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EFFECT OF LASER ETCHING VERSUS SELF-ETCHING ADHESIVE AND CONVENTIONAL ACID-ETCHING TECHNIQUES ON SHEAR BOND STRENGTH, MICROLEAKAGE AND PENETRATION DEPTH OF A RESIN BASED PIT AND FISSURE SEALANT (AN IN VITRO STUDY)

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ABSTRACT

Objectives: The aim of the present study was to compare the effect of enamel pre-treatment with: 37% phosphoric acid-etching or self-etching adhesive and Er:YAG Laser (2940nm) on shear bond strength, microleakage and penetration depth of a fluoride-releasing resin-based pit and fissure sealant applied to sound premolars.

Methods: Seventy-two sound premolars were allocated randomly into 3 groups according to enamel pretreatment protocol. The experimental groups included: Group I: 37% phosphoric acid-etching, Group II: self-etching adhesive, Group III: Er:YAG Laser treatment. Each group was divided into two subgroups (A: shear bond strength, B: microleakage and penetration depth). Shear bond strength test was performed using Universal testing machine. Microleakage and penetration depth were tested after thermocycling (500×, 5-55°C, dwell time: 30s) and dye penetration method. Samples were assessed under Stereo-microscope with a magnification of ×40. One-way ANOVA followed by Tukey's post hoc test was used to analyze data.

Results: Shear bond strength and microleakage results showed that there was statistically significant difference among different groups while penetration depth results showed there was no statistically significant difference among all groups.

Conclusions: Conventional acid etching was the best pretreatment method prior to resin-based fissure sealants in terms of bond strength and microleakage.

KEYWORDS: Laser etching, self-etching adhesive, pit and fissure sealant, universal adhesive, shear bond strength, microleakage, penetration depth.

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INTRODUCTION

Modern dentistry has recently focused on caries-risk reduction, procedures of prevention and minimally invasive techniques, employing approaches such as: public water fluoridation, topical and systemic fluoride application, plaque and sugar control, as well as fissure sealants [(1)].

Fissure sealants have been widely used for pit and fissure caries prevention over the past three decades [(2)]. Their success rate depends on how well they can work as a physical barrier separating occlusal fissures from the oral environment [(3)].

Several surface treatment techniques, mainly mechanical preparation and acid-etching, have previously been used in enamel treatment prior to the application of sealant material. As a result of there are some drawbacks to acid-etching such as superficial enamel removal, various etching depths formation and high water or saliva contamination sensitivity which may result in unsatisfactory bonding, alternative enamel pretreatment methods have been proposed [(4)].

Laser treatment has been advocated as an enamel roughening method. It has the advantage of cleaning, conditioning, and decontaminating in a single step even in inaccessible fissures. Additionally, it is a painless technique with no vibration or heat [(4,5)]. Some studies claimed that laser treatment may result in better resin based pit and fissure sealants bonding and retention [(6,7)].

Self-etching adhesives have been introduced in modern dentistry because of their advantages such as simplifying the bonding process by elimination of washing step and reducing procedure time. This advantage renders them a good alternative to the conventional acid etching system, especially for children [(8)].

Therefore, it is worthwhile to evaluate the outcome of applying pit and fissure sealant after treatment by laser or self-etching adhesive compared to the conventional acid-etching technique.

MATERIALS AND METHODS

Materials used in the present study are listed in table 1.

TABLE (1) Materials used in the study

Brand Name	Manufacturers	Nature	Composition
Eco-Etch etching gel (Figure 7)	Te-Econom plus, Ivoclar, vivadent, Liechtenstein	Acid-etching gel	37% phosphoric acid
Single Bond Universal Adhesive, (Figure 8)	3M ESPE, USA	Self-etching adhesive (Universal adhesive)	10 MDP[1], HEMA[2], dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane
Fissurit F (Figure 9)	Voco, Gmbh, Germany	Opaqe white color fourth-generation fluoridated resin-based pit and fissure sealant (FRBS)	**Matrix:** Bis-GMA[3](25–50%), UDMA[4] (10–25%), 1,6-hexanediylbismethacrylate (10–25%) Fumed silica (5–10%) **Filler:** 9.5% wt. silicon dioxide **Fluoride**: Benzotriazole derivatives sodium fluoride (2.5–5%)
Er: YAG Laser (Figure 10)	Fotona, twinlight, Fotona, Ljubljana, Slovenia	Laser device	Wavelength: 2940 nm

b. Methods:

Study setting:

An in-vitro study was performed in Pediatric Dentistry & Dental Puplic Health Department, Faculty of Dentistry, Ain Shams University, Egypt.

The study protocol received an ethical approval from Faculty of Dentistry Ain Shams University with reference number (FDASU_ RECEM121909) on December 2019.

