Original Article

The Effectiveness of Erbium: Yttrium-Aluminum-Garnet Laser on Enamel Conditioning: An *In vitro* Study

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Objectives: The purpose of this *in vitro* study was to investigate the effect of three different enamel surface conditioning procedures on the bonding strength of two resin-based filled fissure sealants. **Material and Methods:** Freshly extracted, 48 third molar teeth were used in this study. Teeth were randomly divided into three main groups as the phosphoric acid etched, erbium: Yttrium–aluminum–garnet (ER: YAG) laser etched, and the phosphoric acid plus ER: YAG laser-etched groups. The main groups further divided into two subgroups as Clinpro or Fissurit FX applied. After preparation of the enamel surfaces and application of the sealants, the samples were subjected to shear bond strength test. **Results:** According to statistical analysis with one-way ANOVA, the bonding strength values of the phosphoric acid groups were found significantly higher than those values obtained from the ER: YAG laser and ER: YAG laser plus phosphoric acid groups (P < 0.001). **Conclusion:** As a result of the study, it has been concluded that the laser application alone has no additional benefit to the acid application in terms of bonding strength.

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KEYWORDS: Bonding strength, laser, pit and fissure sealant

Introduction

he anatomy of pits and fissures, which provide shelter for microorganisms, prevents adequate plaque control, and cause unsatisfactory hygiene on the occlusal surfaces. These make the posterior teeth vulnerable to carious lesions.^[1,2] Despite the advances in the preventive measures, caries is still the most common chronic childhood disease in pediatric dentistry.^[3,4] It was reported that about 90% of carious lesions originate from pits and fissures in the occlusal surfaces of the posterior teeth.[2] Fissure sealants are considered as a preventive strategy to decrease the occurrence and/or progression of caries originating from the occlusal surfaces.^[5,6] The success of the fissure sealant is strongly associated with biocompatibility, abrasion resistance, and as well as the retention of the sealant.[5] Marković et al.[7] noted that the success of fissure sealant is closely related with the bonding strength of the sealant to the enamel surface.

Acid etching is considered as the standard protocol in removing the smear layer to provide successful bonding.

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This protocol performed by the application of an etchant such as citric acid, phosphoric acid, or maleic acid to isolate the tooth surface. Roughened surface creates with acid etching increases the mechanical retention and bonding strength of the dental materials.^[8,9] However, the removal of superficial enamel, formation of various etching depths, and high sensitivity to water or saliva contamination and demineralization which cause the enamel to be susceptible to caries cause unsatisfactory bonding procedure.^[9] Since these drawbacks of the acid etching jeopardize the bonding quality, alternative enamel conditioning protocols which can create more satisfactory results is needed.^[10]

Lasers have been suggested as a successful option to acid etching in terms of the pretreatment of the enamel surfaces.^[11] Laser irradiation does not cause vibration

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or heat and is a painless procedure which suitable for routine clinical use. Furthermore, laser etching creates resistant enamel surfaces to acid attacks and provides an optimum surface for adhesion.^[9] Various types of lasers such as argon, carbon dioxide (CO₂), and neodymium: Yttrium-aluminum- garnet (Nd: YAG) have been used since the introduction of the ruby laser in 1960s. However, these lasers have thermal side effects such as the creation of fissures and cracks, increasing in pulp temperature, and carbonization. On the other hand, erbium: Yttrium-aluminum-garnet (Er: YAG) caused minimal adverse effects and reported to be more effective in removal of dental hard tissues. [8,9] Er: YAG laser beam is selectively absorbed by water and hydroxyapatite at 2940 nm. The absorption of laser energy by water and hydroxyapatite result with microexplosion, and ablation of the hard tissues.[3]

There seems no consensus about the efficacy of the Er: YAG laser etching in the literature. In terms of shear bond strength, many researchers favor the Er: YAG laser as an appropriate alternative for acid etching; [12-14] however, some other researchers have claimed the opposite. [8,15,16] The purpose of this study was therefore to evaluate the bond strength of two different resin-based fissure sealants to enamel surfaces which conditioned with Er: YAG laser and/or phosphoric acid *in vitro*.

