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The effect of different enamel surface treatments on microleakage of fissure sealants

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Objective. The aim of this study was to evaluate the effects of different techniques of surface treatment on the microleakage of fissure sealants in permanent molar teeth in vitro. Materials and methods. 96 extracted impacted human third molars were randomly divided into 8 surface treatment groups (n=12/group) as 1. Er: YAG laser; (Fidelis II, Fotona, Ljubljana, Slovenia) (125 mj, 20Hz). 2. Er: YAG laser + 37% H₃PO₄ (15s); 3. ER: AG laser + 37% H₃PO₄+Prime&Bond NT; 4. Er: YAG laser + G Bond; 5. Er: YAG laser + Prime&Bond NT; 6. 37% H₃PO₄; 7 37% H₃PO₄ + Prime &Bond NT; 8. G Bond. Sealant material (Clinpro, 3M ESPE, Seefeld, Germany), was applied into the fissures and light-cured for 20s with LED (Bluephase C5, Ivoclar-Vivadent, Schaan, Liechtenstein). Specimens were subjected to thermocycling (1000×, 5-55°C, dwell time: 15s) and immersed in 0.5% basic fuchsin solution for 24h at 37°C. The samples were sectioned and scored on a 3 point rating scale using a light microscope with a magnification of ×20. One-way analysis of variance was used to analyze data. Multiple comparisons were analyzed using Bonferroni test (p=0.05). Results. Er:YAG laser showed the highest microleakage scores whereas Er YAG laser + 37% H₃PO₄ showed the lowest. Although 37% H₃PO₄ group showed higher scores than Er:YAG laser + 37% H₃PO₄, the difference was not statistically significant. Conclusion. Etching fissures with phosphoric acid is sufficient prior to fissure sealant application

Key words: Fissure sealant, Laser, Preventive dentistry.

Introduction
Pit and fissure sealants constitute one of the preventive interventions in paediatric dentistry. Their effectiveness for caries management on occlusal surfaces has been documented in numerous clinical studies (1-3). However, their preventive benefits rely directly upon the ability of the sealing material to thoroughly fill pits and fissures so that it remains completely intact. Subsequently, caries development underneath the sealant restoration is avoided (4). However, undetectable caries in fissures and saliva contamination prior to sealant application are two main problems that can lead to poor bonding to enamel. An invasive approach of widening the fissures before sealing application helps the sealant retention (5). Yet, purposeful removal of enamel in a sound tooth may disturb the equilibrium of the fissure system and expose the patient to drilling (6, 7). The latter problem, saliva contamination, is frequently faced after the pretreatment of
enamel with phosphoric acid to create microporosites for retention. This could occur at the very critical step, when the cotton rolls are changed after rinsing off the etchant. When the microporosites are coated with saliva, the retention and effectiveness of the fissure sealants are jeopardized (8). Several studies have shown the benefits of adding a bonding agent layer between the etched enamel and the sealant to increase the bond strength in the face of moisture and salivary contamination (9, 10).

Currently, various enamel treatment procedures are still under discussion for the optimization of fissure sealant penetration. In recent years there has been significant progress in the use of lasers in dentistry. Lasers can be a useful device for dental care in children, particularly for those with dental fear by eliminating stressors such as the sight and sensation of a drill (11). Laser etching does not require tooth isolation. The lasered enamel surface becomes fractured and uneven, which helps the adhesion of resin based materials. Furthermore, laser produces an acid resistant surface, which could avoid secondary caries formation (11-13). With all these beneficial features, this technique seems to be promising in overcoming the problems faced during fissure sealant application. To date, laser etching of fissures prior to fissure sealant application has been investigated in various studies (14-19). However, it is still questionable whether laser etching alone eliminates the need for acid etching of the enamel surface prior to placement of fissure sealants. There is scarce information about the use of laser pretreatment in combination with acid etching and bonding agents in dental literature. Microleakage under fissure sealants is only one of the several ways of assessing the success or failure.

Therefore, the aim of the present study was to assess the effectiveness of different enamel surface treatments on microleakage of fissure sealants applied to sound permanent molars in vitro. The hypothesis of the study was that lasered and/or bonded fissure sealants showed less microleakage than sealants prepared with the acid etching technique.

