

# Effect of Er:YAG and Burs on Coronal Dentin Bond Strength Stability

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**Purpose:** To evaluate the immediate and aged bond strength and interfacial nanoleakage of different adhesives and protocols on dental elements prepared with diamond burs and Er:YAG laser.

**Materials and Methods:** Forty molar crowns were flattened and a standardized smear layer was created. Teeth were divided into two main groups according to the dentin cutting technique: 1. Er:YAG laser for 30 s at 30 Hz repetition rate, 250 mJ energy per pulse, and water spray irrigation set at level 8; and 2. diamond bur. Each group was then divided into subgroups according to the adhesive protocol: SG1: dentin etching for 15 s followed by universal adhesive application (All Bond Universal, Bisco); SG2: universal adhesive application (All Bond Universal); SG3: two-step self-etch adhesive application (Clearfil SE Bond 2, Kuraray Noritake); SG4: etching followed by 3-step etch-and-rinse adhesive application (Optibond FL, Kerr). After curing the adhesives, resin composite buildups of 4 mm were made and specimens were sectioned to obtain 1-mm-thick sticks in accordance with the  $\mu$ TBS test technique. Sticks were stressed to failure at baseline and after 6 months of storage in artificial saliva. Three teeth per group were prepared for nanoleakage interfacial analyses. Data were statistically analyzed with three-way ANOVA and Tukey's post-hoc test ( $p < 0.05$ ).

**Results:** A significant difference in bond strengths was found for treatment, aging, and adhesive protocol. Nanoleakage analysis showed higher marginal infiltration in Er:YAG-treated groups both at baseline and after aging.

**Conclusions:** Surfaces prepared with diamond burs presented higher bond strengths than did those prepared with Er:YAG laser. Adhesive protocols and aging could influence the adhesive-dentin interface. Further studies are necessary to validate the results obtained.

**Keywords:** adhesion, adhesive interface, bond strength testing, Er:YAG laser.

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Dental caries is an infectious disease that can lead to pain, loss of tooth structure, infection, and, in severe cases, pulp necrosis. Thus, operative dentistry has an important function in caries control by removing the infected

dentin and re-establishing the integrity of the dental structure, so that the patient can clean effectively to slow or stop lesion progression.<sup>26,36</sup>

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For many years, conventional mechanical drilling has been considered the gold standard to remove carious tissue. This procedure, even if efficient and relatively inexpensive, is uncomfortable for the patient, because the vibrations and sharp noises produced during the removal of infected dental substrate can generate pain and stress.<sup>21,29</sup>

In 1990, laser technology was introduced in conservative dentistry by Hibst and Keller,<sup>18</sup> who described the possibility of using Er:YAG laser as an alternative to drills and burs for cavity preparation. Er:YAG was claimed to be more comfortable for the patients and often eliminate the needs for anesthesia.<sup>5</sup>

Thanks to the absorption affinity of the Er:YAG laser wavelength (2940 nm) for water (absorption peak = 3000 nm) and hydroxyapatite (absorption peak 2800 nm), laser technology allows efficient ablation of hard dental tissues without direct side effects on the pulp and surrounding tissues.<sup>5,7</sup> Er:YAG ablation is the result of a high absorption of laser energy during irradiation, which causes heating, water vaporization and micro-explosions, leading to the removal of carious dental tissues.<sup>11</sup>

With the introduction of adhesive dentistry, it became a concern that the method of excavation may affect the surface properties of dental hard tissues as well as the bond between adhesive restorative materials and dental structure in a manner which compromises the longevity of the restoration.<sup>19</sup> Thus, the goal of caries treatment is not merely caries removal but also to remove it employing an excavation method that creates a substrate appropriate for adhesion, in order to improve the long-term performance of composite restorations.<sup>29</sup>

Adhesive mechanical theories state that adhesives interlock micromechanically with irregularities on the surface of the dental substrate.<sup>27</sup> Regarding bond strengths of composite bonded to elements traditionally prepared with burs, the literature is unanimous in affirming that 3-step etch-and-rinse and 2-step self-etch adhesives must be considered the gold standard for adhesion.<sup>42</sup> On the other hand, The literature contains varying results on adhesion to Er:YAG laser-prepared surfaces and the optimal etching strategy for laser-treated dental substrate.<sup>8,9</sup> Some studies recommend acid etching with etch-and-rinse adhesives after laser ablation to eliminate the negative effects of the laser on dentin and to strengthen the bonding of composite resins to dentin surfaces; conversely, other studies suggest that self-etch adhesives produce higher bond strengths than do etch-and-rinse adhesives.<sup>10,13</sup>

Considering the current controversy on this topic, the aim of this in vitro study was to evaluate the immediate and aged bond strength and interfacial nanoleakage of different adhesives and protocols on dental elements prepared with traditional diamond burs and Er:YAG laser. The null hypothesis was that there is no difference in immediate and aged bond strength to dentin and nanoleakage when Er:YAG laser- and bur-prepared samples are treated with different adhesive protocols.

