Laser-prepared and bonding-filled fissure sealing: SEM and OCT analysis of marginal and internal adaptation

Tissiana BORTOLOTTO, Pascal MAST and Ivo KREJCI

Division of Cariology and Endodontology, University Clinic of Dental Medicine, University of Geneva, Rue Barthelemy-Menn, 19, 1205 Geneva, Switzerland

Corresponding author, Tissiana BORTOLOTTO; E-mail: Tissiana.Bortolotto@unige.ch

This study assessed the effect of bur or Er:YAG laser preparation on marginal and internal adaptation of conventional and extended fissure sealing (FS) with a 3-component etch-and-rinse (Optibond FL; OFL) and a 1-component self-etch (Scotchbond Universal; SB) adhesive system. Scanning electron microscope analysis was performed before and after thermocycling/occlusal load and additional optical coherence tomography evaluation was carried out for internal marginal assessment. Significant differences were observed between the groups (ANOVA, $p<0.05$). Laser-prepared and non-etched FS suffered from marginal degradation after fatigue. When enamel was etched with H_3PO_4 and independently of the adhesive system, laser technique was equally effective to bur-preparation with percentages of continuous margins ranging from 96 to 99%, being laser less invasive than bur preparation. This is clinically relevant in paediatric dentistry as minimally invasive FS can be performed with laser and adhesive systems used as fissure sealants. Nevertheless, enamel etch with phosphoric acid is still necessary.

Keywords: Universal adhesive, Fissure sealing, Laser

INTRODUCTION

Modern dental medicine follows a conservative approach and therefore minimizes invasive treatments during tooth restoration[1]. In this context, the prevention of cavity decay has come to play a major role. Its goal is to prevent the appearance of cavities (primary prevention) or their progress (secondary prevention)[2]. Public health measures such as improvement in bucco-dental hygiene, nutritional advice or the daily use of fluoride have proven to be effective. Nevertheless, in high-risk patients, collective measures must be supplemented by individual measures, and it is at this point where fissure sealants become an interesting option[3].

Statistically, 84% of cavities in children between ages 5 and 17 start in pits and grooves; and resin-based fissure sealants (FS) are globally recognized as being an effective preventive method[4]. FS consist of inserting a thin layer of resin into dental grooves and pits in order to prevent the accumulation of food debris at the bottom of the grooves, prevent demineralization caused by bacteria, and facilitate the cleaning of tooth surfaces. They are recommended based on patient's needs, carious index, age, and type of tooth to be treated[5]. This technique can be performed in two ways: conventional (FS) and extended fissure sealants (EFS). FS are primarily recommended for marked grooves in children at high carious risk. In cases where a doubt exists in respect to a carious lesion that is starting to develop, it is important to excavate the carious pits and grooves using a micro invasive fissure sealant technique, *i.e.* an EFS. It is quite common to perform both types of fissure sealing in the same tooth; in this sense, the way the fissure is prepared and the choice of material are important factors influencing long-term success.

Several techniques can be used to adequately prepare the tooth surface for fissure sealing, such as rotating drill, blasting, etching with phosphoric acid, or by using laser technology. Erbium YAG (Er:YAG) laser therapy is an innovative technology with several uses in paediatric dental medicine. It provides an alternative to blasting and rotating instruments in the preparation of the surface prior to performing extended fissure sealants. As such, it offers several advantages: minimally invasive therapy, noise reduction during treatment, the absence of contact with or vibrations at the surface of the tooth and reduced need for anesthesia. Furthermore, the absence of the use of rotating instruments increases the safety of the treatment when applied to young children and makes it more acceptable to patients[6].

