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Cover Page Footnote

The authors would like to thank Bezmialem Vakif University for providing permission to use the ER:YAG laser device and Mehmet Özveren for conducting statistical analysis.

ORIGINAL ARTICLE

Effect of Acid Etching and Er:YAG Laser Enamel Conditioning on the Microleakage of Glass Carbomer Fissure Sealants

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ABSTRACT

Objective: This study aimed to evaluate the effects of Er:YAG laser used alone or in combination with acid etching as surface conditioners on the microleakage of a glass carbomer fissure sealant for permanent molar teeth. **Methods:** Forty sound human permanent molar teeth were randomly divided into four experimental groups based on enamel etching methods: group I, no surface conditioning; group II, 37% phosphoric acid etching; group III, Er:YAG laser etching; and group IV, sequential laser etching and acid etching. After surface conditioning procedures were conducted, the teeth were sealed with a glass carbomer seal. The teeth were subjected to thermocycling with 2500 cycles at 5±2 °C to 55±2 °C for 24 h. Subsequent microleakage was assessed via dye penetration under a stereomicroscope. **Results:** Group II showed the lowest microleakage scores. Groups III and IV exhibited similar score distribution, which was lower than that of group I. **Conclusions:** Laser etching and acid etching decrease the microleakage probability of glass carbomer sealants, and acid etching alone provides a more suitable surface for these sealants. Sequential laser etching and acid etching have no additional benefit in comparison with acid etching alone and yield worse results than those of the other tested methods.

Key words: Er:YAG laser, fissure sealant, glass carbomer, permanent molar, microleakage

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INTRODUCTION

Modern dentistry has focused on preventive measures and turned to conservative treatments instead of invasive treatments.¹ Pits and fissures on occlusal surfaces are particularly prone to dental caries formation, and dental caries mostly develops in these areas.^{2,3} Occlusal pit and fissure sealing is a frequently used and effective method for the prevention of caries formation. Sealing prevents the accumulation of plaque microflora and food residues in fissures, buffers acids, and contributes to the remineralization of initial caries lesions.⁴ The success of fissure sealants is mainly dependent on the marginal sealing abilities of these materials. Any breach in marginal integrity can lead to the development of bacterial colonization and initial caries lesion under the restoration.⁵

Another well-known and important step to increase sealing abilities is acid etching of the enamel prior to

resin-based fissure sealant application. Physicochemical interactions between sealants and acid-etched enamel are the main forces providing sealant retention.⁶ Etching causes the production of microscopic pores on the enamel surface, and an unpolymerized sealant flows through them and hardens in tag-like projections connecting the material to the tooth structure.⁴ However, acid conditioning may cause the demineralization of enamel structures and make the enamel surface more vulnerable to caries formation.¹ The effects of different occlusal surface preparation techniques on the microleakage of fissure sealant materials have been investigated to improve the retention of sealants and to overcome the disadvantages of acid etching. Some of these techniques include diamond-bur application, air abrasion, and laser beam application.⁷

Lasers are used on hard dental tissues for various procedures, including enamel conditioning. In previous studies, erbium:yttrium aluminum garnet (Er:YAG,

wavelength = 2.94 μm) laser is suitable for cutting, ablating teeth, removing caries, and preparing cavities.⁸ Other studies have also evaluated enamel conditioning with Er:YAG laser prior to fissure sealant application.³ Er:YAG laser energy is absorbed by water in hard tissues, thereby causing rapid volume expansion with evaporation due to a significant temperature rise in the interaction zone.¹¹ Enamel surface conditioning leads to the formation of microcraters, such as porosities, on the enamel surface.¹² Laser etching provides a theoretical advantage over acid etching because it increases the resistance of the enamel to acid.¹³ The effectiveness of laser conditioning may vary in terms of different types of fissure sealants.¹⁴

Resin- and glass ionomer-based materials have been traditionally used to seal pits and fissures. For example, glass carbomer is a glass ionomer-based material containing fluorapatite as a secondary filler. The liquid in this glass is polyacrylic acid. This material, which is marketed with nano-sized powder particles, has a better retention than other materials.¹⁵ Calcium fluorapatite nanocrystals act as the core for remineralization. These particles also provide an increased surface area interacting with the glass carbomer liquid, which helps strengthen the material. Glass carbomer resembles the natural tooth enamel with time, so it becomes an esthetic material. This feature is also attributed to material mineralization. A significant advantage of this material in pediatric dentistry is its moisture tolerance that facilitates placement in children.¹⁶ In vitro evaluation models, including thermal cycling, are used to simulate oral cavity conditions, such as thermal effects and aging, and to evaluate the performance of dental materials.¹⁷