## MATERIALS AND METHODS

### Microtensile Bond Strength Test ( $\mu$ TBS)

Forty caries-free extracted human permanent molars were selected for this in vitro study. Teeth were cleaned and stored in distilled water at room temperature until use. Flat coronal dentin surfaces were prepared by removing the roots and the occlusal surface of the teeth with a low-speed diamond saw (Micromet; Remet, Bologna, Italy) under water irrigation. The absence of enamel and/or pulp tissue exposure on the dentin surfaces was verified under a stereomicroscope (Stemi 2000-C; Carl Zeiss Jena; Jena, Germany). The dentinal substrate was wet polished with 600-grit silica paper followed by sonication in distilled water for 30 s to create a uniformly flat surface and remove any superficial debris created during cutting and polishing.

Then, the sectioned teeth were randomly divided into two groups ( $n = 20$ ) according to surface preparation: 1. dentin surface irradiated with Er:YAG laser for 30 s (LiteTouch, Syneron Dental Lasers, Sweden&Martina; Padova, Italia) with a sapphire tip (1.3 x 19 mm) used perpendicular to the surface and not in contact with the dental tissue. The following parameters were selected: 30 Hz repetition rate, 250 mJ of energy per pulse and water spray irrigation set at level 8;<sup>34</sup> 2. dentin surface treated with a medium-grit (100- $\mu$ m) diamond bur (880 Komet; Lemgo, Germany) mounted in a water-cooled highspeed handpiece. A power meter was employed at the beginning of each session to check the power of the laser before dentinal treatment.

After dentinal substrate preparation, groups were further divided into 4 subgroups ( $n = 5$ ) according to the adhesive protocol employed: SG1: dentin etched for 15 s (36% phosphoric acid, Dentsply DeTrey; Konstanz, Germany) followed by universal adhesive application (ER mode) (All Bond Universal, Bisco; Schaumburg, IL, USA); SG2: universal adhesive application (in self-etching mode) (All Bond Universal, Bisco); SG3: two-step self-etch (SE) adhesive application (Clearfil SE Bond 2, Kuraray Noritake; Tokyo, Japan); SG4: dentin etched for 15 s (36% phosphoric acid, Dentsply DeTrey) followed by 3-step etch-and-rinse (ER) adhesive application (Optibond FL, Kerr; Orange, CA, USA).

All adhesives were applied following the manufacturer's instruction and were light cured for 60 s with a multi-LED curing light (VALO, Ultradent; South Jordan, UT, USA).

After adhesive procedures, two 2-mm-thick layers of microhybrid resin composite (Clearfil AP-X, Kuraray Noritake) were placed and polymerized individually for 20 s. Specimens were then sectioned to obtain approximately 1-mm-thick ( $\pm 0.01$  mm) sticks in accordance with the non-trimming microtensile bond-strength technique. The dimension of each stick was recorded using a digital caliper and the bonded area was calculated for subsequent conversion of microtensile bond strength values from N to MPa. Sticks were stressed to failure after 24 h ( $T_0$ ) or 6 months ( $T_6$ ) of storage in artificial saliva at 37°C,<sup>24</sup> using a simplified universal testing machine (Bisco) at a crosshead speed of 1 mm/min. The number of prematurely debonded sticks in each test group was recorded. However, these values were



