Laser tooth preparation for pit and fissure sealing

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Abstract

Background Considerable evidence shows that sealing dental pits and fissures contributes to caries prevention in children and adolescents. Various approaches are available for pit and fissure sealing. Sealants can be inserted with or without mechanical preparation; etching can be applied, with or without bonding. Lasers can be used as an alternative for mechanical preparation. In this study, we systematically evaluated the retention and micro-leakage of pit and fissure sealants, comparing mechanical preparation and Er:Yag laser enamel preparation. For each of these preparations we compared sealants without etching to those with etching, with and without bonding.

Methods Sixty extracted sound third molars were classified into 6 groups: A. bur mechanical preparation and sealant application; B. bur mechanical preparation, etching and sealant; C. bur mechanical preparation, etching, bonding and sealant; D. laser mechanical preparation and sealant; E. laser mechanical preparation, etching and sealant application; F. laser mechanical preparation, etching, bonding and sealant. Pit and fissure sealing were evaluated by assessing the retention and micro-leakage of the sealant.

Results All the groups showed dye micro-leakage beneath the sealants. Less micro-leakage was observed for the groups that used bur (groups A, B, C) than laser (groups D, E, F): 41 versus 44 specimens. The number of specimens without micro-leakage decreased as follows: group E (24), group A (18), groups B and F (17), group C (14) and group D (5). The difference in penetration between group D and the other groups was statistically significant, p<0.001, chi square.

Conclusion Mechanical preparation is important and increases the retention of sealants, especially when using etching material; bonding can help the retention. We recommend preparing sealant with laser, without etching and bonding before its application.
Background

Dental pit and fissure sealing is considered an effective noninvasive approach to preventing caries in children and adolescents. Differences reported in effectiveness may be due to differences in caries prevalence in the populations examined. According to a Cochrane review published in 2013, sealing of the occlusal surfaces in permanent molars in children and adolescents reduces caries for up to 48 months when compared to no sealing.\(^1\) Further, a number of other publications recommended sealants for people with several caries, with high caries risk assessment, deep narrow pits and fissures of deciduous and permanent molars and premolars, and non-cavitated lesions.\(^2-6\) In their review of the literature, Beauchamp and his colleagues reported that sealants can reduce caries by 59-96%, during follow-up periods of 1-9 years.\(^5\)

Studies with longer follow-up showed reduced quantity and quality of evidence.\(^1\)

Over 85% of the lesions in the permanent dentition involve surfaces with pits and fissures, despite the availability of preventive measures such as sealants.\(^7\) Molars and premolars are the teeth most susceptible to caries development, due to their occlusal surface morphology, which limits both saliva and toothbrush mechanical cleaning. Pits and fissures are therefore the areas most prone to caries and need particular protection to prevent carious lesions.\(^2\)

Sealants are composed of various materials and are inserted by a number of techniques. Resin, one of the most common materials used for dental sealant, undoubtedly contributes to preserving the integrity of the occlusal surface and acts as an effective mechanical obstacle for plaque retention, thereby reducing the incidence of fissure caries.\(^8-10\) The efficacy of sealing procedures depends on correct application technique. A number of operative protocols are suggested in the literature, which prolong protection against
For example, sealant can be inserted without any mechanical preparation, only using etching (with or without bonding). Alternatively, sealant can be inserted using mechanical preparation, via air abrasion and other protocols. For sealants placed on maxillary molar teeth, the retention rate was higher when the insertion involved mechanical preparation.\textsuperscript{11}

Lasers can be used as an alternative for mechanical preparation (enameloplasty), before sealing pits and fissures. One study reported that Er:YAG laser was perceived as more comfortable and preferred by adolescents for carious tissue removal, compared to conventional mechanical preparation.\textsuperscript{12} Evidence shows a higher sealing outcome when bur preparation, acid etching, or air abrasion is performed prior to application of the sealant.\textsuperscript{13} Adding a bonding agent layer between the sealant and the enamel that was contaminated with saliva was shown to increase bond strength and retention of resin sealants, and possibly to improve the success of all sealant applications.\textsuperscript{14}

The relative effectiveness of the available approaches for enamel sealing has yet to be established. The aim of the present study was to assess retention and micro-leakage of pit and fissure sealants following various methods of preparation and application of materials. We hypothesized that less retention and more micro-leakage would be attained following a protocol based on laser enamel preparation, yet without the use of etching or adhesive material prior to sealant placement.

