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Effect of acid etching and different Er:YAG laser procedures on microleakage of three different fissure sealants in primary teeth after aging

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The purpose of this study was to evaluate the effects of different surface conditioning procedures on microleakage and unfilled area proportions of three fissure sealants(Aegis, Helioseal and Helioseal F) in primary molar teeth. One hundred and fifty teeth were randomly divided into five main groups according to surface conditioning procedures. Main groups were; acid-etching(Group A); laser 3.75 W(Group B) and 5 W(Group D); acid-etching combined with laser 3.75 W(Group C) and 5 W(Group E). Samples were aged by thermocycling and water bath. Afterwards, they were immersed in basic fuchsin solution for 24 h and bucco-lingual sections were obtained from each tooth. Digital images were taken using a stereo-microscope and the microleakage and unfilled area proportions were assessed by using a software system. Consequently, Aegis FS containing amorphous calcium phosphate was found to exhibit the lowest microleakage in all surface conditioning procedures. Unfilled areas were not detected in Groups C and E of all FSs.

Keywords: Primary molar teeth, Er:YAG laser, Fissure sealant, Aging

INTRODUCTION

In pediatric dentistry, pit and fissure sealants (PFSs) have gained an important and effective role in the prevention of dental caries. PFSs, covering all pits and fissures prevent the accumulation of plaque and the cariogenic microflora along with the incipient caries in the teeth. Some of their advantages include: reducing the microorganism formation, remineralizing incipient caries, protection from plaque accumulation, prevention of food debris accumulation in deep pits and fissures[1]. Generally, types of PFSs may be classified based on whether they are resin based or glass ionomer based and their fluoride content[2,3].

Recently, a new generation of fissure sealant has been developed with amorphous calcium phosphate (ACP). One of the most crucial features of ACP is the significant capacity to release calcium and phosphate. For this reason, interest in and research on ACP increased in recent years. For instance, a number of studies[4,5] have investigated the microleakage and penetrability of pit and fissure sealants containing ACP in permanent teeth and emphasized the importance of this type of sealant. Another study indicated that; PFSs containing ACP may promote remineralization of artificially induced carious lesion on smooth enamel surfaces[6].

Acid etching of the enamel is one of the most common and important stages in resin-based PFS application in clinical practice. Sealant retention is mainly mechanical through a small physicochemical interaction between the resin and etched enamel[7]. An additional, important stage for successful treatment is isolation that protects the teeth from contamination to saliva and water. Cotton rolls and the rubber dam are the mostly commonly used materials during this stage. However, this procedure takes much more time during treatment and may cause difficulty with non-cooperative children[8-10].

In recent years, hard-tissue laser systems have gained popularity in pediatric dentistry, such as Er:YAG and Er,Cr:YSGG lasers. These lasers have been used for pulpotomy, pulpectomy, dental caries removal and enamel pretreatment[11]. Laser systems have some important advantages for traditional approaches of dental treatment for children. Namely, they may require less anesthetic, allow for painless treatment, and decreased of dental fears and anxiety[12,13].

Some studies have demonstrated that the micro-roughened appearance of the surface morphology after laser irradiation on permanent enamel has indicated similarities to those obtained with conventional acid etching[14,15]. Furthermore, the irradiation of fissure enamel of permanent teeth with Er:YAG laser produced some morphological changes such as melting and recrystallization of fissure enamel[16].

In-vivo evaluation of dental materials has caused difficulties in clinical trials. For this reason, *in-vitro* evaluation has become more suitable for obtaining simulations of oral cavity conditions (thermocycling, water aging and chewing forces) in a laboratory environment to mimic the natural aging process. The present study differs from the studies mentioned above[17,18], in that all specimens were aged using

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thermocycling (10,000 times) and distilled water (at 37°C for six months). In the current body of literature, no study has thus far concentrated on the microleakage of fissure sealant containing ACP in primary teeth. The purpose of the present study was to evaluate the effects of the Er:YAG laser, used alone or in conjunction with acid-etching techniques for surface treatment, on the microleakage of three FSs after an aging procedure for the primary molar teeth.