**Table 1** Means and standard deviations of  $\mu$ TBS (in MPa) for each bur- and laser-treated group

Adhesive system	Diamond bur		Er:YAG	
	T <sub>0</sub>	T <sub>6</sub>	T <sub>0</sub>	T <sub>6</sub>
SG1 H <sub>3</sub> PO <sub>4</sub> +All Bond Universal	31.3 $\pm$ 7.4 <sup>aA</sup> (%70A/10CC/10CD/10M)	30.3 $\pm$ 3.0 <sup>aA</sup> (%85A/0CC/10CD/5M)	22.5 $\pm$ 9.1 <sup>aB</sup> (%67A/8CC/25CD/0M)	20.9 $\pm$ 5.6 <sup>aB</sup> (%50A/0CC/25CD/25M)
SG2 All Bond Universal	28.8 $\pm$ 9.2 <sup>aA</sup> (%80A/15CC/5CD/0M)	22.5 $\pm$ 8.2 <sup>ba</sup> (%78A/5CC/12CD/5M)	25.7 $\pm$ 7.4 <sup>aA</sup> (%65A/20CC/5CD/10M)	29.1 $\pm$ 7.7 <sup>ba</sup> (%70A/15CC/10CD/5M)
SG3 Clearfil SE Bond 2	33.8 $\pm$ 6.6 <sup>aA</sup> (%80A/10CC/0CD/10M)	27.0 $\pm$ 9.4 <sup>ba</sup> (%100A/0CC/0CD/0M)	36.8 $\pm$ 8.6 <sup>ba</sup> (%70A/20CC/10CD/0M)	30.2 $\pm$ 4.9 <sup>ba</sup> (%65A/15CC/7CD/13M)
SG4 Optibond FL	38.4 $\pm$ 8.0 <sup>ba</sup> (%28A/39CC/11CD/22M)	34.4 $\pm$ 11.2 <sup>aA</sup> (%87A/0CC/13CD/0M)	17.2 $\pm$ 3.5 <sup>cB</sup> (%75A/7CC/0CD/18M)	16.8 $\pm$ 3.9 <sup>cB</sup> (%90A/5CC/5CD/0M)

Same superscript uppercase letters in rows indicate no difference between storage times ( $p > 0.05$ ); same superscript lowercase letters in columns indicate no difference between luting procedures ( $p > 0.05$ ). Percentages of fracture pattern are listed in the table. Failure types: A: adhesive; CC: cohesive in composite; CD: cohesive in dentin; M: mixed.

not included in the statistical analysis because all premature failures occurred during the cutting procedure, did not exceed the 3% of the total number of tested specimens, and were similarly distributed within the groups. A single observer evaluated the failure modes under a stereomicroscope (Stemi 2000-C; Carl Zeiss Jena) at magnifications up to 50X and classified them as adhesive, cohesive in dentin, cohesive in composite, or mixed failures.

The microtensile bond strength data were statistically analyzed with three-way ANOVA (variables: treatment, aging, and adhesive protocol) and Tukey's post-hoc test to evaluate the influence of the variables on dentin bond strength. Statistical significance was set for  $p < 0.05$ .

### Interfacial Nanoleakage Analysis

Three additional teeth were prepared for each tested subgroup SG1–SG4, cut vertically into 1-mm-thick slabs to expose the bonded surfaces, and tested after the two storage times (T<sub>0</sub> and T<sub>6</sub>) mentioned above. Specimens were covered with nail varnish, leaving 1 mm of exposed dentin at the bonded interface, and processed for interfacial nanoleakage evaluation. Nanoleakage analysis was performed by light microscopy. Bonded interfaces were immersed in 50 wt% ammoniacal AgNO<sub>3</sub> solution in a dark environment for 24 h according to the protocol described by Tay et al.<sup>38</sup> After immersion in the tracer solution, specimens were rinsed in distilled water and immersed in a photodeveloping solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grain within voids along the bonded interfaces.

The specimens were then fixed on glass slides (Menzel; Bielefeld, Germany) using cyanoacrylate glue (Super Cyanolit, Panacol-Elosol; Steinbach, Switzerland) and ground with a series of abrasives paper disks (180-, 600-, 1200-, 2400-, and 4000-grit SiC) under water irrigation using a grinding device (LS2 Remet; Bologna, Italy). Bonded interfaces were

analyzed using a light microscope (E800, Nikon; Tokyo, Japan) at 20X magnification. Interfacial nanoleakage was scored based on the percentage of adhesive surface showing AgNO<sub>3</sub> deposition: 0 = no nanoleakage; 1 = nanoleakage on <25% of the surface; 2 = nanoleakage on 25% to 50% of the surface; 3 = nanoleakage on 50% to 75% of the surface; 4 = nanoleakage on >75% of the surface.

Statistical differences between different subgroups' nanoleakage scores were analyzed with the Kruskal-Wallis ANOVA. Pair-wise differences between group means were analyzed using the Mann-Whitney U-test (level of significance,  $p < 0.05$ ). The level of significance was adjusted according to Bonferroni's correction.

### RESULTS

Means and standard deviations of microtensile bond strength (in MPa) at times T<sub>0</sub> and T<sub>6</sub> months are reported in Table 1 and Fig 1.

