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COMPARISON BETWEEN ER, CR: YSSG LASER AND CONVENTIONAL ETCHING METHODS AS SURFACE TREATMENT FOR PITS FISSURE SEALANTS REGARDING MARGINAL ADAPTATION: AN IN VITRO STUDY

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ABSTRACT

Background: Proper marginal adaptation of pit and fissure sealant can prevent occlusal caries via prevention of the accumulation of biofilm and the penetration of microorganisms. Hence, the aim of this study was to evaluate the marginal adaptation of the resin-based sealant after fissure pretreatment with Er, Cr: YSSG Laser and compare it to the conventional etching method.

Methods: In two equal groups (n=9), 18 extracted premolars were randomly enrolled in the study and pretreated with Erbium, Chromium: Yttrium Scandium Gallium Garnet (ER, CR: YSSG) laser etching in Group I and conventional acid etch in Group II as a surface treatment before application of pits and fissure sealants. After thermocycling and sectioning of the teeth, the Scanning Electron Microscope (SEM) was used to measure the sealant marginal adaption.

Results: ER, CR: YSSG laser etching showed higher adaptation of the pits and fissure sealant in comparison to conventional acid etching with a statistically significant difference (*p*-value=0.0001).

Conclusions: ER, CR: YSSG laser etching could be used as an alternative to the conventional acid etching prior to the application of pits and fissure sealant.

KEYWORDS: Adaptation, Laser, Pit and fissure sealant, Resin-based sealant

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INTRODUCTION

Pit and fissure caries make up 90% of tooth surfaces affected by caries, which can be attributed to their retentive morphology compared to smooth surfaces [1, 2]. Pit and fissure caries can be prevented by applying pit and fissure sealant which prevent the accumulation of biofilm and the penetration of microorganisms [2].

The quality of the adhesion between the sealant and enamel affects the success of pit and fissure sealant, which ensures continual resistance to microleakage from saliva and microorganisms at the interface [3, 4]. Sealant adhesion and retention are essentially derived from the micromechanical interlocking, as there are only a few chemical interactions between resin and enamel [4].

Several surface treatment techniques have previously been used for sealant application, mainly mechanical preparation, air abrasion, and acid etching [5-7]. Acid etching is a generally accepted and standard method for pretreating the enamel surface to allow the adhesion of restorative materials [5,6].

Acid etch is an admitted and standard technique for pretreatment of enamel surface for adhesion of restorative materials, however in case of sealant placement, residual remnants and pellicle could not be removed from the deepest layer of fissure by conventional etching procedure, thus other methods have been suggested for surface treatment of fissure for sealants [8, 9]. Lasers have been used on hard tooth tissue where previous research has shown the ability of the Erbium: yttrium-aluminum-garnet (Er: YAG) laser to ablate or cut tooth structure, remove carious lesions, cavities preparation, and dentin and enamel surfaces modifications to increase bond strength [7, 10].

This study aimed to compare Erbium, Chromium: Yttrium Scandium Gallium Garnet (ER, CR: YSSG) laser and conventional acid etching when used as surface treatment of pits and fissures on the occlusal surface of premolar regarding the marginal adaptation of pits and fissure sealants.

MATERIAL AND METHODS

Study design

The present study was a randomized, two arms, in vitro study with a 1:1 allocation ratio.

Ethical Approval

The study protocol was approved by the Research Ethics Committees of the Faculty of Dentistry, Cairo University, Egypt on 30/4/2020 with approval number 17/4/20.

Study setting

- The premolar teeth used in the current study were collected from the orthodontic department, Faculty of Dentistry, Cairo University.
- Surface preparation using ER, CR: YSSG laser and conventional acid etching were performed at the laser research center, Faculty of Dentistry, Misr University for Science and Technology.
- Measurements of marginal adaptation using scanning electron microscopy were carried out at National Research Center.