MATERIALS AND METHODS

One hundred fifty primary molars that were free of caries, macroscopic defects, and restoration which were and pulled out for orthodontic reasons were selected for this study. The present study was approved by the Local Ethics Committee on Human Research of Cumhuriyet University (2010-02/04). After extraction, all teeth were cleaned of debris and stored in distilled water at 4°C for up to one month. The occlusal surfaces of the teeth were cleaned by a low-speed handpiece and a polishing brush, without using pumice or any other materials, for 20 s. A probe was used to clean debris from the fissures. The teeth were rinsed with an air-water spray for 30 s and dried with an oil-free compressed air jet for 10 s.

All of the teeth were randomly divided into five main groups according to surface conditioning procedures (Acid-etching, 3.75 W laser, 3.75 W laser+ Acid etching, 5 W laser, and 5 W laser+Acid etching).

Main groups (surface conditioning procedures)

Acid-etching (Group A): The occlusal enamel surfaces were etched with 37% phosphoric acid for 30 s, rinsed thoroughly using the air-water spray for 30 s, and gently air dried to obtain a uniform chalky-white appearance.

Laser irradiation (3.75 W) (Group B): The occlusal enamel surfaces were irradiated using a very short-pulsed Er:YAG laser device (Smart 2940D Plus; DEKA Laser, Firenza, Italy) emitted at a 2.94 μm wavelength, with a pulse energy of 150 mJ and a repetition rate of 25 Hz (3.75 W) for 30 s under air and water cooling. The laser was used with a handpiece head positioned 1 mm above and perpendicular to the fissures[11,19].

Laser irradiation (3.75 W)+Acid-etching (Group C): After the 3.75 W application as stated above, acid etching was performed.

Laser irradiation (5 W) (Group D): The occlusal enamel surfaces were irradiated with a very short-pulsed Er:YAG laser with a pulse energy of 200 mJ and a repetition rate of 25 Hz (5 W) for 30 s under air and water cooling.

Laser irradiation (5 W)+Acid etching (Group E): After the 5 W application, as stated above, acid etching was performed.

Each main group of teeth was further randomly divided into three subgroups according to type of pit and fissure sealants (n=10 for each). These subgroups were as follows:

Subgroup 1: Aegis ACP was applied to conditioned fissures, according to the manufacturer's instructions and photopolymerized with an LED curing unit (Bluephase Led, Ivoclar Vivadent, Schaan, Liechtenstein) for 20 s.

Subgroup 2: Helioseal was used on conditioned fissures, according to the manufacturer's instructions.

Subgroup 3: Helioseal F was applied to conditioned fissures, according to the manufacturer's instructions.

Thermocycling and water aging procedures

Just after the curing procedure, the teeth were stored in distilled water for 24 h and thermocycled 10,000 times at 5–55°C in water baths with an immersion time of one minute. Following thermocycling, the teeth were stored in a water bath at 37°C (Nüve BM 402, Ankara, Turkey) for six months with the water being replaced week. After the aging methods were applied, the apical parts of the teeth were sealed with a composite resin (Filtek Z250, 3M ESPE).

Microleakage assessment

The specimens were covered with two layers of nail varnish, except for the sealant and up to one mm from the sealant margins. Then, they were immersed in a 0.5% basic fuchsin solution for 24 h. The samples were rinsed with water to remove excess dye and embedded in a chemically activated acrylic resin. Each specimen was sectioned longitudinally by a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) with water-cooling in the bucco-lingual direction through the line connecting the buccal and lingual cusp tips to provide three sections. However, only the mesial and distal sections were selected and included in present study. Subsequently, two sections of each tooth were digitally photographed using a stereo-microscope with a magnification of 20× (Stemi DV4, Carl Zeiss, Jena, Germany) (Fig. 1). The images were transmitted to a computer. Clemex Vision-Lite 5.0 software (Clemex Technologies, Quebec, Canada) was used to perform all measurements during this experiment. In each sample, the microleakage proportion was expressed as the length of dye penetration (μm) divided by the length of the sealant-

