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Short-pulse Er:YAG laser increases bond strength of composite resin to sound and eroded dentin

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Abstract. This study evaluated the influence of the irradiation with a short-pulse Er:YAG laser on the adhesion of composite resin to sound and eroded dentin (SD and ED). Forty-six samples of occlusal dentine, obtained from human molars, had half of their surface protected, while the other half was submitted to erosive cycles. Afterward, 23 samples were irradiated with Er:YAG laser, resulting in four experimental groups: SD, sound irradiated dentine (SID—Er:YAG, 50 μ s, 2 Hz, 80 mJ, and 12.6 J/cm²), ED, and eroded irradiated dentin (EID—erosion + Er:YAG laser). A self-etching adhesive system was used, and then cylinders of composite resin were prepared. A microshear bond strength test was performed after 24 h storage ($n = 20$). The morphology of SD and ED, with or without Er:YAG laser irradiation, was evaluated under scanning electron microscopy ($n = 3$). Bond strength values (MPa) were subjected to analysis of variance followed by Tukey's test. Statistically significant differences were found among the experimental groups: SD (9.76 ± 3.39 B), SID (12.77 ± 5.09 A), ED (5.12 ± 1.72 D), and EID (7.62 ± 3.39 C). Even though erosion reduces the adhesion to dentin, the surface irradiation with a short-pulse Er:YAG laser increases adhesion to both ED and SD. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.21.4.048001]

Keywords: bond strength; erosion; Er:YAG; human dentin; short pulse laser.

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1 Introduction

Dental erosion has been a major concern of dentists, as its prevalence has increased significantly in the population, even among children and youths.^{1–3}

The etiology of tooth erosion is multifactorial, and after diagnosis, measures must be taken to prevent its progression or, regarding severe lesions with great surface loss, to restore the teeth's function or aesthetics, and/or treat dentin hypersensitivity.⁴ When tooth restoration becomes necessary, a direct restorative technique with composite resin is considered a useful conservative procedure.^{4,5}

However, the eroded dentin (ED) is a challenging substrate to bond to.^{5,6} The dissolution of hydroxyapatite crystals by erosion causes the exposure of long collagen fibrils and the increase of the inner diameter of dentinal tubules.⁷ This fibril network is very resistant to mechanical impact, such as brushing,⁸ and can compromise the permeability of adhesive systems and hybrid layer formation. Consequently, to reach a greater bond strength, it is necessary to prepare the tooth surface before the use of adhesive systems^{5,9} in order to selectively remove the outer demineralized dentin.

Techniques to treat the ED surface prior to bonding procedures have been investigated,^{5,9} and high-power lasers have been recently reported for that purpose.¹⁰ As the wavelength of the Er:YAG laser ($\lambda = 2.94 \mu\text{m}$) coincides with the absorption band of water and hydroxyapatite, specific parameters can conservatively remove dental hard tissue (ablation process) and promote the selective removal of carious dentin, microbial reduction, and

pretreatment (prior to adhesive procedures) of the tooth surface.^{11–14}

Most studies on the use of Er:YAG lasers for tooth preparation have used equipment with a pulse width in the range from 100 to 350 μ s. However, there is already commercially available equipment with shorter pulse widths that are reported to cause less or no thermal damage to the tooth surface.^{15–17} Although there are many studies on the use of Er:YAG laser on dentin prior to restorative procedures,^{12,13,16,18–22} little is known about its effect on ED.¹⁰ To date, no studies about the effects of short pulse Er:YAG laser on the bond strength of ED to composite resin have been conducted.

Given the information above, this in vitro study aimed to evaluate the effect of the dentin surface's (sound and eroded) irradiation with a short pulse width Er:YAG laser on the adhesion to composite resin.

2 Materials and Methods

2.1 Ethical Aspects

This study protocol was approved by the Committee of Ethics on Research of the School of Dentistry of the University of Sao Paulo (Protocol n. 944.762). The authors declare no conflicts of interest on this research.

2.2 Sample Preparation

Forty-six recently extracted third human molars were used.