MATERIAL AND METHODS

This *in vitro* study was carried out with the approval of Inonu University Local Ethics Committee (protocol number 2017/120). Written consent was obtained from the patients before tooth extraction by explaining the study procedure.

Preparation of samples and study groups

In this study, 48 lower third molar teeth which completed the root development, caries free, without other microscopic defects, and nonrestored were used. The teeth were cleaned and rinsed with fluoride-free pumice after extraction and stored in distilled water until the day of the experiment. The teeth were cut under the cementoenamel junction and half the teeth's buccolingual distance horizontally embedded into auto-curing acrylic resin. The buccal enamel surface was polished with 400 grit sandpaper underwater to obtain smooth and uniform enamel surface 3 mm in diameter. Teeth were randomly divided into three main groups as the phosphoric acid etched, ER: YAG laser etched, and the phosphoric acid and ER: YAG laser etched. The main groups further divided into two subgroups that applied either Clinpro or Fissurit Fx (Laser + Clinpro [LC]; Acid + Clinpro [AC]; Laser + Fissurit [LF]; Acid + Fissurit [AF]; Acid + LC [ALC]; and Acid + LF [ALF]), [Table 1].

Surface preparation

Er: YAG laser, 37% phosphoric acid, or Er: YAG laser plus phosphoric acid were applied during enamel conditioning. For chemical etching, 37% phosphoric acid gel (Panora 200, Imicryl, Turkey) applied to the surface of the enamel for 30 s, washed for 15 s, and dried them using an air spray for 10 s to get a chalky-white appearance. Er: YAG laser system (Fidelis Plus II, Fotona Medical Lasers, Ljubljana, Slovenia), with a power output of 1.2 W, pulse energy of 120 mJ, and frequency of 10 Hz was used during the laser-etching process at 2940 nm wavelengths, and the procedure was performed in the contact mode with water irrigation (50 ml/min) according to the instructions of the manufacturer.

Application of sealant

Cylindrical transparent gelatins with a diameter of 3 mm and height of 2 mm placed on conditioned enamel, to limit the sealant precisely during application. Then, fissure sealants were applied and polymerized with LED light source (Valo, Ultradent Products Inc., South Jordan, UT, USA) for 20 s, and then, the transparent gelatins were carefully removed.

Biomechanical testing

The prepared samples were kept in 37°C distilled water for 24 h. After that, for the application of shear bond strength test (1 mm/min), the specimens placed to test machine (TSTM 02500 Elista Ltd., Istanbul, Turkey) and chisel edge was placed on the fissure sealant parallel to the bonding surface [Figure 1]. After the test, the fracture that observed in the specimens was recorded in Newtons. Then, it was converted to MPa using the following formula: Megapascal (MPa) = Newton (N)/connection surface area (mm²).

Statistical analysis

The data were analyzed using the IBM SPSS Version 20.0. (Armonk, NY: IBM Corp., Chicago, USA) package program. All data were characterized using descriptive statistics (n, mean, standard deviation [SD], median, minimum, maximum, and ranges). Normal distribution of the data was examined by Shapiro–Wilk test. One-way ANOVA and Tamhane tests were used in the analysis of normally distributed data. Results were presented as mean \pm standard deviation (M \pm SD) and were assessed with 0.05 level of significance.

RESULTS

In this *in vitro* study, the effects of three different etching protocols on the bonding strength of two different fissure sealants were explored. Descriptive statistics of the groups were shown in Table 2. The statistical analysis showed significant differences (P < 0.05) among groups.