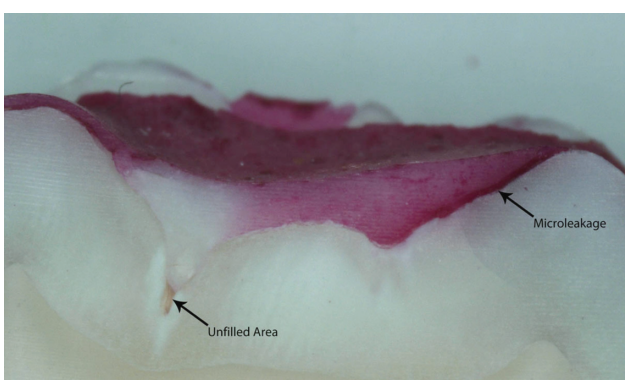


Fig. 1 The digitally photographs of a specimen using a stereo-microscope.

tooth interface[19] (μm). Unfilled area proportions were expressed as the unfilled area (μm²) divided by sum of the sealant/fissure area (μm²) and the unfilled areas[20] (Fig. 2). The measurements were made by one qualified and blinded person.

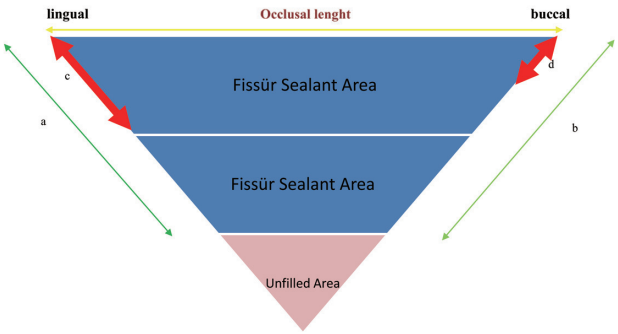


Fig. 2 Scoring system used for evaluation of microleakage and penetration proportion.
c, d: dye penetration lenght
a, b: lenght of tooth-fissure sealant interface

Scanning electron microscope analysis

Samples from each surface conditioning procedure group were coated with gold using a sputter coater (SCD 005; Bal-Tec, Balzers, Liechtenstein), evaluated and photographed under a scanning electron microscope (SEM) LEO 1430 VP, Carl Zeiss (Cambridge, England) to determine the morphological differences of the enamel surfaces. The samples were examined at 3,000× magnification (Fig. 3).

Statistical analysis

Microleakage and unfilled area proportions were analyzed with using a one-way ANOVA. Multiple comparisons were performed with using a Tukey test with the significance level set at 0.05. SPSS for Windows, Version14.0 (SPSS Inc, Chicago, IL, USA) was used to conduct statistical analysis.