Sample size calculation

Sample size calculation was performed using G*Power version 3.1.9.21. An effect size (d) of (8.56) was calculated based on the results of **Youssef et al**. [11] in which the (Mean ±SD) values of the test and the control groups were (6.87±0.43) and (4.93±2.28) respectively. By adopting an alpha (α) level of 0.05 (5%), and beta (β) level of 0.20 (20%) where power=70, the predicted sample size (n) was found to be a total of (18) samples (9) samples for each group.

Sample selection

A total of 18 freshly sound premolars extracted for orthodontic treatment were selected for the present study. The teeth were free from cracks or developmental defects, and their occlusal surface have deep and fissures. The teeth with macroscopic attrition or fracture were excluded [7,12]. Also, the teeth with previous restoration or sealant were not included in this study [13]. Teeth were examined teeth under the light microscope to confirm their adherence to eligibility criteria.

Sample randomization

The samples were randomly divided into two equal groups, where Group I was treated with ER, CR: YSSG laser etching and Group II was treated with conventional acid etching. Sample randomization was carried by "Random.org" to generate the sequence by one of the co-authors [14]. Teeth were placed in a sterile separate numbered glass container covered with opaque paper, and the generated random sequences for the containers were kept with the same co-author.

Blinding

The laboratory assessor and statistician were blinded.

Sample preparation

The selected teeth were cleaned from any gross debris using ultrasonic cleaner (Woodpecker, Guilin Woodpecker Medical Instrument, China), and were disinfected with 5.25% Sodium Hypochlorite (Clorox, Cairo, Egypt) by immersion for 7 days in a glass container [15]. Then, the occlusal surfaces were cleaned by tap water using a disposable soft brush and low-speed air motor handpiece (Sirona, Dentsply Sirona, North Carolina, USA). Teeth were stored in distilled water at room temperature [16] in a sealed and sterile separate transparent glass container labeled with numbers from 1 to 26.

Pit and fissure etching procedure

Group I

Pits and fissures of each premolar were dried with air-water spray for 15 s and treated with hard tissue Er, Cr: YSSG laser (Biolase, USA) [8]. Laser parameters were set at a 2780 wavelength with 140-µs pulse duration and a pulse repetition rate of 20 Hz [8, 17]. The power output was determined to be two watts. According to the manufacturer's instructions, the air and water sprays were adjusted to 90% and 80%, respectively, and the used fiber was an MZ6 fiber tip with a 600-µm diameter and applied the laser radiation two mm from the fissure surfaces with a LED for 20 s [18]. Afterward, teeth surfaces were dried for 15 s until a white, frosty, and porous appearance was seen [8, 18].

Group II

Pits and fissures of each premolar were dried with air-water spray for 15 s and treated with 37% phosphoric acid gel (Scotch bond TM, 3M ESPE, USA). Acid was rinsed with an air-water spray for 15 s, and the tooth surface was let dry for another 15 s until a chalky white appearance was obtained [7, 8].

Sealant application

Fluoride releasing resin-based pit and fissure sealant (Clinpro TM Sealant, 3M ESPE, USA) was applied as a small drop in deep part with scrubbing motion then another drop was applied to the final shape of the fissure. Sealant was polymerized using the LED light cure (Woodpecker, Guilin Woodpecker Medical Instrument, China) for 20 s [1,7]. After curing, sealant was tested with an explorer for complete coverage and retention. Finally, all samples were stored in distilled water at 37°C for 24 h before thermocycling [1,12].

Thermocycling

All teeth were exposed to thermal cycles (Robota automated thermal cycle; BILGE, Turkey) in water baths at a temperature range between 5°C and 55°C. The duration of each bath was 15 s with a 10 s transfer time. The thermal cycles were repeated 1200 times and a dwell time of 5s according to Morresi et al., 2014 [18, 19].

Evaluation of sealant marginal adaptation

Adaptation of pits and fissures sealant was assessed using SEM (Quanta FEG 250, Hillsboro, USA). Teeth were sectioned in a buccolingual direction parallel to the long axis of the tooth up to the cementoenamel junction following the direction of enamel rods. The sample surfaces were then coated with a thin layer of pure gold (5-10 nm) using an ion sputtering unit (BOC Edwards, UK). Samples were examined using 450X magnifications and then evaluated according to the method described by **Kane et al.** [20] where score 1 indicates a smooth adaptation where sealants flow with enamel with no ledge and score 2 indicates that sealant is not well adapted and a ledge may be present.