**Methods**

The protocol of the study was approved by the institutional Helsinki Committee for Human Clinical Trials (Identifier: 0483-15-HMO). Sixty sound extracted third molars (were originally acquired from the department of oral maxillofacial surgery) and were stored in saline solution, then cleaned with water and a soft brush (low speed air motor hand
The apices were sealed with self-cured glass ionomer. Teeth were arbitrarily assigned to six equal groups of ten specimens each (Figure 1). The groups included the following preparation protocols: (A) mechanical preparation with high speed bur (330 tungsten burr) and sealant application; (B) mechanical preparation with high speed bur (330 tungsten), etching and sealant application; (C) mechanical preparation with high speed bur (330 tungsten), etching, bonding and sealant application; (D) mechanical preparation with Er-YAG laser and sealant application; (E) mechanical preparation with Er-YAG laser, etching and sealant application; and (F) mechanical preparation with Er-YAG laser, etching, bonding and sealant application.

The sealant application was performed by homogenous spreading of the material using a dycalon and then polymerization for 20 seconds. Etching was performed for 20 seconds, followed by 10 seconds washing and 5 seconds drying using a triple syringe. Two layers of bonding were applied and followed by 5 seconds of air application using a triple syringe.

All materials and instruments used in the present study are listed in Table 1.

Retention of the sealants was tested with a dental explorer by four specialists in paediatric dentistry who were blind to the groups of the teeth examined. Results were recorded as total retention, partial loss or total loss. Sealant failure was defined when the material was partially or totally lost, as previously described. To assess inter-examiner reliability (kappa between examiners), before conducting the experiment, each examiner evaluated retention of the same 10 teeth.

The teeth were then subjected to 500 thermo-cycles in water baths with a temperature in the range of 7-55°C (the duration of each bath was 30 seconds and the time between baths was 5 seconds). The teeth were dried, then sealed entirely with three layers of nail varnish, separate from the sealant and 1mm beyond the sealant margins. All teeth were immersed in 1% methylene blue solution for 24 hours to allow dye to penetrate into
possible gaps between the tooth substances and the sealant. After the teeth were removed from the dye, the nail varnish was removed using a sharp instrument and the specimens were dried, and embedded in self-cured acrylic resin. The teeth were each divided into three longitudinal bucco-lingual sections with a water-cooled diamond saw. Retention and micro-leakage were evaluated: (1) clinically, using a dental explorer; (2) using a Stereo Microscope (NIKON SMZ25) for examination of the teeth, and micro-leakage penetration depth was recorded in micro-millimeters; and (3) by computer measurements of the photographic depiction of the dye penetration.

Dye penetration was evaluated in the enamel and sealant interface. The length of the sealant and the length of the dye penetration were recorded, and the percent of dye penetration was calculated.

Statistical analysis

All statistical analyses were performed using SASâ™ software (SAS Enterprise Guide 7.1). Descriptive statistics were performed to assess differences between preparation methods. Differences between the methods were assessed by applying Fisher’s exact test to evaluate the presence or absence of micro-leakage (categorical parameter). A general liner model was used for one-way analysis of variance (ANOVA) of multiple comparisons of the means of continuous parameters (length of the sealant, length of dye penetration and percent of dye penetration) between preparation methods. Bonferroni multiple comparison tests were used to determine statistically significant differences between all methods. The level of significance for all tests was set to 0.05.

Results

In all groups except for group D, retention of the sealants was scored as 100%. Group D was characterized by mechanical preparation with Er-YAG laser and sealant application. Nine of the teeth in this group were scored as total retention and one tooth was scored as
partial sealant retention. Between the four examiners, all the evaluations were the same, thus yielding 100% inter-examiner reliability.

Microscope depiction demonstrated dye penetration in all 6 groups. From 60 teeth, 180 specimens were prepared. Of them, 95 were without penetration at all, and 85 were with partial penetration, including some with total penetration of the dye beneath the sealants. No statistically significant differences were observed between groups A, B, C, E and F. However, a statistically significant difference was found in dye penetration between the preparation methods; the highest dye penetration was found in group D, compared to the other 5 groups (p-value<0.0001).

Retention of the sealants was not found to differ significantly between the groups with high speed bur preparation (groups A+B+C) and those with Er:YAG laser preparation (C+D+E), p-value =0.20. However, when high speed mechanical preparation was used, dye penetration was less beneath the sealant: 46 versus 49 teeth. Penetration of the dye from the highest to the lowest group was in the following order D>C>F/B>A>E (table 2).

Figure 2 depicts the mean percent of dye penetration for each group. For groups A and B, penetration of the dye was 30% of the length of the sealant; in group C, 20%; and in groups E and F, 10%. The worst dye penetration was in group D, with a mean of 70%.

Table 3 shows results of the Bonferroni multiple comparison test, which indicates statistically significant differences in dye penetration between the preparation methods (p-value<0.0001). Treatment D demonstrated the highest dye penetration. Differences between the other preparation methods were not statistically significant (p-value>0.05).

