Shear Bond Strength of A Sealant to Contaminated-Enamel Surface: Influence of Erbium : Yttrium–Aluminum–Garnet Laser Pretreatment

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ABSTRACT

Background: Salivary contamination is one of the factors that can disturb the sealing process and interfere in the longevity of pit and fissure sealants. Erbium : yttrium-aluminum-garnet (Er : YAG) laser could influence the bond strength of enamel and increase the acid resistance.

Purpose: To evaluate the influence of Er : YAG laser on the shear bond strength of a sealant to a salivary contaminated enamel surface.

Methods: Twenty-four third molars had the roots sectioned 2 mm coronal to the cementoenamel junction. The crowns were mesiodistally sectioned providing 48 halves that were embedded in polyester resin. Enamel was flattened and a 2-mm diameter bonding area was demarcated. Specimens were randomly assigned to two groups according to the superficial pretreatment—37% phosphoric acid (A) and Er : YAG laser (80 mJ/2 Hz) + phosphoric acid (L), which were subdivided into two groups (N = 12), without salivary contamination (C) and with salivary contamination (SC). To contaminate the specimens, 0.25 mL of human fresh saliva was applied for 20 seconds and then dried. Fluroshield sealant was applied in all specimens. After storage, shear bond strength of samples were tested in a universal testing machine.

Results: Means in MPa were: AC—14.61 (± 2.52); ASC—6.66 (± 2.34); LC—11.91 (± 1.34); and LSC—2.22 (± 0.66). Statistical analysis revealed that surfaces without salivary contamination and with acid treatment had the highest mean (p < 0.05). The group with salivary contamination treated by Er : YAG laser followed by phosphoric acid application presented the lowest bond values (p < 0.05).

Conclusions: The phosphoric acid etching under dry condition yielded better bonding performance. Er : YAG laser was not able to increase the effectiveness of conventional acid etching of enamel in the bond of sealants in both dry and wet conditions.

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CLINICAL SIGNIFICANCE

Under the conditions of this study, the conventional etching protocol (phosphoric acid without salivary contamination) is still preferable to laser-conditioning enamel surface prior to sealant application.

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INTRODUCTION

Tissure sealants used on occlusal L'tooth surfaces were introduced in the 1970s for protecting pit and fissures from dental caries.¹⁻⁴ Pitand-fissure sealants have been considered an outstanding adjunct to oral health preventive strategies in the decrease of occlusal caries onset and/or progression.^{1,4} The purpose of sealing the pits and fissures is to prevent plaque microflora and food debris from accumulating in the fissures where saliva cannot reach, to remineralize initial lesions, and to buffer acid produced by cariogenic bacteria.^{3,5,6} The properties of an ideal sealing material include biocompatibility, retention, and resistance to abrasion and wear.⁷

Several factors influence the strength of the sealant-etched enamel bond. Bond strength appears to be sensitive to quite small variations in etching, washing, and drying time.^{1,8} Accidental salivary contamination of etched enamel can disturb the sealing process.^{2,9,10} Brief contact of etched enamel with saliva can cause the formation of an adherent coating that covers many of the

pores created in the enamel,^{11,12} and, as a result, the resin tags responsible for the mechanical interlocking to dental surface are not formed.¹⁰ Indeed, salivary contamination of conditioned surfaces can affect the micromechanical retention of the sealant to enamel because of the formation of an organic film on the etched area.^{6,8} Therefore, if the microporosities created by acid etching are filled, sealant retention will be further undermined, thereby compromising the marginal integrity and favoring the occurrence of microleakage phenomenon, which will inherently interfere with its clinical performance.^{2,3,12}

Some studies reported that salivacontaminated and unwashed enamel provided significantly lower bond strength values of resin composite to enamel.^{2,11,13} Others have sought ways to reestablish the etched enamel surface morphology after salivary contamination, with the goal of restoring bond strength and eliminating microleakage.^{5,6,10} Vigorous washing has not shown favorable results,¹² whereas re-etching seems to produce better results.¹¹ Laboratorial¹⁰ and clinical⁵ studies showed that adding an adhesive layer on contaminated and previously etched enamel prior to sealant placement reestablished better bonding.

Recently, several studies^{14–20} have focused on investigating the efficiency of erbium : yttrium– aluminum–garnet (Er : YAG) laser on the removal of carious tissue, surface pretreatment, and cavity preparation, thereby searching for an alternative technique to the conventional air-turbine handpiece.