**Materials and methods**

Recently extracted sound impacted third molars were kept in 0.2% sodium azide solution at a temperature of 4°C prior to the study. The fissures were cleaned with a pumice, using a soft brush and air-water jet. Subsequently, fissures were examined at 20× under a dissecting microscope to exclude ones with cracks, structural defects or incipient caries lesion. A total of 96 teeth were assigned randomly to eight groups (n=12/each) (Table 1), in which different pretreatments were carried out according to the manufacturer’s instructions (Table 2).

In the laser groups, occlusal fissures were irradiated with an Er: YAG laser (Fidelis II, Fotona, Ljubljana, Slovenia). Before operation the power output was set at 2.5 W. The pulse energy was set at 125 mj and the repetition rate was 20 Hz. The laser beam was delivered in non-contact mode with the hand piece positioned perpendicularly to the fissures.

After each of the pretreatments, the sealant (Clinpro, 3M ESPE, Seefeld, Germany) was applied. The sealants were polymerized using a curing light (Bluephase, Ivoclar Vivadent, Schaflatt, Switzerland). The microleakage was assessed under an optical microscope (20×) after 7 days. The teeth were divided into three groups according to the sealant type: group 1 (Clinpro), group 2 (Clinpro + Bond), and group 3 (Clinpro + Bond + Light Cure). The results were statistically analyzed using ANOVA and Tukey’s post hoc test.

**Table 1** Different pretreatment of fissures prior to sealant application

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Er: YAG laser irradiation</td>
</tr>
<tr>
<td>2</td>
<td>Er: YAG laser irradiation + 37% orthophosphoric acid etching</td>
</tr>
<tr>
<td>3</td>
<td>Er: YAG laser irradiation + 37% orthophosphoric acid etching + Prime &amp;Bond NT</td>
</tr>
<tr>
<td>4</td>
<td>Er: YAG laser irradiation + G Bond</td>
</tr>
<tr>
<td>5</td>
<td>Er: YAG laser irradiation + Prime &amp;Bond NT</td>
</tr>
<tr>
<td>6</td>
<td>37% orthophosphoric acid etching</td>
</tr>
<tr>
<td>7</td>
<td>37% orthophosphoric acid etching + Prime &amp;Bond NT</td>
</tr>
<tr>
<td>8</td>
<td>Self etching adhesive (G Bond)</td>
</tr>
</tbody>
</table>

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(many) was applied into the fissures with a tip syringe and spread with a dental probe to prevent air entrapment. The sealant was light-cured for 20 s using LED (Bluephase C5, Ivoclar Vivadent) with an output of 500 mw/cm². All teeth were treated by the same operator. After light curing, the specimens were subjected to thermocycling. The teeth were subjected to a thermocycling regimen of 1000 cycles between 5°C and 55°C water baths. Dwell time was 15 seconds with a 10 second transfer time between baths. Thereafter, microleakage was assessed by the conventional dye-penetration method. The apices of the teeth were covered with composite to avoid dye penetration, and after that, the whole tooth surface, apart from the area within 2 mm of the sealant varnish interface. Specimens were then immersed in 0.5% basic fuchsin solution (Wako Pure Chemical Industry; Osaka, Japan) for 24 h at 37°C. Afterwards the specimens were rinsed thoroughly with water and had their roots cut out using a diamond bur. The sealed crowns were embedded in self-curing acrylic resin and sectioned along the bucco-lingual direction through the mesial, central and distal fissures, resulting in four tooth fragments with six section sides available for inspection. Microleakage was scored by two blinded independent observers, using a light microscope with magnification of × 20 (Leicamicrosystems stereo microscope, Ltd. Stereo and microscope systems; Heerburg, Switzerland). Microleakage per section side was scored on a 3 point rating scale. Score 0 indicated no microleakage visible, score 1 revealed microleakage in up to half of the fissure, while score 2 meant microleakage reaching more than half of the fissure. In case of disagreement between the observers, a third independent observer was consulted to make the final decision. To determine the intra-examination reliability, 10 randomly selected molar fragments were re-evaluated for microleakage. The intra kappa value was found to be 1 by both of the examiners.

### Statistical analysis

Statistical analysis was carried out by using Statistical Packages for Social Sciences (SPSS) 15.0 for Windows. One-way analysis of variance was used to compare the microleakage measures of different groups. Multiple comparisons between groups were analyzed using the Bonferroni test. Statistical analysis was conducted at a significance level of p<0.05.

### Results

The mean microleakage scores and standard deviations of all groups are given in Table 3. No tooth section was lost during the preparation of the specimens.