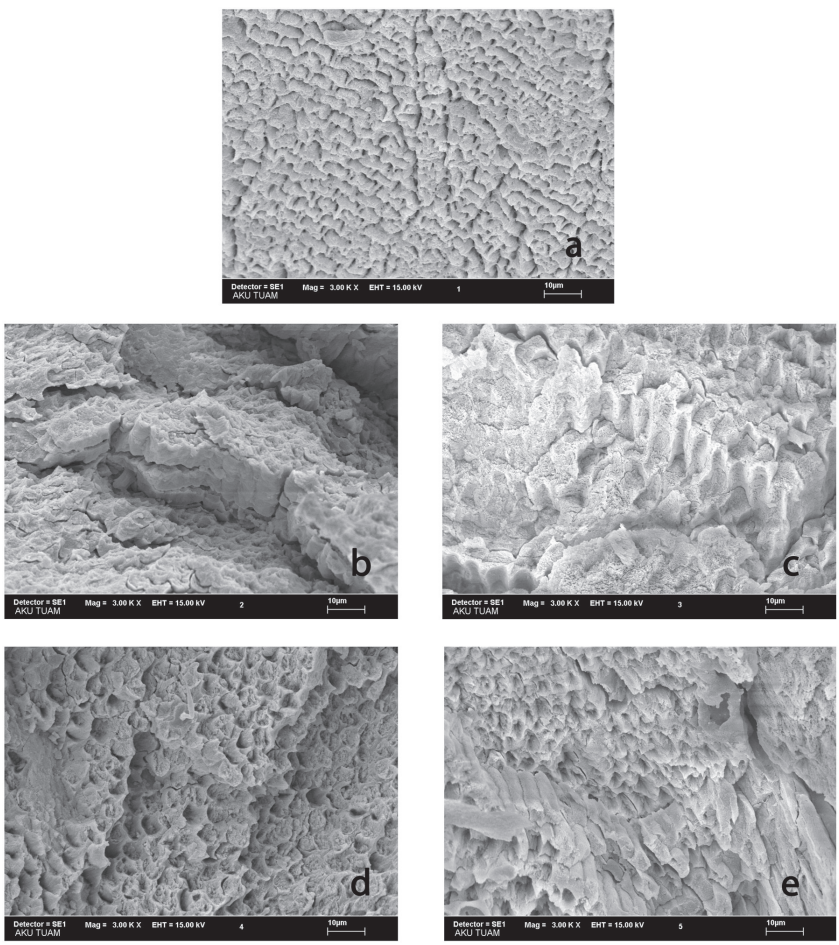


Fig. 3 SEM images after surface conditioning procedures; a) Group A, b) Group B, c) Group C, d) Group D, e) Group E.

RESULTS

Tables 2 and 3 illustrate the microleakage and unfilled area proportions with standard deviation.

In all surface conditioning procedures, although there were statistically significant differences among Aegis and all other fissure sealants ($P<0.05$), there was no statistically significant difference between Helioseal and Helioseal F FS ($P>0.05$).

In subgroups with Aegis FS applied; although there was no statistically significant difference between Groups A and C ($P>0.05$), there were statistically significant differences between Group A and all other groups ($P<0.05$). Moreover, there was no statistically significant difference between Groups B and D ($P>0.05$). The lowest microleakage was found in Group E when compared with all other groups.

In the Helioseal and Helioseal F fissure sealants; although there were statistically significant differences between Group A and respectively Groups C and D, there were also statistically significant differences in Groups B and D when compared with all other groups.

Although there were no statistically significant differences among the unfilled area proportions of the three fissure sealants (all surface conditioning procedures) ($P>0.05$), unfilled areas were not observed in each of the three fissure sealants of Groups C and E (Table 3).

Stereo-microscopic image obtained from after enamel surface conditioning procedure is shown in Fig. 1.

Table 1 Materials used in the study

Materials	Composition	Application procedures	Lot No.
AEGİS Bosworth®Company (USA)	UDMA, mono-and di methacrylates resins TLV-TWA: 15 mg/m3 TWA for ACP N/A for resin	1.After etching of enamel 2.Apply sealant 3.Wait for approximately 15 s 4.Light-cure (Bluephase, İvoclar Vivadent, Liechtenstein) for 20 s	0608-398
HELİOSEAL İvoclar Vivadent Ets., (Schaan, Liechtenstein)	Bis-GMA, TEGDMA(>99 wt%) Additional contents are stabilizers and catalysts(<1 wt%)	1.After etching of enamel 2.Apply sealant 3.Wait for approximately 15 s 4.Light-cure (Bluephase, İvoclar Vivadent, Liechtenstein) for 20 s	L24213
HELİOSEAL F İvoclar Vivadent Ets., (Schaan, Liechtenstein)	Monomer matrix: Bis-GMA(11.8%), UDMA (23.4%), TEGDMA(23.4%) Fillers: Fluorosilicate glass(20.3%), highly dispersed silicon dioxide(20.2%). Pigments, titanium dioxide, initiators, stabilizers	1.After etching of enamel 2.Apply sealant 3.Wait for approximately 15 s 4.Light-cure (Bluephase, İvoclar Vivadent, Liechtenstein) for 20 s	H23186

Bluephase, İvoclar Vivadent, Liechtenstein; light intensity: 1,200 mw/cm^2.
Abbreviations: Bis-GMA=bisphenol-A-glycidyl methacrylate; N/A=Not available; TEGDMA=triethyleneglycol dimethacrylate; TLV-TWA=Threshold Limit Value-Time-Weighted Average; UDMA=urethane dimethacrylate.