Six dentin discs, obtained from six teeth, were used to compose the samples for the scanning electron microscope (SEM)

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test. For this purpose, discs were cut in the middle, and 12 hemi-discs of dentin were obtained ($n = 3$). The remaining 40 teeth were also cut in discs, and 80 hemi-discs of dentin were obtained and used for bond strength analysis ($n = 20$) (Table 1). They were stored in chloramine T solution at 0.5%, under refrigeration (4°C) until the beginning of the experiment (up to three months). Experimental groups were divided according to the factors of substrate and surface treatment and were evaluated by bond strength and SEM.

The occlusal enamel was removed with a low-speed diamond saw (Isomet 1000, Buehler Ltd., Lake Buff, Illinois), to obtain dentine discs that were included on acrylic resin (JET Clássico, Sao Paulo, SP, Brazil). Dentine was polished (Ecomet 3, Buehler Ltd.) with 120, 240, 400, and 600-grit silicon carbide sandpaper discs (1 min each).

A fragment of an adhesive tape (Scotch 3M, 3M ESPE, Americana, SP, Brazil) of $\sim 3 \times 3$ mm was placed on the occlusal surface of each disc, protecting the area that corresponded to the sound dentin (SD) (not exposed to erosive cycling) to delimit the SD area. The samples were then individually submitted to erosive cycling, consisting of the immersion of the samples in 10 mL of 0.05 M citric acid solution (Merck Dermstadt, Germany), pH 2.3, six times per day (10 min each), for five days.²³ Between each immersion in acid solution, with an interval of 1 h each, and during the remaining time of the day, the specimens were stored in 10 mL of remineralizing solution (H_3PO_4 4.8 mM, KCl 20:10 mM, Na_2CO_3 11.90 mM, CaCl_2 1.98 mM, pH 6.7).²⁴ The solutions were renewed daily at the beginning of the experiment, and the pH value of all solutions was checked at the beginning and end of each experimental day.

2.3 Surface Treatment with the Er:YAG Laser

After the erosive challenge, the adhesive tape was removed from the dentin samples' surfaces, exposing the SD area. Twenty-six specimens were irradiated with the short pulse Er:YAG laser (flashlamp-pumped solid-state laser, Fidelis III, Fotona, Slovenia), with almost square-shaped pulses of adjustable duration, working at 2.94 μm , fitted with an R02 noncontact hand-piece (beam spot size in focus: 0.9 mm).

Irradiation was performed using a precision translation platform driven by a stepping motor (ESP301, Newport Corporation, Irvine, California), in such a manner that the laser beam was delivered perpendicular to the dentin surface and at a distance of 7 mm (focused mode) from the sample's surface. A base, with a fixed dentin sample, was automatically moved (0.8 mm/s) based on commands previously established through a computer connected to the scanning device, allowing the irradiation to reach the entire dentin area.

The parameters consider for irradiation were 50 μs , 2 pulses/s, 80 mJ, and 13.3 J/cm². The area to be irradiated was standardized with dimensions of 8 \times 8 mm, covering both sides (sound and eroded) of the sample.

2.4 Dentin Surface Morphology

Three samples of each experimental group (dentin hemi-discs) were prepared for analysis under SEM. Samples' preparation for SEM was conducted according to the protocol described by Trevelin et al.²⁵ The surface analysis was performed using SEM images (Philips XL30, The Netherlands) with 1000 \times and 3000 \times magnification.

2.5 Microshear Bond Strength

Eighty samples ($n = 20$, dentin hemi-discs) were used, and half of them underwent laser surface treatment following the erosive cycling and prior to the restorative procedure. A 0.05-mm-thick double-sided adhesive tape piece was placed (Tectape, Manaus, AM, Brazil) on the dentine disc. The tape presented four circular perforations of 1.0 mm diameter each, delimiting the area of application of the adhesive and the resin cylinder on both SD and ED (two composite resin cylinders for each dentin substrate). These procedures were performed as described by Shimaoka et al.²⁶

A self-etching adhesive system (Clearfil SE Bond, Kuraray Medical Inc., Osaka, Japan, Lot. 051 550), used according to the manufacturer's instructions, was applied on the dentin surface. A silicone microtube (Tygon, Norton Performance Plastic Co., Cleveland, Ohio), with an internal diameter of 1.0 and height of 0.5 mm, was used as template for the construction

Table 1 Study design.

