

BOND STRENGTH ON ERODED DENTIN: A PILOT STUDY USING AN ER: YAG LASER**Vivian Colucci¹, Rodrigo Alexandre Valério², Tiago Gilioli Varise³, Taísa Penazzo Lepri³, Cecília Pedrosa Turssi⁴, *Silmará Aparecida Milori Corona³**¹DDS, MS, PhD, Assistant Professor. Department of Restorative Dentistry, Dental School, University of Ribeirão Preto, Avenida Costábile Romano, 2201, Ribeirão, CEP: 14096-900 Ribeirão Preto - SP, Brazil.²DDS, MS, PhD Student. Department of Clinical Pediatric Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Av do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brazil.³Scientific Initiation Scholarship. Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Av do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brazil.³DDS, MS, PhD Student. Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Ribeirão Preto, Av do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brazil.⁴DDS, MS, PhD, Assistant Professor. Department of Restorative Dentistry, São Leopoldo Mandic Institute and Dental Research Center, Av. José Rocha Junqueira, 13 CEP: 13045-755 Campinas – SP, Brazil.^{3*}DDS, MS, PhD, Associate Professor. Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Av do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brazil.***Correspondence for Author: Silmará Aparecida Milori Corona**

DDS, MS, PhD, Associate Professor. Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Av do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brazil.

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ABSTRACT

Objectives: The aim of the present *in vitro* study was to evaluate the effect of an Er:YAG laser on the bond strength of eroded dentin. **Materials and Methods:** Fifteen bovine incisors (n = 10) were sectioned crowns, resulting in 60 samples (4 x 4 x 2 mm). Samples were randomly assigned in 3 surface treatment: A. 5% fluoride varnish; B. Er:YAG laser irradiation and C. no treatment (control) - and 2 bond systems: (I) Total etching and (II) Self-etching. Specimens were immersed in 0.3% citric acid solution (pH 3.2). The 5% fluoride varnish and an Er:YAG laser (80 mJ, 3 Hz) were applied at dentin surface. After performing the treatments on eroded dentin, specimens were randomly subdivided according to the bond system (I or II). All specimens were individually fixed at a metallic device, forming a cylinder (3 x 4 mm), to be subjected to the shear bond strength test (MPa). **Results:** ANOVA showed that there was no statistically difference between the employed surface treatments (p < 0.05). With respect to the bond systems, it was observed greater shear bond strength to the self-etching bond system (Adhese – 11.89 MPa) compared to the total-etching (Excite – 8.45 MPa) (p < 0.05). **Conclusion:** It can be concluded that the treatments performed at eroded dentin did not interfere on the bond strength of the composite resin to this tissue. The self-etching adhesive system provided greater adhesion to the eroded dentin, compared to the total-etching.

KEYWORDS: Erosion, Dentin, Lasers, Fluoride varnish, Bond strength.**INTRODUCTION**

As people are retaining their natural teeth longer ^[1] and the consumption of dietary acids has increased, ^[2] the risk of developing non-carious lesions, such as dental erosion, is growing ^[3]. These lesions consist of the irreversible loss of dental tissue due to chemical processes without the involvement of microorganisms ^[4-6] and can be caused by intrinsic agents, such as recurrent vomiting or regurgitation of gastric content ^[6-10] or by extrinsic factors, including the intake of acidic substances in drinks, foods or medicines ^[6-8,11].

In early stages, the dentin erosion is similar to that described for the enamel, involving chemical corrosion of dental substrate with demineralization and consequent reduction of superficial hardness ^[12]. It is remarkable that, first of all, the peritubular dentin is eroded and, as the lesion progresses, the dentinal tubules then exhibit greater diameter and, subsequently, intertubular dentin areas are also affected, exposing the organic matrix, resulting in a porous and rough surface ^[13]. Thus, clinically, apart from the possibility of compromising the esthetic appearance and masticatory function, dental erosion can also cause dentin hypersensitivity ^[14-15].

To be effective, the management of dentin hypersensitivity needs to begin with a proper diagnosis, in order to identify and eliminate its causative factors. Among the strategies to treat dentin hypersensitivity, are fluoride varnishes^[16] and Er:YAG laser^[17]. The Er:YAG laser occludes the dentinal tubules^[18] and interferes on the hydrodynamics conduction of dentin^[19].

In some clinical circumstances, after therapeutic approaches have provided ineffective results, restorative intervention of the tooth may be necessary. The self-etching bond systems could provide a greater adhesion to the eroded dentin due to changes and partial removal of smear layer, preventing the breakdown of collagen fibers^[20], besides promoting decalcification of the dentinal surface^[21]. In this context, there is a lack in the literature of studies that evaluated the bond strength of adhesive restorative materials to dentin previously exposed to therapeutic agents to manage hypersensitivity.