Optimizing laser parameters seems to be the key for achieving optimum efficiency while limiting damage during cavity preparation procedures. In the case of Er:YAG laser (LiteTouch®, Syneron Medical, Yokneam Illit, Israel), thanks to its 2,940 nm wavelength, this laser is very well absorbed by water and hydroxyapatite. This is why Er:YAG lasers have become the preferred option for cavity preparation[7]. Er:YAG lasers must use a water spray to reduce the secondary thermal effects which constitutes another factor that can influence laser effects[8,9]. It has been demonstrated that the spray is not only important for cooling purposes but also plays a role in tissue ablation. The volume of water must also be taken into consideration, as too much water can reduce ablation rate.

Regarding restorative materials for FS; some

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Received May 2, 2016: Accepted Jan 5, 2017
doi:10.4012/dmj.2016-025 JOI JST.JSTAGE/dmj/2016-025

studies have evaluated adhesive systems when used as sealing agents[10]. Filler-containing adhesive systems such as the 3-step etch-and-rinse Optibond FL (OFL: Kerr, Orange, CA, USA) have obtained the best results both in terms of marginal adaptation and resistance against occlusal disintegration. In addition, etch-and-rinse systems provide a better microstructure for micromechanical retention. Nevertheless, non-charged resins, which are less viscous, may penetrate into the fissures more effectively. Resin that persists properly ensures treatment effectiveness over time[11]. Moreover, universal mono-component self-etch adhesive systems (SB: Scotchbond Universal, 3M ESPE, St. Paul, MN, USA) hold a considerable advantage from a practical and clinical point of view, as they are faster and simpler to apply compared to etch-and-rinse systems. The goal is to obtain a sealant that is perfectly adapted to the fissure space and long lasting in order to prevent any loss of adhesion, marginal discolouration, or bacterial infiltration.

To determine if Er:YAG laser technology can be used as an alternative to well-known surface preparation methods prior to performing fissure sealants, the marginal adaptation of bur and laser-prepared FS filled with two different bonding agents (mono-component self-etch and multi-component etch&rinse) were compared before and after the application of occlusal stress and warm-cold thermal cycles with a fatigue test. Following the fatigue test, the internal adaptation was evaluated on loaded samples by using optical coherence tomography (OCT), allowing a non-destructive tissue analysis. The null hypothesis tested was that: there is no significant difference between the two surface preparation techniques when using two adhesive systems in terms of continuous margins either before or after fatigue.

MATERIALS AND METHODS

Sixty intact anonymous human upper molars free of any decay were collected and preserved in a 0.1% thymol solution from the time of extraction until their use for this investigation. The research protocol for the present study was prepared in accordance to the regulations for the anonymous collection of biological samples approved by the ethical committee of the Canton of Geneva, Switzerland (Human Research Act, article 2, alinea 2) which stipulates that such anonymous tooth collection does not need to be submitted to approval by an ethical committee. These teeth were free of decay as confirmed by visual inspection under an optical microscope (Wild M5, Wild, Heerbrugg, Switzerland) and measurement with a caries-detecting device (DIAGNOdent Classic, KaVo, Warthausen, Germany). After polishing using nylon brushes and a polishing paste, the teeth were mounted onto metal holders, keeping their roots in the center, and fixed with cold-curing resin (Paladur, Heraeus-Kulzer, Werheim, Germany). These teeth were randomly divided into 6 study groups (n=10). The materials to be tested consisted of two adhesive systems (SB: Scotchbond Universal, OFL: Optibond FL) and a nano hybrid composite (Filtek Supreme, 3MESPE), as shown in Table 1. Each sample contained both, a conventional (FS) and extended fissure sealing (EFS); conventional FS was determined by fissure's anatomy. For the EFS, a micro cavity 3 mm deep and 0.6 mm diameter was drilled at the middle groove.

