

Influence of Etching with Er,Cr:YSGG Laser on the Microtensile Bond Strength of Adhesives to Dentin

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Purpose: The aim of this study was to investigate the bond strength of a composite to dentin treated with an Er,Cr:YSGG laser and different bonding systems.

Materials and Methods: Thirty-three extracted human teeth were longitudinally sectioned to obtain 65 specimens. After coronary dentin exposure and smear layer standardization, samples were divided into 5 groups (n = 13) and etched according to the Er,Cr:YSGG laser (20 Hz, 600 μm tip, 30%/30% air/water spray) parameters used: G1 (no laser), G2 (0.25 W), G3 (0.5 W), G4 (0.75 W), and G5 (1.0 W). Then, one sample per group was prepared for SEM observation. The remaining specimens were divided in two subgroups for the application of adhesive systems (Single Bond [3M] and Clearfil SE Bond [Kuraray]) followed by the application of a 5-mm buildup of Z250. The microtensile bond strength (μTBS) was determined after 24 h of storage in water at 37°C. The obtained data were compared using the two-way ANOVA and Tukey's test (p ≤ 0.05).

Results: No statistical differences were found among the samples treated with Single Bond. The μTBS was significantly lower for samples treated with Clearfil and laser with powers over 0.75 W. SEM observation showed that the morphological characteristics of the irradiated dentin were similar for all parameters used.

Conclusion: The bonding effectiveness of adhesives to laser-irradiated dentin may be influenced not only by the structural substrate alterations induced by the laser, but also by the characteristics of the adhesive employed.

Keywords: laser, Er,Cr:YSGG, dentin, bond strength, adhesives, composite resin.

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Since the introduction of the first ruby laser by Goldman et al¹ and Stern and Sognaes,² many wavelengths have been studied for their clinical applications in dentistry. At that time, unsatisfactory results were obtained, which included destruction of enamel and dentin as well as increased pulpal temperature to critical levels when using CO₂, Nd:YAG, and ruby lasers for cavity preparation and caries removal. In the late 1980s, desirable results started to be obtained,

but using different wavelengths. Hibst and Keller³ showed that tooth structure could be removed by the Er:YAG laser without causing major thermal damage. Furthermore, these studies showed that thermal damage to enamel and dentin was minimal when proper settings were used and an adequate water cooling spray was employed.^{3,4} After that, two wavelengths were developed for use in dental hard tissues. These include the Er:YAG (2.94 μm) and the Er,Cr:YSGG

(2.78 μm), which by many scientific accounts have very similar properties.⁵

The potential applications of Er,Cr:YSGG laser for dental hard tissue treatment have been explored by a number of investigators.⁶⁻¹⁵ This laser, which uses a pulsed-beam system, fiber delivery, and a sapphire tip bathed in a mixture of air and water vapor, has been shown to be effective for soft tissue surgery as well as for cutting enamel, dentin, and bone.⁶⁻⁸ The Er,Cr:YSGG laser removes hard tissue via laser energy interaction with water molecules situated above and in the tissue interface, and it has therefore been termed a “hydrokinetic” system, which refers to the process of removing biological materials through the use of controlled high-speed water droplets. Strong absorption of laser energy at 2.78 μm by fine water droplets results in a violent yet controlled microexplosion, exerting strong mechanical forces on target tissue surface. The resulting hydrokinetic forces mechanically separate the calcified tissue surface, removing tissue quickly and cleanly.⁶

Preliminary studies stated that Er,Cr:YSGG laser is a precise tool for dental hard tissues.^{9,10} Furthermore, it was considered more comfortable for patients than the conventional method of caries removal, because it requires less or no anesthesia during clinical procedures.^{11,12}

Scanning electron microscopic analyses of dentin surfaces after erbium laser irradiation showed microirregularities, absence of smear layer, opened dentinal tubules, and a scaly surface.^{10,13} These characteristics may promote a micromechanical interlocking between dental restorative materials and tooth surface. Hence, several studies evaluated the adhesion of composites to lasered dentin, but the data obtained in these studies are conflicting and inconclusive.¹⁴⁻¹⁹

For this reason, the purpose of this *in vitro* study was to measure and compare the bond strength of a composite to dentin treated with Er,Cr:YSGG laser at different power densities and with two adhesive techniques.