Whereas surface treatments applied in erosive lesions that simulate dentinal hypersensitivity could interfere with the bond strength of composite resin to dentin, the present study aimed to evaluate whether the shear bond strength of total- and self-etching adhesive materials to eroded dentin would be affected by the use of fluoride varnish or Er:YAG laser, applied as previous steps before adhesive restoration.

MATERIALS AND METHODS

Experimental design

The study factor was the surface treatment of eroded bovine substrate in 3 levels - A. 5% fluoride varnish; B. Er:YAG laser irradiation and C. no treatment (control) – and bond system at 2 levels (I) Total-etching and (II) Self-etching. The experimental sample consisted of 60 slabs of eroded bovine dentin (n=10), randomly assigned: 5% fluoride varnish/total-etching adhesive (AI); 5% fluoride varnish/self-etching adhesive (AII); Er:YAG laser irradiation/total-etching adhesive (BI); Er:YAG laser irradiation/self-etching adhesive (BII); no treatment (control)/total-etching adhesive (CI); no treatment (control)/self-etching adhesive (CII), according to a complete and randomized block design. The quantitative response variable was the shear bond strength (MPa) of the eroded bovine substrate subjected to the surface treatment.

Tooth Selection

Fifteen sound bovine incisors, freshly extracted from Nelore cattle (five years old and 250 kg) slaughtered for human consumption, treated within quality standards strict under the supervision of the Ministry of Agriculture of Brazil (SIF 1758), were provided by Mondelli Indústria de Alimentos company (Bauru, São Paulo, Brazil), to perform *in vitro* studies. Teeth were stored in 0.1% thymol solution at 4°C cleaned and examined under a 20x magnification, using a stereomicroscope (Leica S6 D Stereozoom, Microsystems Leica AG, Switzerland).

Roots were sectioned 2 mm below the cement-enamel junction. Crowns were sectioned at the mesial-distal and cervical-incisal direction, using a low-speed water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA), obtaining 60 slabs (4 x 4 mm). The slabs were individually embedded in acrylic resin (JET, Classico, São Paulo, SP, Brazil) and included in polyvinyl chloride rings (2.0 cm diameter, 1.0 cm height), with the enamel surfaces facing upwards. After that, samples were flattened and polished on a water-cooled polishing machine (DP-9U2; Struers S/A, Copenhagen, Denmark), with 180-, 400-, 600- and 1200-grit of Al₂O₃ papers (Hermes Abrasives Ltd., VA, USA) and 0.3 µm alumina suspension (Arotec S/A Ind. Com., São Paulo, SP, Brazil) to exposure and flattening of dentin surface. After polishing, samples were coated with acid-resistant nail varnish (Colorama Maybelline Ultra Duração; Cosbra Cosméticos LTDA., São Paulo, SP, Brazil) to isolate dentin from the acidic challenge, maintaining only the diameter of 3 mm uncovered.

Erosive Challenge

The specimens were individually immersed in 20 mL of 0.3% citric acid (pH 3.2) at room temperature, in an Erlenmeyer flask placed in an orbital shaker (CT155, Cientec, Piracicaba, São Paulo, SP, Brazil) for 2 hours^[22], to simulate *in vitro* erosion-like lesions. After challenge, specimens were stored in plastic flasks containing 10 mL of artificial saliva, for 24 hours at 37°C.

Surface Treatment

Specimens were randomly assigned according to the surface treatment (n=10): A. 5% fluoride varnish; B. Er:YAG laser irradiation and C. no treatment – control. An Er:YAG laser (Kavo Key Laser 2, Kavo; Biberach, Germany), with a 2.94 µm wavelength, 250 to 500 msec pulse duration, spot size of 0.63 mm, frequency of 3 Hz and an energy density of 80 mJ was used. The laser was applied in non-contact, unfocused mode, 17 mm distant from the dentin surface^[23] during 20 seconds, with constant water flow of 2.0 mL/min^[24].

Dentin surfaces were irradiated using an automatic custom designed device (MPC ElEquip, São Carlos, SP, Brazil) that affix the laser handpiece, in such a manner that the laser beam was delivered perpendicular to the specimen surface and at a constant distance from the target site. A semi-adjustable base, with fixed specimen, was automatically moved based on commands previously established through a computer connected to the scanning machine, allowing the irradiation to reach the entire enamel area.