Figure 3 shows a specimen of each group, with the dye leakage beneath the dental sealant. The length of sealant was 1019.98 µm, 1639.55 µm, 3344.3 µm, 1859.74 µm, 2502.92 µm and 2149.77 µm, for specimens A-F, respectively. The percentages of penetration were 100% in groups A, B and D; 39.3% in group C; 78.2% in group E; and
Discussion

The American Academy of Paediatric Dentistry and the American Dental Association concluded that dental sealants are underused by practitioners in the prevention and treatment of early stages of dental caries.\textsuperscript{15} Accordingly, they published guidelines that strongly recommend the application of sealants on the occlusal surfaces of primary and permanent teeth. Resin-based sealant showed better retention than did other materials. The exception to this was the application of glass-ionomer sealants for erupting teeth, thus serving as temporary sealants until full exposure of the teeth. The guidelines state that sealants should be placed according to the instructions of the manufacturer of the material used. This includes the recommendation of etching the surface before application of the resin-based material and the use of enameloplasty or adhesive materials.\textsuperscript{15} Enameloplasty and adhesive materials applied prior to the sealant increase the retention of the sealant and decrease the micro-leakage between the sealant and the pit. However, some reports concluded that mechanical preparation was not beneficial.\textsuperscript{5,16} With regard to primer placement before sealant application, one randomized clinical trial found that acetone or ethanol solvent-based primers, especially the single bottle system, enhanced the retention of sealants, whereas water-based primers drastically reduced the retention of sealants.\textsuperscript{17} With regard to self-etch bonding agents that do not involve a separate step for etching, a systematic review concluded that self-etch bonding agents may provide a lower quality of retention than the acid etch technique.\textsuperscript{18} However, a randomized clinical trial reported similar retention rates of self-etching and acid etching.\textsuperscript{19} Hence, adhering to the manufacturer’s instructions for each sealant material is important.\textsuperscript{20} Interestingly, we found lower retention of sealant following mechanical preparation with
Er-YAG laser, without etching and bonding (90%), compared to other protocols of mechanical preparation. The differences between the groups were not statistically significant, except for the difference between the group with laser mechanical preparation and sealant, and the group with the highest micro-leakage. This supports our hypothesis of less retention and more micro-leakage following a protocol that did not include etching or adhesive material prior to sealant placement. Retention of the dental sealants that were placed on sound teeth was high in all groups, and our findings regarding sealant retention are in agreement with previous in-vitro and in-vivo studies.\textsuperscript{21-23}

A possible explanation for our findings relates to the lesser penetration of the sealant into the enamel prisms when using phosphoric acid. From this we can assume that without the use of etching material, the sealant material would harden better after enameloplasty with bur than with Er:YAG laser.\textsuperscript{24}

We observed more micro-leakage following the use of Er:YAG than bur (44 vs. 41). This concurs with the research of Borsatto and colleges and Yossef et al.\textsuperscript{8,25} In both these studies, as in ours, the protocol consisting of Er-YAG laser, without etching and bonding was the only one without full retention. We suggest that that this may be due to insufficient ablation by the laser of the tooth surface and the lack of extension of the enamel prisms. In contrast, etching increases retention of the sealant. Therefore, we do not recommend using this protocol, and advocate the use of etching.

Of note, Topaloglu-Akand and colleagues\textsuperscript{26} reported leakage with all the protocols they examined. However, they concluded that only etching without enameloplasty is sufficient. In contrast, Javadinejad\textsuperscript{27} and colleagues found that mechanical preparation with pre-sealant application does not improve the mechanism of the adhesive. Nevertheless, they upheld their support for the use of etching. Memarpour and colleagues showed less micro-
leakage in the group treated with Er-YAG laser than following etching, bonding and sealant application. However, they did not find a statistically significant difference between bur mechanical preparation with and without bonding. Thus they concluded that Er:YAG laser can be an alternative method before sealant application.28

Our findings contrast with those of Nahvi and colleagues.29 They reported significant reduction in micro-leakage with the use of a pre-sealant bonding agent. Elsewhere, the bonding agent beneath the sealant was shown to increase the bond strength even in moisture-contaminated conditions, and to decrease micro-leakage.30 This is similar to our findings, though our results did not show statistical significance. Notably, Tehrani et al recommended the use of a combination of etching and bonding agents prior to sealant placement, and concluded that this is the best technique for sealing pits and fissures.31

Conclusions

This systematic evaluation of sealants showed high performance and no statistically significant differences between high speed mechanical preparation using laser and all other protocols examined. Nevertheless, the technique of laser mechanical preparation without etching and bonding is not recommended.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study was approved by the committee on research involving Human Subjects of the Hebrew University- Hadassah Medical School Jerusalem Israel (0483-15)
Consent for publication
Not applicable