Sample size calculation

a) Shear Bond Strength:

A power analysis was devised to have enough power to conduct a 2-sided statistical test of the study hypothesis (null hypothesis) that there is no difference in shear bond strength among the different tested enamel preparation methods. According to the findings of Moslemi et al., (2010) [9], the impact size (f) was (0.542). Using an alpha (α) level of 5% (0.05), and a beta (β) level of 20% (0.20), i.e. power=80%, the expected sample size (n) was 36 specimens, with 12 specimens per group. G*Power version 3.1.9.4 was used to calculate sample size.[10]

b) Microleakage and penetration depth:

A power analysis was devised to have enough power to conduct a 2-sided statistical test of the study hypothesis (null hypothesis) that there is no difference in microleakage across the various evaluated enamel preparation methods. According to Mehran et al., (2014) [11], the impact size (f) was found to be (0.542). Using an alpha (α) level of 5% (0.05), and a beta (β) level of 20% (0.20), i.e. power=80%, the expected sample size (n) was 36 premolars, with 12 premolars per group. G*Power version 3.1.9.4 was used to calculate sample size.[10]

Teeth Selection:

Seventy-two freshly-extracted premolars for orthodontic purposes in children aging from 13 to 15, were selected basing on the following criteria:

***Inclusion criteria:** [8]

1. Premolars with deep pits and fissures.
2. Sound premolars free from any decay.
3. Premolars free from cracks and developmental defects.

***Exclusion criteria:** [8]

1. Previously restored premolars.
2. Premolars with pit and fissure sealant.
3. Premolars with macroscopic fractures or attrition

The premolars, were cleaned, scaled with ultra-sonic scaler and fluoride-free pumice was used for polishing. Premolars were then examined by previously trained technician under a stereo-microscope with 40X magnification (BX60, Olympus, Japan) to detect any cracks, pittings, or enamel lesions.

* All teeth were kept in a container of distilled water in the refrigerator for no more than a month.

Sample grouping

- Seventy-two premolars were serially numbered and allocated randomly into 3 groups according to the type of enamel treatment as follows: (Figure 1)

* Group I: Conventional acid-etching (n = 24 premolars)

* Group II: Self-Etching adhesive (n = 24 premolars)

* Group III: Laser Treatment (n = 24 premolars)

- Based on the type of assessment, samples from each group were sub-divided into two subgroups as follows:
- Subgroups IA, IIA, IIIA: Shear Bond Strength (n = 12 premolars).
- Subgroups IB, IIB, IIIB: Microleakage & Penetration Depth (n = 12 premolars).