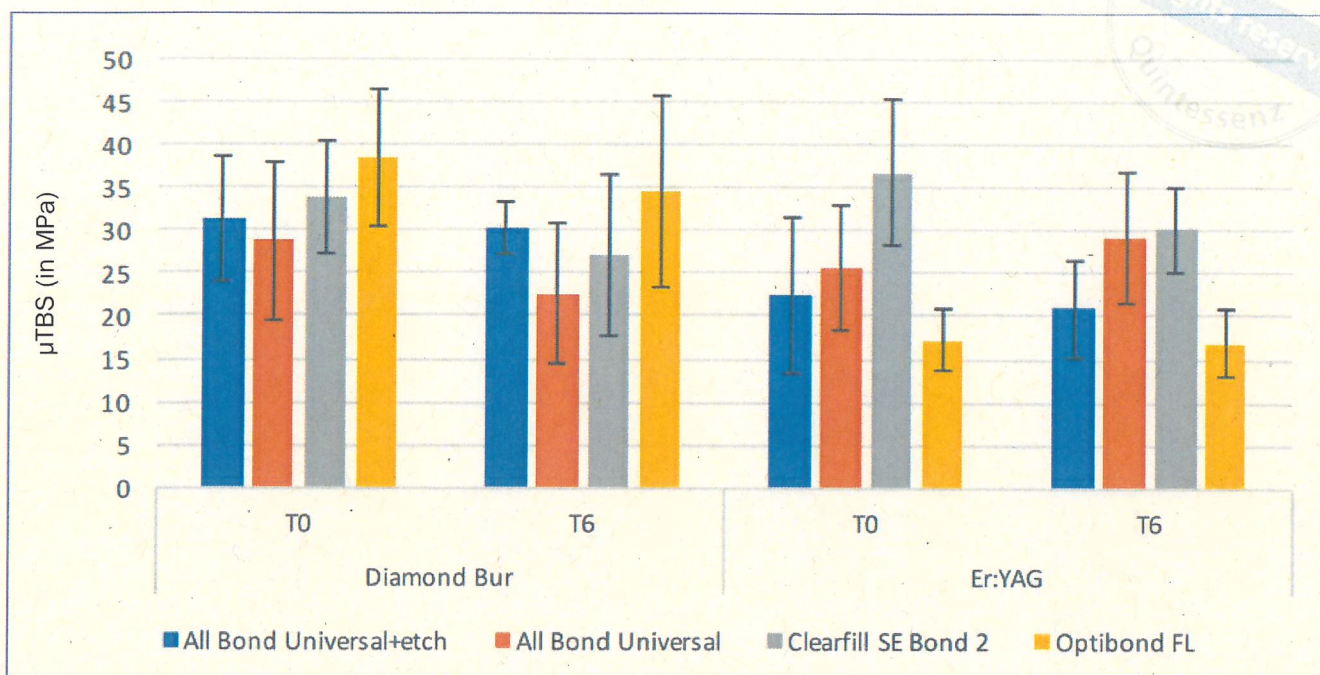
Three-way ANOVA detected a significant difference for the variables treatment ( $p = 0.01$ ), aging ( $p = 0.02$ ), and adhesive protocol ( $p = 0.01$ ).

Er:YAG laser-prepared specimens showed statistically significantly lower bond strength compared to diamond bur-prepared specimens, both immediately and after storage in artificial saliva at 37°C for six months.

In terms of adhesive protocols, Clearfil SE Bond 2 produced significantly higher bond strengths than did All Bond Universal employed in SE and ER mode and Optibond FL. However, when dentin was prepared with diamond burs, the 3-step ER approach was more effective than the other adhesives employed.

Nanoleakage analysis showed significantly higher marginal infiltration in Er:YAG treated groups than in diamond





**Fig 1**  $\mu$ TBS means and standard deviations in MPa for each bur- and laser-treated group.

bur prepared groups, independent of the aging and adhesive variables (Fig 2). However, no differences between subgroups of the two different dentin preparation modes were found considering aging time and adhesive employed.

A predominance of adhesive failures was detected in all groups at T<sub>0</sub> and T<sub>6</sub>, except for bur-treated SG4 at T<sub>0</sub>, which resulted in increased cohesive-in-composite fractures (39%) at the bonded interface.

## DISCUSSION

The results of the present in vitro study showed that bur-treated specimens performed better in terms of  $\mu$ TBS and interfacial nanoleakage at the adhesive-dentin interface, regardless of storage in artificial saliva. Thus, the first null hypothesis was rejected.

