CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

DOI: 10.5152/cjms.2018.569

In Vitro Comparative Evaluation of Er,Cr:YSSG Laser and Conventional Etching Methods on the Microleakage and Adaptation of Pit and Fissure Sealants

Aylin İslam[1], Zerhan Kızılelma[2], Serap Çetiner[1,3]

[1]Department of Pediatric Dentistry,, Near East University, Nicosia, Cyprus
[2]Darıca Oral and Dental Health Center, Kocaeli, Turkey
[3]Pediatric Dentistry Department, Kyrenia University, Kyrenia, Cyprus

ORCID IDs of the authors: A.İ.: 0000-0001-6338-4505, Z.E.: 0000-0002-5082-9541, S.Ç: 0000-0003-1793-1840

Cite this article as: İslam A, Kızılelma Z, Çetiner S. *In Vitro* Comparative Evaluation of Er,Cr:YSSG Laser and Conventional Etching Methods on the Microleakage and Adaptation of Pit and Fissure Sealants. Cyprus J Med Sci 2018; 3: 85-9.

BACKGROUND/AIMS
The main objectives of the current research were to compare the conventional etching and alternative laser etching techniques and to evaluate the microleakage and Scanning Electron Microscope (SEM) analysis of pit and fissure sealants.

METHODS and MATERIALS
The extracted teeth were randomly selected, and 120 extracted teeth were divided into four experimental groups as follows: Group 1: Bur invasion and Conventional acid etching; Group 2: Bur invasion and Laser etching; Group 3: Laser etching; and Group 4: Laser etching and Conventional acid etching. According to experimental groups, teeth were treated with 37% phosphoric acid and laser parameters were set at a 2780 nm wavelength with a 140-μs pulse duration and a pulse repetition rate of 20 Hz. The power output was determined to be 1.75 watts. Then, a thermal cycle was applied to all samples to imitate an oral environment. After the thermal cycle procedure, each experimental group was randomly divided into two groups for microleakage tests and SEM analysis.

RESULTS
No statistically significant difference in microleakage scores was determined among the experimental groups ($p>0.05$). The fissure sealant adaptation was detected to be higher in Group 1 than in Group 2 and Group 3, and this difference was found to be statistically significant ($p<0.05$). The same trend was observed in Group 4, Group 3, and Group 2, with Group 4 having the higher fissure sealant adaptation ($p<0.05$).

CONCLUSION
Er,Cr:YSSG laser and acid etching combination can be a good choice and is comparable to bur invasion from the standpoint of the high pit and fissure adaptation.

Keywords: Er,Cr:YSGG laser, etching, microleakage, sealants

INTRODUCTION

Currently, dental caries is the most prevalent chronic infectious disease among children and adolescents. According to a World Health Organization report, approximately 90% of the world's population suffers from dental caries (1). Due to their complex morphologic structure, pits and fissures of occlusal surfaces are the most caries-affected surfaces and susceptible to decay (2, 3). In the preventive dentistry field, there have been many advances in treatments and preventive techniques for dental caries, such as topical or systemic fluoride applications, sugar substitutes, pit and fissure sealants (mechanical barriers), and antimicrobials (4).

Pit and fissure sealants have been applied for the prevention of occlusal caries since the 1970s (5-7). The success, effectiveness, and retention of pit and fissure sealants depend on several factors, including micromechanical interlocking and adhesion accomplishment between the sealant and enamel surface, surface pretreatment of enamel before sealant application, and pellicle and debris removal (8-10).

This study was presented at the 2nd International Biomedical Engineering Congress (IBMEC), May 24-27, 2018, Nicosia, Cyprus.
Corresponding Author: Aylin İslam
E-mail: aylin.islam89@gmail.com
Received: 16.06.2018
Accepted: 18.07.2018

Recently, various enamel surface pretreatment preparative techniques have been developed for increasing the effectiveness, retention, and success of pit and fissure sealants. The main accepted and standard procedure for this is the conventional acid etching method. However, a few clinical and practical disadvantages have been observed for this technique. For example, remaining pellicle and debris might not be totally removed from the base of fissures; demineralized enamel surface following acid etching creates a low-resistant enamel prone to acid attacks, and the application required for good tooth isolation is time-consuming (11-13).