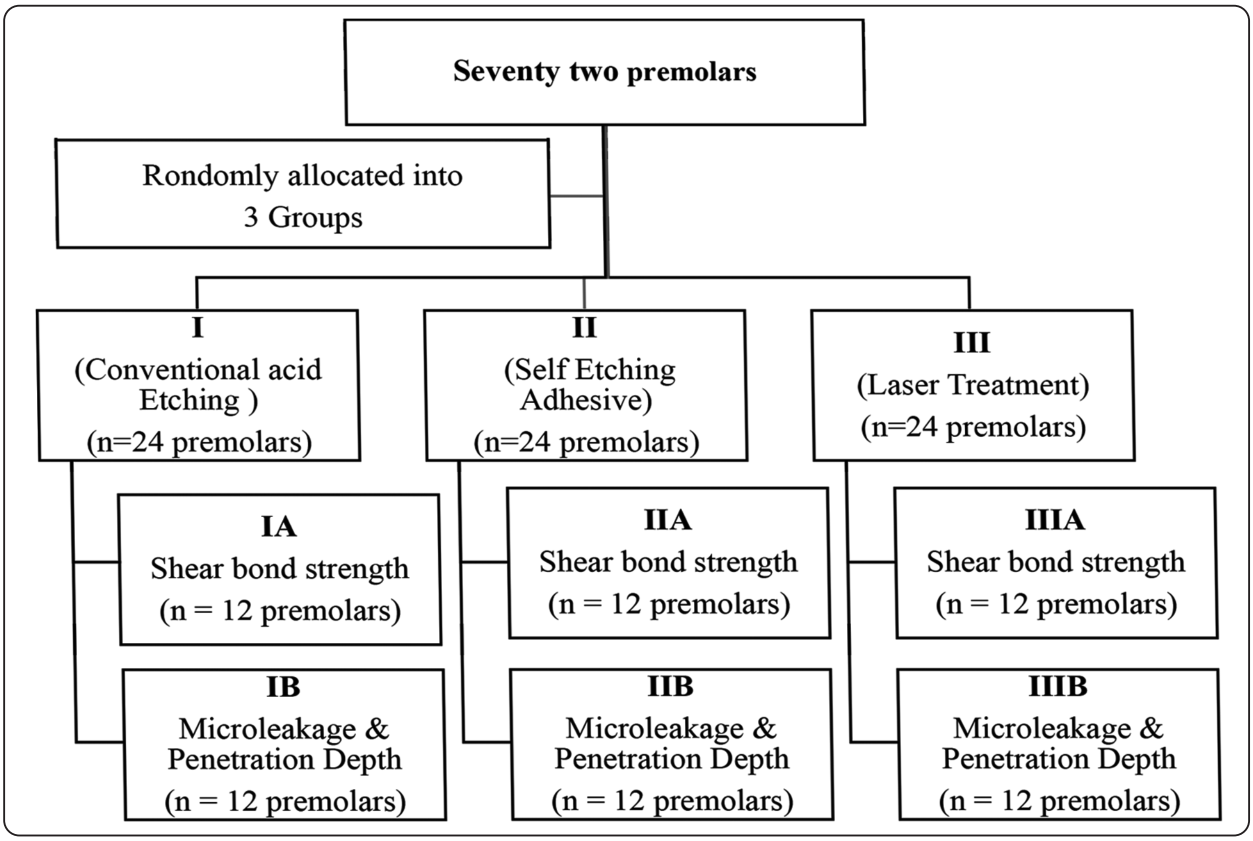

Fig. (1) Study groups & subgroups

1- Shear Bond Strength Testing:

a. Specimens Preparation:

- All teeth's roots were cut 2 mm below the cementoenamel junction by previously trained technician. The crowns were then longitudinally bisected in mesiodistal direction using a water-cooled diamond disc in low-speed handpiece. As a result, 36 buccal tooth halves (12 buccal tooth halves for each subgroup) [12].
- Samples were horizontally-embedded into self-curing acrylic resin (Acroston, Acroston, Egypt) surrounded by polyvinyl chloride rings (2 cm in diameter, 1 cm high) [13]
- The rings were removed after polymerization of the acrylic resin.[13]
- Under running water, the enamel surface was polished with 120- and 400-grade silicon carbide sandpapers to obtain a smooth, flattened and uniform enamel surface of at least 3 mm in diameter for bonding of the sealant material.[14]
- Polishing was performed by a study independent blinded operator.
- Teeth were checked with a probe to make sure that no dentin was exposed.

b. Surface Treatment:

In Group IA (Conventional acid-etching): 37% phosphoric acid gel was applied for 15 seconds to the enamel surface, rinsed for 15 seconds using air-water spray, and dried for 10 seconds to achieve a chalky-white appearance. [12]

In Group IIA (Self-Etching Adhesive): Single drop of self-etching adhesive was dispensed into dispensed dish. A single adhesive layer was applied for 15 seconds of active application using a bond brush. The bond layer was air dried gently using air-flow spray and cured for 20 seconds according to the manufacturer's instructions. [11]

In Group IIIA (Laser Treatment): A 2.94-µm Er: YAG laser was used to perform laser irradiation. (Fotona Twinlight, Fotona Medical Lasers, Ljubljana, Slovenia). The device was set to the following parameters:

- 1.6 W of power output.
- 400 mJ of pulse energy.
- 4-Hz repetition rate.
- Pulse width 50µs with 40% air and 40%water.
- The delivery of laser beam was in a non-contact and pulsated mode of super short pulse and perpendicular to the specimen surface through tipless hand piece at a constant working distance of approximately 12 mm and with spot size of 0.9mm for 15 seconds.[15]

c) Application of the sealant material:

- A cylindrical transparent gelatin tube 3 mm in diameter and 4 mm in height was placed on the treated enamel to ensure standardization of the sealant size and shape during sealant application. [15]
- Fissurit F (Voco, Gmbh, Germany) fissure sealant was applied into the tube in excess through the syringe tip. A gentle pressure was applied with a microscopic glass slab to eliminate any air bubbles. Therefore, sealant light cured using (RTA Light Emmiting Diode Device, Woodpecker, China) light curing unit with light intensity 1000-1200 mw/cm^2 for 20 seconds. Curing was applied on the top and sides of the sealant specimen.
- The glass slab and any excess sealant were removed after curing and transparent gelatin tube was then carefully sectioned by a lancet tip and removed providing a cylinder of sealant material applied on the tooth surface. After that, all specimens were examined to ensure adequate configuration.[16]

d) Measuring Shear Bond Strength:

- Before testing, the prepared samples were stored in 37°C distilled water for 24 hours.[17]
- Each specimen was placed into a Universal Testing Machine (LLOYD Instruments, Ametek, UK) and the edge of chisel was applied on the sealant loaded to the adhesion interface running at a 1 mm/min crosshead speed till the fracture occurred. The force necessary to fracture the specimen was measured in Newtons and was transformed into MPa using the following equation: Megapascal (MPa) = Newton (N) / surface area of the connection (mm2).[18]