To define the ideal parameters for the Er:YAG laser (LiteTouch, Syneron®, Serial-No: LI-FG0001A) that would be used in this study, some pre-tests were performed on extracted molars by evaluating various laser parameters to determine the optimal ones for tooth preparation. As the lasers' pulse length is constant, sapphire diameter, distance, energy, frequency and water spray were separately evaluated. After several tests we concluded that the ideal parameters for conventional fissure sealing (FS) were: sapphire with a 1 mm diameter, 1 mm distance, 50 mJ, 20 Hz, and maximum water spray. For extended fissure sealing

Table 1 List of materials used in this study

Product	Manufacturer	LOT	Composition	Properties
Scotchbond™ Universal Adhesive	3M ESPE, St. Paul, MN, USA	Lot: 457855	10-MDP Phosphate Monomer Dimethacrylate resins A-methacrylate-modified polyalkenoic acid copolymer (Vitrebond™ Copolymer) HEMA, Filler, Ethanol, Water, Initiators, Silane	- 1-component adhesive - Self Etch
OptiBond FL™	Kerr, Orange, CA, USA	Lot: 4462763	Primer: HEMA, GPDM, MMEP, Water, Ethanol, CQ, BHT Bond: Bis-GMA, HEMA, GDMA, CQ, ODMAB, Filler (fumed SiO_2, barium aluminoborosilicate, Na_2SiF_6), Coupling factor A174 (approximately 48 wt% filled)	- 3-component adhesive - Etch & rince - Fluoride release - Opaque
Filtek™ XTE Supreme Universal	3M ESPE	Lot: N324452	Bis-GMA, UDMA, TEGDMA, Bis-EMA, Filler	- Nano hybride composite

(EFS) the ideal parameters were: sapphire with a 0.6 mm diameter, distance of 1 mm, 300 mJ, 30 Hz and maximum water spray followed by treatment of the surface using the conventional FS parameters to obtain a regular surface. During surface preparations for both, FS and EFS, two different hand tools were used: a hand tool No 1855 for surface ablation at 50 mJ, and a hand tool No 1823 for surface ablation at 300 mJ, respectively.

The experimental groups are described in Table 2. The occlusal surfaces were meticulously cleaned using a blaster that emitted particles of aluminium oxide (27 microns) with a pressure of 2 bars (DENTO-PREP Microblaster, Ronvig Dental Mfg, Daugaard, Denmark). In groups 1, 2 and 4, 5, fissures were treated with highly concentrated phosphoric acid (Kerr, Etching gel 37.5% Phosphoric acid Lot 4466220 and 3M ESPE, Scotchbond Universal Etchant Lot 454767) according to manufacturer recommendations. During the etching procedure, acid penetration was enhanced using an ultrasound insert[12]. After etching, the teeth were thoroughly rinsed with water for at least 30 s, dried with compressed air but without desiccating, as recommended by the manufacturer. The adhesive systems to be used as fissure sealants (OFL and SB) were then meticulously applied on occlusal surfaces following manufacturer recommendations. The ultrasound insert was then applied to the occlusal surface so as to increase penetration of the resin to the bottom of the grooves. After allowing the product 30 s of latency, polymerization was carried out for 40 s using a LED curing unit pre-set at a power density of 1,000 mW/cm^2 (Demi Plus 230 V, Kerr, Middleton, WI, USA). Following polymerization, the surfaces were covered with glycerine gel and then again polymerized for 40 s in order to eliminate the oxygen inhibition layer. Given the highly precise application of the material, there was no need to polish the resin. The morphology of fissure sealing as well as the aspect of the margins after restorative procedures are shown in Fig. 1.

The sealed teeth were kept in the dark and in water at 37°C for a week and then subjected to a fatigue test consisting of a combination of occlusal load and thermocycling in a chewing simulator[13]. Thermocycling was performed 500× by using water jets with temperatures between 5 and 50°C and vice-versa, with an immersion time of 2 min. Mechanical stress comprised 200,000 load cycles transferred to the center of the occlusal surface with a frequency of 1.7 Hz and a maximum load of 49 N[14]. This load was applied *via* an antagonistic natural molar cusp in contact with the central surface of the EFS.

