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Effect of enamel preparation method on *in vitro* marginal microleakage of a flowable composite used as pit and fissure sealant

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Summary. *Objectives.* The aim of this *in vitro* study was to evaluate the microleakage in occlusal surfaces, after preparation with Er:YAG laser and compared to the diamond-bur conventional technique.
Methods. Thirty premolars were divided into three groups: I – high-speed handpiece + 37% phosphoric acid; II – Er:YAG laser (350 mJ, 4 Hz and 112 J/cm^2) + 37% phosphoric acid; and III – Er:YAG laser (350 mJ, 4 Hz and 112 J/cm^2) + Er:YAG laser (80 mJ, 4 Hz, and 25 mJ/cm^2). All cavities received the same adhesive system and were restored with flowable composite according to manufacturer's instructions. Teeth were submitted to thermal cycling and immersed in 50% silver nitrate solutions for 8 h in total darkness. Specimens were sectioned longitudinally in the bucco-lingual direction, in slices of 1 mm thick. Each slice was immersed into photo developing solution and was photographed, and microleakage was scored from 0 to 7, by three calibrated examiners.
Results. A statistically significant difference ($P < 0{\cdot}0001$) was observed between Er:YAG laser prepared and etched specimens and those in the other groups.
Conclusions. It can be concluded that no significant difference was noted between the two types of enamel preparation when etching was performed. Preparing and treating the enamel surface exclusively by Er:YAG laser resulted in the highest degree of leakage.

Introduction

Pits and fissures sealants have been widely employed since the 1970s, and are considered an efficient preventive method, as they can block the development of caries lesions on occlusal surfaces [1].

The effectiveness of sealants hinges on their ability to isolate pits and fissures from the combination of bacteria, their nutrients, and acidic metabolic products [2–5]. Consequently, poor sealing ability may cause secondary caries and interfere in long-term success of this technique.

Since its introduction, acid etching has become a crucial and indispensable step in sealant applications. Etching produces microscopic porosities in the enamel surface into which the unpolymerized sealant flows and hardens in tag-like projections that attach the material to the tooth structure [6].

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In an attempt to improve the retention of sealants, a number of studies have examined the influence of occlusal surface preparation, such as diamond-bur [7–9], air-abrasion [6,10], or laser irradiation [11,12], on microleakage of pit and fissure sealants.

One such laser is the erbium:yttrium aluminium garnet (Er:YAG, wavelength = 2·94 µm), which has its laser energy absorbed in the water of the hard tissues, promoting a rapid volume expansion of the vaporizing water that occurs as a result of a substantial temperature elevation in the interaction site [13]. Microexplosions are produced, causing hard tissue disintegration. Short, high-energy pulses allow effective tissue removal with almost no temperature elevation to the surrounding tissues [14].

The Er:YAG laser has been widely used because of its ability to cut or ablate tooth structure for removing carious lesions, cavity preparations, and modifying dentin and enamel surfaces as an alternative treatment instead of acid etching [13,15–17].

There is little reported research, however, concerning the effects of laser irradiation for pit and fissure preparation associated or not to conventional acid etching on marginal sealing of flowable resin composite used as sealant.

One of the indications of the flowable composites is their application as pits and fissures sealants on occlusal enameloplasty [18], because of their low filler loading, which promotes an appropriate consistency that permits the resin to flow into the fissures, enhanced wetting of the tooth surface, and a low modulus of elasticity [19].

In this context, the purpose of this study was to assess the degree of marginal leakage of this material on enamel occlusal surfaces prepared by high-speed diamond bur or Er:YAG laser.

Materials and methods

Thirty sound human premolars, stored in a 0·4% sodium azide solution at 4 °C, were selected, cleaned with scaler and water/pumice slurry in a dental prophylactic cup for 20 s. The teeth were then randomly assigned into three equal groups ($n = 10$).

For Group I, the occlusal surfaces were prepared using a #1191F diamond bur (K.G., Sorensen, Barueri, São Paulo, Brazil) at high speed with air–water spray. New burs were used after every five preparations. For Groups II and III, the occlusal surfaces were prepared by a Er:YAG laser (Kavo Key III, 1243 – Kavo Co., Biberach, Germany) at 400 mJ/pulse and 4 Hz, resulting in an energy density of 128,61 J/cm^2, emitted at a wavelength of 2·94 μm under water spray coolant. The diameter of the laser beam at the tooth surface was 0·63 mm. Irradiation was performed in a non-contact mode with a focused beam at 12 mm of working distance.