Table 1: Study groups							
Groups	Co	nditionin	g protocol	Sealant			
	Acid	Laser	Acid + laser	Clinpro	Fissurite FX		
AC	X			X			
AF	X				X		
LC		X		X			
LF		X			X		
ALC			X	X			
ALF			X		X		

AC=Acid+Clinpro; LC=Laser + Clinpro; AF=Acid + Fissurit; LF=Laser + Fissurit; ALC=Acid + laser + Clinpro; ALF=Acid + laser + Fissurit; X=Conditioning protocol

Table 2: Descriptive statistics of the groups							
Groups	n	Mean	SD	Minimum	Maximum	P	
Laser + Clinpro	8	7.1225	1.1628	5.72	8.6	0.000*	
Acid + Clinpro	8	23.4025	2.0758	20.95	26.49		
Laser + Fissurit	8	14.3275	2.0108	11.15	17.32		
Acid + Fissurit	8	37.345	5.4633	29.46	44.4		
Acid + laser +	8	14.5513	3.0158	9.79	17.29		
Clinpro							
Acid + laser + Fissurit	8	20.95	3.5415	15.4	24.52		

**P*<0.05. SD=Standard deviation; *n*=Number of samples; AC=Acid + Clinpro; LC=Laser + Clinpro; AF=Acid + Fissurit; LF=Laser + Fissurit; ALC=Acid + laser + clinpro; ALF=Acid + laser + Fissurit

Table 3: Multiple comparisons among the groups						
Groups	AC	AF	LC	LF	ALC	ALF
AC		0.001	0.000	0.000	0.000	0.849*
AF			0.000	0.000	0.000	0.000
LC				0.000	0.025	0.000
LF					1.00*	0.011
ALC						0.025

*Not significant *P*>0.05. AC=Acid + Clinpro; LC=Laser + Clinpro; AF=Acid + Fissurit; LF=Laser + Fissurit; ALC=Acid + laser + Clinpro; ALF=Acid + laser + Fissurit

According to statistical analysis, significant difference found between LC group and AC, AF, LF, AF, ALC, and ALF groups. Significant difference observed between AC group and LF, AF, ALC, and ALF groups, while no difference found between AC and ALF groups (P = 0.849). There was a significant difference between LF group and AF and ALF groups. However, a significant difference not found between LF and ALC groups (P = 1.00). Significant difference observed between AF group and ALC and ALF groups. Significant difference found between ALC and ALF groups. Multiple comparisons of the data [Table 3] revealed that the highest bond strength value was seen in AF group (37% phosphoric acid etching + Fissurit FX). The lowest mean bond strength was seen LC group (Er: YAG laser conditioning + Clinpro) [Figures 2 and 3].

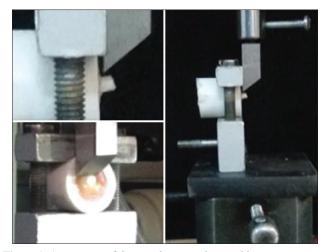


Figure 1: Appearance of the samples on testing machine

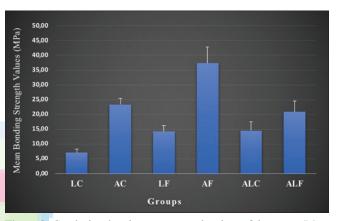


Figure 2: Graph showing the mean strength values of the group (Mpa: Megapascal)

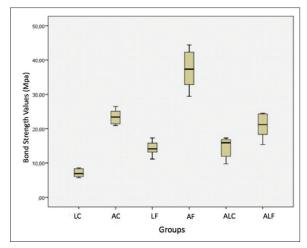


Figure 3: Box plot graphic showing the difference among the groups in terms of bond strength

DISCUSSION

The preventive capacity of fissure sealants is influenced by various factors. However, the chemical and mechanical interactions between the sealant and the conditioned enamel are the most critical factor among them.^[17] In a review conducted by Ahovuo-Saloranta *et al.*,^[18] it has denoted that the efficiency of the fissure sealants is strongly associated with sufficient retention of the sealant.