46 human molars—46 dentin discs				
Microshear bond strength ($n = 20$)	40 dentin discs (80 hemi-discs)			
	40 hemi-discs		40 hemi-discs	
	Protected surface	Eroded surface	Protected surface	Eroded surface
	20 hemi-discs of SD	20 hemi-discs of ED	20 hemi-discs of SD	20 hemi-discs of ED
Groups	No surface treatment		Er:YAG laser irradiation	
	SD	ED	SID	EID
Surface morphology ($n = 3$)	6 dentin discs (12 hemi-discs)			
	3 hemi-discs of SD	3 hemi-discs of ED	3 hemi-discs of SD	3 hemi-discs of ED
Group	No surface treatment		Er:YAG laser irradiation	
	SD	ED	SID	EID

of the composite resin cylinders. After the positioning of four tubes on each dentin disc (two on SD and two on ED), they were filled with composite resin (A3 color, Filtek Z350 XT, 3M ESPE Dental Products, St. Paul, Minnesota, Lot: 14339020560 HB004134092) with the aid of a ballpoint probe, also known as a CPI probe (Trinity® Periodontics, Sao Paulo, SP, Brazil). After filling the silicone tubes, the composite resin excesses were removed, with subsequent curing for 20 s (light-emitting diode source, Radi-lime, SDI Limited, Bayswater, Victoria, Australia) with a power intensity of 1200 mW/cm².

After curing, silicone tubes were removed immediately, and all specimens were observed under a stereoscopic lens with 40× magnification for certifying the absence of defects in the bond interface. The specimens were then stored in artificial saliva²⁷ in an incubator at 37°C for 24 h, before undergoing the mechanical microshear test. The artificial saliva used for sample storage consisted of CaCl₂ (0.7 mmol/L), MgCl₂·6H₂O (0.2 mmol/L), KH₂PO₄ (4.0 mmol/L), KCl (30 mmol/L), Na₂CO₃ (0.3 mmol/L), and HEPES buffer (20 mmol/L).

The specimens were submitted to the microshear test on a universal testing machine (Instron 5942, Canton, Massachusetts) (1 mm/min and 50 N load). The maximum load values supported by the dentin/restorative material interface were obtained in newtons and later converted to megapascals (MPa).

The results of the bond strength test (average of bond strength obtained from the two resin cylinders of each sample) were analyzed using the statistical software Biostat (AnalystSoft Inc., Walnut, Canada), considering the significance level of 5%. As the distribution of the mean values was normal and homogeneous (Lilliefors normality test), analysis of variance was performed, followed by Tukey's test.

2.6 Failure Mode

After completion of the microshear test, all the specimens were analyzed under stereoscopy (Carl Zeiss Citoval 2, Jena, Germany) at 20× magnification. The failure mode was classified as follows: type I—adhesive; type II—cohesive in dentin; type III—cohesive in composite resin; type IV—mixed.

3 Results

3.1 Dentin Surface Morphology

Morphological analysis of the specimens showed different characteristics according to the surface treatment.

The SD appeared homogeneous, with grooves caused by the abrasion of the silicon carbide polishing paper and a smear layer covering the whole surface with the obliteration of the dentinal tubules [Figs. 1(a) and 1(b)]. The sound dentin submitted to the laser irradiation (SID) revealed a microretentive pattern with an irregular surface, absence of smear layer, open dentinal tubules, and prominent peritubular dentin [Figs. 1(c) and 1(d)].