All the groups demonstrated microleakage regardless of the surface pretreatments. The lowest microleakage values were obtained in group 2 (laser etching in combi-
The highest microleakage scoring was observed in group 1 (laser etching). Based on the levels of statistical significance, the following ranking was achieved in terms of lowest to highest microleakage values: Laser + acid etching = laser + GBond = laser + acid etching + Prime&Bond NT = acid etching < acid etching + Prime&Bond NT = GBond = laser ("<" denotes significantly lower value at p<0.001; and "=" denotes no significant difference at p>0.05).

**Discussion**

Microleakage in fissure sealants is often faced, unless the material is handled properly and saliva contamination is controlled (20). The latter problem derives from the sensitivity of the technique of placement of fissure sealants (21). The multi-step requirements such as drying, acid etching, rinsing and drying again increase the saliva contamination risk. Many pretreatment protocols have been developed to overcome this problem. One of them was the bonded sealant technique which supported the idea of using an adhesive system prior to sealant applications (22, 23). Both in vitro and in vivo studies substantiated the benefits of use of adhesive materials as an intermediate layer under the sealant materials (9, 10). With the introduction of self-etching adhesives, this technique has become even more attractive due to the elimination of the separate etching, rinsing and drying steps. Moreover, a reduction in chair time is another advantage for dentists, especially in treating uncooperative children (24).

Laser etching is another alternative to be used with fissure sealant application. It leads to the formation of more stable and less acid soluble compounds, thus reducing susceptibility to secondary caries (25, 26). In addition, enamel treatment with laser irradiation is claimed to facilitate the receptiveness to adhesive procedures (27, 28). However, in the dental literature there is scarce information about the combined use of laser etching and adhesive systems prior to the placement of resin based sealant material. Microleakage tests are useful methods to evaluate the sealing performance of adhesive systems (29). Dye penetration measurements are the most commonly used techniques. However, in the dental literature, microleakage studies are often quite incomparable due to the different study designs and dye materials used. Various particle sizes of dye materials can affect the dye penetration between the enamel and the resin material, leading to different microleakage results (17). Moshonov et al. (30) reported no microleakage in both laser etched and acid etched samples. They concluded that 1% methylene blue dye solution might have produced these results because the particle sizes were bigger than other solutions, such as 0.2% rhodamine, 50% silver nitrate or 0.5% basic fuchsine solutions. In the present study, 0.5% basic fuchsine solution was used to overcome the dye penetration problem. Three parallel cuts were made in the bucco-lingual direction through the mesial, central and distal fissures, resulting in four molar fragments with six section
sides available for inspection, to increase the reliability of measurements. To date, comparative studies regarding laser etching versus acid etching have yielded conflicting results (30-33). Our results are in line with studies reporting that laser etching does not eliminate the necessity for additional acid etching (33, 34), in contrast to those reporting similar etching performance (30). Within the limitations of these in vitro conditions, the current study showed that laser etching alone performed the worst, whereas laser etching in combination with acid etching performed the best among all groups.

However, conventional phosphoric acid was found sufficient to etch the enamel surface to create the required retention of the resin based fissure sealant material.

Based on our results, lasered v. non-lasered fissures prior to application of a total etch adhesive system did not make a significant difference in terms of microleakage. Our finding was supported by Cehreli et al. (35). They reported that the use of Er, Cr: YSGG laser prior to bonded fissure sealant application did not improve microleakage resistance (35). However, laser etching in combination with self-etching adhesive provided less microleakage compared to self etching alone. This may be explained by previous studies, stating the lower bond strengths, greater microleakage and shallow etching patterns of self-etching adhesives (20, 22, 36, 37). It may be suggested that laser etching might promote stronger resistance to microleakage of self etching adhesives.

Conclusion

Within the limitations of the present study, etching fissures with phosphoric acid was sufficient prior to fissure sealant application. Further studies are required to test the effectiveness of laser pretreatments in preventing microleakage and secondary caries formation under resin based sealant material.

Authors' contributions: Conception and design: ATA, OO, BG, BB; Acquisition of data: ATA, OO, BG, BB; Analysis and interpretation of data: ATA, OO, BG, BB; Drafting the article: ATA, OO, BG, BB; Revising it critically for important intellectual content: ATA, OO, BG, BB.

Conflict of interest: The authors declare that they have no conflict of interest.

References


