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EFFECT OF LASER TOOTH SURFACE PRETREATMENT ON SOME MECHANICAL PROPERTIES OF PIT AND FISSURE SEALANT (AN IN VITRO STUDY)

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ABSTRACT

Objectives: This in vitro study was directed to evaluate the effect of laser surface pretreatment alone or in combination with acid etching on resin penetration, microleakage, and micro-tensile bond strength of resin-based pit-and-fissure sealant. **Subjects and Methods:** A total of 60 permanent mandibular molars were divided into four equal main groups (n= 15) according to the enamel surface pretreatment protocol. Group I; occlusal pit-and-fissure pretreated with acid etch alone (control group), Group II; occlusal pit-and-fissure pretreated with laser alone, Group III: occlusal pit-and-fissure pretreated with acid etch followed by laser; and Group VI: occlusal pit-and-fissure pretreated with laser followed by acid etch. Assessment includes, resin penetration, microleakage, and micro-tensile bond strength of resin-based pit-and-fissure sealant. **Results:** The results of the present study revealed that the use of acid etching pretreatment alone of the occlusal pit and fissures resulted in significantly better resin penetration, microleakage resistance and better micro-tensile bond strength when compared to laser pretreatment alone. Also, the results of the present study showed that the samples which pretreated with laser in combination with acid etching resulted in insignificant better resin penetration, microleakage resistance and better micro-tensile bond strength when compared to acid etch pretreatment alone. **Conclusion:** laser etching alone is not an alternative therapy to conventional acid etching. But the laser and acid etching combination can be a good choice and is comparable to bur invasion.

KEYWORDS: Pit-and-fissure sealant, resin penetration, microleakage, micro-tensile bond strength.

INTRODUCTION

Dental caries is a multifactorial disease that develops in both primary and permanent dentitions due to changes in the bacterial biofilm's composition, which create an imbalance between the processes of demineralization and remineralization. About 90% of caries in permanent posterior teeth and 44% in children's and teenagers' primary teeth are caused by pit and fissure caries [(1)].

Pits and fissures are more prone to caries than smooth surfaces because of their plaque-retentive nature, which makes them difficult to clean. They may also not be protected by fluoride treatment [(2)].

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Pit and fissure sealants are a preventive conservative approach involving the application of sealants into the pits and fissures of caries prone teeth; this sealant bonds to the tooth micromechanically, providing a physical barrier that keeps bacteria away from their source of nutrients [3].

The clinical success of a fissure sealant depends on effective bonding to the enamel area around the entrance of the fissure, as well as on complete penetration and sufficient adhesion to fissure walls. acid conditioning of enamel before the application of a resin-based fissure sealant increases its sealing abilities, penetration, and retention[4]. Enamel pretreatment with various concentrations of phosphoric acid has been the standard method for creating microporosities that serve for the retention of the sealant material. However, Acid conditioning is a well-accepted and standard method for pretreatment of enamel surfaces for adhesion of restorative materials. The standard conditioning techniques, however, might not be able to remove any leftover debris and pellicle from the base of cracks in the case of sealant installation [4].

Moreover, acid conditioning may cause the demineralization of enamel structures and make the enamel surface more vulnerable to caries formation. As a result, alternatives to acid conditioning for fissure preparation, such as air abrasion and laser, have been suggested. Laser beam application is used on hard dental tissues for various procedures, including enamel conditioning [5].

However, there are controversial results about the efficacy of laser beam alone or in combination with acid conditioning in enamel surface pretreatment. Therefore, the aim of this study was to test the effect of laser surface preparation for tooth pits and fissures on the resin penetration, microleakage, and micro-tensile bond strength of resin-based pit and fissure sealant.

SUBJECTS AND METHODS

Study design: An experimental in vitro study.

Study setting: The study was carried out in Laser Research Center, Faculty of Dentistry Misr University for Science and Technology. Measurement of sealant marginal leakage and shear strength were carried out at National Research Center. Measurement resin penetration with SEM (scanning electron microscope) carried out at Egyptian atomic energy authority .

Inclusion criteria:

1. No restoration or prior sealant present on teeth.
2. Sound tooth structure.
3. Deep pit and fissure.
4. Absence of cavitated carious lesions.

Exclusion criteria:

1. Teeth with visible caries lesion.
2. Teeth with any signs of anatomical abnormalities.
3. Teeth with any signs of surface pigment.
4. Teeth with fracture or attrition.