Table 2 Microleakage proportion of fissure sealants

	Microleakage Scores				
Fissure Sealant	Group A	Group B	Group C	Group D	Group E
Aegis	0.25 (0.02)^{A,B,a,b,c}	0.32 (0.07)^{C,D,a,d}	0.28 (0.07)^{E,F,e,f}	0.37 (0.12)^{G,H,b,e,g}	0.16 (0.03)^{J,K,c,d,f,g}
Helioseal	0.33 (0.02)^{A,h,i}	0.41 (0.08)^{C,h,l}	0.36 (0.08)^{E,m}	0.47 (0.03)^{G,i,m,n}	0.30 (0.05)^{J,l,n}
Helioseal F	0.34 (0.05)^{B,o,p}	0.42 (0.08)^{D,o,q}	0.37 (0.04)^{F,r}	0.48 (0.03)^{H,p,r,s}	0.31 (0.03)^{K,q,s}

n=20 specimens per experimental condition. Standard deviations are shown in parentheses.
By the one-way ANOVA: F=38.125 P=0.000, $p<0.05$ Tukey's test indicates statistical difference ($p<0.05$) for means followed by the same letters; capital letters in the column are for the comparison of different fissure sealant with the same surface pretreatment; small letters in the rows are for the comparison of different surface pretreatment with the same fissure sealant.

Table 3 Unfilled area proportion of fissure sealants

	Unfilled Area Proportional				
Fissure Sealant	Group A	Group B	Group C	Group D	Group E
Aegis	0.019 (0.04)	0.028 (0.06)	0.000	0.015 (0.04)	0.000
Helioseal	0.025 (0.05)	0.047 (0.07)	0.000	0.059 (0.12)	0.000
Helioseal F	0.029 (0.06)	0.054 (0.08)	0.000	0.074 (0.11)	0.000

n=20 specimens per experimental condition.
Standard deviations are shown in parentheses. By the one-way ANOVA: F=3,360 P=0.000, p>0.05.

The acid-etching (Group A) SEM image obtained after enamel surface is shown in Fig. 3a. This SEM image shows a chalky surface following organic debris cleaning. The SEM images of groups that used lasers alone (Groups B and D) are indicated in Figs. 3b and 3d. These SEM images exhibit cone-shaped enamel rods, furthermore they exhibit unevenness and irregularity of the enamel surface. The SEM images of the Er:YAG laser combined with acid etching groups (Groups C and E) are presented in Figs. 3c and 3e. These SEM images showed chalky surfaces with higher porosity according to the laser groups used alone.

DISCUSSION

In preventive dentistry, the success rate of fissure sealants is affected by various factors such as enamel surface properties, duration of acid-etching, and the type of acid and surface pretreatment techniques. Furthermore, the most important factor is the retantion of sealants, which essentially occurs through micro-mechanical and physico-chemical interactions between the resin and the etched enamel[21]. The dye penetration score technique has been commonly used in research to evaluate microleakage[4,5,11,19]. However, in the present study, image analysis software was used in order to obtain quantitative results instead of a conventional subjective scoring.

The Er:YAG laser is an effective device for roughing the surface of enamel including hydroxyapatite and water. One of the superiorities of this laser system lies in the rapid evaporation of hard tissue during irradiation[22]. The Er:YAG laser also incorporates water cooling and thereby ablates the surrounding tissue with minimal thermal side effects[23]. We preferred PFSs application, which is a non-invasive technique like Er:YAG laser.

Amorphous calcium phosphate (ACP) containing PFS that renews day by day, has recently gained popularity in pediatric dentistry. A few studies have conducted the microleakage of this material. For instance, Marks *et al.*[5] investigated the microleakage of various fissure sealants (Aegis, Admira Seal and Conseal F). In their study following sealant placement the specimens were thermocycled for 1,000 cycles. As a result, the lowest microleakage was shown in the Aegis fissure sealant group. Another study, conducted by Selecman *et al.*[4] investigated the microleakage of occlusal surfaces treated with air-abrasion and pumice after the five different fissure sealants application (Aegis, Admira Seal, Conseal F, Fuji Triage, and Delton Opaque). In their study, the lowest microleakage values were obtained in both air-abrasion and the pumice surface treatments with Aegis. In the present study, Aegis indicated the lowest microleakage of all surface conditioning procedures (P<0.05). This result shows similarities to previously mentioned studies[4,5].