To the best of our knowledge, few studies have evaluated the microleakage of glass carbomer fissure sealants, and no studies have analyzed the effect of laser etching on the microleakage of glass carbomer fissure sealants. As such, this study aimed to examine the effects of Er:YAG laser used alone or in combination with acid etching as surface conditioners on the microleakage of the glass carbomer fissure sealant for permanent molar teeth. The tested null hypothesis was that the microleakage of the glass carbomer fissure sealant did not differ among the methods that involved no preconditioning and preconditioning with acid etching and/or laser ablation.

METHODS

Ethical approval with the decision number TUTF-BAEK 2017/129 was obtained from the Research Ethics Committee of the Trakya University Faculty of Medicine. This study was performed in compliance with the ethical standards determined by the Declaration of Helsinki and its later amendments.

Table 1. Experimental groups.

Group	N	Enamel pretreatment
I	10	No
II	10	Acid etching
III	10	Laser conditioning
IV	10	Acid etching + Laser conditioning

Sample preparation

Permanent molars with deep pits and fissures in accordance with the criteria proposed by Symons et al.¹⁸ were included in this study. Teeth with visible carious lesions were excluded. Forty sound human molar teeth extracted due to orthodontic or periodontal problems were used. Written informed consent was obtained from all the patients.

Manual scaling tools were used for debridement. A bristle brush and pumice paste were applied to clean the teeth. Afterward, the teeth were stored in distilled water for 5 days and randomly divided into four experimental groups based on the surface conditioning procedures (Table 1). All the samples were prepared by the same operator.

The groups were: Group I: No surface conditioning methods were used prior to sealant application in compliance with the manufacturer's instructions on glass carbomer seal (GCP Dental, The Netherlands). Group II: The occlusal surfaces of the teeth were acid etched with 37% phosphoric acid gel (Ultra-Etch, Ultradent Products, USA) for 20 s, rinsed for 15 s, and air dried for 10 s. Group III: The conditioning procedures of the occlusal surfaces of the teeth were performed using an Er:YAG laser system (LightWalker®, Fotona, Slovenia) operating at a wavelength of 2940 nm with a power output of 1.2 W, a pulse energy of 120 mJ, and a frequency of 10 Hz. The teeth were then rinsed and dried as described for group II. Groups IV: The teeth were sequentially laser exposed and acid etched as described for groups III and II, respectively. Then, they were rinsed and dried as conducted for Group II.

After surface conditioning procedures were performed, the glass carbomer seal (GCP Dental, The Netherlands) was activated, mixed for 15 s in a GCP CarboMix (GCP Dental, The Netherlands), extruded onto the tooth surface within 1 min from the start of mixing, and spread as a thin film to ensure that no air bubbles were included. With the help of a cotton pellet, a thin layer of GCP gloss (GCP Dental, The Netherlands) was applied over it and cured using an LED light curing unit (VALO LED Curing Light, Ultradent Products, USA) with an output of 1400 mw/cm² for 60 s.

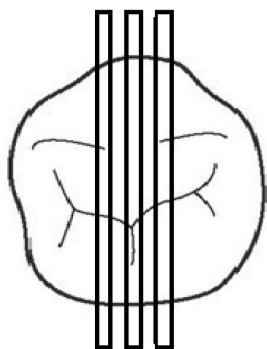


Figure 1. Schematic of tooth sectioning

Table 2. Microleakage scoring criteria

Score	Definition
0	No dye penetration
1	Dye penetration up to half of the fissure
2	Dye penetration beyond half of the fissure without total involvement
3	Dye penetration to the sealant base