Sample size:

The sample size was calculated using a freeware program (G*Power 3.1.9.3 for Mac OS X) from the results of a previously published study AlHumaid et al. [2]. The effect size (dz=0.630) and the required sample size were calculated for a=0.05 and a power of 0.95 (1−b error probability), assuming a normal distribution. For this study, a sample size of 15 was obtained per group.

Ethical Consideration:

This work was approved by the Ethical Committee of the Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo), with the permission number EC Ref. No. (545/3116)

Intervention:

Pretreatment protocol:

The collected teeth were cleaned with a prophylaxis brush in a low-speed micromotor handpiece and a dental explorer was used to clean debris from the pits and fissures so that deposits and soft tissue residues were removed [6] . Then they were examined to presence of caries according to clinical parameters using a sharp explorer and visual inspection.

The extracted teeth were disinfected with 0.5% chloramine T solution for 1 week [2] . Chloramine T solution has been recommended because it doesn't show adverse effect on organic phase of dentin.

Prior to the experiment, the chosen teeth were rehydrated in distilled water at 4°C in a single container for no more than two weeks [1].

Sample preparation:

Each tooth's pit and fissure were thoroughly cleaned with a dry brush prior to applying the sealant. The occlusal surfaces of the teeth were then gently dried with oil-free compressed air for 10 seconds after being washed with water for 15 seconds. Before testing, the teeth roots were cut off 2-mm below the cementoenamel junction (CEJ) and the root apices were sealed with sticky wax .Each tooth was mounted in self-curing acrylic resin block (Acrostone). [1, 2]

Grouping of Sample:

A total of 60 permanent mandibular molars (n=15 for each group) were used during this study **Group I:** Pretreatment using orthophosphoric acid gel for 30 seconds (Bisco Etchant, Schaumburg ,USA) then pit and fissure sealant(ClinproTM Sealant, 3M ESPE, USA)

Group II: Pretreatment using laser (Er Cr YSGG) at 250 mJ and a frequency of 4 Hz under air-water cooling. The laser beam of Er Cr YSGG was delivered in noncontact mode, with the hand piece positioned 1 mm above and perpendicular to the fissures before restoration with pit and fissure sealant.

Group III: Pretreatment using acid etchant gel for 30 seconds After that, the occlusal surface treated with laser

Group IV: Pretreatment using laser. Then the enamel treated with acid etchant gel for 30 seconds.

Then, each main group was subdivided into three equal subgroups (n=5) according to the type of test (resin penetration, marginal adaptation, and retention).

Testing procedures:

1. Evaluation of resin penetration:

5 sample from each group were used for this test. The restored tooth sample in each group was sectioned in three parallel planes (mesial, central, and distal) in a buccolingual direction using a water-cooled low-speed diamond saw . Scanner electron microscopy (SEM) was used to measure the level of resin penetration in each group's sectioned samples. The penetration ability was assessed as the proportion of area of the fissure which is unfilled by the sealant relative to the whole fissure area [7].

2. Evaluation of microleakage:

Five sample from each group were used for this test. The microleakage of the teeth samples was assessed with the dye penetration technique. After that, the teeth were thermocycled for 2,500 alternating thermal cycles (5°C/55°C, dwell time 30 seconds) in a thermocycling apparatus. Teeth were dried and sectioned with a water-cooled diamond saw in a buccolingual direction through the sealant resulting in three sections for each specimen. A digital photograph of each section was obtained at 20X (1280 x 1024 resolution) under a stereomicroscope (Olympus; Tokyo, Japan) The images were transferred to an IBM-compatible PC. The microleakage value for each section was calculated by dividing the sum of buccal and lingual dye penetration values by the sum of the lengths of buccal and lingual enamel-sealant interfaces.

3. Evaluation of micro-tensile bond strength:

5 sample from each group were used for this test .The tooth sample in each group was sectioned in three parallel planes (mesial, central, and dista l) in a buccolingual direction using a water-cooled low-speed diamond saw . Lloyd universal testing machine was used to assess the micro-tensile bond strength of the teeth samples. they were exposed to a tension load with a cross-head speed at 1 mm/min until the sample failure ensued The load at failure (F) in newton (N) and the cross-sectional area (A) in mm2 at the fracture were recorded to calculate the bond strength P [MPa]) as follows: P (MPa) = F (N)/A (mm2).

Data management and analysis:

Preliminary analysis of the data showed that not all data for the experimental groups conformed to a normal distribution (Kolmogorov-Smirnov test). The collected data during the study were tabulated and statistically analyzed using the One-way ANOVA test using SPSS version 22. The ANOVA level of significant was at p-value < 0. Comparison among the groups was done using Post-Hock's test.