Over the last decades, laser etching especially has been developed as a suggested alternative technique for enamel surface pretreatment in response to the disadvantages of the conventional acid etching method (14-16). According to the American Academy of Pediatric Dentistry's report in 2013, the advantages of laser therapy for pediatric patients were listed as follows: 1. No need for local anesthesia; 2. No pain; 3. Less noise and vibration; and 4. More comfortable. Also, laser etching has an important advantage in that it creates an etching on the acid-resistant prismless superficial layer of the primary enamel for pretreatment prior to fissure sealant application (2, 17).

The main objectives of the current research were to compare the conventional etching and alternative laser etching techniques and to evaluate the microleakage and SEM analysis of pit and fissure sealants.

MATERIAL and METHODS

In the present study, 120 healthy impacted third molars that had been extracted for different reasons were collected. All extracted teeth were stored in 0.1% thymol solution at 25°C for up to 3 months. In the present study, we used a total of 120 human impacted third molars.

The current clinical experimental study was approved by Ankara University Institutional Review Board (No:128/5), and consent forms were taken from patients for extracted teeth used in this study.

Determination of Experimental Groups

The extracted teeth were selected randomly, and 120 extracted teeth were divided into four experimental groups (n=30).

Group 1: Bur invasion and Conventional acid etching
In this group, enamel surfaces of teeth were roughened with a diamond bur kept within enamel. Later, teeth were dried and treated with 37% phosphoric acid (ScotchbondTM, 3M ESPE, USA) for 30 s. Following this, acid was rinsed with an air-water spray for an additional 15 s, and the tooth surface was dried for another 15 s. Lastly, fissure sealant (ClinproTM Sealant, 3M ESPE, USA) was applied via a probe to microporosities on the enamel surface and was polymerized for 20 s.

Group 2: Bur invasion and Laser etching
The enamel surfaces of the teeth in Group 2 were roughened with the same bur invasion as Group 1. Following the bur invasion, enamel surfaces were irradiated with an Er,Cr:YSSG laser (Waterlase MD, BIOLASE Technology, USA). Laser parameters were set at a 2780 wavelength with a 140-µs pulse duration and a pulse repetition rate of 20 Hz. The power output was determined to be 1.75 watts. According to the manufacturer's instructions, the air and water sprays were adjusted to 90% and 80%, respectively. We used an MZ6 fiber tip with a 600-µm diameter and applied the laser radiation at 2 mm from the enamel surfaces for 20 s. Then, the enamel surfaces were dried for 15 s, and fissure sealants were applied with the same technique as in Group 1.

Group 3: Laser etching
Fissure surfaces of selected teeth were roughened with an Er,Cr:YSSG laser. Laser parameters were set at a 2780 wavelength with a 140-µs pulse duration and a pulse repetition rate of 20 Hz. The power output was determined to be 2 watts. According to the manufacturer's instructions, the air and water sprays were adjusted to 90% and 80%, respectively. We used an MZ6 fiber tip with a 600-µm diameter and applied the laser radiation at 2 mm from the fissure surfaces for 20 s. Later, the roughness of the enamel surfaces was done using the same laser parameters as in Group 2. Afterward, teeth surfaces were dried for 15 s and fissure sealants were applied and polymerized with an LED light (Elipar™ Freelight 2, 3M) for 15 s.

Group 4: Laser etching and Conventional acid etching
Fissure surfaces of teeth were roughened with the same laser parameters as in Group 3. After fissure surfaces were roughened with Er,Cr:YSSG laser, teeth surfaces were dried for 15 s and fissure sealants were applied and polymerized with an LED light (Elipar™ Freelight 2, 3M) for 15 s.

All samples have waited in distilled water at 37°C for 24 h. Then, a thermal cycle (Nüve BM, 302 hot water device and Nüve BS 302 cold water device, Nüve Ankara, Turkey) was applied to all samples to imitate an oral environment. The thermal cycle was repeated 1000 times between 5°C and 55°C with a 10-s transfer time and a dwell time of 30 s.