Before and after the fatigue test, occlusal surfaces

Table 2 Description of the different groups according to the type of fissure treatment, enamel etching and restorative material

Groups	Fissure treatment	Etching with H_3PO_4	FS filled with
1	Control: Al_2O_3+bur	Yes	OFL+Filtek
2	Er:YAG	Yes	OFL+Filtek
3	Er:YAG	No	OFL+Filtek
4	Control: Al_2O_3+bur	Yes	SB+Filtek
5	Er:YAG	Yes	SB+Filtek
6	Er:YAG	No	SB+Filtek

OFL: Optibond FL, SB: Scotchbond Universal.

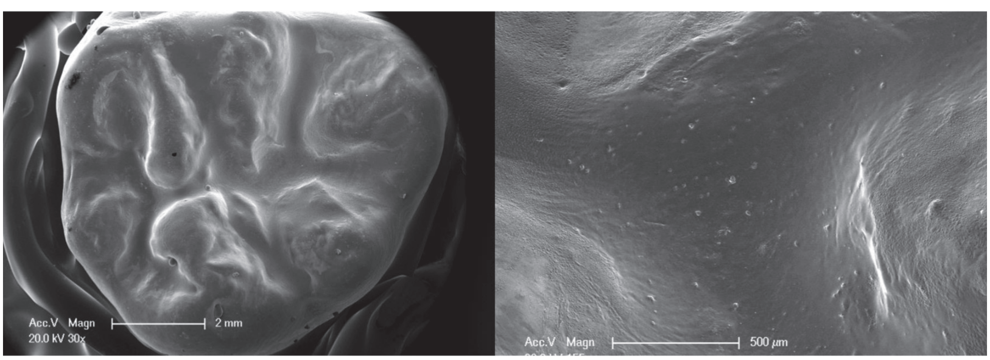

Fig. 1 Representative image of a fissure sealing (left, magnification 30×) and higher magnification of the margins to be quantitatively analyzed by SEM (right).

were cleaned with rotating brushes embedded in toothpaste and impressions were obtained by using low viscosity impression silicone (President Plus Light Body, ISO 4823 Type 3, Coltène-Whaledent, Altstätten, Switzerland). Using these silicone molds, replicas made out of an epoxy resin (Epofix Kit, Struers, Rödovre, Denmark) were then prepared and subjected to a quantitative margin analysis using a scanning electron microscope (SEM, XL 20, Phillips, Eindhoven, The Netherlands)[13,15]. Micromorphology of the margins was assessed as percentages of continuous margins at the total margin length (Fig. 1). After loading, the internal adaptation was qualitatively evaluated with optical coherence tomography (OCT, Cirrus 4000 High definition OCT, Zeiss, Serial No. 4000-1793) according to a previously described protocol[16]. The purpose of this qualitative evaluation was to detect any differences in surface appearance between bur or laser-prepared cavities.

Statistical analysis was performed with specific software (SPSS for Mac, version 21). The differences in "percentages of continuous margins" between groups were statistically assessed with ANOVA and Duncan *post-hoc* test at a level of confidence of 95%.

RESULTS

Percentages of continuous margins (CM) before and after loading are shown in Table 3. Thermo mechanical loading significantly influenced marginal adaptation ($p<0.05$). Before loading, all groups presented near 100% CM and no significant differences were observed between the six groups ($p=0.273$). After loading, significant differences in %CM were observed between the groups ($p=0.000$). The two control groups (1 and 4) restored with SB and OFL achieved the highest results in terms of %CM. When using OFL with previous enamel etch on laser prepared cavities (Gr. 2), laser was as effective as bur preparations and no significant differences were observed between this group and the controls. The lowest %CM was obtained in the groups prepared by laser but omitting phosphoric acid etch, independently of the adhesive system (Gr. 3 and 6).