2- Microleakage and Penetration Depth Testing:

a) Specimens Preparation:

A total of 36 premolars were selected, cleaned and stored as previously described. According to enamel treatment, all premolars were allocated randomly into three groups (12 premolars each).

b) Enamel treatment:

Group IB (Conventional Acid Etching): 37% phosphoric acid gel was applied for 15 seconds, then rinsed for 15 seconds and dried for 10 seconds, resulting in a chalky white appearance. [19]

Group IIB (Self-Etching Adhesive): one drop of self-etching adhesive was applied on the tooth surface using bond brush for 15 seconds as previously described. The adhesive was then dried and cured for 20 seconds at room temperature, as recommended by the manufacturer.[20]

Group IIIB (Laser Treatment): An Er: YAG laser was used to irradiate the occlusal surface with the same parameters described before for 15 seconds.[19]

c) Application of sealant material:

- In Groups IB, IIB and IIIB, One drop of sealant material was dispensed in the center of the fissures using syringe tip and spreaded on all fissure system using a bond brush with a brushing motion.
- Any excess of sealant was removed by a dry bond brush.
- The presence of any voids was checked using an explorer.
- Finally, Fissurit F was cured for 20 seconds.
- After curing, an explorer was used to check the complete coverage and retention of the sealant material. [8]

d) Thermocycling:

- Before thermocycling process, the specimens were kept in a container of distilled water at 37°C for 24 hours. [8]
- All teeth were thermocycled for 500 cycles in two water baths held at 5-55°C for 30 seconds each (dwell time) and 10 seconds (transfer time).[21]

e) Microleakage and Penetration Depth assessment:

- After thermocycling, The specimens' apices were sealed with sticky wax (Hiflex, Prevest Direct, India).[22]
- Each sample's surface was covered by a double layer of nail polish applied 1mm away of tooth-sealant conjunction. [23]
- All of the samples were immersed in 2% methylene blue dye solution (SD Fine-Chem limited, Mumbai, India) for 24 hours at room temperature. [24]
- After that, the teeth were rinsed under running water to remove any excess of the solution.
- All specimens were buccolingually bisected at the middle of occlusal surface into two fragments using a low-speed diamond saw (Top Dent, Edenta Golden, Swiss) to obtain two sections from each tooth for microleakage evaluation. [24]
- Each cross section was observed under Stereo-microscope with 40X magnification (BX60, Olympus, Japan) and the image was taken by digital camera (EOS 650D, Canon, Japan). [25]
- Both cross sections were examined by previously trained operator and the section with the worst score was selected to be included in the analysis. [24,25]

After that, Images were transferred to the computer system for analysis. This was performed in the Precision Measurement Unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. The measurement of dye penetration and sealant penetration within the fissure in relation to the depth of the whole fissure was carried out using Image J, 1.41a, (NIH, USA) image analysis software.

Outcome Assessment:

-Microleakage[12] and the depth of sealant penetration [23] were evaluated according to the following scoring:

A- Microleakage: [12]

- Score 0: No dye penetration.
- Score 1: Dye penetrated up to 1/3 of the sealant-tooth interface length.
- Score 2: Dye penetrated from 1/3 to 2/3 of the sealant- tooth interface length.
- Score 3: Dye penetrated more than 2/3 of the sealant-tooth interface length.

B-Penetration Depth: [23]

- Score 1: Sealant penetrated to about one-third of the entire fissure's length.

- Score 2: Sealant penetrated to one-half of the entire fissure's length.
- Score 3: Sealant penetrated to the entire fissure's length.
- Microleakage and penetration depth of pit and fissure sealant were scored by the two senior supervisors who were blinded to type of enamel treatment.
- In case of disagreement, the worst score was documented.
- Both outcomes were assessed by supervisors of thesis twice with a 2-weeks interval to assess intra-examiner reliability while inter-examiner reliability was measured by comparing the scores of both assessors at the first assessment.[22] The inter-examiner and intra-examiner Cohen's kappa scores were 0.85 and 0.88 respectively.

Statistical analysis

Ordinal data (microleakage and penetration depth) were presented as frequency and percentage values and were analyzed using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. Shear bond strength data were presented as mean and standard deviation values. They were explored for normality by checking the data distribution using Shapiro-Wilk test. They were normally distributed and were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows. (26)

RESULTS

The present study compared the effect of various enamel preparation techniques; 37% phosphoric acid etching, self-etching adhesive and Er:YAG laser etching prior to application of a fluoridated resin-based pit and fissure sealant on caries-free premolars in regard to shear bond strength, microleakage and penetration depth.