Statistical Analysis

Statistical analysis was performed using the Graph Pad Instat 20 software for windows. Categorical data were expressed as numbers and percentages and were analyzed using the chi-square test. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Regarding the marginal adaptation of pit and fissure sealants, there was a statistically significant difference between ER, CR: YSSG laser (Group I), and conventional acid etching (Group II) using the chi-square test with (P= 0.0001) as shown in Fig. (1). In group I, 4 teeth (44.4%) recorded score 1 where it showed a smooth adaptation, and sealants flowed with enamel with no ledge. While five teeth (55.6%) recorded a score 2 where sealants weren't well-adapted and ledge as shown in Fig. (2). However, one tooth (11%) in group II recorded a score 1 where it showed a smooth adaptation, and sealants flowed with enamel with no ledge and eight teeth (89%) recorded a score 2 where sealants weren't well-adapted and ledge as presented in Fig. (3).

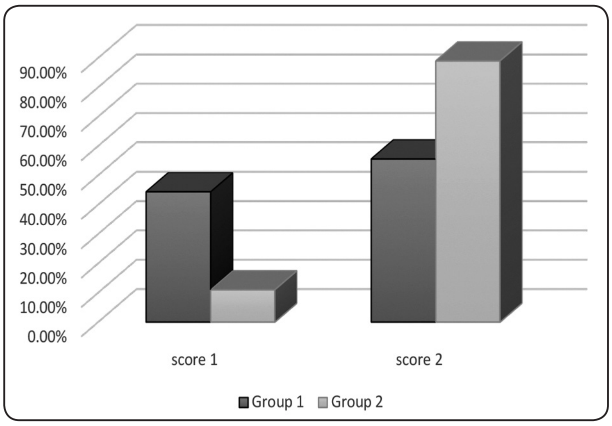

Fig. (1): Bar chart showing the distribution of the adaptability scores between two groups.

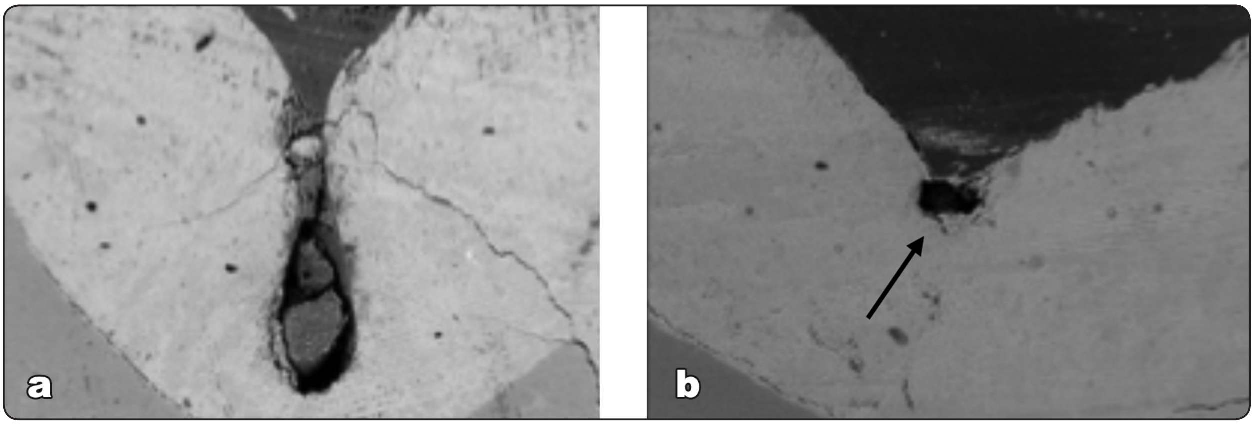

Fig. (2): SEM photos showing the pit and fissure sealant adaptability score among Group I; a: Photo showing adaptability score 1. (arrow showing smooth adaptation of sealants along fissure wall); b: Photo showing adaptability score 2. (arrow showing ledge between sealants and fissure wall).