Availability of data and materials
Not applicable

Competing interests
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Abbreviations
Er:YAG laser-erbium-doped yttrium aluminium garnet laser, erbium YAG laser

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Not applicable

Authors' contributions
YS collected data, carried out the initial analyses, and reviewed and revised the manuscript.
NB conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript.
DR conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript.
ED conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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**Tables**

*Table 1.* Materials and instruments used in the present study.
| Company                                           | Instruments / Materials |
|--------------------------------------------------|-------------------------|
| Clinpro™ Sealant (3M™ ESPE™, Minnesota, USA)     | Sealant                 |
| Best-Etch™ 37% Phosphoric Acid Etch               | Etching                 |
| Single Bond Universal (3M ESPE St Paul, MN, USA)  | Bonding                 |
| Syneron Dental Lasers (Opus 20 by Opus Dent, Netanya, Israel) with 7.5w; 300mj; 25Hz per pulse and 12 pulses per second | Er:YAG LASER            |
| ADA HEALTH FOINDATION                            | Thermocycling           |
| Unifast trad® (UT), Tokuyama® Rebase II Fast (TR), Ufi gel hard® (UG) | Self-cured acryl resin  |
| NIKON SMZ-25                                     | Scanning Stereo microscope |

**Table 2** – Penetration of the dye beneath the dental sealant. The table shows differences between the groups in the number of teeth with and without penetration. Penetration was compared between the groups using Fisher’s Exact Test.
| Penetration | Treatment |
|------------|-----------|
| Frequency  |  A  |  B  |  C  |  D  |  E  |  F  | Total |
| Expected   | 18  | 17  | 14  | 5   | 24  | 17  | 95    |
| Cell Chi-Square | 15.8 | 15.8 | 15.8 | 15.8 | 15.8 | 15.8 |
| Percent    | 0.30| 0.09| 0.21| 7.4123| 4.21| 0.09| 52.78 |
| Total      | 10.00| 9.44| 7.78| 2.78| 13.33| 9.44| 52.78 |
| Penetration* | 12  | 13  | 16  | 25  | 6   | 13  | 85    |
| Expected   | 14.2| 14.2| 14.2| 14.2| 14.2| 14.2|
| Percent    | 0.33| 0.10| 0.24| 8.28| 4.71| 0.10|
| Total      | 6.67| 7.22| 8.89| 13.89| 3.33| 7.22| 47.22 |
| Total      | 30  | 30  | 30  | 30  | 30  | 30  | 180   |
| Expected   | 16.7| 16.7| 16.7| 16.7| 167 | 16.7|
| Percent    | 100.00| 100.00| 100.00| 100.00| 100.00| 100.00|

Table 3 – Mean values, standard error and the Bonferroni multiple comparison test for means of length of dye penetration between preparation methods (ANOVA tests, Bonferroni multiple comparison test)
| Preparation Method | Mean+/SE  | Comparison of Preparation Method | P-Value       |
|---------------------|----------|----------------------------------|---------------|
| A                   | 563.3+/- | A vs B                           | 1.00          |
|                     |          | A vs C                           | 1.00          |
|                     |          | A vs D                           | <0.0001*      |
|                     |          | A vs E                           | 0.8932        |
|                     |          | A vs F                           | 1.00          |
| B                   | 460.4+/- | B vs A                           | 1.00          |
|                     |          | B vs C                           | 1.00          |
|                     |          | B vs D                           | <0.0001*      |
|                     |          | B vs E                           | 1.00          |
|                     |          | B vs F                           | 1.00          |
| C                   | 421.5+/- | C vs A                           | 1.00          |
|                     |          | C vs B                           | 1.00          |
|                     |          | C vs D                           | <0.0001*      |
|                     |          | C vs E                           | 1.00          |
|                     |          | C vs F                           | 1.00          |
| D                   | 1456.0+/-| D vs A                           | <0.0001*      |
|                     |          | D vs B                           | <0.0001*      |
|                     |          | D vs E                           | <0.0001*      |
|                     |          | D vs F                           | <0.0001*      |
| E                   | 216.7+/- | E vs A                           | 0.8932        |
|                     |          | E vs B                           | 1.00          |
|                     |          | E vs C                           | 1.00          |
|                     |          | E vs D                           | <0.0001*      |
|                     |          | E vs F                           | 1.00          |
| F                   | 236.8+/- | F vs A                           | 1.00          |
|                     |          | F vs B                           | 1.00          |
|                     |          | F vs C                           | 1.00          |
|                     |          | F vs D                           | <0.0001*      |
|                     |          | F vs E                           | 1.00          |

**Figures**
Figure 1

Group design.
Figure 2

Dye penetrations following different protocols (mean percent calculated as dye penetration).

Figure 3 – Representative depiction for each tested group.
Figure 3

Representative depiction for each tested group.