MATERIALS AND METHODS

Sampling

Thirty-three extracted human third molars were used in this study. This research was conducted with the approval of the Ethics Committee of the School of Dentistry of the University of São Paulo. Crowns with

caries, restorations, or fractures were discarded. The teeth were stored at 4°C in distilled water containing 0.2% thymol to inhibit microbial growth until use. To minimize deterioration, the storage medium was replaced every week. First, each molar was cut just below the occlusal pits and fissures, perpendicular to the long axis of the tooth, by means of a low-speed diamond disk (Isomet, series 15HC diamond; Buehler, Lake Bluff, IL, USA). Second, each tooth was divided into two parts by a mesio-distal longitudinal cut. Then, the occlusal surface of each specimen was wet sanded with a 120-grit silica paper to remove the superficial enamel. Then, to create a uniformly flat surface, a 400-grit silica paper was used. Finally, to standardize the smear layer, each specimen was polished with a 600-grit silica paper for 1 min.

The specimens ($n = 65$) were divided into 5 groups (13 specimens per group) according to the different laser parameters tested.

Laser Irradiation

The occlusal dentin surface was treated with an Er,Cr:YSGG laser (MillenniumTM II, Biolase Technology; San Clemente, CA, USA), which emits a wavelength of 2.78 μm with a repetition rate of 20 Hz. The output power used varied from 0.25 to 1 W (Table 1). The laser beam was used in noncontact mode within 2 mm from the target area, and the tip with a 600- μm -diameter fiber was held perpendicular to the lasered surface during irradiation (focused mode). The air/water spray was set on 30% air and 30% water. The irradiation was done for 30 s in each sample. To perform standardized irradiation, a mechanical device (Marcelo Nucci ME; São Carlos, SP, Brazil), which allowed a uniform handling of both the laser handpiece and the focal distance, was used. After laser irradiation, one specimen per group was used for morphological observation in scanning electron microscopy (SEM).

Sample Preparation

After laser application, the samples were divided into 2 subgroups, according to adhesive system applied. Half of the samples were treated with an etch-and-rinse adhesive (Single Bond, 3M ESPE; St. Paul, MN, USA) and the other half with a self-etching adhesive system (Clearfil SE Bond, Kuraray; New York, NY, USA). The adhesive systems were applied according to the manufacturer's instructions. When necessary, a visible light

Table I Experimental groups

| Group | n | Power Output (W) | Fluence (J/cm ²) | Adhesive System |
|-------|---|------------------|------------------------------|-----------------|
| 1 | 6 | 0 | - | SB |
| | 6 | 0 | - | SE |
| 2 | 6 | 0.25 | 4.46 | SB |
| | 6 | 0.25 | 4.46 | SE |
| 3 | 6 | 0.5 | 8.92 | SB |
| | 6 | 0.5 | 8.92 | SE |
| 4 | 6 | 0.75 | 13.38 | SB |
| | 6 | 0.75 | 13.38 | SE |
| 5 | 6 | 1.0 | 17.85 | SB |
| | 6 | 1.0 | 17.85 | SE |

SB= Single Bond; SE: Clearfil Se Bond.

curing unit XL 3000 (3M ESPE) with an output of 400 mW/cm² was used. After adhesive system application, a 5-mm-high composite resin (Filtek Z250, 3M ESPE) block was built up incrementally on the dentin surface. Each increment was light cured for 20 s following the manufacturer's instructions. After storage in distilled water (37°C/24 h), the specimens were vertically and serially sectioned in mesiodistal and buccolingual directions with a diamond saw to obtain beams with a cross-sectional area of approximately 0.8 mm². Prior to the microtensile bond strength test, the beams were observed under light microscopy at 40X magnification to detect possible defects in the adhesive interface.

Microtensile Bond Strength Test

The beams were fixed to a microtensile device (Geraldini's jig¹⁹) with the adhesive interface perpendicular to the long axis of the applied tensile strength.²⁰ The tests were carried out in a Mini Instron (model 4442; Canton, MA, USA) at a constant speed of 0.5 mm/min. After the test, the fractured surface area was measured using a digital caliper (Mitutoyo; Kawasaki, Japan) with a precision of 0.001 mm. Then, the final values of bond strength were obtained and expressed in MPa using

the following mathematical equation: Bond strength (MPa) = strength (N)/area (mm²).