Microleakage analysis

After the sealing procedure was conducted, the teeth were placed in deionized water at 37 °C, and thermocycling was performed for 2500 cycles at 5±2 °C to 55±2 °C for 24 h with a dwell time of 30 s and a transfer time of 10 s. Dye penetration was selected to assess the microleakage, and 0.5% basic fuchsin was chosen for this procedure. The teeth were coated with nail varnish, and a 2 mm window was retained around the sealant. The roots were embedded in an acrylic resin block (Meliodent, Bayer Dental, UK), and the teeth were held in a dye solution for 24 h. Afterward, the teeth were rinsed with tap water for 5 min to remove the dye residues and sectioned in the buccolingual direction by using a water-cooled diamond saw (Mecatome T180, Presi, France) to obtain three slices (Figure 1). Each of the slices was then examined by two blind investigators under a stereomicroscope (SMZ 800, Nikon, Japan) at 40× magnification and scored in accordance with the criteria listed in Table 2.² Lastly, the microleakage of each tooth specimen was recorded by calculating the mean microleakage values of the three sections.

Intraobserver and interobserver reproducibilities were evaluated on the basis of 15 randomly selected tooth slices that were re-evaluated after an interval of 2 h.

Scanning electron microscopy

One sample from each group was randomly selected, and scanning electron microscopy (SEM) was carried out on the central slice of the three obtained slices of the samples. These slices were attached to carbon tabs on a Zeiss EVO LS10 electron microscope with an accelerating voltage of 20 kV with a magnification of 1000×.

Statistical Analysis

Intraexaminer and interexaminer reproducibilities were analyzed with Cohen's kappa statistic. Differences between groups for microleakage score distributions were tested using Chi-square statistic. $P < 0.05$ was accepted as statistically significant.

RESULTS

Microleakage analysis

The results of intraobserver and interobserver reproducibility tests were almost completely in agreement with Landis and Koch's guidelines (K:0.903 for intraobserver and K:0.803 for interobserver).¹⁹

The distributions of microleakage scores for each experimental group are listed in Table 3. Group II showed the lowest microleakage scores. Groups III and IV exhibited similar microleakage score distributions, which were lower than those of group I.

The presence of microleakage is shown in Table 4. Group II showed no microleakage in 66.7% of the sections, and this percentage was significantly better than that of the other groups. In Group I, 6.7% of the sections were microleakage free. By comparison, 23.3% of the sections in groups III and IV were microleakage free.

Light microscopy

The representative light microscopy images of the groups with the highest and lowest microleakage scores are shown in Figure 2.

Scanning Electron Microscopy

The SEM images showing the sealant–enamel interface for each group are presented in Figure 3. They revealed a more relatively irregular surface pattern in laser-exposed groups (groups III and IV) than in the exclusively acid-etched group (group II).

DISCUSSION

The tested null hypothesis was partially rejected except the differences between preconditioning with laser-etched and sequentially laser- and acid-etched groups. The microleakage scores of laser etching, acid etching, and sequential laser etching and acid etching were lower than those of the group that included no preconditioning methods.

The sealants provided a physical barrier that prevented the bacterial passage and colonization of cariogenic microorganisms. The effectiveness of pit and fissure sealants depends on various factors, but microleakage is accepted as the most detrimental one.^{16, 20} Glass carbomer sealant is a moisture-tolerant material that

Table 3. Microleakage scores according to enamel conditioning

Score	0	1	2	3	Chi-square significance	Median	Mean±SD
Group I	2	11	10	7	A	2	1.7±0.9
Group II	20	6	4	0	C	0	0.5±0.7
Group III	7	15	7	1	B	1	1.1±0.8
Group IV	7	16	6	1	B	1	1.0±0.8

Different capital letters indicate significant differences among the groups ($p < 0.05$).

Table 4. Microleakage presence according to enamel conditioning

Score	No	Present	Chi square significance
Group I	2 (6.7%)	29	A
Group II	20 (66.7%)	10	B
Group III	7 (23.3%)	23	A
Group IV	7 (23.3%)	23	A

Different capital letters indicate significant differences among the groups ($p < 0.05$).