After the thermal cycle procedure, each experimental group was randomly divided into two groups (n=15) for microleakage tests and SEM analysis. These groups are summarized in Table 1.

Microleakage Assessment

Microleakage was assessed according to Pardi et al.'s (18) microleakage criteria via the dye penetration test. The teeth surfaces were isolated with double-layer nail varnish, leaving a 1 mm window around the sealant, and the roots were embeded in an acrylic resin block (Orthocryl EQ, Dentautum, Germany). Following this procedure, the teeth were set in 0.5% basic fuchsin solution for 24h at 37 °C. Later, the teeth were rinsed and cleaned under tap water for 5 min, and all teeth were sectioned in the buccolingual/palatal position with a sensitive slice device (Micracut Precision Cutter, Metkon Instruments Ltd, Bursa, Turkey). Lastly, each of the tooth sample slices was evaluated under a stereomicroscope (Leica MZ 12, Meyer Instruments, Houston, TX, USA) at 25× magnification. The microleakage criteria of Pardi et al. (18) are given in Table 2.

SEM Analysis

For SEM analysis, seven teeth were randomly selected from each experimental group. Crown parts of selected teeth were separated from roots and sectioned in the buccolingual posi-

TABLE 1. Experimental Groups used in Microleakage Tests and SEM Analysis

Experimental Groups	Microleakage Test	SEM Analysis
Group1 (Bur invasion+Acid)	n=15	n=15
Group2 (Bur invasion+Laser)	n=15	n=15
Group3 (Laser+Laser)	n=15	n=15
Group4 (Laser+Acid)	n=15	n=15

TABLE 2. Microleakage Criteria

Score	Explanation
0	No microleakage
1	Microleakage restricted with ½ external side of sealant
2	Microleakage restricted with ½ internal side of sealant
3	Microleakage through sealant base

TABLE 3. Distribution of Microleakage Scores According to Experimental Groups

Penetration Depth	Group 1	Group 2	Group 3	Group 4
0	14	14	13	15
1	1	1	2	0
2	0	0	0	0
3	0	0	0	0
Total	15	15	15	15

tion. Samples were fixed with TRUMP solution, which includes 37% formaldehyde, 25% glutaraldehyde, NaH_2PO_4, NaOH, and distilled water, for 24h at 37°C. After fixation of samples, they were coated with gold-palladium. Sealed samples were examined using SEM (JSM, 6400, Tokyo, JAPAN) with energy-dispersive X-ray analysis at 250X and 1000X magnifications. Images were evaluated according to Kane et al.'s (19) SEM criteria.

Statistical Analysis

Statistical analysis of experimental data was analyzed with the Statistical Package for the Social Sciences (SPSS) Version 17 (SPSS Inc.; Chicago, Illinois, USA) for Windows. The Shapiro-Wilk test was used to analyze the normality of the data. Since there was a non-normal distribution, the microleakage data score was determined using the Kruskal-Wallis test for multiple comparisons. $p<0.05$ values were accepted as statistically significant.

RESULTS

The microleakage scores of all experimental groups are given in Table 3. No statistically significant difference in microleakage scores was determined among the experimental groups ($p>0.05$).

Representative SEM images of sealed and sectioned teeth from the experimental groups are shown in Figure 1. The fissure sealant adaptation was detected to be higher in Group 1 compared with Group 2 and Group 3, and this difference was found to be statistically significant ($p<0.05$). The same trend was observed in Group 4, Group 3, and Group 2, with Group 4 having the higher fissure sealant adaptation ($p<0.05$). Conversely, between Groups 1 and 4, there was no statistical difference in fissure sealant adaptation ($p>0.05$).

DISCUSSION

Traditional treatment procedures, materials, and devices in dentistry have undergone changes along with scientific and technological developments. Over the last 20 years, minimally invasive approaches have been popularized with developments in dental materials (20, 21). Currently, more effective preventive dental procedures, rather than operative dental treatments, have become dentists' primary aim (22). Hence, from the pediatric dentistry perspective, a preventive treatment's success depends on the ideal environment for the cooperation of children (23).