Failure types at the dentin/resin interface were analyzed using a digital stereoscope (Leica MZ8; Heeburg, Switzerland) at 40X magnification. To determine the failure mode, the classification used by Trajtenberg et al²¹ was adopted: type 1, complete adhesive failure between resin and dentin; type 2, partial adhesive failure between resin and dentin and partial cohesive failure within resin; type 3, complete cohesive failure within the resin; type 4, partial cohesive failure within dentin.

Scanning Electron Microscopy (SEM)

One specimen of each group was used for morphological observation of the laser irradiated dentin. Immediately after irradiation, one sample from each group was fixed in 2.5% glutaraldehyde solution in sodium phosphate buffered solution 0.1 M for 2 h at room temperature. After fixation, the specimens were chemically dehydrated in ascending grades of ethanol and HMDS. After drying procedures, the samples were mounted in aluminum stubs, gold sputter coated (Baltec DCS 050; Balzers, Liechtenstein) and observed in the scanning electron microscope (LEO 4309; Cambridge, England).

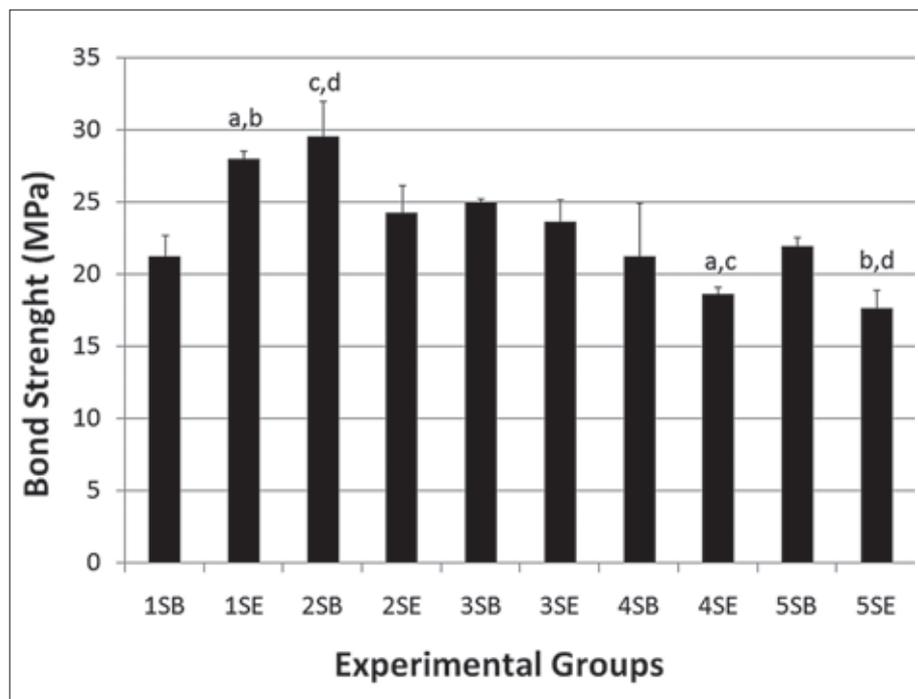


Fig 1 Means and standard deviations of the means of microtensile bond strength values in all groups. Same letters mean significant statistical differences ($p < 0.05$). SB= Single Bond and SE= Clearfil SE Bond.

Statistical Analysis

The bond strength data were compared using two-way ANOVA followed by Tukey's test. The level of significance was 5% ($p \leq 0.05$). All data were analyzed by means of SPSS 10.0 for Windows (SPSS; Chicago, IL, USA).

RESULTS

The mean microtensile bond strengths and standard error of the means for all groups are graphically represented in Fig 1. The bond strength of the Single Bond (SB) treated specimens varied from 21.3 ± 3.69 MPa for group 4SB, where samples were irradiated with 0.75 W, to 29.6 ± 2.43 MPa for group 2SB. There were no significant differences among the experimental groups, or compared to the control group (1SB: 21.3 ± 1.47 MPa).

The bond strength of the Clearfil SE Bond specimens varied from 28.0 ± 0.6 MPa for the control group (1SE) to 17.66 ± 3.14 MPa for group 5SE. There were no significant differences among the bond strengths of the laser groups. The groups treated with 0.75 W

(group 4) and 1 W (group 5) presented similar bond strengths, which were significantly lower than those of the control group ($p < 0.05$).