After surface preparation, enamel was conditioned according to the experimental group. Groups I and II were etched with 37% phosphoric acid gel (Tooth Conditioner Gel, DENTSPLY Indústria e Comércio Ltda, Petrópolis, Brazil) for 15 s, rinsed with air–water spray for 15 s and air-dried for 10 s; for Group III, the surfaces were treated by Er:YAG laser with 80 mJ/pulse at 4 Hz for 30 s.

A uniform layer of a single component bonding system (Single Bond, 3M Dental Products, St Paul, MN, USA) was applied to all specimen preparations, air-thinned and light-cured for 30 s with a LED curing unit with an output of 600 mW/cm^2 (Bright Lec-MMOptics LTDA, São Carlos, São Paulo, Brazil). A flowable composite resin (Fill Magic Flow – Vigodent, Rio de Janeiro, Brazil) was placed in bulk and light-cured for 40 s.

The samples were stored in distilled water in stove at 37 °C for 7 days. After this period, the apexes were sealed with a cyanocrylate glue (Super Bonder, Loctite Brazil Ltda, Diadema, Brazil), and the teeth were entirely covered with three layers of nail varnish, leaving a 2-mm window around the sealant.

The specimens were submitted to a thermocycling regimen (AMN Instrumental, MCT2, São Paulo, Brazil), of 700 cycles between 5 °C and 55 °C water-baths. Time of immersion was of 1 min, with a 3-s transfer time between baths.

After thermocycling, the specimens were immersed in a 50% silver nitrate solution at room temperature and darkness for 8 h [20]. Then, they were embedded in a chemically activated acrylic resin (JET, Clássico, São Paulo, Brazil) and sectioned longitudinally in a buccal-lingual direction with a water-cooled diamond saw in the sectioning machine Labcut 1010 (Extec, Enfield, CT, USA), obtaining three sections of 1-mm thick from each tooth.

Following sectioning, each slice was immersed into photo developing solution under 16 h of fluorescent light. The sections were identified, carefully fixed on slides, and analysed for leakage, by photographing them with a digital video camera (Nikon D70, Nikon Imaging Co. Ltd, Jiangsu, China), with 120-mm medical lens (Nikkor, Nikon Co. Ltd, Ayuttaya, Thailand) under a 2 × magnification. The images obtained were transmitted to a personal computer and after recording in a CD-R (SL 80, 700MB – NIPPONIC) they were analysed by three calibrated examiners. The following criterion were used to evaluate microleakage (Fig. 1): 0 = no dye penetration; 1 = dye penetration restricted to occlusal third of one of the sealant's walls (buccal or lingual); 2 = dye penetration restricted to occlusal third of both sealant's walls; 3 = dye penetration restricted to medium third of one of the sealant's walls; 4 = dye penetration restricted to medium third of both sealant's walls; 5 = dye penetration restricted to pulpal third of one of the sealant's walls; 6 = dye penetration restricted to pulpal third of both sealant's walls; and 7 = total dye penetration along the cavity walls, including the pulpal wall.

The score of each slice, instead of the mean score for each specimen, from the total of the specimen per group was obtained and compared to the total of slices from the other groups. The total number of slices examined for dye penetration was 90.