Non-destructive analysis of samples using OCT (Figs. 2 and 3) revealed very clear opaque X-ray lines representing internal spaces, especially in groups 3 and 6 that were not subjected to enamel conditioning with H_3PO_4. A noticeable difference in opacity was observed between the filler-containing (OFL, Fig. 2) and no-filler-containing (SB, Fig. 3) adhesive systems as well.

DISCUSSION

As fissure sealants remain an effective measure for the prevention of cavities[17], this study compared two adhesive systems used as sealing agents. Scotchbond Universal (SB) was selected for this study because it is composed by resin modified with glass ionomer (Vitrebond copolymer) that renders the material more tolerant to humidity; this is an advantage in terms of manipulation during paediatric dental treatment. It also contains 10-MDP, a phosphate-based monomer that favors not only bonding of hydroxyapatite to enamel and dentine, but also bonding to non-tooth surfaces such as ionomer glass, zirconia and metals[18]. This adhesive system does not contain fillers and can be applied on enamel in a thin layer; this is an advantage when performing fissure sealants. Furthermore, it can be used with or without treatment of enamel, as it is a self-etching adhesive; this characteristic may be desirable to save time during paediatric dental treatments. As for Optibond FL, it is a standard adhesive system having three components, that is, H_3PO_4 gel, a primer and a bond. It is considered the gold standard in terms of bonding. In more recent publications, this product yielded excellent *in vitro* results and long-term clinical data[19]. Meanwhile, due to the presence of the acidic monomer glycerol phosphate dimethacrylate (GPDM) within the primer in OFL, a self-etching effect on enamel and dentin has been observed when using OFL without previous etch with H_3PO_4[20]. Therefore, an additional group (Gr. 3) was included to determine if the self-

Table 3 Percentages of continuous margins (%CM) before and after the fatigue test, expressed as Mean (SD)

Groups	%CM Before fatigue		%CM After fatigue	
1. bur, etch, OFL (control)	99.5 (0.9)	A	99.2 (1)	a
2. laser, etch, OFL	99.3 (1.3)	A	98.8 (1.6)	a
3. laser, no etch, OFL	100	A	93.1 (4)	d
4. bur, etch, SB (control)	98.5 (2.3)	A	97.8 (1.2)	a,b
5. laser, etch, SB	99.2 (1)	A	95.6 (1.6)	b,c
6. laser, no etch, SB	99.4 (1)	A	91.6 (4.9)	c,d

Values connected by different letters are significantly different at the $p=0.05$ level and apply to each column. Upper case letters show the differences between groups before fatigue and lower case letters show the differences between groups after fatigue test.