RESULTS

1. Resin Penetration:

There was *statistically significant* difference in the resin penetration between all tested groups as indicated by One-way ANOVA test (p=0.00002). The results showed that the pretreatment of enamel of pit and fissures with laser alone (group II) resulted in significant decrease in the resin penetration with larger unfilled resin area when compared to acid etch alone (group I) (control group). However, the pretreatment of enamel of pit and fissures with laser in combination with acid etch (group III and IV) resulted in insignificant increase in the resin penetration with smaller unfilled areas when compared to acid etch alone (group I) (control group).

TABLE (1) Comparison of resin penetration (unfilled area) among all groups.

Variable	Mean ± SD	*f*-ratio	p-value
Acid etch	$0.0146{\pm}0.0005^{A}$	18.311	0.00002*
Laser	$0.0172{\pm}0.0113^{B}$		
AE/Laser	$0.0138{\pm}0.0008^{A}$		
Laser/AE	$0.0136{\pm}0.0005^{A}$		

**; The results statistically at p<0.05.*

; different capital litters in the same column indicted statistically significant.

; ns= non-significant.

2. Microleakage:

The statistical analysis of microleakage of all tested groups revealed that; there was *statistically significant* difference in the proportion of microleakage between all tested groups as indicated by One-way ANOVA test (p=0.000056). The results showed that the pretreatment of enamel of pit and fissures with laser alone (group II) resulted in significant increase in the proportion of microleakage when compared to acid etch alone (group I) (control group). However, the pretreatment of enamel of pit and fissures with laser in combination with acid etch (group III and IV) resulted in insignificant decrease in the proportion of microleakage when compared to acid etch alone (group I) (control group).

TABLE (2) Comparison of proportion of microleakage among all groups.

Variable	Mean ± SD	*f*-ratio	p-value
Acid etch	0.118±0.008^{A}	15.431	0.000056*
Laser	0.144±0.011^{B}		
AE/Laser	0.112±0.008^{A}		
Laser/AE	0.108±0.008^{A}		

**; The results statistically at p<0.05.*

; different capital litters in the same column indicted statistically significant.

; ns= non-significant.

3. Micro-tensile Bond strength:

The statistical analysis of micro-tensile bond strength of all tested groups revealed that; there was *statistically significant* difference in the micro-tensile bond strength between all tested groups as indicated by One-way ANOVA test (p=0.000074). The results showed that the pretreatment of enamel of pit and fissures with laser alone (group II) resulted in significant decrease in the micro-tensile bond strength when compared to acid etch alone (group I) (control group). However, the pretreatment of enamel of pit and fissures with laser in combination with acid etch (group III and IV) resulted in insignificant increase in the micro-tensile bond strength when compared to acid etch alone (group I) (control group).

TABLE (3) Comparison of micro-tensile bond strength among all groups.

Variable	Mean ± SD	*f*-ratio	p-value
Acid etch	14.8± 0.158^{A}	14.690	0.000074*
Laser	13.6± 0.291^{B}		
AE/Laser	15.08± 0.712^{A}		
Laser/AE	15.13±0.286^{A}		

**; The results statistically at p<0.05.*

; different capital litters in the same column indicted statistically significant.

; ns= non-significant.