The microtensile bond strength test is a well-established method to evaluate the adhesive performance of bonding agents on dentin. The application of a force perpendicular to the adhesive interface makes it possible to test the efficacy of an adhesive material on the dentinal substrate.<sup>30</sup>

The test is usually conducted on coronal dentin, excluding enamel, as it is considered the dental tissue with the greatest influence on adhesive performance.<sup>35</sup>

The aim of this study was to evaluate the effect of two validated dentin preparation methods on bond strength with four different adhesives. Laser irradiation has been proposed for modifying enamel and dentin surfaces to improve

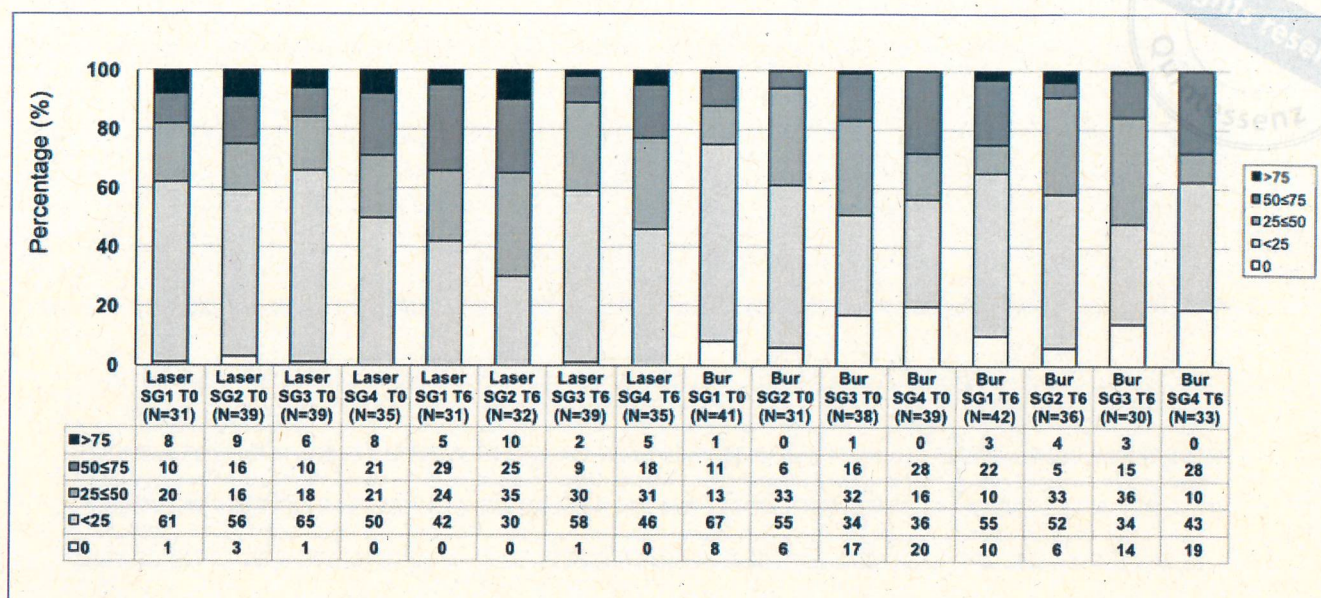
the adhesion of resin materials to those substrates. Some laser systems have the ability to treat dental surfaces, resulting in a rough microretentive pattern. Many types of lasers, such as Nd:YAG and CO<sub>2</sub>, have been examined over time, but the initial results were not encouraging due to thermally induced tissue damage, including that of the pulp.<sup>3,17</sup> In contrast, Er:YAG laser showed a better interaction with dental hard tissues and improved bond strength in association with adhesive treatment if compared to Nd:YAG and CO<sub>2</sub> lasers.<sup>10</sup>

During conventional preparation with rotating instruments, a smear layer is produced on the cavity surface. It mainly consists of pulverized enamel and dentin, caries debris, and bacteria.<sup>10,41</sup>

On the other hand, Er:YAG laser excavation mode produces a smear layer free of debris, with a microcrater-like topography<sup>7</sup> and a superficial layer of ca 5  $\mu$ m thickness where no collagen fibrils are detectable.<sup>5</sup>

Considering that all adhesives should interact with cavity walls and smear layer to create the hybrid layer, it is crucial to analyze the effect of cavity preparation tools on the bond strength of modern adhesives. The results of this study demonstrated that bond strengths to mid-coronal dentin were significantly lower when tooth surfaces were prepared with the Er:YAG laser, independent of the adhesive employed. These findings are in accordance with those of other authors, stating that laser ablation decreased adhesive bond strength.<sup>1,23</sup> Er:YAG laser vaporizes water and organic components, causing thermomechanical ablation of the inorganic