I- Shear bond strength

Mean and standard deviation of shear bond strength values in different groups:

One-way ANOVA followed by Tukey's post hoc test results showed statistically significant difference between different groups (p=0.007). The highest value was found in group (I) conventional acid etching (10.80±3.66), followed by group (II) self-etching adhesive (8.82±2.45), while the lowest value was found in group (III) Er:YAG laser treatment (6.42±0.69). Post Hoc pairwise comparisons showed group (I) conventional acid etching to have a significantly higher value than group (III) Er: YAG laser treatment (p<0.001), while there was no statistically significant difference between group (I) conventional acid etching group and group (II) self-etching adhesive and there was no significant difference between group (II) self-etching adhesive and group (III) Er: YAG laser treatment.

Mean and standard deviation values for shear bond strength (MPa) in different groups are presented in Table (2).

TABLE (2) Mean and Standard deviation (SD) of shear bond strength values in different groups

Shear bond strength (MPa) (Mean±SD)			p-value
Group (I)	Group (II)	Group (III)	
10.80 ± 3.66^{A}	8.82 ± 2.45^{AB}	6.42 ± 0.69^{B}	**0.007***

II- Microleakage

Mean and standard deviation of microleakage values in different groups:

Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction results showed a statistically significant difference between different groups (p=0.011). The highest value was found in group (III) Er: YAG laser treatment (1.67±1.37), followed by group (II) self-etching adhesive

(0.58±1.00), while the lowest value was found in group (I) conventional acid etching (0.17±0.39). Post Hoc pairwise comparisons showed significant difference between group (III) Er: YAG laser treatment and group (I) conventional acid etching (p<0.001). However, there was no statistically significant difference between group (I) conventional acid etching and group (II) self-etching adhesive and there was no significant difference between group (II) self-etching adhesive and group (III) Er: YAG laser treatment.

Mean and standard deviation of microleakage values in different groups were presented in Table (3).

TABLE (3) Mean and Standard deviation (SD) of microleakage values in different groups

Microleakage (Mean±SD)			p-value
Group (I)	Group (II)	Group (III)	
0.17±0.39 A	0.58±1.00 AB	1.67±1.37 B	**0.011***

Frequency and percentage of microleakage values in different groups

There was a significant difference between mean values of different groups; with group (III) having statistically significant higher value than group (I) (p=0.011). The majority of group (I) (83.3%) and group (II) (66.7%) had score (0) while the majority of group (III) (41.7%) had score (3) as indicated by Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction.

The majority of group (I) samples had score (0) [10 (83.3%)], while [2 (16.7%)] had a score of (1). Similarly, most of group (II) samples had score (0) [8 (66.7%)], lower percentage had score (1) [2 (16.7%)], while scores (2) and (3) were only found in [1 (7.1%)] sample. For group (III), most of the samples had score (3) [5 (41.7%)], fewer percentage [4(33.3%)] had score (0), two samples have score (2) and only one sample has score (1).

Frequency and percentage of microleakage values in different groups are presented in Table (4).

Scores of microleakage under SEM are shown in Figures (2, 3, 4, 5)

TABLE (4) Frequency and percentage of microleakage values in different groups

Microleakage		Group (I)	Group (II)	Group (III)	p-value
Score (0)	n	10 A	8 AB	4 B	**0.011***
	%	83.3%	66.7%	33.3%	
Score (1)	n	2 A	2 AB	1 B	
	%	16.7%	16.7%	8.3%	
Score (2)	n	0 A	1 AB	2 B	
	%	0.0%	8.3%	16.7%	
Score (3)	n	0 A	1 AB	5 B	
	%	0.0%	8.3%	41.7%	

III- Penetration depth

Mean and Standard deviation (SD) of penetration depth values in different groups:

All groups showed the same score (3.00±0.00). that indicates that the sealant in all groups penetrated to the full length of fissures.

Mean and standard deviation values for penetration depth in different groups were presented in table (5).

TABLE (5) Mean and Standard deviation (SD) of penetration depth values in different groups

Penetration depth (Mean±SD)			p-value
Group (I)	Group (II)	Group (III)	
3.00±0.00 A	3.00±0.00 A	3.00±0.00 A	**1.000ns**

