducted a study to investigate the impact of a SPR-G filler containing fissure sealant and 2 different resin based fissure sealant on the prevention of demineralization and the promotion of remineralization in the intact enamel surrounding the sealant material. Following a 10-day pH cycling period, the enamel surface proximate to the sealant material was examined through scanning electron and confocal laser microscopies. They also prepeared sealant disks and immersed in demineralization solution and measured ph change during 10 days by using ph electrodes. Researchers have reported that S-PRG containing fissure sealant has buffering capacity and is more effective in preventing demineralization on the surrounding enamel surface compared to fluoride-containing fissure sealants. They also noted that the high potential for promoting remineralization is attributed to the ions present in the S-PRG content.

Our findings that Beautisealant is superior to resin based fissure sealants in inhibiting demineralization agrees with previous in-vitro studies. When microhardness scores were examined in our study, it was shown that the values on the buccal surface were higher than the values on the lingual surface at all depths in all fissure sealant groups but this was statistically important only in the BS group. Likewise when the buccal surfaces were evaluated the highest microhardness values were observed in the BS group and the difference was statistically important especially at 25, 50, 75 and 100 µm depths when compared to Hel and Hel-F Groups.

It is known that fluoride ion has an anti-caries effect by inhibiting bacterial metabolism and growth, preventing plaque, pellicle formation and demineralization, and supporting remineralization. This caries preventive effect of fluoride is achieved by incorporating fluoroapatite to the structure of the enamel, thereby reducing the solubility of the enamel. Fluoride-releasing materials are like a fluoride reservoir, increasing the fluoride concentration in saliva, plaque, and dental hard tissues.[26] The intricate structure of pits and fissures poses a challenge for sealant materials to fully penetrate these areas. Consequently, the use of phosphoric acid etching before sealant application enhances sealant retention. However, over-etching may lead to demineralization and potentially contribute to the development of caries on the surfaces of permanent teeth.4 In this study, phosphoric acid etchant was employed as a pretreatment for Hel and Hel-F, while self-etching primer was used for BS. Despite Hel-F being a fluoride-containing sealant, cross-sectional microhardness scores were lower than BS group, indicating that the demineralization induced by the phosphoric acid etchant could not be effectively remineralized. The fact that BTS provides this effect may be related to the use of a soft self-etch primer, that is, it does not require phosphoric acid pretreatment, and the functional effects of the S-PRG filler to protect the enamel structure.

Alsaffar *et al*.[12] evaluated the demineralization inhibitory properties of pit and fissure sealants on the adjacent tooth surface. Researchers found that one of the fluoride-containing resin-based fissure sealants was superior in preventing demineralization compared to the non-fluoride resin-based fissure sealant, while the other fluoride-containing resin-based fissure sealant showed no significant difference. Salar *et al*.[13] stated in their study that fluoride-containing resin-based fissure sealants had better demineralization inhibition properties than fluoride-free conventional resin-based fissure sealants, but this effectiveness was lower than glass ionomer cements. Similarly, Zawaideh *et al*.[11] reported that the fluoride-containing resin-based fissure sealant was superior in their study in which they compared the demineralization inhibition properties of fluoride-containing and non-fluoride resin-based fissure sealants.

In our study, it was observed that the fluoride-containing resin-based fissure sealant Helioseal-F gave higher microhardness values than the fluoride-free resin-based fissure sealant Helioseal at all depths. Furthermore, it was observed that the microhardness values of the buccal surface were higher than the lingual surface, in both conventional resin-based fissure sealants. But these differences were not statistically important. We think that this can be due to the fact that both fissure sealants form an effective physical barrier and protect the tooth surface against acid attack, regardless of fluoride content. The results of our study are similar to the results of Vatanatham *et al.*'s[27] study in which they evaluated the effect of fluoride-containing and non-fluoride resin-based fissure sealants on mineral loss in initial caries lesions. Researchers reported that there was no important difference among the demineralization inhibition properties of fluoride-containing and non-fluoride resin-based fissure sealants.