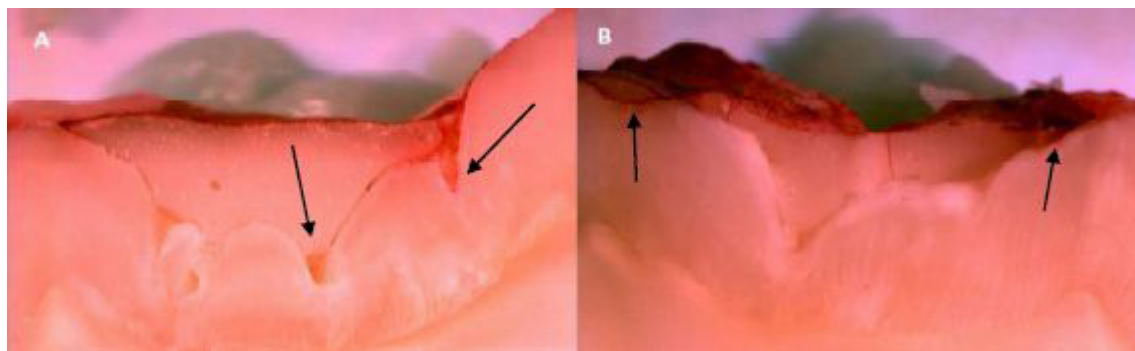


Figure 2. Light microscopy images of (A) control (highest microleakage) and (B) acid-etched (lowest microleakage) groups

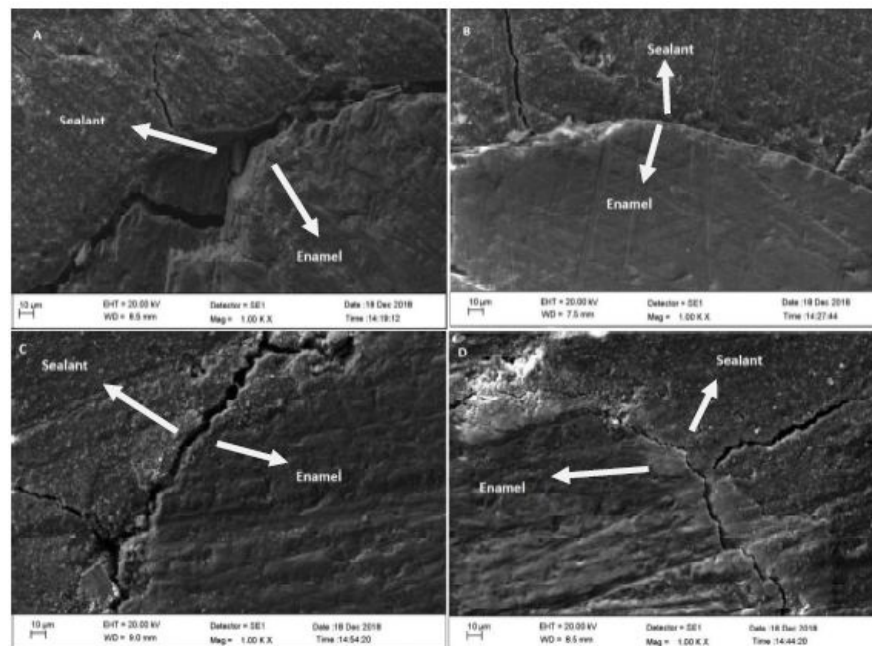


Figure 3. SEM images of (A) control, (B) acid-etched, (C) laser-etched, and (D) laser- and acid-etched groups

has not been studied for enamel preconditioning. Therefore, it was chosen for this *in vitro* study.

The *in vivo* evaluation of dental materials has some difficulties, so *in vitro* evaluation models have been

developed to simulate oral cavity conditions, such as thermal effects, water aging, and chewing forces.¹⁷ In the present study, an *in vitro* model was used, and all the samples were aged through thermocycling. Thermocycling was implemented because sealants

have one of the highest coefficients of thermal expansion among dental materials, and changes in temperature in the oral cavity can affect the bonding interface that can cause microleakage.²¹ However, no standardized thermal cycling protocol has been established. Temperature is typically set at 5 °C and 55 °C, but dwell time (ranging from 15 s to 120 s) and the number of cycles (ranging from 100 to 100,000) widely vary in experimental studies. Gale and Darvell²¹ suggested that 10,000 cycles correspond to 1 year of aging, but fewer cycle numbers have been chosen in most studies; as such, many studies have disagreed with their proposal. Therefore, in the present study, all the samples were subjected to 2500 cycles for the thermally aging procedure.