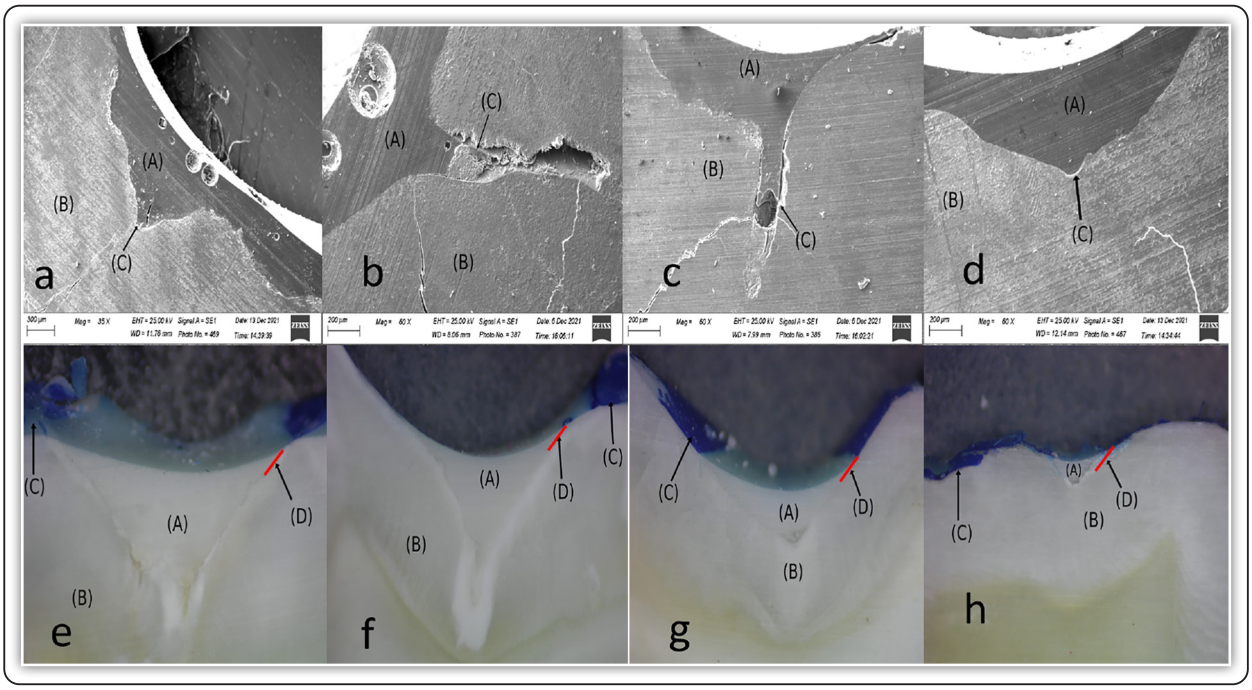

FIG (1) **Resin Penetration**, a, Acid etching, b, Laser, c, Acid-etch followed by laser and d, Laser followed by acid etching. A): Fissure sealant (B): Enamel (C): area of unfilled of tooth with sealant. **Microleakage**, e, Acid etching, f, Laser, g, Acid-etch followed by laser and h, Laser followed by acid etching. (A): fissure sealant, (B): Enamel (C): methylene blue dye, (D): area of leakage, (E): leakaged area

DISCUSSION

The use of laser in dentistry has been considered for over 30 years. In recent years, there has been a growing debate on the use of lasers for conditioning enamel and dentin as a possible alternative to acid etching. Er,Cr:YSGG, which has a high absorption coefficient in water (6,500/cm) and enamel (400/cm), leading researchers to explore its use in enamel conditioning [7].

Pit and fissure sealants involve the application of sealants into the pits and fissures of teeth that are prone to caries. This sealant attaches to the tooth micromechanically and creates a physical barrier that prevents bacteria from growing near their source of sustenance[3]. An efficient binding to the enamel at the fissure's entry, as well as thorough penetration and enough adhesion to the fissure walls, are necessary for a fissure sealer to be successful in clinical settings. A resin-based fissure sealer has better sealing capabilities, penetration, and retention when enamel is acid-conditioned before being applied [4].

Additionally, acid conditioning may demineralize enamel structures and increase the susceptibility of the enamel surface to caries development. Laser have so been proposed as alternatives to acid conditioning for the preparation of fissures. A laser beam is applied to hard dental tissues for a variety of operations, such as conditioning the enamel[5]. This study was conducted to evaluate the effect of laser surface preparation for tooth pits and fissures on the resin penetration, microleakage, and micro-tensile bond strength of resin-based pit and fissure sealant.

Regarding penetration, the results showed that the pretreatment of pit and fissures with laser alone (group II) showed a significant decrease in the resin penetration when compared to acid etch alone (group I). In accordance, Abou El-Yazeed et al, [8] stated that, Traditional pit and fissure sealing maneuver using acid etching technique showed significantly higher penetration scores than the other two groups which advocates the application of laser to enhance the sealing quality.

In agreement with our results Abou El-Yazeed et al.[8], the pretreatment of pit and fissures with laser in combination with acid etch resulted in non-significant increase in the resin penetration with smaller unfilled areas when compared to acid etch alone (group I). The non significant differences between Laser etching groups and Laser etching followed by acid etching show that either laser pretreatment to enamel surface or application of laser firstly followed by acid etching appear to have the same efficiency and are superior to using acid etching only in sealing of pits and fissures. This is in accordance with Zervou et al,[9] and Mohamed[10]; however better results were demonstrated by using laser plus etching which might be due to the prismatic nature of the enamel in permanent teeth rather than that of the deciduous.