As an answer to these requirements, laser technology and dental lasers have been developed and have rapidly replaced dental treatment procedures (22, 24).

In the present *in vitro* comparative study, the microleakage and enamel surface effects of the laser etching and conventional acid etching techniques (with/without bur invasion) prior to fissure sealant application were evaluated.

When the microleakage results of our study were evaluated, microleakage rates of all experimental groups were low. According to Pardi et al's (18) criteria, no "score 2" or "score 3" was found in any groups. Group 1 (bur invasion + acid etching) and Group 2 (bur invasion+Er,Cr:YSGG etching) showed similar microleakage rates, while no microleakage was observed in Group 4 (Er,Cr:YSSG etching+acid etching) and (Er,Cr:YSGG etching) only one sample showed microleakage in Group 3. When these differences were compared, they were not found to be statistically significant. In light of these first microleakage evaluation test results, Er,Cr:YSSG only or combined with the conventional acid etching method was evaluated as an alternative therapy compared to the conventional acid etching method only.

Although the microleakage test results found laser etching to be an effective and accomplished method, according to the SEM results, a half fissure sealant adaptations to pits and fissures were detected in all samples that only used laser etching in the present study. This difference between the microleakage test and the SEM analysis showed that only the microleakage test can provide wrong results regarding laser etching and conventional acid etching comparison.

Hatırlı et al.'s (25) latest report in 2018 regarding the effect of non-invasive and enameloplasty pretreatment of enamel surfaces and different materials on microleakage were compared, and the difference between tested materials was found to be significant in spite of the difference in preparation techniques not being significant.

Studies conducted in 2013 and 2016 used similar methods and reported parallel results with our study's microleakage data (16, 26). Accordingly, an Er,Cr:YSSG laser etching pretreatment method was accepted as an alternative to the conventional acid etching method with its retention and patient acceptability features. In addition, no statistical difference was found be-