To increase the success of the retention several protocols have been used for the conditioning of enamel surface before sealant application.[16,17,19] Enamel etching with phosphoric acid is the conventional protocol that was originally introduced by Buonocore in 1955 to facilitate the retention. [4,8] Acid etching creates a uniform surface pattern suitable for adhesion by selective dissolution/removal of hydroxyapatite crystals and removing the smear layer. After acid etching, a surface which is easily wetted by the sealant occur, and this provides a favorable condition for the effective penetration of the sealant.[5,20] However, it is reported that remaining debris on the surface cannot be removed completely with the use of conventional acid etching.^[17] In addition to the need for detailed technique, rigorous isolation, and excessive time, the etching process with phosphoric acid also has other potential drawbacks such as the demineralization which creates more susceptible enamel surface to acid and carious lesions.[14,21]

In the past two decades, laser applications have been gained more popularity in the field of pediatric dentistry.[12,21] Among the lasers that have been introduced, the Er: YAG laser is one of the most commonly used laser for etching purposes. Unlike the acid etching, tissue removal by Er: YAG laser is not provided with demineralization. Laser irradiation causes vaporization of organic and water content of the teeth and triggers the microexplosive destruction of inorganic components such as hydroxyapatite crystals.[16] Microexplosions as a result of this ablation inside the material, create craters. In contrast to CO, and Nd: YAG lasers, Er: YAG laser create a strong bonding without causing any structural change in enamel orientation.[22] The Er: YAG laser also can perform with water cooling and cause minimal thermal side effects to the surrounding tissues. [12,14] After etching with Er: YAG laser, physicochemical changes occur in the enamel surface, and these changes decrease susceptibility of the enamel surface to carious lesions and acid. This change in enamel surface is considered to originate from the changes in calcium/phosphorus ratio, increase in the fluoride uptake, and as well as with the reduced water and organic contents of the enamel. It is also suggested that the remineralization microspaces that occur after laser etching have an antimicrobial effect by entrapping free ions. [3,16,21] On the other hand, the surfaces etching with laser are generally have lower surface energy because surface melting and resolidification that occur after the laser application. As a result of laser etching, water and organic content of the tooth evaporate. Thus, after laser etching, the tooth has less water content than it had before the application of laser. These limit sealant penetration into the enamel surface effectively and cause reduction on bond strengths. [20] Lepri *et al.* [5] stated that the Er: YAG laser irradiation block the interprismatic spaces and prevent the sealant diffusion into the enamel surface. Furthermore, as the Er: YAG cause intermittent emission of the laser beam, irregular microstructure with subsurface fissures which triggers bond failure, occur. [5,16,17]

There seems no consensus in terms of the efficacy of the Er: YAG laser etching in the literature. Keller and Hibst^[12] reported that the Er: YAG laser abrasion process yields a tensile strength corresponding to 92.5% of that obtained by the acid-etching process. Topcuoglu *et al.*^[13] compared the effect of Er: YAG laser and phosphoric acid etching on bond strength of orthodontic brackets, and the authors reported that laser etching was effective protocol to enhance the bond strength. According to Cozean et al.[23] Er: YAG laser modifying the enamel surface, improving the bonding forces, and promoting a better junction between the bonding agent and the enamel. Unal et al.[14] compared microtensile values of fissure sealant after various enamel-etching methods. They found that Er: YAG laser-etching may be an efficient option to acid-etching for enamel conditioning. Mirhashemi et al. [22] in their study compared the effect of Er: YAG and Er, Cr: YSGG lasers on bonding strength of the composite to orthodontic brackets. They reported that both Er, Cr: YSGG, and Er: YAG lasers can be used as an alternative to acid etching.