Unlike our findings, Khogli[7] discovered no appreciable variations in sealant penetration between laser and traditional acid etching approaches. According to Memarpour et al.[11] conventional acid etching produced an occlusal seal that was comparable to that produced by teeth that had been prepared with Er, Cr:YSGG laser. This is due to the fact that the traditional procedure for producing microporosities that allow the sealant material to penetrate enamel has been to pretreat it with phosphoric acid. Additionally, phosphoric acid pretreatment increases the wettability of enamel [12]. Moreover, this may be because of the uniform etching pattern that was usually observed at a greater depth on the prismless section of the fissure wall when use acid etching [13]. However, laser etching does not create an even, uniform etching pattern; instead, laser ablation yields a random fragmentation and removal of dental substances with a real cleavage of the enamel prism pathway [15].

The pulsed nature of Er:YAG laser beam emission and small malpositions of the tip placement and angle may be factors contributing to irregular etching patterns [12]. Therefore, the results from the present investigation imply that treatment with the appropriate acid-etching agent significantly improves the ability of the material to penetrate deep into the fissure when compared with laser pretreatment alone.

Regarding Microleakage, Microleakage is a laboratory phenomenon and may not precisely reflect the clinical situation [11]. However, the in vitro microleakage tests are useful methods to evaluate the sealing performance of adhesive systems. The success of preventive procedure such as sealant application may be undermined if the applied material cannot resist microleakage [11].

The results showed that the pretreatment of pit and fissures with laser alone (group II) showed a significant decrease in the resin penetration when compared to acid etch alone (group I).

In agreement with our results, in accordance to Abou El-Yazeed et al.[8] stated that, Traditional pit and fissure sealing maneuver using acid etching technique showed significantly higher penetration scores than the other two groups which advocates the application of laser to enhance the sealing quality. However, in the present study, the pretreatment of pit and fissures with laser in combination with acid etch resulted in non-significant increase in the resin penetration with smaller unfilled areas when compared to acid etch alone (group I). The non significant differences between Laser etching groups and Laser etching followed by acid etching show that either laser pretreatment to enamel surface or application of laser firstly followed by acid etching appear to have the same efficiency and are superior to using acid etching only in sealing of pits and fissures. This is in accordance with Zervou et al.[9] and Mohamed et al.[10].

The results showed that the pretreatment of pit and fissures with laser alone (group II) resulted in significant increase in the proportion of microleakage when compared to acid etch alone (group I). However, the pretreatment of pit and fissures with laser in combination with acid etch (group III and IV) resulted decrease in the proportion of microleakage when compared to acid etch alone (group I) (control group). In agreement with the results of the present study Baygin et al.[13], and Khogli et al.[7], showed that laser pretreatment followed by etching resulted in less microleakage than acid etching pretreatment prior to sealant application. These findings could be explained by the fact that laser ablation, which creates imperfections on the enamel surface and therefore increases its roughness and surface area more than acid etching alone, promotes greater sealant adaptation to the enamel walls. Additionally, laser therapy may reach the deepest portions of fissures and remove material without creating a smear layer [14].

On the other hand, Kumar et al.[15], examined the retention rates of sealants applied either with traditional acid etching or with Er, Cr:YSGG laser preparation of the enamel surface. Regarding retention and patient acceptance, Er,Cr:YSGG laser etching is equivalent to acid etching. Similarly, Sungurtekin and Oztas [16] reported that the micro-leakage values in their laser plus acid etching alone group were similar to those of their acid-etching group.

Regarding Micro-tensile bond strength, the results showed that the pretreatment of pit and fissures with laser alone resulted in significant decrease in the micro-tensile bond strength when compared to acid etch alone (group I) (control group). However, the pretreatment of pit and fissures with laser in combination with acid etch resulted in insignificant increase in the micro-tensile bond strength when compared to acid etch alone. This study showed that the micro-tensile bond strength produced between the enamel and sealant is significantly higher with acid etching when compared to Er, Cr:YSGG laser pretreatment alone.

In line with our results, Drummond et al.[17], and Shahabi et al.[18] found that acid etching significantly increases the bond strengths compared with laser treatment.

However, in contrast, studies have also demonstrated that laser treatment produces bond strengths that were comparable or higher than those produced by acid etching[2,11]. This may be because the lower power (2.5 W) produces inferior micro-tensile bond strength[2]. Also, this could be attributed to formation of deeper microprosities with uniform etching pattern of acid when compared to laser alone [20].

CONCLUSION

The results of the current study showed etching enamel prior to sealant application with conventional acid etching remains the most effective and simplest technique in sealants success. Laser alone was inadequate for etching enamel prior to sealant application and conventional acid etching remains the most effective and simplest technique in sealants success.

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