Fissure sealants containing S-PRG fillers have the advantage of releasing a large number of bioactive ions in neutral and acidic conditions, in addition to their fluoride (F) release properties like fluoride-containing resin-based fissure sealants. These ions are strontium (Sr), sodium (Na), silicon (Si), aluminum (Al) and boron (B) ions. F and Sr ions form an acid-resistant layer and transform hydroxyapatite into fluoroapatite, strengthening the tooth structure. It also plays an important role in remineralization. Al ions also combine with F to form aluminofluoro complexes. Thus, in the recharging of the material with fluoride, F is incorporated into the structure of the material.[28]

As a result of acid production by bacteria, prolonged pH below the critical value of 5.5 initiates demineralization, leading to the formation and progression of enamel caries. It has been shown that the pH of the solution plays a more critical role in regulating mineral loss in dentin compared to the concentration of fluoride.[21] For this reason, it is important for the materials used as fissure sealants to have a high buffering capacity as well as their F content in order to prevent demineralization. Wang *et al.*[15] compared the buffering capacity of BeautiSealant, two different fluoride-containing resin-based fissure sealants and glass ionomer cement. Researchers have observed that the pH of lactic acid, which has a pH of 4.0, changes in the neutral direction with the effect of Si, Sr, Al, B, Na and F ions released when BeautiSealant comes into contact with lactic acid solution. As a result, the researchers reported that BeautiSealant has a strong buffering capacity. Kakuda *et al.*[16] evaluated the buffering capacities of a fluoride-containing resin-based fissure sealant, BeautiSealant and a glass ionomer-based fissure sealant. They reported that the buffering capacities of BeautiSealant and glass ionomer-based fissure sealant are similar and higher than that of fluoride-containing resin-based fissure sealant. Kaga *et al.*[21] evaluated the buffering capacity and inhibitory effects on enamel demineralization of BeautiSealant, a fluoride-free resin-based fissure sealant and a fluoride-containing resin-based fissure sealant. Researchers examined the ions released from the materials and the pH change. They found no significant difference between the Beautisealant and the F-containing fissure sealant group in the amount of F ions released while the pH value increased for BeautiSealant and decreased for the F-containing fissure sealant.

İn in-vivo studies, it has been reported that although S-PRG containing fissure sealants show less clinical retention when compared with conventionel resin fissure sealants, caries does not progress or does not occur at all, even with partial or total loss. Researchers have documented that this factor could be linked to heightened remineralization by giomer, attributed to the ionic potential of SPR-G particles.[5,29] This primarily takes place in the early stages of caries, where the Sr presence transforms hydroxyapatite into strontium-apatite, fortifying the enamel and forming a demineralization-resistant layer.[21]

In our study, when the microhardness values of the BS group were examined, statistically significantly higher values were observed at 25, 50, 75 and 100 micron depths, while the difference was not significant at depths deeper than 100 microns. The reason for the higher detection of microhardness at these depths, especially close to the surface where the material is applied, may be that the destructive effect of demineralization is more on the surface and the buffering capacity of the material is observed more at these depths due to this effect.

Nevertheless, predicting the prognosis of sealants is challenging, given the influence of various factors such as the oral environment, adherence to tooth fissures, tooth preparation method, eruption status of the tooth, and the released fluoride amount.One of the limitations of this study is the use of the lingual surface of the teeth as a control group in the study, where the morphologies and prismatic structures of the buccal and lingual surfaces of a tooth may differ from each other. Hence, additional research is necessary, considering various factors such as the morphological structure of tooth surfaces and the eruption status of teeth.

CONCLUSION

Based on the results obtained from this in-vitro study, it is possible to draw the following conclusion:

-When compared to other resin-based fissure sealants, S-PRG filler containing fissure sealant has been found to be the most effective in preventing demineralization.

-Resin-based fissure sealants may have some protective effect on enamel demineralization regardless of fluoride content.

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