The ED samples showed a homogeneous porous surface with large and open dentinal tubules, demineralized peritubular dentin, and a rough intertubular dentin, showing the exposed network of collagen fibers [Figs. 1(e) and 1(f)]. Samples of ED that were treated with the Er:YAG laser (EID) revealed a regular flat surface with obliterated tubules, highlighting a collapse aspect of the collagen fibers [Figs. 1(g) and 1(h)].

3.2 Microshear Bond Strength

The microshear bond strength values found for the SD samples, regardless of being irradiated (SID-12.77 ± 5.09 A) or not (SD-9.76 ± 3.39 B), were higher than the bond strength values of the eroded samples (ED-5.12 ± 1.72 D; EID-7.62 ± 3.39 C). Also, the irradiated samples resulted in higher bond strength values than their respective untreated controls (nonirradiated) as described in Fig. 2.

In all groups, the failure mode was predominantly adhesive (between adhesive and dentine), followed by mixed failure and cohesive failure as depicted in Fig. 3.

4 Discussion

Without control of the etiologic factors, the loss of dental mineralized structure by erosion can result in dentin exposure.²⁸ In such cases, restorative treatments are indicated to reduce the thermal sensitivity, to prevent injuries to the pulp, and to rehabilitate the contour, function, and aesthetics of the affected elements.²⁹ In order to understand the behavior of restorative materials in different dentinal substrates, some authors seek to simulate dental erosion in *in vitro* protocols, although laboratory studies present limitations to accurately replicate the *in vivo* conditions.³⁰ In *in vitro* studies, however, isolated conditions in different tooth substrates exposed to erosive challenges can be investigated under destructive/nondestructive methods.

In the case of dentin exposed to erosive challenges, the acids may remove the plugs that seal the dentinal tubules and demineralize both inter- and peritubular dentin, exposing its organic matrix and increasing the diameter of the tubules.^{31,32} Regarding this aspect, the erosive cycling considered for the present study succeeded in reproducing the characteristics of eroded dentin as seen in SEM images, with open tubules, without peritubular dentin, and having intertubular dentin with a decalcified and fibrous appearance.^{7,33}

As adhesive materials must adhere to different surfaces other than those for which they were originally created and tested, studies on ED are mandatory, but adhesion to this substrate has been poorly investigated. Studies have so far found lower bond strength to this substrate compared to SD,^{6,9,10,34,35} corroborating our findings.

The high level of demineralization on ED submitted to etching results in a softened surface layer that, after the penetration of the adhesive, leads to the formation of a thin hybrid layer when compared to an etched SD. These layers are structurally imperfect and contain pores that generate predominantly hydrophilic areas and demineralized zones without resin reinforcement.³⁶ These factors can contribute to the low bond strength values found for ED, because resinous monomers' penetration into the demineralized substrate is compromised.^{34,36,37}

The irradiated ED showed higher bond strength values than ED, even though their bond strength values were lower than those found for SD (irradiated or not). These findings are consistent with the morphological analysis of the ED submitted to irradiation. The Er:YAG laser irradiation of the ED resulted in a homogeneous demineralized surface with occluded tubules and collapsed collagen fibrils. These characteristics are possibly a result of the residual heat generated during the ablation process, which could have negatively influenced the penetration of the adhesive and the formation of the hybrid layer.³⁸ Therefore, treating the surface with the laser parameters tested in the present study was not effective in removing the softened outer dentin layer and improving the adhesion to composite resin.