0.1 g of 5% fluoride varnish (Duraphat; Colgate-Palmolive Ind. Com. Ltda, São Paulo, SP, Brazil, lot 11. 07-02, pH 4,5) was applied with a microbrush (Dentsply Ind. Com. Ltda, Rio de Janeiro, RJ, Brazil), during 1 minute on the dentin surface. Twenty-four hours after the storage in artificial saliva at 37°C, the fluoride varnish

was removed from the dentin surface with a scalpel blade^[25] (Free-Bac, China).

During surface treatments of A and B groups, specimens from C group were maintained in relative humidity at 37°C.

Restorative Protocol

Specimens were randomly subdivided according to the adhesive protocol: (I) total-etching and (II) self-etching. The specimens restored with total-etching adhesive system had their dentinal surfaces conditioned with 35% phosphoric acid (Ivoclar Vivadent, Ellwangen, Jagst, Germany) for 15 seconds. The dentin surface was washed with distilled and deionized water for 1 minute and dried with absorbing paper. After that, the total-etching bond system Excite (Ivoclar Vivadent, Germany) was applied on the specimens according to the manufacturer's instructions and light-cured for 10 seconds using a photo-curing unit with 750 mW/cm² power (Ultralux, Dabi Atlante, Ribeirão Preto, SP, Brazil). The specimens restored with the self-etching system received the application of the AdheSe bond system (Ivoclar Vivadent, Ellwangen, Jagst, Germany), without acid conditioning according to the manufacturer's instructions and it was light-cured as described above.

All specimens were individually fixed on a metallic device (Houston Biomaterial Research Center, USA), in which specimen kept the dentin surface parallel to the horizontal plane. A split teflon matrix (3 mm inner diameter, 4 mm height) was placed under the specimen, to obtain restorative material cylinders with measures described above. In each specimen, a light-cured hybrid composite resin (Four Seasons – Ivoclar Vivadent, Germany) was inserted in 2 increments, both photo-

activated during 20 seconds. The material excesses were removed with a #15 scalpel blade (Free-Bac, China) and the mold was opened and separated, forming a composite resin restoration shaped as a cylinder with 3 mm of diameter and 4 mm of height adhered to the dentin substrate. Restored specimens were stored in artificial saliva, at 37°C, for 24 hours and then subjected to the shear bond strength test using the Universal Testing Machine (MEM- 2000 model, EMIC, São José dos Pinhais, PR, Brazil), with a speed of 0.5 mm/min until fracture. Values were recorded in Kgf and converted into MPa.

Statistical Analysis

After evaluating the assumptions of normality and homoscedasticity, data were analyzed using a two-way analysis of variance (ANOVA). Statistical analysis was performed using Statgraphics Centurion XV.

RESULTS

Two-way ANOVA revealed that there was no significant interaction between the surface treatment applied to the eroded dentin and the bond system employed ($p=0.143$). Surface treatment did not affected the bond strength between eroded dentin and composite resin, regardless of the adhesive system used ($p=0.8012$). Significant difference ($p=0.021$) was observed between the adhesive systems, with the self-etching adhesive providing the highest bond strength, regardless the treatment previously applied on dentin. The means and standard deviations of shear bond strength are showed in Table 1.

Table 1 – Shear bond strength means and standard deviations of experimental groups.

Surface Treatment	Adhesive System		Grand Mean
	Total-etch	Self-etch	
Fluoride Varnish	8.54 (3.56)	16.05 (4.34)	12.30 (4.88) A
Er:YAG Laser Irradiation	13.70 (7.19)	12.99 (4.92)	13.34 (6.00) A
None (Control)	10.43 (4.62)	16.91 (5.22)	12.97 (5.45) A
Grand Mean	10.89 (5.42) B	15.31 (4.85) A	—

Means followed by identical capital letter did not differ from each other.

Means followed by identical lower cases did not differ from one another.

DISCUSSION

The dentin hypersensitivity has been considered an increasingly frequent clinical condition in the general population^[26], causing great discomfort to patients. The occurrence of dentinal hypersensitivity is a process with multifactorial etiology^[27], in which dentin erosion is considered the most important factor^[6].

To simulate the real clinical condition as close as possible, in the present study, erosion-like lesions were

developed on dentin surfaces prior to application of desensitizing treatments. Specimens were immersed in citric acid for 2 hours under agitation, as proposed by Vanuspong et al.^[22]. After the development of the erosive lesions, the surface treatments were performed on the eroded dentin.