When comparing the SB groups with the SE groups, we found that the bond strength obtained for group 2SB was significantly higher than that obtained for groups 4SE and 5SE ($p < 0.05$).

The percentage of failure patterns are presented in Fig 2. For both groups, most failures observed were adhesive, followed by type 2 failures.

The scanning electron micrographs in Fig 3 illustrate the morphology of the laser treated dentin prior to acid etching or adhesive system treatment. Independent of the laser power output used, the surfaces presented an irregular aspect (Figs 5A and 5C), indicating that dentin was ablated even using lower parameters. A clear peritubular dentin collar protrudes from the dentin surface (Figs 5B and 5D). The laser irradiation created an overall similar appearance of a scaled surface, free of smear layer and exhibiting opened dentinal tubules. These tubules were homogeneously distributed across the dentin surface. Signs of cracking and carbonization were not observed in any specimen.

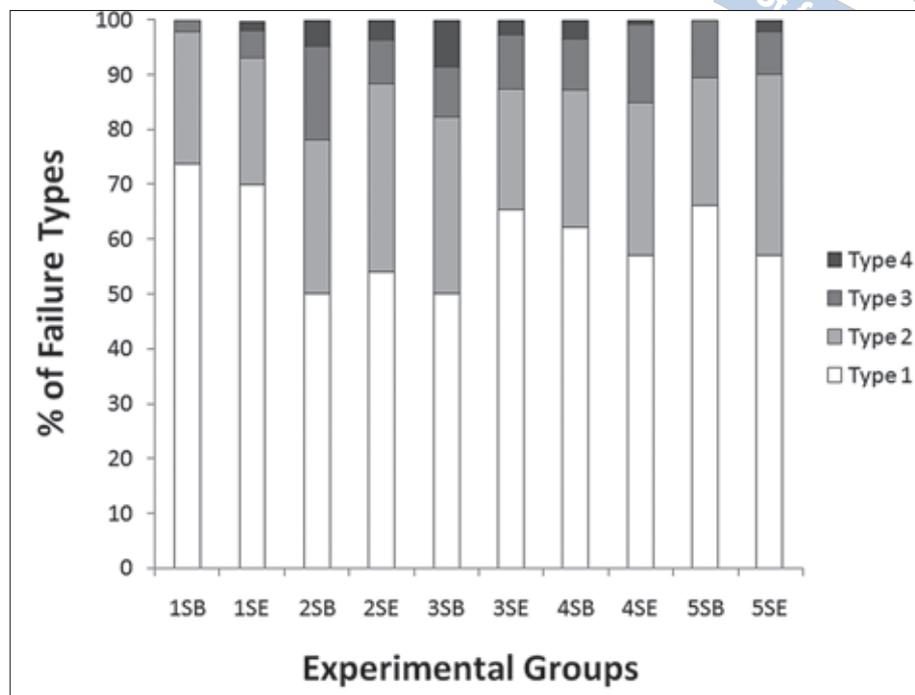


Fig 2 Percent values of failure types for all groups. SB= Single Bond and SE= Clearfil SE Bond.