The ability of Er : YAG laser to effectively ablate dental tissues is ascribed to its 2.94-µm wavelength emission, which is coincident with the main absorption band of water (~3.0 µm) and OH- groups in hydroxyapatite (~2.8 μm).^{17,21,22} The incident radiation is highly absorbed by water molecules in the dental hard structures,^{15,21,23} causing sudden heating and water evaporation. The resulting highstream pressure leads to the occurrence of successive microexplosions with ejection of tissue particles,^{21,23,24} which are characteristic of the ablation process and

determine the microcrater-like appearance of lased surfaces.^{25,26}

Once the Er : YAG laser mechanism of action occurs in dental hydrated substrate, the application of this energy in a salivary contaminated surface could improve the sealant performance.¹⁴ Furthermore, Er : YAG laser has been considered a promising alternative for preventive dentistry because of its ability to increase fluoride uptake²⁷ and decrease acid dissolution, thus creating a surface more resistant to acid attack.¹⁶ Therefore, it could be assumed that if the sealant falls out from a laser-pretreated tooth, the surface would be more resistant to acid dissolution

Considering these facts, the purpose of this in vitro study was to evaluate the shear bond strength of a sealant to Er : YAG laser irradiated-enamel after the salivary contamination.

MATERIALS AND METHODS

Experimental Design

The factors under study were the type of surface treatment at two levels—37% phosphoric acid (A) and Er : YAG laser (80 mJ/ 2 Hz) + phosphoric acid (L); and the salivary contamination at two levels—without salivary contamination (C) and with salivary contamination (SC). The sealant material was Fluroshield (Caulk/ Dentsply, Milford, DE, USA). The association between surface treatment and salivary contamination resulted in four groups. The experimental sample consists of 48 specimens (N = 12) made in a random sequence. The response variable was shear bond strength evaluated in MPa. Failure type was qualitatively analyzed in stereoscopic microscope.

Teeth Selection

Twenty-four sound human third molars extracted within a 6-month period and stored in chloramine solution at 4°C were selected and cleaned with a scaler and pumice/water slurry in dental prophylactic cups.

Specimens Preparation

Roots were sectioned 2 mm below the cementoenamel junction. The 24 crowns were bisected longitudinally in mesiodistal direction with a water-cooled diamond disc (Minitom Struers A/S, Copenhagen, Denmark) in low-speed handpiece, providing 48 halves. Halves were individually embedded in polyester resin (Milflex Indústria Química, São Bernardo do Campo, São Paulo, Brazil) surrounded by polyvinyl chloride rings (2 cm in diameter, 1 cm high). After resin polymerization, the rings were removed and the teeth/resin blocks were ground in a water-cooled polishing machine (DP-9U2; Struers A/S) with 400- and 600-grit silicon carbide papers (Buehler Ltd., Lake

Bluff, IL, USA) until the overlying enamel was flattened. To demarcate the bonding site, a piece of insulating tape with a 2-mm diameter central hole, made by means of a modified Ainsworth rubberdam punch, was attached to the specimen surface. This procedure had a double aim: to define a fixed test surface, and ensure that the sealant would be precisely adhered to the treated enamel surface.

The specimens were randomly assigned to two groups of equal size according to the surface treatment: A—phosphoric acid (control) and L—Er : YAG laser irradiation + phosphoric acid. To perform the conventional treatment, phosphoric acid at 37% (etching gel; 3M ESPE, St. Paul, MN, USA) was applied for 15 seconds, followed by rinsing for the same time and removing the excess of water with absorbing paper.

Groups were divided into two subgroups (N = 12): C—without salivary contamination (control) and SC—with salivary contamination. In the subgroup with salivary contamination, after the surface treatment, specimens were contaminated with 0.25 mL of fresh human saliva from the same person and then gently air dried for 10 seconds.⁶

The Er : YAG laser device used was the Kavo Key Laser 2 (Kavo

TABLE 1. MEAN VALUES AND STANDARD DEVIATIONS OF SHEAR BOND STRENGTHS OF EACH GROUP (MPa).			
Tested conditions	Erbium : yttrium-	Phosphoric	
	aluminum–garnet laser +	acid (A)	
	phosphoric acid (L)		
With salivary contamination	$2.22 \ (\pm 0.66)^{d}$	6.66 (±2.34) ^c	
Without salivary contamination	11.91 (±1.34) ^b	14.61 $(\pm 2.52)^{a}$	
Different superscript letters indicate statist	tical difference ($p < 0.05$).		