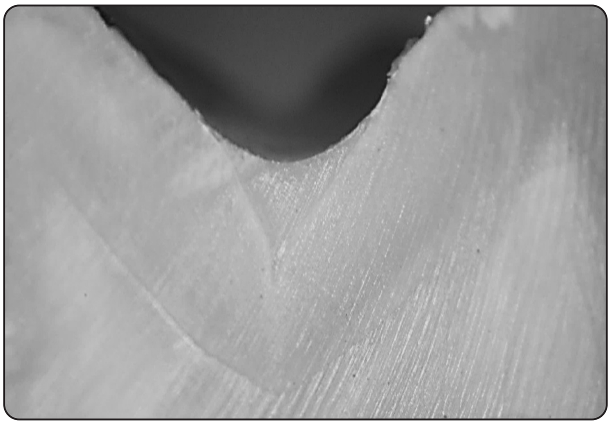

Fig. (2): Score 0 microleakage and score 3 penetration depth for the resin sealant

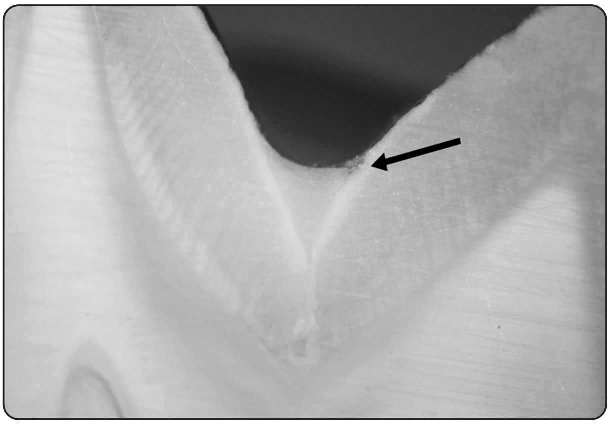

Fig. (3): Score 1 microleakage

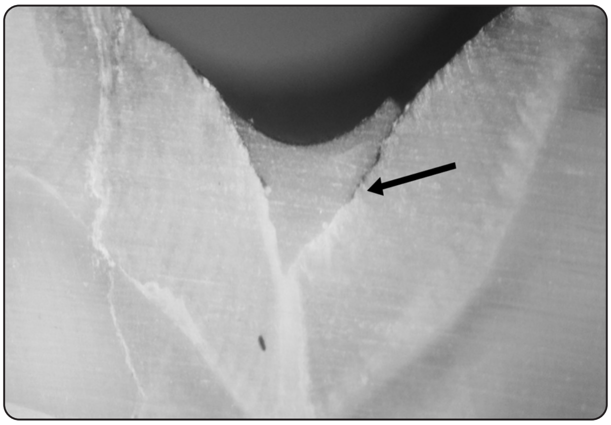

Fig. (4): Score 2 microleakage

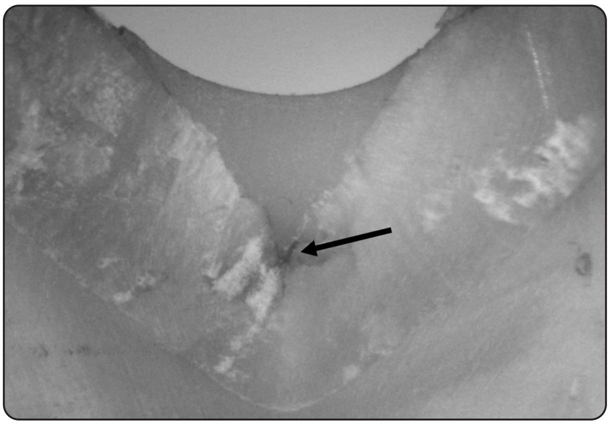

Fig. (5): Score 3 microleakage

Frequency and percentage of penetration depth values in different groups

All samples of different groups showed score (3) with no statistically significant difference between them (p=1) as indicated by Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction, figure (5)

Frequency and percentage values for penetration depth in different groups were presented in Table (6).

TABLE (6): Frequency and percentage of penetration depth values in different groups

Penetration depth		Group (I)	Group (II)	Group (III)	p-value
Score (1)	**n**	0	0	0	**1.000ns**
	%	0%	0%	0%	
Score (2)	**n**	0	0	0	
	%	0%	0%	0%	
Score (3)	**n**	12 A	12 A	12 A	
	%	100.0%	100.0%	100.0%	

DISCUSSION

Pit and fissure sealants are one of the most important preventive methods which can be used as a primary prevention measure to prevent the development of dental caries, or as a secondary prevention measure to inhibit disease progression.[27] The success rate of pits and fissures sealant rely on the retention durability and marginal adaptation to the tooth surface because penetration of bacteria beneath the sealant might allow caries onset and progression.[28]

Thus, the present study aimed at evaluating and comparing the effect of various enamel preparation techniques; 37% phosphoric acid etching, self-etching adhesive and Er:YAG laser etching before application of a fluoridated resin-based pit and fissure sealant on caries-free premolars in regard to shear bond strength, microleakage and penetration depth. Up to our knowledge, no studies could be found in the literature that compared effect of those three different enamel treatment modalities on shear bond strength, microleakage and penetration depth.