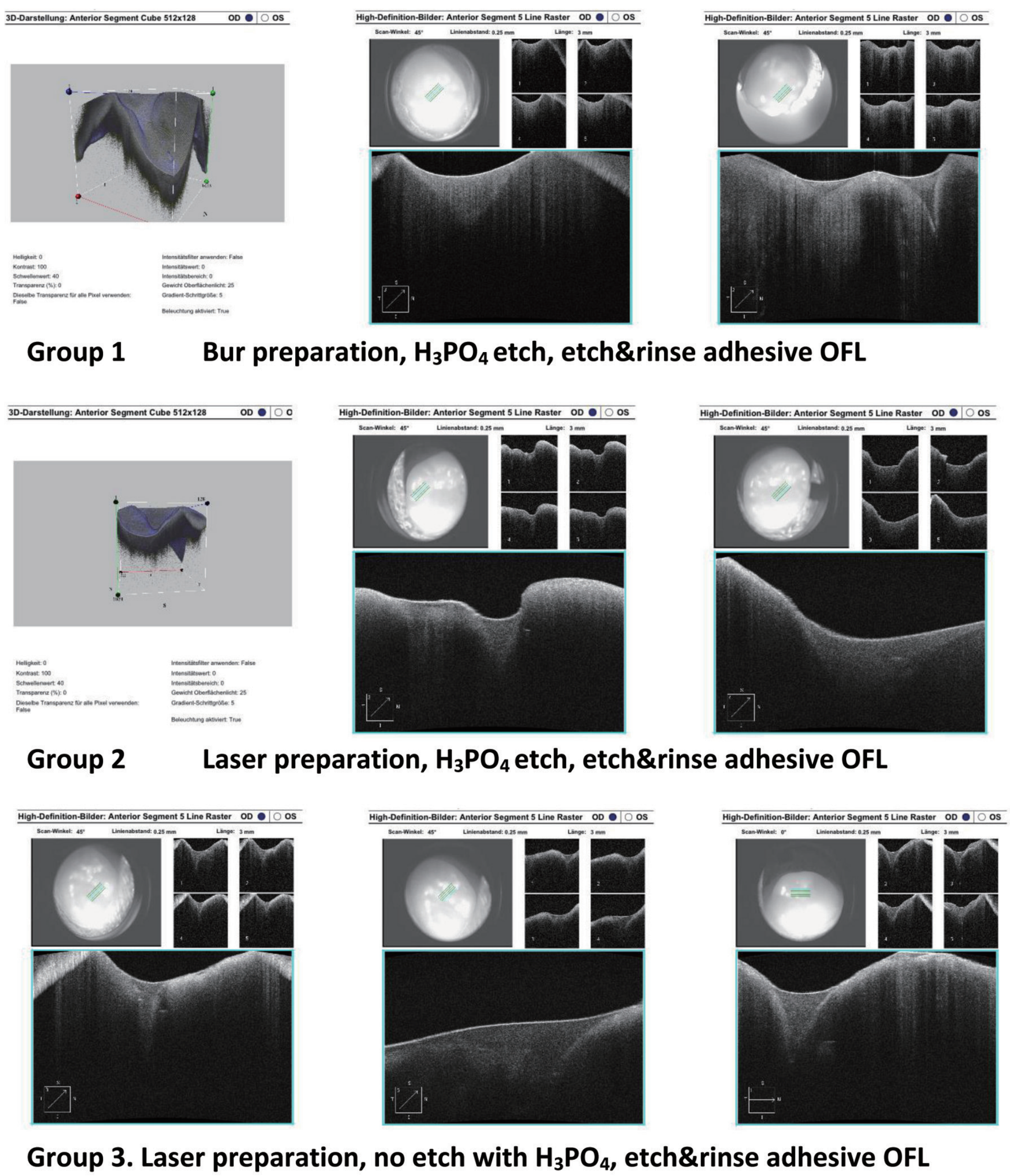

Fig. 2 Representative OCT micrographs of fissure sealings restored with the etch&rinse adhesive OFL (Gr. 1 to Gr. 3).
OFL: Optibond FL, SB: Scotchbond Universal. A similar quality of internal adaptation, without the presence of white opaque interfacial lines, is observed in groups 1 (bur preparation) and 2 (laser preparation). A white and opaque line is observed on enamel surface in the group in which phosphoric acid etch was omitted (Gr. 3), indicating a poor internal adaptation of the fissure sealing to enamel.

etching effect of OFL would be effective on laser-treated enamel, with the objective of decreasing clinical time.

An Er:YAG laser was used to prepare enamel surface as an alternative to bur preparation. Its emitted wavelength is in the infrared spectrum and matches the absorption peak of water and also highly that of hydroxyapatite, making it an excellent tool and also the most efficient type of laser for localized surface removal of enamel and dentin. In addition, one interesting feature of Er:YAG laser on hard tissues is its bactericidal effect, as enamel fissures can be cleaned and sterilized simultaneously[7]. Lastly, a thorough drying using alcohol was performed to increase the wettability of enamel before sealant application[12].