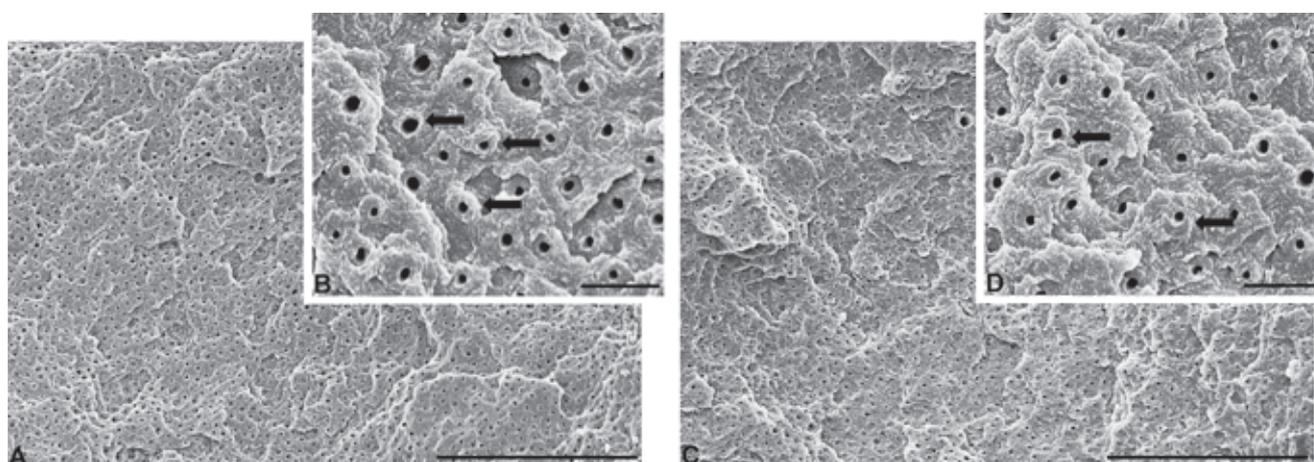


Fig 3 Representative scanning electron micrograph of dentin surface after laser irradiation using 0.25 W (A and B) and 1.0 W (C and D). Even using different power outputs, the Er,Cr:YSGG laser produced ablated dentin surfaces with similar characteristics. In A and C, note the irregular surface with a scaly appearance, lack of smear layer, and clearly visible dentinal tubules. Peritubular dentin collars (arrows) protrude from the dentin surface. No carbonization or melting were observed in any samples. Magnification: A and C 1000X, bar= 100 μ m; B and D 4000X, bar= 10 μ m.

DISCUSSION

The purpose of this study was to evaluate the microtensile bond strength of a composite to human dentin treated with an Er,Cr:YSGG laser using different parameters and two different adhesives: one etch-

and-rinse and one self-etching adhesive system. The microtensile test results showed that the four powers used (0.25, 0.5, 0.75 and 1.0 W) followed by the application of 37% phosphoric acid and Single Bond adhesive system did not influence the bond strength of composite restorations. When the self-etching adhesive

was used, powers over 0.75 W led to lower μ TBS values when compared to the non-irradiated group. The observation of images obtained by scanning electron microscopy showed that laser, with all the parameters studied here, promoted an irregular dentin surface, free of smear layer and with open dentinal tubules. The irregularities found in samples irradiated with 0.25 W showed that the dentin can be ablated even using low power densities.

Basically, the bonding of restorative materials to dental tissues is possible because of the action of an adhesive system in this substrate. This process is, in effect, an exchange process involving replacement of minerals removed from the hard dental tissue with resin monomers, which, upon curing, become micro-mechanically interlocked in the created porosities.²² This interlocking was first described by Nakabayashi et al²³ and is known as hybridization. Based upon the underlying adhesion strategy, the two most important mechanisms of adhesion are currently in use with contemporary adhesive systems: the etch-and-rinse and the self-etching techniques.

Etch-and-rinse adhesives involve a separate etch-and-rinse phase. In their most common configuration, an acid (mostly 30% to 40% phosphoric acid) is applied and rinsed off. This step is followed by a priming step and application of the adhesive resin, resulting in a three-step application procedure. Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin into one application. An alternative approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin, the so-called self-etching adhesives. This technique eliminates the rinsing phase, which not only decreases the clinical application time, but also significantly reduces the technique sensitivity (the risk of making errors) during application.²²

These procedures were developed primarily for use in cavities prepared with burs, which is the most commonly used method for both caries removal and cavity preparations. However, the development of new preparation methods and caries removal procedures has increased doubts about the efficacy of those adhesive systems in areas where burs were not used. For this reason and particularly because of the dentin characteristics created by erbium lasers (eg, irregular surface, absence of smear layer, with opened dentinal tubules) that are, theoretically, ideal for adhesive procedures, several studies have been performed to analyze the interaction between laser surfaces and restorative materials.¹³ However, analyses of the bond strength and the quality of adhesive restorations placed on irradiated

dentin substrate have shown conflicting results, and have thus been widely debated.¹⁴⁻²⁷

Studies have shown that laser therapy induced surface roughness comparable with that of acid etching, and facilitated or even improved bond strength.²⁸⁻³⁰ Visuri et al²⁸ mentioned that the greater presence of peritubular dentin, which has more mineral content than intertubular dentin, might result in better bonding to the dentin. In their study, they obtained higher shear bond strength of composite when it was bonded to Er:YAG laser-prepared dentin than to acid-etched dentin. Another difference between acid etchant and laser actions related to dentin is their effect on the structure of dentin tubules. When an acid etchant is applied, the peritubular dentin is preferentially etched, resulting in funnel-shaped openings of the tubules. Along with polymerization shrinkage, this structure may contribute to pulling the tags away from the walls. Laser irradiation produces no demineralization of peritubular dentin, and the dentinal tubules remain open, with no widening.³¹