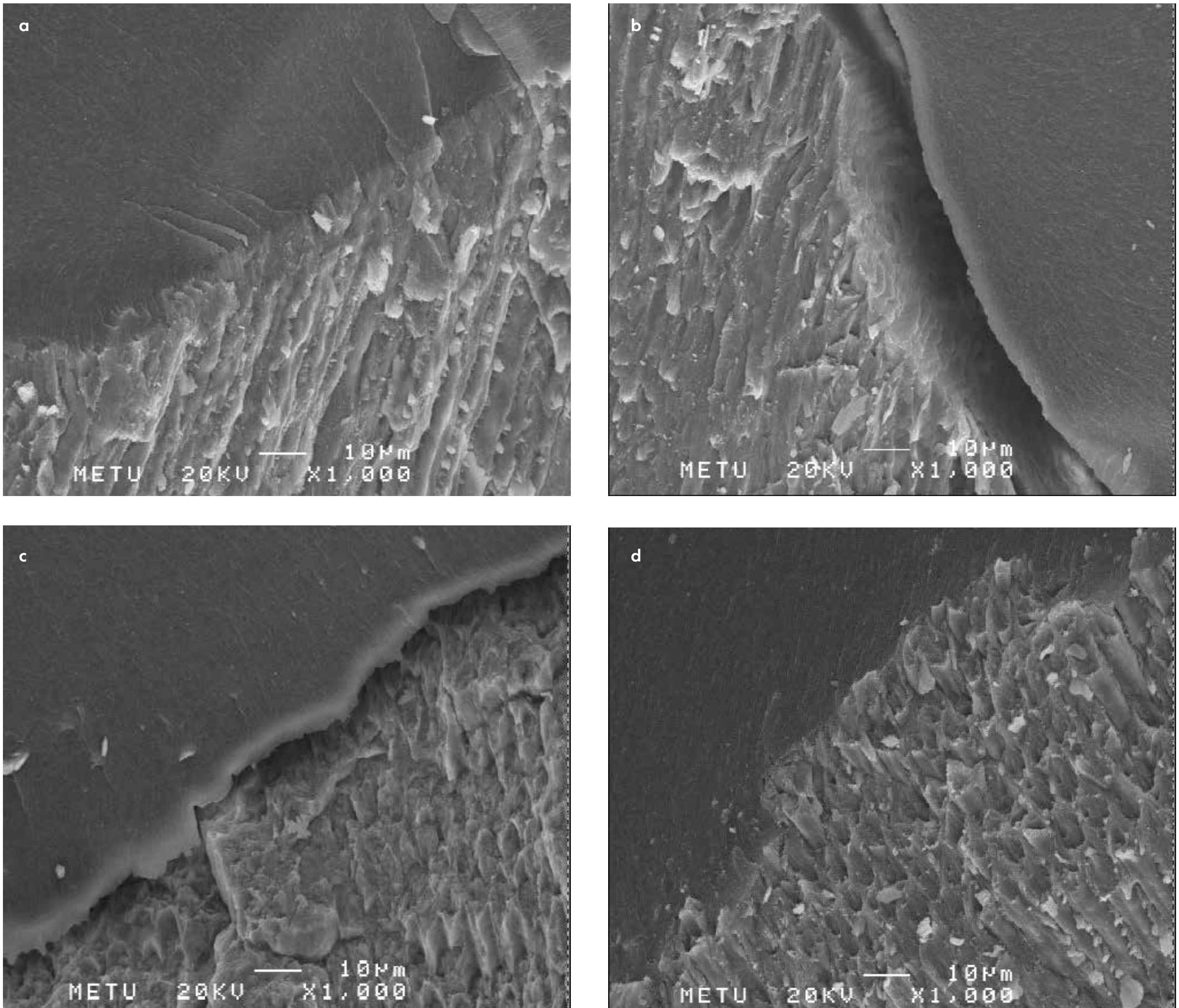

FIGURE 1. a-d. SEM images of each group (x1000) and fissure sealant adaptation rates. A) Group 1-Score 3 (a) Group 2-Score 2 (b) Group 3-Score 2 (c) Group 4-Score 3 (d)

tween the Er:YAG laser and acid etching combination and the conventional acid etching only, as in our study.

Dental literature research about microleakage studies of fissure sealants shows variable interpretations. Firstly, Cehreli and co-workers (2) reported that there was no influence of the Er,Cr:YSSG laser etching pretreatment method on fissure sealants in primary teeth. A supportive research to Cehreli et al's (2) report found that laser irradiation alone or combined with acid etching showed higher microleakage rates (27). In our study, these results were supported particularly by SEM analysis and found that laser etching alone was not adequate for fissure sealant adaptation. However, the laser and acid etching combination (group 4) showed the same effect as the bur invasion and acid etching combination (group 1).

In our study, both Group 1 and Group 4 were not found to be statistically different when comparing the microleakage test and SEM analysis. This statistical similarity can be explained using Sungurtekin and Oztas's study (15). The Er,Cr:YSSG laser mechanism has a pulse feature, and this may create unaffected irregularly formed enamel surfaces between pulses. Following the laser irradiation, conventional acid etching may tolerate these unaffected surfaces and lead to uniformly etched surfaces similar to those resulting from acid etching.

In vivo experiments need to be tested for a better understanding of the laser etching mechanism on pits and fissures. Also, long-term effects of laser etching and its combinations should be followed.

As a result, Er,Cr:YSSG laser etching alone is not an alternative therapy to conventional acid etching. But the Er,Cr:YSSG laser and acid etching combination can be a good choice and is comparable to bur invasion from the standpoint of the high pit and fissure adaptation, with no microleakage and with pediatric patient acceptability.

Further investigations are required in this field to develop an alternative to minimally invasive treatment to replace conventional acid etching in clinical practice.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board of Ankara University (Approval Date: 14.03.2008, Approval Number: 128/5)

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept – A.İ., Z.K., S.Ç.; Design – A.İ., Z.K.; Supervision – S.Ç.; Resource – A.İ., S.Ç.; Materials – A.İ., Z.K.; Data Collection and/or Processing – A.İ., Z.K.; Analysis and/or Interpretation – A.İ., S.Ç.; Literature Search – A.İ., Z.K.; Writing – A.İ., S.Ç.; Critical Reviews –A.İ., S.Ç..

Acknowledgements: This research was supported by Near East University.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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