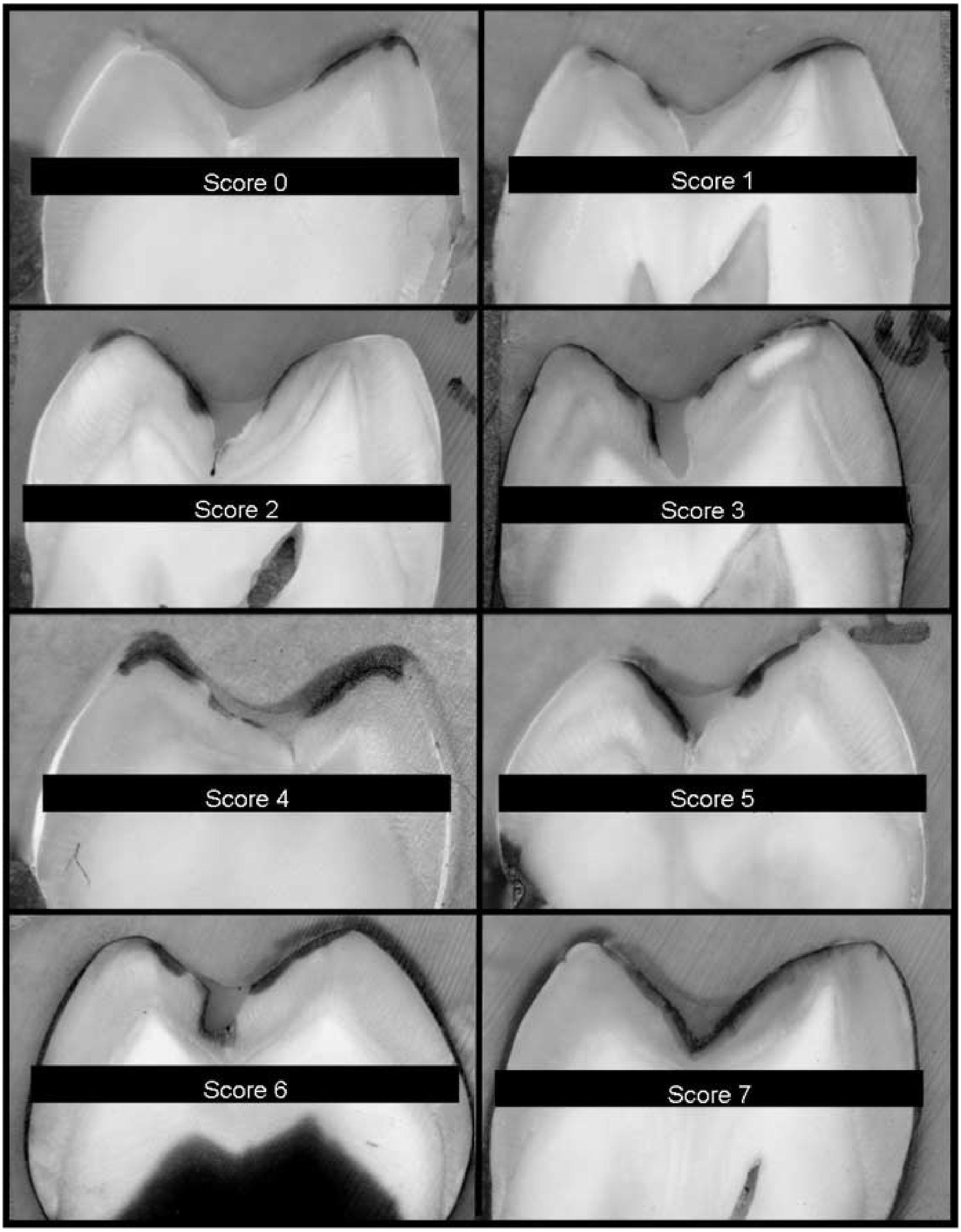

Fig. 1. Scores from 0 to 7 used to evaluate microleakage.

The data were submitted to statistical analysis using Kruskal–Wallis and chi-squared test ($P < 0{\cdot}05$). For the chi-squared test the scores were grouped as follows: low scores = scores 1 + scores 2; medium scores = scores 3 + scores 4; and high scores = scores 5 + scores 6 + scores 7.

Results

Table 1 shows the dye penetration for all slices.

The results of the Kruskal–Wallis test are presented in Table 2. Analysing the data obtained, it was observed that there was no statistical difference

Table 1. Dye penetration score obtained for all sections in each group.

	Dye penetration scores								
Group	0	1	2	3	4	5	6	7	Total
I	0	5	0	4	0	2	8	9	28
II	0	3	4	2	2	1	5	14	31
III	0	0	0	0	0	1	2	28	31

Table 2. Kruskal–Wallis tests for ranks.

Group	Mean rank	*
I	34·6607	A
II	39·2903	A
III	61·5000	B

*Mean ranks with the same letter are not significantly different in pair comparison ($\alpha = 0{\cdot}01$).

Table 3. Chi-squared test: frequency that grouped scores (low scores = scores 1 + scores 2; medium scores = scores 3 + scores 4; and high scores = scores 5 + scores 6 + scores 7) occurred in each group.