In contrast, other studies showed that Er,Cr:YSGG laser irradiation adversely influenced the bonding effectiveness of adhesives to dentin.^{5,14,15,25} These results suggest that the Er,Cr:YSGG laser is incapable of removing the hydroxyapatite crystals without causing any damage to the collagen network. Although these aspects have not yet been described for the Er,Cr:YSGG laser, Ceballos et al²⁶ found that the Er:YAG laser alters the dentin subsurface so that the collagen fibrils lose their cross-banding and fuse together, eliminating interfibrillar spaces and impairing hybridization. The study done by Aranha et al²⁷ demonstrated gaps between the dentin irradiated with Er,Cr:YSGG laser and the adhesive interface, suggesting collagen alteration. It is important to note that these studies used powers for cavity preparation, which are higher than those used in this research.

In the present study, the lowest possible parameters were primarily used to not cause ablation, but only to etch the dentin. However, as seen in the SEM images (Figs 3A and 3B), the ablation occurred even when the lowest power output (0.25 W) was used. The protrusion of the peritubular dentin, a characteristic that occurs when higher parameters are used, was also observed here.¹³ This indicates that the peritubular dentin was more resistant to laser action than intertubular dentin, since the peritubular tissue has higher mineral and lower water content than the intertubular tissue.³² In addition, thermal changes such as melting or carbonization were not observed.

Thus, we believe that the parameters used here, although they caused ablation, probably did not damage the collagen network, which permitted an adequate hybridization. This is particularly evident in those samples treated with the etch-and-rinse adhesive.

Although not evaluated in this study, one factor that may affect the dentin/adhesive system interaction is the fact that laser irradiation increases the acid resistance of dental hard tissue.^{33,34} The surface produced by laser etching is acid resistant, because laser radiation of dental hard tissues modifies the calcium-to-phosphorus ratio (Ca:P ratio), reduces the carbonate-to-phosphate ratio, and leads to the formation of more stable and less acid-soluble compounds, thus theoretically reducing susceptibility to acid attack and caries.³⁵ The alterations in chemical composition may affect the permeability and solubility characteristics of dentin and the adhesion of dental materials to dental hard tissues.

The results observed in groups treated with laser at outputs higher than 0.75 W and Clearfil SE Bond suggest that this might have occurred. The primer of this adhesive system is mildly acidic (pH 1.8),³⁶ and thus is unable to condition the dentin surface in the same way as the control group, where Er,Cr:YSGG laser was not applied, and therefore does not represent an acid resistance that would hinder the primer action. For this reason, the control group would present higher bond strength values. When a surface is etched with a substance having a lower pH than Clearfil SE Bond primer (eg, 37% phosphoric acid),³⁷ it is possible that the acid resistance promoted by the Er,Cr:YSGG laser, under the present irradiation conditions, was not great enough to hinder the action of the phosphoric acid and, thus, the dentin could be properly conditioned.

Visual inspection found the types of failure in this study to be predominantly adhesive (type I) for all tested groups, which means that the cavity preparation techniques did not change the stress concentration during the test. This finding is in accordance with previous studies.^{38,39}

CONCLUSION

Taking into consideration the experimental conditions of the present study, it can be concluded that the use of the Er,Cr:YSGG laser prior to the application of an etch-and-rinse adhesive did not negatively influence the action of this adhesive system in the dentin surface, since the results obtained for irradiated samples were not statistically different from those of control groups.

On the other hand, when the self-etching adhesive system was used, the μ TBS was significantly lower for samples irradiated with parameters higher than 0.75 W. For lower parameters, the samples behaved in a manner similar to the control group. Therefore, these results indicate that the bonding effectiveness of adhesives to laser-irradiated dentin may be influenced not only by the structural substrate alterations induced by the laser, but also by the characteristics of the adhesive employed.

To validate the results shown here, further studies should be carried out in order to clarify the Er,Cr:YSGG laser action on the dentin tissue, mainly in the collagen network. This would allow the choice of safe parameters for both etching and cavity preparation, which improve the action of adhesive systems in dental hard tissue.

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