Percentages of marginal adaptation higher than 90% after fatigue were observed for each group, evidencing in general a good performance for both materials. A similarly statistical performance was

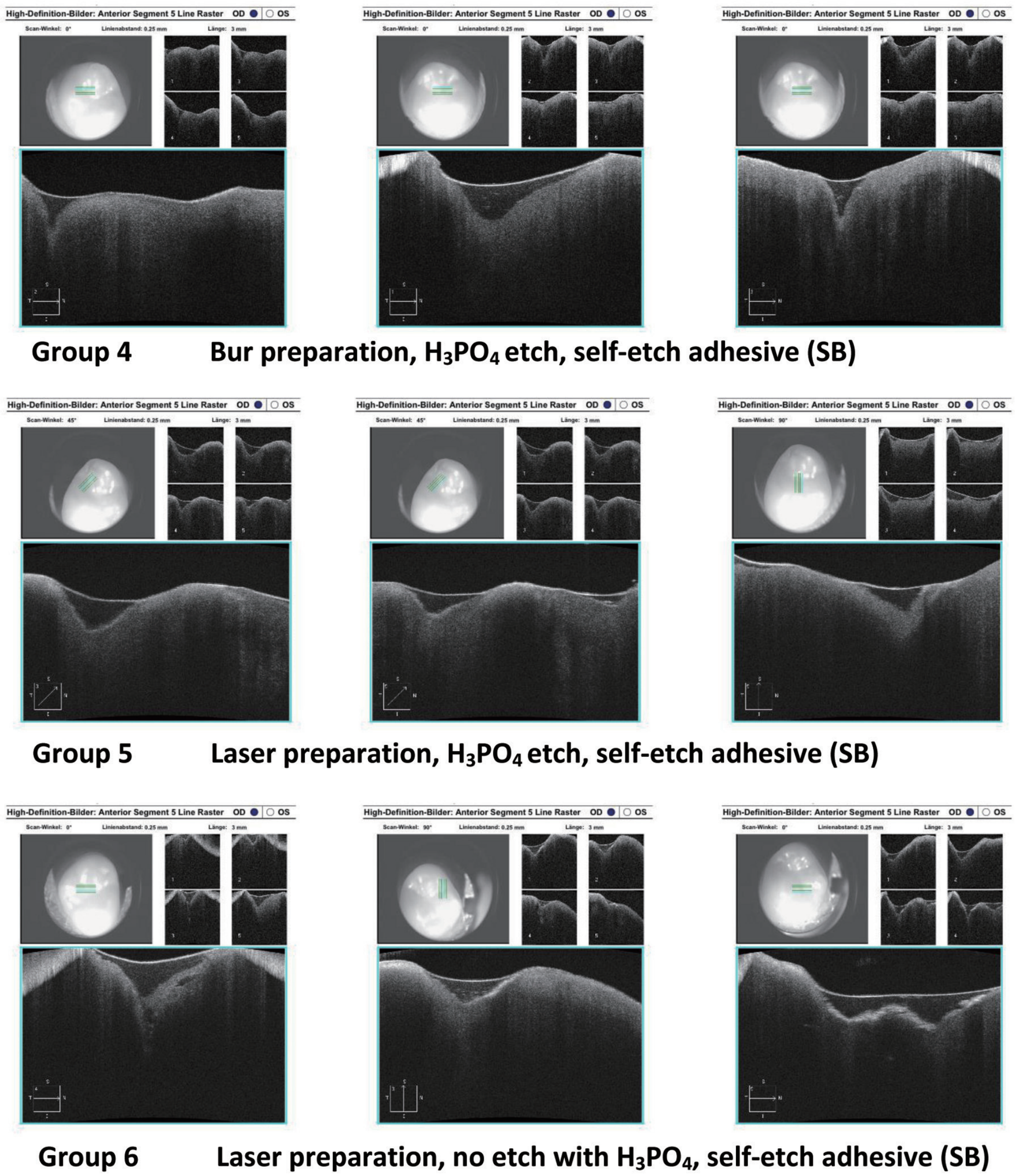

Fig. 3 Representative OCT micrographs of fissure sealings restored with the self-etch adhesive SB (Gr. 4 to Gr. 6).
OFL: Optibond FL, SB: Scotchbond Universal. See that a similar quality of internal adaptation is observed in group 4 (bur preparation) in respect to Gr. 1 and 2. A white and opaque line is observed on enamel surface in the group in which phosphoric acid etch was omitted (Gr. 6), indicating a poor internal adaptation of the fissure sealing to enamel.