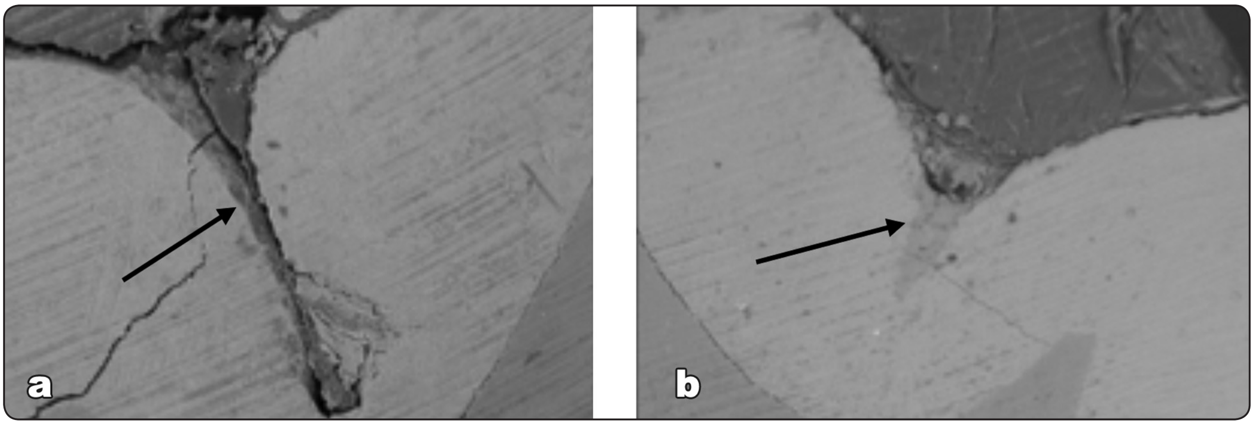

Fig. (3): SEM photos showing the pit and fissure sealant adaptability score among Group II; a: Photo showing adaptability score 1(arrow showing smooth adaptation of sealants along fissure wall); b: Photo showing adaptability score 2. (arrow showing ledge between sealants and fissure wall).

DISCUSSION

Pits and fissures of posterior teeth are the most susceptible for caries incidence; due to the high bacterial colonization, morphologically susceptible areas due to the bacterial colonization can increase the demineralization as in young molar teeth [7]. Preventing pits and fissures is vital since bacteria can colonize enamel through the pits and fissures causing occlusal caries [7,21].

The sealant's cariostatic properties are accredited to the pits and fissures physical obstruction which prevents the pits and fissures colonization with new bacteria and the pits and fissures remaining bacteria from any fermentable carbohydrates supply [22]. Therefore, the comparison of acid etching and laser etching on the marginal adaptation of fissure sealant was the aim of the study.

This comparison was made because it has long been claimed in the literature that the acid- etch conventional technique for sealant placement does not allow the complete cleaning of the pits and fissures prior to sealant placement [23]. Also, acid etching may cause the demineralization of enamel structures and make the enamel surface more vulnerable to caries formation [23,24].

The freshly extracted premolars were included in the study sample as they were the most commonly extracted sound teeth for orthodontic reasons. Also, they remained in the oral cavity for the least amount of time, providing the best possible standardized conductive environment for the application of the sealant based on histology of fissures regarding the cellular element of enamel organ in the fissure and enamel porosities [12].

To allow complete sealant penetration into pit and fissure, the process of fissure cleaning using an ultrasonic cleaner that produces a surface without any remaining plaque and debris is the most important key for successful application of the pits and fissures sealant [25].