For the conventional method of sealant application, phosphoric acid in the range of 32-37% is routinely used to etch enamel. It may be considered as the gold standard protocol for sealant placement.[3,29] Nevertheless, this conventional acid etching method removes several microns from the enamel surface which could become a disadvantage if sealants lost their retention or marginal integrity.[30] Several studies claimed that the use of other preparation techniques as self-etching adhesive and Er:YAG laser etching may enhance retention rates and reduce microleakage of pit and fissure sealants and may overcome the conventional acid etching technique drawbacks.[8,15,19,31–37]

Self-etching adhesive systems were introduced to simplify the clinical procedures of adhesive application.[8]

The adhesion strength of an adhesive depends on its enamel etching capacity, bond type, and mechanical properties.[20] In the present study, **Single Bond Universal Adhesive** contains the acidic monomer10-methacryloyloxydecyl (10-MDP), which interacts chemically with calcium in the hydroxyapatite of enamel. [38–40]

The third study group assessed laser enamel pretreatment.[41] Laser produces micro explosions that provide microscopic and macroscopic irregularities in enamel that may eliminate the need for the use of conventional acid etching. Furthermore, laser decreases the percentage of calcium ion dissolution and so may increase enamel resistance to acid decalcification, and at the same time does not yield a smear layer.[42]

Erbium:Yttrium-Aluminum Garnet (Er:YAG) laser (2,940 μm) was used in the present study due to its properties of interaction with hard dental tissues caused by its affinity to water and hydroxyapatite allowing for “cold ablation”[43,44]. The Er:YAG laser produces a small spot of ablated tissue less than 1 mm in diameter so it is useful as a tool for enamel pretreatment and prevents unnecessary etching of enamel [45]**.**

In acid etching group, 37% phosphoric acid was used because of its effectiveness in elimination the smear layer. In addition, it provides a relatively rough surface to create a better interface upon the application of sealants.[46]

The etching time was performed at 15 seconds, as the literature recommendation for both primary and permanent teeth for more enamel preservation without compromising sealant clinical adhesion.[46]

In self-etching adhesive group, active self-etching application was performed to enhance etching pattern and improve the micromechanical interaction of the adhesive with the underlying enamel. This application method was reported by **Loguercio et al, (2015).** [47]

In laser group, enamel was irradiated with a pulsed Er: YAG laser at 1.6 watt where the tipless hand piece was held perpendicular to the enamel surface at a distance of 12 mm with 40% air and 40% water for only 15 seconds.[19] These parameters and method of application allow for only micro roughness without cavitation nor collapse or damage to the tooth surface.[19]

In the present study, three mechanical properties were evaluated; shear bond strength, microleakage and penetration depth as these tests reflect the stability of the material under masticatory force and adhesion effectiveness with the tooth surface.[48]

The results of the present study regarding SBS revealed a significant difference among different groups. Conventional acid etching showed the highest shear bond strength (10.80±3.66) followed by self-etching adhesive (8.82±2.45), while laser treatment showed the lowest value (6.42±0.69). There was significant difference between group I (Conventional acid etching) and group III (Er: YAG laser). However, there was no significant difference between group I (Conventional acid etching) and group II (self-etching adhesive). There was no significant difference between group II (self-etching adhesive) and group III (Er: YAG laser). The high SBS of conventional acid etching compared to other groups may be related to the creation of microscopic spaces or pores in enamel that increase surface roughness and bonding area and thus produce proper micromechanical retention.[49] Self-etching adhesive also showed satisfactory results regarding SBS which may be related to its content of acidic monomer (10-MDP) which is also able to form an ionic bond with calcium ions.[8] The lower SBS of self-etching adhesive (8.82±2.45) compared to conventional acid etching (10.80±3.66) , although non-significant , may be due to the higher pH of self-etching adhesive (pH 2.7) compared to that of phosphoric acid (pH 0.1-0.4). [32,50]

On the other hand, the low SBS values of Er: YAG group (6.42±0.69) may be explained by the fact that Er: YAG laser causes subsurface fissuring or cracks that may not be suitable for optimal adhesion. [51]

However, SBS results of the three groups are considered acceptable and capable of withstanding masticatory forces, as they all exceed the minimum SBS (5.9-7.8) MPa suggested by Reynolds.[52]

Similar results were reported by **Mézquita-Rodrigo et al, in 2017** [53] who found the highest SBS with conventional acid etching when compared to self-etching adhesive and laser. There was significant difference between conventional acid etching and Er: YAG laser. However, there was no significant difference between conventional acid etching and self-etching adhesive and there was no significant difference between self-etching adhesive and Er: YAG laser. However, in that study, only SBS was evaluated.