Microleakage was measured with several techniques, including dye penetration, bacterial, chemical, and radioactive markers, compressed air application, electrochemical investigations, SEM, and micro-CT imaging.²² In the present study, dye penetration was conducted to test *in vitro* microleakage because this method is easy, cheap, and nontoxic. Several dyes are used for this method, but the type of dye plays a negligible role except methylene blue, which is unstable at room temperature and under exposure to ambient light.²³ Furthermore, 0.5% basic fuchsin solution with 24 h immersion time is the mostly used one, as in this study.^{2, 3, 14, 15, 24}

Dye penetration has been criticized because it is not considered a standardized test. The clinical relevance of *in vitro* microleakage tests is also questionable.²³ Microleakage refers to the ingress of oral bacteria, which have an approximate diameter of 0–5 µm, but sizes of dye traces are 1–2 nm.¹⁰ Therefore, dye leakage tends to be a severe test for microleakage.²⁵ Nevertheless, leakage can provide useful information on a dental sealant's capacity to maintain good marginal adaptation and the effects of etching primers on the microleakage of pit and fissure sealants.²³

Few studies have explored the microleakage of glass carbomer sealants. Submaranian et al.¹⁶ investigated a glass carbomer sealant without any enamel preconditioning method by using milder thermocycling parameters and 5% methylene blue for dye penetrance. They reported that the dye penetrated two-thirds of the fissure depth of the majority of the specimens.¹⁶ In our study, the median microleakage score of the no preconditioning group (group I) was 2, which implied that the dye penetrated more than half of the fissure without a total involvement of the majority of the specimens. This finding was consistent with that of Submaranian et al.¹⁶

Cehreli et al.¹⁵ investigated the microleakage of glass carbomer cement in primary teeth without using any preconditioning methods. They found that surface coating significantly lowers the microleakage of

glass carbomer cement. They also reported similar microleakage of a glass carbomer, a conventional GIC with surface coating, and a compomer without surface coating. In our study, the surface coating gloss in all our groups was used in accordance with the manufacturer's instructions, and acceptable results were acquired when these instructions were combined with enamel conditioning methods.

Chen et al.²⁶ investigated a glass carbomer sealant applied to samples with micro-CT. They used stiffer thermocycling parameters and reported fracture lines and cracks in all the samples. However, they also found that the glass carbomer remains viscous after it is cured and suggested that this viscosity is the potential cause of the unexpected result. Our SEM images also revealed cracks and fracture lines to the applied glass carbomer sealant, but the sealant enamel interface was maintained. The interface of the exclusively acid-etched group was relatively smoother and its contact was better than those of the laser-etched groups, which showed gaps of several microns at some sites of the restoration interface.

Our results implied that laser etching and acid etching decrease the microleakage probability of glass carbomer sealants. The results of acid etching alone were better than those of laser etching alone or sequential laser etching and acid etching. Acid etching increases the sealing strength for resin-based fissure sealants, but laser etching alone or its combination with acid etching yield conflicting results.⁶ Lasers have the following possible advantages over acid etching: laser etching provides resistance to acid attacks, does not cause demineralization, and does not need avoidance of moisture contamination.^{2, 14, 24, 27} However, some studies have shown no benefits of laser etching to microleakage and suggested that 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.² 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.^{1, 3} These differences occurred likely because sealant materials had hydrophilic components that seemed to benefit from laser enamel conditioning. More viscous materials adapt poorly to the enhanced roughness of lased enamel, and this characteristic may account for conflicting findings.^{14, 24} In other studies, the same sealants are used, but contradictory results are still obtained.^{1, 27} These differences may be due to variations in the chosen *in vitro* aging methods and parameters, microleakage assessment models, laser devices, and parameters used.

Further *in vitro* and *in vivo* studies should be performed to show the sealing ability of glass carbomer materials with and without enamel preconditioning. Standardized thermocycling and microleakage evaluation models

should be developed to reliably compare and discuss different studies. The physicochemical interaction of laser-conditioned enamel and different sealant materials should also be investigated, and optimal laser etching parameters and tip sizes should be validated.

CONCLUSION

This study is the first to investigate the effect of Er:YAG laser and acid conditioning on the microleakage of a glass carbomer sealant. Acid conditioning can be applied to enhance the sealing success of glass carbomer sealants and significantly cause a lower degree of microleakage than laser etching or sequential laser and acid etching do. SEM reveals the cracking of the sealant body in all the groups and more porosity at the interface of the laser-etched groups than that of the other groups.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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