According to the results obtained in the present study, no significant difference on the bond strength of composite

resin to the eroded dentin previously treated with Er:YAG laser or 5% fluoride varnish was found.

The available literature about bond strength between restorative material and laser-irradiated dental surfaces remains contradictory. According to some authors, irradiated surface patterns such as occlusion of dentinal tubules [28-29], reduction in interfibrillar spaces [30] and melting areas [18], can cause lower adhesion of restorative materials, since difficult the infiltration of the adhesive systems, and consequently, the formation of the hybrid layer [30]. However, Esteves Oliveira *et al.* [31] and Malkoc *et al.* [32] found that irradiation with the Er:YAG laser provides a micro-retentive pattern which possible favor the adhesive procedures due to the absence of the smear layer, presence of open dentinal tubules and prominent peritubular dentin, arising from structural changes induced by thermal effects in irradiated dentin. Laser irradiation can originate different organic and inorganic compounds which present different levels of acid solubility [33]. Laser-modified dentin is not completely etched and this could partially obstruct the micromechanical attachment of adhesives [34]. In previous studies irradiation was performed on sound dental substrate, however, in this study, irradiation was performed on eroded dentin aiming to simulate treatment for dentin hypersensitivity. In this way, the response from the tissue to the treatment could be affect by different characteristics of the irradiated substrate, since laser irradiation interacts to the target-tissue and with their compounds [35]. Additionally, Er:YAG laser was employed with subablative parameters and changes induced in the dentin surface did interfere with bond strength of composite resin.

The 5% fluoride varnish presents high content of fluoride, which forms a calcium fluoride protective layer on the dentinal surface, preventing fluid movement within the tubules, reducing the dentin solubility, responsible for decreasing the discomfort caused by hypersensitivity [36-37]. A previous *in vitro* study evaluated the use of fluoride varnish previously to the restorative procedure and found that the surface treatment performed provided a reduction on the bond strength of the composite resin to sound root dentin [38]. At eroded dentine, the treatment with tin-containing fluoride mouthrinse increased the adhesive resistance of self-etching system [39]. Titanium tetrafluoride (TiF₄) modifies the micromorphology of dentin surface and produces a surface resistant to erosion, by means of a surface layer of precipitates on intertubular and intratubular dentin [40].

It must be considered that, in the available studies, both Er:YAG laser irradiation and fluoride varnish application were performed on sound dentin, which presents a morphological pattern different from eroded dentin. The eroded dentin presents several dentinal tubules with increased diameter and reduced intertubular dentin area [41]. Besides, the organic matrix is demineralized [6,42] and

the collagen fibers are denatured [43]. The efficacy of dentin adhesives to sclerotic dentin is reduced for most of the dentin adhesives [44]. As laser, the fluoride varnish directly interacts with the treated dental tissues where chemical, morphological and structural changes could interfere with the response to different treatments. The fluoride varnish application on the demineralized dentin limits the penetration of the material to approximately 40% of the depth of the lesion, due to its hydrophobic nature [45].

Regarding adhesive systems, in the present study, the use of self-etching bond (AdheSE) promoted greater adhesion. These results are in agreement with Ramos *et al.* [42] and Flury *et al.* [39], who verified higher bond strength of composite resin to eroded dentin provided by a self-etching bond system (Clearfil SE Bond). The application of phosphoric acid on previously eroded dentin, results in a superficial zone totally demineralized which difficult the penetration of the adhesive through the collagen fibers network [46]. Possibly, the resin monomers not completely diffuse throughout demineralized dentin in depth [46] which could complicates the formation of a suitable hybrid layer [42]. The eroded dentin irradiated with CO₂ laser presented small μ TBS and large gaps using the total-etching adhesive system, probably due to a thick layer of exposed collagen on eroded dentin surfaces and its higher water content [47].

The self-etching bond system AdheSE has a component, an acidic primer, which allows, at the same time, dentin demineralization and monomers diffusion, preventing the breakdown of collagen fibers and improving the dentinal sealing [20,42]. Furthermore, the molecules of methacrylamide present in the AdheSE bond system promote the formation of hydrogen bridges between the carboxyl groups and the amide of monomers with the carboxyl groups from the collagen [48] that could explain the greater bond values.

CONCLUSION

It may be concluded that the treatments performed on eroded dentin, Er:YAG laser irradiation and 5% fluoride varnish, did not interfere on bond strength of composite resin. The self-etching bond system (AdheSE) provided greater adhesion of composite resin to eroded dentin when compared to the total-etching system (Excite).

Conflict of interest: The authors declare that they have no conflicts of interest.

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