Scores	Group I	Group II	Group III
Low	5	7	0
Medium	4	4	0
High	19	20	31

between the Groups I and II. Only Group III exhibited significantly more specimens with high scores compared to Groups I and II ($P < 0{\cdot}0001$). Occlusal surface preparations conditioned with phosphoric acid leaked significantly less than did those in laser etched preparations.

Group III where the surfaces were treated with Er:YAG laser only exhibited significantly more specimens with high scores ($P = 0{\cdot}0002$) compared with Groups I and II (Table 3).

Discussion

The clinically undetectable passage of bacteria, fluids, molecules, or ions between the cavity wall and the applied restorative material, known as microleakage, is an important concern in restorative dentistry because of its clinical damages, such as secondary caries lesions, pulpal pathologies, postoperative pain and sensitivity and, consequently, the failure of the restorative procedure. In the case of pit and fissure sealants, the success of this technique can be hindered if the applied material cannot resist microleakage, resulting in the initiation and/or progression of caries under sealed surfaces, as well as increasing the difficulty of diagnosing and treating this lesion.

In an attempt to improve the retention of sealants and decrease microleakage, mainly when the control of humidity is unsatisfactory, the association of adhesive systems and pits and fissures sealants was proposed [21–26]. Therefore, in this study, the association of an adhesive system with a flowable composite resin was chosen over a traditional sealant.

This study, however, showed that all the specimens exhibited some degree of leakage. This finding may be explained by the type of dye material used, 50% silver nitrate, which has smaller particle size than other dye solutions (e.g. 1% methylene blue, 0·2% rhodamine, 0·5% solution of basic fuchsin) and, consequently, has a higher capacity to penetrate along the cavity walls. Such penetration of silver nitrate solution, which has silver ions of 0·059 nm [24], may be considered even worst than the clinical penetration of typical bacteria (0·1–1 μm) along cavity walls, but similar to the penetration of bacteria products, permitting a closer simulation of clinical microleakage.

Nevertheless, it can be clearly observed that acid etching the enamel prior to the placement of a pit and fissure sealant remains mandatory for the achievement of better marginal sealing, regardless of the method for occlusal surface preparation. This finding is consistent with the results of Lupi-Pegurier *et al.* [25] that used the Er:YAG laser to prepare occlusal surfaces followed or not by acid etching and compared it to conventional bur preparation and acid-etching technique. In such study, no significant difference was noted between the two types of enamel preparation when etching was performed. On the other hand, laser alone showed the highest number of specimens with microleakage.

These results were also found by other studies [11,17,26], where Er:YAG laser was used as a tooth conditioner alone or associated to acid etching and compared to conventional acid etching alone. Those studies concluded that laser irradiation did not eliminate the need for etching the enamel surface before applying the sealant, because of the capacity of acid-etching technique to ensure a complete surface conditioning and effective adhesion of the restorative material to cavity preparation.

The employment of laser technique for preparing and conditioning enamel surfaces resulted in the highest scores of microleakage and the highest frequency of score 7, which represents the total dye penetration along the cavity walls. A possible explanation for these results would be that the laser does not create the uniform microporosities characteristic of acid conditioning on the enamel surface. Instead, it promotes a disorganized destruction of enamel prisms. The resultant microretention clearly varies from acid-etching patterns, and this irregular microstructure results in poor sealing and a higher degree of marginal leakage [16].

The laser and conventional surface preparation and etching, however, were compared only with respect to marginal leakage. In this context, other parameters must be considered when comparing both techniques, such as long-term retention, the integrity of the sealant or the shear strength of the sealant. Nevertheless, despite its limitations, this study provides some data to support further research into the use of lasers in the performance of more dental procedures.

What this paper adds
- The use of a Er:YAG laser for preparing and treating pits and fisures did not eliminate the need for acid etching this surface prior to the placement of sealants.

Why this paper is important to paediatric dentists
- Complementing either conventional bur or Er:YAG laser preparation with a subsequent acid-conditioning enhances the marginal retention of pit and fissure sealants.

Conclusion

According to the results obtained, and considering the limitations of an *in vitro* study, it may be concluded that:

- No significant difference was noted between the two types of enamel preparation when etching was performed.
- Preparing and treating the enamel surface exclusively by Er:YAG laser resulted in the highest degree of leakage.

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