observed, in terms of marginal adaptation, between the etch&rinse 3-component OFL adhesive (Gr. 1) and the mono-component SB adhesive (Gr. 4) on bur-prepared fissures, provided that enamel was previously etched with phosphoric acid. This confirms recently published data showing an equivalent performance of multi- and mono-component adhesive systems[21].

The groups that were prepared by laser, and for which no enamel treatment was performed, yielded lower results (Gr. 3 and 6, score d). Previous studies have shown that when preparing enamel by laser with an energy of 4 J, enamel temperature increased dramatically, inducing a chemical change of enamel composition[22,23]. In our study enamel was laser-prepared by using energies of 0.05 J (50 mJ) and 0.3 J (300 mJ); so it is little probable that at such low energies, a chemical change of enamel composition would occur. Meanwhile, it is well known that after laser treatment of enamel, an acid resistant layer is formed on enamel surface[24]. In theory, this layer is formed due to laser irradiation effects at low energy densities, like the ones used in our study, that heat enamel to temperatures lower than 400ºC. This heating would cause a partial decomposition of the organic matrix of enamel, leading to a blockage of inter- and intra prismatic spaces. This "blocked spaces" within enamel would reduce ion diffusion and therefore, demineralization[25,26]. In the context of our study, it is highly possible that if an acid-resistant layer was formed on enamel as a result of laser irradiation, the etching effect of the self-adhesive system SB on enamel was decreased, explaining why the results were lower in the groups irradiated by laser and in which enamel was not etched with phosphoric acid. The same might have occurred in Gr. 3 when using OFL as a self-etching adhesive, as the acidic monomer present within the primer of OFL was not aggressive enough to condition the acid-resistant enamel layer. Based on these results, the hypothesis stating that no differences existed between the groups in terms of marginal adaptation before and after the fatigue test had to be rejected.

An appropriate use of phosphoric acid to condition enamel, combined with a separate hydrophobic and charged bonding layer, could explain the highest results that were observed in Gr. 1 and 2. Phosphoric acid effect might have increased the wettability of resin on enamel as well as surface energy and capillary pressure[27]. While a charged bonding agent provided with a thick and more fatigue resistant layer of FS. For the above mentioned reasons, treating enamel with an acid before the application of a sealing material is still necessary.

OCT analysis enabled a quick and non-destructive 3D analysis of the internal margins of the FS. Despite the similar results in terms of marginal adaptation between laser and bur-preparations, OCT images showed an increased ablation of enamel with the bur technique. In this sense, one clinical advantage of laser preparation would be maximum preservation of healthy enamel[27]. Therefore, within the context of micro invasive dental medicine, this observation has clinical relevance and supports the use of laser as a non-invasive method for surface preparation before the application of a fissure sealant. It is also possible that the opaque lines on enamel observed by OCT corresponded to the thickness of the acid-resistant layer resulted from laser preparation (Gr. 3 and 6). In this sense, further studies are being carried out in our laboratory to determine the nature of this layer.

CONCLUSIONS

Based on the results of this *in vitro* study, the following conclusions can be drawn:

1. When used as sealants, the two adhesive systems (multi-step OFL and one-component SB) yielded results higher than 90% in terms of percentage of continuous margins.
2. The application of an adhesive system without previously etching enamel provided the least favorable results in terms of marginal adaptation.
3. Surface preparation for FS could be efficiently performed with a laser device and was less invasive than bur preparation.
4. The laser technique proved to be as effective as bur preparation, provided that enamel is previously etched with phosphoric acid.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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