Dental GmBH Co. KG, Biberach, Germany) emitting at 2.94-µm wavelength. The parameter settings used were: 80 mJ of energy and 2 Hz of pulse repetition rate. The laser beam was delivered on a noncontact mode, with a fine water mist of 1.5 mL/minute for 20 seconds, 17 mm desfocado.⁶ Laser beam spot size was 0.63 mm and the 2,051 handpiece with a removable tip attached to a flexible fiber delivery system was used. The irradiation distance was standardized by using a custom-designed apparatus consisting of two parts: a holder to fix the laser handpiece in such a way that the laser beam was delivered perpendicular to the specimen surface at a constant working distance from the target site; and a semi-adjustable base, on which the Plexglass® plate with the fragment attached to it was firmly fixed with wax. Two operators manipulated the apparatus' micrometer screws in such a way that the semi-adjustable base was alternately moved in both rightto-left and forward-to-back directions, thus allowing the laser

beam to provide an accurate ablation of the entire enamel site. The irradiation distance was checked with a ruler for each sample.

Specimens were individually fixed in a metallic clamping device (developed at the Houston Biomaterial Research Center and manufactured at the Precision Workshop at Ribeirão Preto School of Dentistry of the University of São Paulo, Brazil), keeping the enamel surface parallel to a flat base. A split bisected polytetrafluoroethylene jig was positioned on the tooth/resin block surface, with the center coincident with the demarcated 2-mm diameter bonding site. FluroShield sealant (Caulk/ Dentsply) was inserted into the jig and was light-cured with a visible light-curing unit (XL 3,000; 3M ESPE) with a light output not less than 450 mW/cm,² for 40 seconds. As the matrix cavity was filled, the specimen was removed from the clamping device and the matrix was opened. After 24-hour storage in distilled water at 37°C, specimens were loaded in tension, using

a universal testing machine (Mod MEM 2002; EMIC Ltd., São José dos Pinhais, Parana, Brazil) at a crosshead speed of 0.5 mm/minute and a 50-kgf load cell until fractures. Bond strengths were recorded in kgf and converted into MPa. Means and standard deviations were calculated, and data were analyzed by analysis of variance and Scheffé test at 0.05 significance level.

Fracture types at the surface/ sealant interface were verified under a stereoscopic microscope (Nikon Inc. Instrument Group, Melville, NY, USA) at 40× magnification. Failure was considered adhesive if it occurred at the substrate/sealant interface, cohesive if it occurred in the material or the substrate, and mixed if it involved both the interface and the material. Bond failure sites were not statistically analyzed.

RESULTS

Means and their respective standard deviations are described in Table 1. The statistical analysis showed significant differences (p < 0.05) among groups.

Multiple comparisons of the data revealed that the control group (phosphoric acid treatment surface without salivary contamination) had the highest mean bond strength (p < 0.05). The lowest shear bond values were obtained in

TABLE 2. FAILURE TYPES (%) OF EACH GROUP.				
Experimental groups	Adhesive	Cohesive	Mixed	
Acid—without contamination	31.25	62.50	6.25	
Er : YAG laser + acid—without contamination	50.00	12.50	37.50	
Acid—with contamination	50.00	37.50	12.50	
Laser Er : YAG + acid—with contamination	81.25	12.50	6.25	
Er : YAG = erbium : yttrium–aluminum–garnet.				

the presence of salivary contamination when the surface was treated with Er : YAG laser followed by phosphoric acid application, which were statistically different from the other groups (p < 0.05).

The analysis of the bonding sites after shear strength test revealed that adhesive-failure mode was predominantly observed in all groups, except for the control group (phosphoric acid treatment surface without salivary contamination) in which cohesive failure was predominantly recorded (Table 2).

DISCUSSION

Based on the fact that the microretentive pattern obtained after Er : YAG laser irradiation has been described as suitable for adhesion^{14,15,25,28} and considering that Er : YAG laser can increase enamel resistance to acid attack,¹⁶ the current study assessed the shear bond strength of a sealant to lased-enamel surface in salivary contamination condition. Strength testing is a laboratory methodology that has been used to evaluate the adhesion capacity of dental materials to teeth surface.^{3,19,22} Shear bond strength test holds great importance for providing insight into the adhesion of these materials and is also a screening mechanism for predicting clinical performance.^{14,20}

The hypothesis that Er : YAG laser combined with the conventional treatment with acid would increase the surface adhesion could not be confirmed in the present research because groups that received laser irradiation were less effective than those pretreated with phosphoric acid solely.