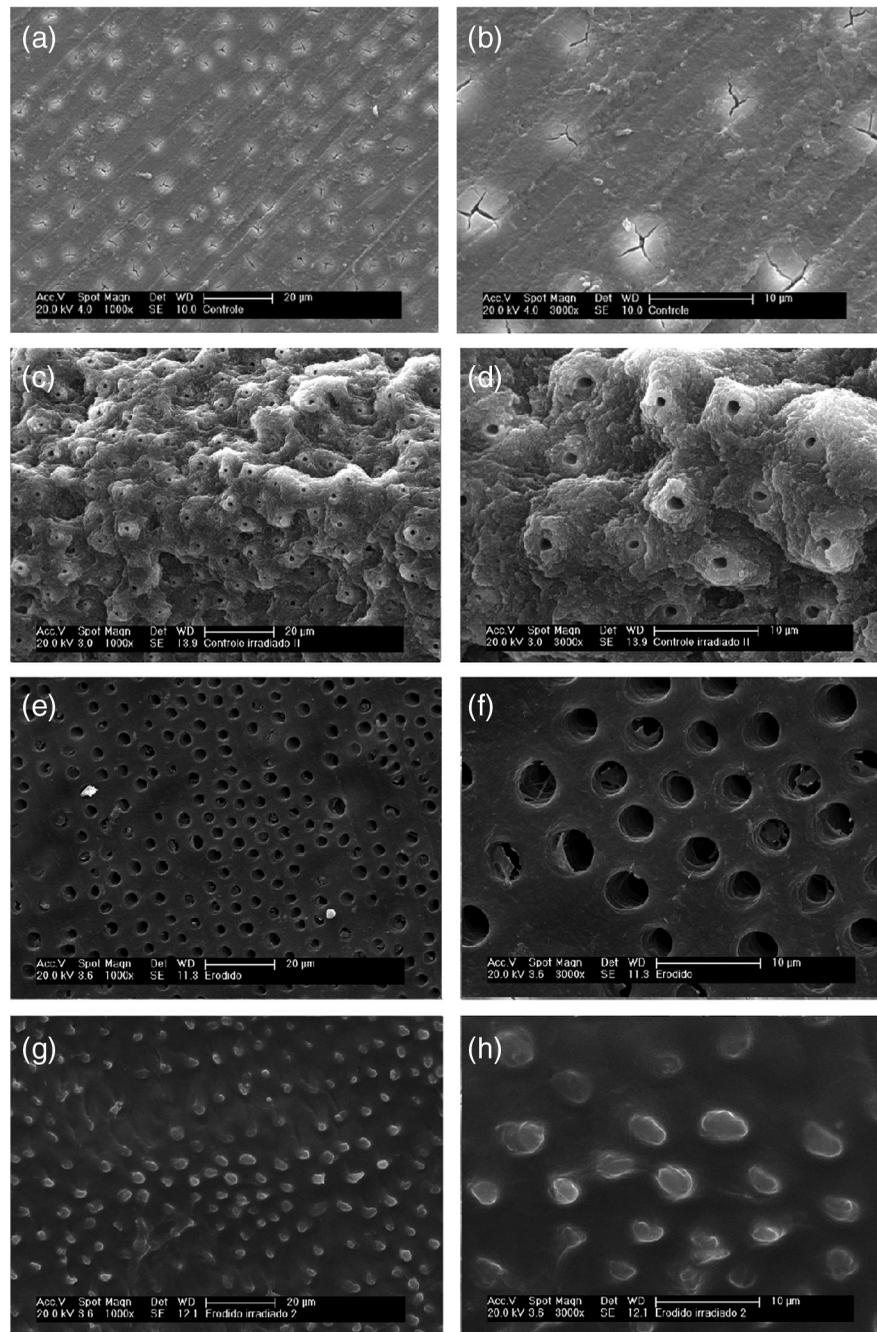


Fig. 1 Electromicrographs obtained by SEM: morphological characteristics of the dentin surfaces after different surface treatments: (a) and (b) SD, (c) and (d) SID, (e) and (f) ED, and (g) and (h) EID.

Ramos et al.¹⁰ reported that the dentin surface treatment with the Er:YAG laser (60 mJ, 2 Hz, 0.12 W, 19.3 J/cm², 150 to 250 μ s) was not able to increase the bond strength values to ED or SD. Their results contrast with our findings that showed that irradiated dentin reached higher bond strength values than those of its nonirradiated counterparts. Even though the energy density considered by Ramos et al.¹⁰ and the one used in the present study were similar, the higher pulse width used by the authors may have led to thermal damages at the target tissue³⁹ and influenced the adhesion to dentin.