Results of the present study also came in agreement with **Coelho et al., (2019)** [54] who found that the SBS of fissure sealant in conventional acid etching group was statistically non-significant to self-etching adhesive group, although the conventional acid etching group showed the highest values.

Our results were also in line with **Magbul et al., (2022)** [55] who clinically evaluated the retention of resin-based fissure sealant applied using self-etching and conventional acid-etching techniques for 24 months in school children and stated that the retention of the conventional acid-etching technique was superior to that of the self-etching technique.

Regarding laser treatment, the present study's results were in line with **Attrill et al, (2000)** [51], **Ciucchi et al., (2015)** [4], **Odabasi et al., (2018)** [56] and **Rattanacharoenthum et al., (2019)** [57] who reported that conventional acid-etching yielded higher SBS results when compared with Er:YAG as an enamel preparation technique prior to sealant application despite the use of different parameters of pulse energy and output power.

However, studies by **Karaman et al., (2013)** [58] and **Kumar et al., (2016)** [59] that clinically evaluated the effect of erbium laser treatment compared to acid etching on the pit and fissure sealant retention, reported no significant differences between the retention rates of sealant in both groups. This may be explained by the relatively acceptable SBS values of laser as stated by Reynolds which may not affect its clinical performance [52].

The present study's microleakage results revealed that Er: YAG laser treatment showed the highest microleakage scores compared to self-etching adhesive and conventional acid-etching. There was significant difference between group I (Conventional acid etching) and group III (Er: YAG laser). However, there was no significant difference between group I (Conventional acid etching) and group II (self-etching adhesive) and there was no significant difference between group II (self-etching adhesive) and group III (Er: YAG laser).

The chemical interaction bonding mechanism of self-etching adhesive systems minimizes hydrolytic degradation allowing restorations to remain marginally sealed for a longer period of time[60]. HEMA in self-etching adhesive improves the wetting properties and prevent the separation of hydrophobic resin components of the sealant material after evaporation of the solvent [61].

However, the lower microleakage values of group II (self-etching adhesive) compared to group I (conventional acid etching) may be caused by the inability of self-etching adhesive containing weak acids to penetrate deep in enamel [62].

The present study's results were comparable with **Nejad et al., (2012)** [63] who found no statistically significant difference in microleakage between acid etching and self-etching adhesive groups. Lower microleakage scores were also observed in the acid etching group.

The current results are also consistent with **Memarpour et al., (2018)** [64] who reported no significant difference in microleakage between self-etching adhesive and conventional acid etching groups prior to pit and fissure sealant application.

However, our results disagreed with, **Suharni et al., (2018)** [65] and **Amend et al., (2021)**[22] who found significant difference in microleakage between conventional acid etching and self-etching adhesive groups. This may be due to the large sample size selected in both studies.

Regarding laser treatment, the results of the present study were comparable with, **Borsatto and colleagues (2001)** [66], **Sancakli et al, (2011)**[67] **, Topaloglu et al., (2013)**[68] **, Fumes et al, (2017)** [69] and **Shingare et al., (2021)** [70] who found that Er: YAG laser treatment showed the highest microleakage scores compared to conventional acid-etching. This finding may be also related to the formation of enamel microcracks.

However, our results were contrasted with those of **Dostálová et al., (1998)**[71] and **Moshonov et al., (2005)**[72] who reported a similar degree of microleakage when comparing laser and acid etching. On the other hand, **Nashaat et al., (2022)** [73] reported laser etching had lower microleakage compared to conventional acid etching. This difference in findings may be related to differences in the use of laser parameters or the use of different laser machines.

The results of penetration depth assessment showed the same score in all groups (Score 3). The high penetration depth in the present study may be due to the high flowability of the tested sealant (Fissurit F). [74] This result was in line with **Muntean et al., 2019** [75] and **Dixit et al., 2021** [24] who reported that the fissure depth and morphology as well as the dental material characteristics are the key factors for sealant penetration depth.

As an in-vitro study, the present study design ensures standardization of testing conditions and criteria of teeth selection. This provides constant and accurate test results and enables the detection of minor differences between groups unlike the clinical studies.

Limitation of the study:

The in-vitro testing methods used in this study was not able to mimic the oral conditions including the masticatory forces and pH changes which may affect the actual serviceability of the sealant material.

CONCOLUSIONS

Within the limitations of this study, the following conclusions could be drawn:

1. Conventional acid etching was the best pretreatment method prior to resin-based fissure sealants in terms of increased bond strength and reduced microleakage.
2. Self-etching adhesives can be an effective alternative to conventional acid-etching prior to the application of pit and fissure sealants especially in children.
3. Enamel pretreatment with laser produced the least Shear Bond Strength and the highest microleakage.

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