The surface conditioning procedures may play a crucial role that affects the microleakage and retention of fissure sealants in clinical approaches. In one of the studies conducted on this issue, Moshonov *et al.*[24] investigated the microleakage of pit and fissure sealant (Helioseal) application on enamel treated with acid-etching and Er:YAG laser (800 mJ, 12 Hz). However, they did not find any differences between the two types of surface treatment. In other study, Borsatto *et al.*[25] evaluated the microleakage of fissure sealant on primary teeth treated with three different etching techniques such as acid-etching, Er:YAG laser (120 mJ; 4 Hz) and Er:YAG laser with acid-etching. Ultimately, the Er:YAG laser used alone increase the microleakage. Moreover, the Er:YAG laser did not achieve significantly better marginal sealing in primary microleakage, compared to conventional acid etching. Another research also evaluated the microleakage of the enamel surface treatment techniques using acid-etching, Er:YAG laser and Er:YAG laser (350 mJ; 4 Hz) with acid-etching that restorated with flowable composite. The findings revealed that, there was no difference between the Er:YAG laser with acid etching and conventional techniques. Moreover, fissures prepared with the Er:YAG laser alone showed the highest degree of microleakage[26]. In the present study, the higher microleakage was found in both Er:YAG laser groups used alone according to all other groups. On the other hand, there is no statistically significant difference between the 3.75 W and 5 W laser power energy. Moreover the deeper craters, cracks and grooves on enamel surfaces that were seen clearly in the SEM images, caused the highest microleakage in the 5 W

group (Figs. 3b and 3d). Although this finding aligns with some of the previously mentioned studies[25,26], it was not in accordance with the findings of Moshonov *et al.* In our opinion, this distinction emerged as a result of higher pulse repetition rate and lower pulse energy. For this reason, the laser groups with a higher pulse repetition rate caused deeper craters and grooves on the enamel surface (Figs. 3b and 3d). Furthermore, although there were not statistically significant differences between acid-etching and laser irradiation with acid etching groups for all fissure sealants (except Aegis Group E), the lowest microleakage was shown in the laser 5 W with acid-etching group. These results were supported by the SEM images in which chalky enamel surfaces with deeper craters, grooves and porosity were shown (Figs. 3c and 3e).

The unfilled area is one of the crucial factors playing an important role in fissure sealant treatment. In a study, Celiberti *et al.*[20] investigated the sealing ability of an unfilled fissure sealant under different humidity conditions and etching times. In the results, the shallow fissures showed higher microleakage and lower unfilled area than the deeper fissures. In another study, Francescut and Lussi[27] evaluated the microleakage of various surface treatment procedures including Er:YAG laser (600 mJ, 6 Hz), diamond bur, Er: YAG laser (200 mJ, 4 Hz) and powder jet cleaner (control group) with acid-etching followed by a fissure sealant (Delton opaque). Resultly, although the lowest microleakage was obtained from diamond bur, the highest microleakage was found in the laser group (200 mJ). Moreover, the proportions of unfilled area were respectively ranked from low to high as diamond bur, the laser groups and the control group. In the present study, although there were no statistically significant differences among the unfilled area proportions in all surface conditioning procedures, the unfilled areas were not seen in laser irradiation combined acid etching groups. This result was different according to upper mentioned study[27]. In our opinion this difference may be caused by the higher pulse repetition rate. Additionally, we prefered the primary teeth unlike above-mentioned studies[26,27].

In pediatric dentistry, we think that PFSs applications in which lasers used alone for the surface conditioning procedure show lower microleakage and unfilled area according to laser combined acid etching. In light of these results, the Er:YAG laser combined acid-etching may be preferable over the laser used alone in clinical approaches. These results could make great contributions for further studies. We suggest that amorphous calcium phosphate containing PFS is superior in terms of microleakage for primary teeth, although the subject requires further studies to investigate the microleakage of surface condition procedures.

CONCLUSIONS

The following conclusions can be obtained based on the results of this study:

- Higher microleakage values were obtained in groups with laser etching.
- Aegis ACP fissure sealant showed lower microleakage in samples compared to other fissure sealants (Helioseal, Helioseal F).
- Unfilled areas were not observed in groups that used laser irradiation combined acid etching.

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