Teeth were disinfected with 10% formalin as it is an effective method of disinfecting both the internal and external structures of the teeth without affecting enamel and dentin microstructure [15]. Teeth were stored in distilled water at room temperature to provide adequate hydration [26]. Moreover, teeth were sealed in a sterile separate glass container covered with opaque paper to prevent selection bias which may affect the internal and the external validity of the analysis [27].

The samples were randomly divided into two groups to provide comparable groups and eliminate all known or unknown confounders that may affect the outcome. This similarity is very important to allow statistical inferences on the treatment effects [14].

A concentration of 37% phosphoric acid etching was utilized in the current study because it is effective in removing the smear layer and provides a relatively rough surface to create a better interface upon the application of sealants [28]. Etching gel was applied instead of etching solution as this consistency provides good handling properties allowing the exact application at the correct position and time. Also, it allows a complete rinse-off with no phosphoric acid left in the fissure [29]. A 37% phosphoric acid gel was used with an etching time of 15 seconds, as supported in literature for both primary teeth and permanent teeth. With this reduction of etching time, more enamel is preserved without affecting the clinical adhesion of the sealant [28].

Er, Cr: YSGG laser system used in the present study is a hydrokinetic system that is capable of suppressing the temperature rise and subsequently preventing inflammatory pulpal response [30]. Also, pulsed-beam system and fiber delivery has been shown to be a valuable tool for removing enamel and dentin and preventing unnecessary etching of the enamel [31]. Laser parameters were set at a 2780 wavelength with 140-μs pulse duration, a pulse repetition rate of 20 Hz and power output was determined to be two watts because that may reduce damage to resin- enamel interfaces [32].

Before sealant application, surface dryness for another 15 seconds was performed to enhance the sealant penetration into pits and fissures [33]. Fluoride releasing resin-based pit and fissure sealant was applied as it possesses high retention rates, good fracture resistance, superior wear resistance, and is a color-changing sealant [23,34].

A small drop of sealant has been applied in the deep parts of the fissure with a scrubbing motion in order to eliminate any microporosities that may lead to failure of the sealant process [1,35]. Sealant was polymerized using an LED light cure for 20 s to permit a better penetration and adaptation of sealant into the fissures resulting in a bond with a deeper enamel layer [35]. Sealants were checked after curing using an explorer to ensure complete coverage and retention of the sealant. Finally, all samples were stored in distilled water at 37c for 24 h before thermocycling in order to prevent specimen dehydration and shrinkage of sealant [1,12].

All teeth were subjected to water baths with thermal cycles at a temperature range between 5°C and 55°C to simulate temperature change that takes place in the oral cavity [19]. Teeth were sectioned in a buccolingual direction parallel to the long axis of the tooth up to the cementoenamel junction following the direction of enamel rods to see fissures in a cross-sectional direction [36].

Sealant adaptation was assessed using SEM as it provides a means for direct visual observation of penetration and adaptation of sealant materials to enamel walls due to its magnification and depth of focus [35].

Regarding the marginal adaptability of the pit and fissure sealant, 11% of Group I and 44.4% of Group II showed a smooth adaptation, and sealants flowed with enamel with no ledge. On the other hand, 89% of Group I and 55.6% of Group II showed sealants weren't well-adapted with a statistically significant difference between the two groups. This finding was in agreement with **Bortolotto et al.** [37] which can be attributed to an acid-resistant layer formed on enamel as a result of laser irradiation, decreasing the etching effect of the self-adhesive system.

These results could be attributed to the ability of laser to produce micro-explosions, providing microscopic and macroscopic irregularities to the hard tissue which makes the enamel surface more retentive and allows the adhesion mechanism without acid-etching [38]. Besides, laser reduces the percentage of calcium ion dissolution, increases enamel resistance to acid decalcification, and it doesn't yield a smear layer [38-40].

CONCLUSIONS

Based on the results of the current study it could be concluded that the use of ER, CR: YSSG laser etching as an alternative to the conventional acid etching prior to the application of pits and fissure sealant.

Limitations of the study

- In vitro studies do not completely predict how sealant will behave in the oral cavity.
- The study did not evaluate the effect of fissure morphology on sealant properties.

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