The probable explanation for these results is derived from the Er : YAG laser's microablasive process that causes vaporization of water and dental organic components, promoting microexplosive destruction of inorganic substances,^{22,28} thus blocking the intraand interprismatic spaces and restricting material interdiffusion

into the enamel surface.^{24,29} The micromorphology of laserirradiated surface exhibits less regular and homogenous aspects,¹⁹ with subsurface fissures resulting from heat generated during irradiation, which might be adverse factors for the bonding process.^{20,22,30} In addition, Er : YAG laser irradiation modifies calciumto-phosphorus ratio, reduces carbon-to-phosphorus ratio, and leads to the formation of more stable and less acid-soluble compounds,^{16,17} which would hamper the adhesion of sealants. This fact can affect the permeability of the surface to the acids and might compromise the diffusion of the adhesive system because of the formation of an acid-resistant enamel surface. Recent studies have also shown that Er : YAG laser irradiation reduced the shear bond strength of noncontaminated enamel; however, these investigations analyzed composite resin²⁶ and glass ionomer cements.²⁰ Delfino and colleagues,²⁹ using ablative parameters, observed that the bond strengths of restorative systems to enamel after Er : YAG laser irradiation were lower than those obtained with of high rotation turbine. Recently, Souza-Gabriel and colleagues³¹ observed, by scanning electron microscope analysis, that the subsequent acid etching on lased-enamel partially removed the disorganized tissue but was unable to completely

eliminate the surface fissures and cracks or expose the enamel prisms, regardless of the irradiation distance. This morphological aspect created by Er : YAG laser on enamel surface might be responsible for the lower shear bond strength values obtained with the lased samples of the current study.

Conversely, the enamel surface without salivary contamination and with the phosphoric acid treatment presented the highest bond strength means. On this concern, it is well known that acid etching provides suitable substrate for adhesion, as it removes the smear layer and creates a uniform microretentive pattern, because of the selective dissolution and removal of hydroxyapatite crystals.³² This aspect is a favorable condition to the deep penetration of the material into the microporosity network, thus forming tags upon light curing.^{4,19} Therefore, it seems feasible to speculate that phosphoric acid conditioning after laser irradiation would be insufficient to effectively remove the laser-altered layer and produce an etching pattern similar to that created by phosphoric acid alone. In microleakage studies, Borsatto and colleagues⁶ verified that Er : YAG laser irradiation did not eliminate the need for acid etching enamel prior to the placement of a pit-andfissure sealant. On the other hand, Manhart and colleagues⁸ reported

that, if Er : YAG laser conditioning was followed by acid etching, the retention of the compomer-based sealant was equal to the acid etch solely, a finding unlike the present study.

In this study, it was observed that salivary contamination negatively affected the adhesion of the Fluroshield sealant to the enamel, regardless of the surface pretreatment. This might have occurred because of the fact that the surface contamination forms an organic film on the etched area.9 Salivary contamination influences the etched enamel morphology quickly. It has been reported that just 1 second of saliva exposure is enough to create an altered layer able to penetrate and close the enamel micropores created by acid etching,^{2,10} decreasing the adhesion strength from 50 to 100%.¹⁰

Regarding the types of failure observed in the fractured specimens, a cohesive-failure pattern (into the substrate) was predominantly observed in the non-lased group, indicating that the adhesive interface was preserved. In contrast, failure mode in the lased groups (contaminated or not) was mostly adhesive, which may be attributed to the fact that the Er : YAG laser beam does not provide a uniform, homogeneous etching pattern, reducing the interface strength.^{18,20,22} Overall, Er : YAG laser was not able to increase the effectiveness of conventional acid etching of enamel in the bond of sealants, nor is the laser helpful in overcoming the negative effects of salivary contamination. However, it is difficult to obtain a more appropriate comparison of the results of this study because of the lack of literature reporting the adhesion of sealant to laser-irradiated surfaces. Hence, there is still little information available concerning the Er : YAG laserirradiated enamel. This amount of information will be required. The great variety of current dental materials is crucial features that also should be considered. Further studies with other laser parameters and long-term clinical trial are required to determine what protocol is preferred to improve the mechanism of adhesion to laser irradiated-surface before this technology becomes routine in dental practice.

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