Among the tested groups, the irradiated SD was the one that reached the highest shear bond strength values. SEM images

showed that, unlike SD (nonirradiated, uniform smear layer, occluded dentinal tubules), SID showed wide opened tubules, irregular surface, and prominent peritubular dentin, creating the microretentive surface that has been suggested by some authors.^{25,40} For Moretto et al.,²⁰ although the resulting microretentive aspect of the irradiated dentin surface apparently favors the bond strength, it did not actually improve it. The authors reported microcracks on the dentin subsurface and suggested that the decrease in bond strength was due to these changes. According to Esteves-Oliveira et al.,¹³ the thermal degradation of collagen may also be a contributing factor to the reduction of bond strength values.

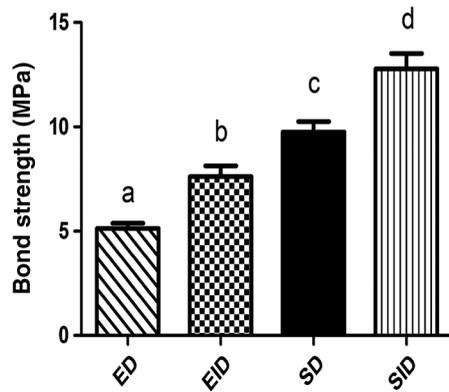


Fig. 2 Microshear bond strength values obtained for all experimental groups.

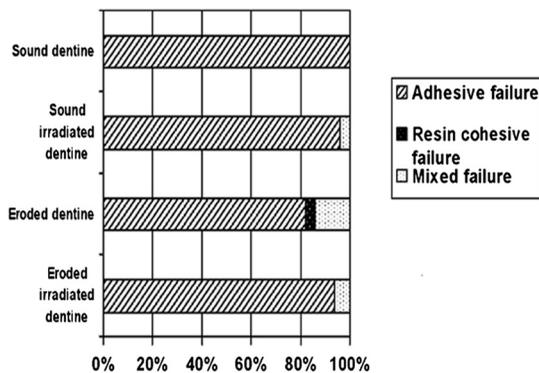


Fig. 3 Prevalence (in percentage) of failure mode for each experimental group.

It is important to highlight that the pulse width of the laser equipment used in previous studies^{13,20,21,41} might have negatively affected the properties of the irradiated tissue, due to the heat diffusion into the inner dentin.³⁹ According to Fried et al.,⁴² the higher the pulse duration, the higher the residual heat in the irradiated tissue. Bodrumlu et al.⁴³ compared the temperature increase during root apicectomy using Er:YAG at three different pulse widths (50, 100, and 300 μ s) and reported the lowest temperature increase for the 50 μ s pulse width.

Staninec et al.⁴⁴ suggested that shorter pulse widths, as used in the present study, might minimize the thermal damage to the dentin surface and subsurface. Their results showed that the SD irradiated with a super short pulse Er:YAG laser (35 μ s) resulted in bond strength similar to the nonirradiated control group. Bahrami et al.⁴⁵ also reported a significant increase in bond strength of the composite resin to dentin when irradiating the substrate with an Er:YAG super short pulse laser (80 mJ, 10 Hz, 50 μ s, 12.58 J/cm²).

In the current study, microcracks on the surface of irradiated SD and ED were not expected.⁴⁶ The treatment with the Er:YAG laser (50 μ s pulse width) with low energy densities appears to be an alternative for tooth pretreatment, without compromising the adhesion to composite resin.

Given the exposures above, we conclude that, regardless of the substrate (SD or ED), the Er:YAG laser with super short pulses favored the dentin bond strength to composite resin. It was the first time that Er:YAG laser was shown to enhance bond strength to ED. Future studies should be conducted in

order to assess different laser parameters, the long-term effect of the laser treatment on SD and ED bond strength, and the quality of the bonding interface prior to adhesive procedures.

5 Conclusion

The short pulse Er:YAG laser (50 μ s), when used prior to the restorative treatment, is able to promote positive changes on both SD and ED surface and contribute to the adhesion to composite resin.

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