On the other hand, Borsatto et al.[15] found that conditioning enamel surface with Er: YAG laser did not provide an optimal penetration into the enamel surfaces. Shahabi et al.[16] who investigated in their study the tensile bond strength of the fissure sealants after using three different etching methods on permanent molar teeth, claimed that the use of Er: YAG laser for etching purpose did not efficient as acid etching. Lepri et al. [5] evaluated the influence of Er: YAG laser on salivary contaminated and dry enamel surface in terms of the shear bond strength of sealant. They reported that in both dry and wet conditions Er: YAG laser did not increase the bond strength of conventional acid etching. Attrill et al.[8] reported according to their result that the shear bond strengths were significantly lower in Er: YAG laser etching than those obtained using conventional acid etching. Martínez -Insua et al.[21] also stated that the enamel surfaces prepared by Er: YAG laser cause subsurface fissuring that is not suitable for optimal adhesion.

In accordance with the studies which suggested that effectiveness of the acid etching on bonding strength comparably higher than the laser etching, in this study, significantly higher bonding strength values were found in the acid-etched groups compared to the laser-etched groups. In this study, also higher bonding strength values in the acid plus laser-etched groups, when compared to the groups which the laser applied alone were observed. According to this result, it can be said that the conditioning of the enamel surfaces acid plus laser facilitate the retention of the sealant. This may explain the closer bond strength values among the AC and ALF groups in this study. Similar to the result of this study, Manhart et al.[24] and Lepri et al.[5] reported that application of Er: YAG laser together with the acid result with the increased retention of the sealants that is nearly equal to achieved with acid etching alone. Furthermore, Sasaki et al.[25] concluded that irradiation with Er: YAG and acid together resulted in a more homogeneous surface pattern, compared to the surfaces treated only with laser.

Fissure sealants with or without filler generally show similar penetration pattern to fissures and have similar binding forces. Some researchers claimed that fissure sealants with filler are advantageous because of less wear.[26] In a study evaluated the retention of filled and unfilled resin-based sealants, it was suggested that the filled sealants showed higher retention rates compared to the unfilled ones. [4] In this study, two types of resin-based sealants were tested. These are Fissurit FX which has high-filler content (55%) and Clinpro which has lower content (6%) of filler. In this study, significantly higher bonding values were found in Fissurit groups in all etching protocols when compared to the Clinpro groups. This may be the possible reason of the similar bonding values observed among the LF and ALC groups in this study.

To evaluate the bonding capacity of the fissure sealants to the enamel, tensile, and shear strength tests have been used frequently. Strength testing is an *in vitro* method used to investigate the adhesion potential of materials to the tooth surface. A shear bond strength test is useful tool for assessing the adhesion performance and predicting the long-term clinical success of the materials. In this study, shear bond strength test was used to investigate the bond strength of two different fissure sealants to the enamel surfaces that etched with three different protocols.

In terms of water cooling, it is reported that laser etching without a coolant, result with cracking which triggered by increased thermal stresses occur during the laser-etching process. Water act as a coolant during the laser-etching procedure and can prevent local thermal stresses. Histologically, the appearance of specimens prepared with the presence of a surface coolant was suitable than those prepared without coolant for adhesion. This suggested that the laser etching should be performed together with a water coolant to prevent iatrogenic thermal damage to tissues.^[8] Therefore, all irradiation is done with water coolant in this study to prevent the iatrogenic damages.

This study is an *in vitro* study. It is difficult to simulate the oral scenarios completely. Thus, this study has limitations in simulation of clinical conditions. Loads applied in the testing machine *in vitro* are different from loads in the oral environment. Thus, a generalization of results in terms of the clinical application must be done with caution. In addition, thermal stresses, moisture, acidity, and plaque could not simulate because of the *in vitro* design.

The difference of the results among the studies in the literature, possibly, originating from the different experimental designs, different parameters, and different evaluation methods used in these studies. Further clinical and laboratory studies with different laser parameters required to determine which protocol and dental material must be preferred to improve the bonding potential to laser-etched surface.

Conclusion

According to the results obtained from this *in vitro* study, it can be concluded that laser did not improve the sealant retention when used alone. Thus, laser etching under the conditions described in this study cannot be recommended as a viable option to the conventional acid-etch protocol.

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Conflicts of interest

There are no conflicts of interest.

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