**Fig 2** Interfacial nanoleakage expression was evaluated in all groups, and the percentage of nanoleakage scores are listed. Interfacial nanoleakage was scored based on the percentage of the adhesive surface showing silver nitrate deposition. 0: no nanoleakage; 1: <25% with nanoleakage; 2: 25% to ≤ 50% with nanoleakage; 3: 50% to ≤ 75% with nanoleakage; 4: > 75% with nanoleakage. Increasing grey shades indicate increasing interfacial nanoleakage expression.

components. The ablation of dentin fuses collagen fibrils, resulting in a lack of interfibrillar space and consequently the lack of resin penetration, causing lower bond strength.<sup>29</sup>

Other authors<sup>10,13,34</sup> tested the efficacy of Er:YAG laser on different dentinal substrates, such as primary teeth or cervical lesions, and documented an improvement of the final bond strength, as opposed to the results of present study. Those authors speculated that, even though laser ablation modifies the dentin, improved adhesion could be attributed to the amount of mineralized tissue around the tubule orifices which could provide additional surface area available for adhesion.<sup>10,13,34</sup>

However,  $\mu$ TBS results are influenced not only by the quality of the surface produced during preparation, but also by the adhesive protocol applied. To better evaluate the effect of surface features on adhesive outcomes, in the present study, four different adhesive approaches were considered, both after laser and bur employment. As the adhesive technique has been shown to be operator dependent,<sup>31</sup> special attention was given to correct application procedures, with careful attention paid to standardizing tooth substrate preparation. Occlusal enamel was flattened perpendicular to the tooth axis to standardize the orientation of dentinal tubules. Only the central portion of the mid-coronal dentin surface was used so that all tubules were oriented perpendicular to the surface to minimize any local effects on the  $\mu$ TBS. Although bonding to such laboratory model substrates may clinically be less relevant, the intention of the present study was to determine optimal bonding

efficacy under ideal circumstances. Hence, the data can be compared with other studies conducted in a similar way.<sup>18</sup>

The analysis of the bond strengths obtained emphasized different behaviors of the adhesives as being strongly related to the qualitative characteristics of the substrate considered. In the laser-treated tissues, the highest bond strengths were observed for specimens treated with a 2-step self-etch adhesive, followed by specimens treated with the universal SE adhesive. Conversely, in the bur treated groups, the highest bond strengths were obtained for the 3-step ER mode. An explanation for these findings may be the paucity of exposed collagen on laser-treated dentin, which may compromise adhesive procedures.<sup>11</sup> Several authors analyzed the morphology of laser-treated surfaces and concluded that Er:YAG-lased dentin also revealed a scaly, rough surface, with no evidence of thermal injuries, lack of smear layer, opened dentinal tubules, and ultra-structurally modified intertubular dentin.<sup>3</sup> A further reason for the decrease in bond strength of ER adhesives to laser-irradiated dentin could be related to the alteration of the acid resistance of treated dentin, as well as the poor diffusion of adhesive monomers within the denatured fibrils, since Er:YAG laser irradiation does not selectively remove hydroxyapatite crystallites without affecting the collagen fiber network.<sup>2,12,16</sup> In other words, laser irradiation can form different organic and inorganic compounds such as melted collagen fibrils, calcium pyrophosphate, calcium metaphosphate, and  $\alpha$ - and  $\beta$ -tricalcium phosphates, which present different levels of acid solubility.<sup>1,8,12</sup>



On the other hand, after conventional preparation of a cavity with diamond burs, an amorphous smear layer with organic and inorganic debris that occludes the tubules is formed on the dentin surface.<sup>4</sup> The subsequent use of phosphoric acid should remove the smear layer and partially dissolve the surrounding peritubular dentin, allowing more bonding resin to infiltrate the demineralized dentin, improving micromechanical retention.<sup>20</sup> Nevertheless, the self-etching adhesives include hydrophilic acidic monomers, which are able to simultaneously demineralize and penetrate dentin. Thus, the smear layer created by those adhesives is not completely resolved or removed, but partially integrated into the hybrid layer.<sup>28</sup> The bond strength of self-etching adhesives could be affected by the quantity and quality of the smear layer on dentin, due to the weaker acidity of self-etching primers.<sup>32</sup> Actually, the thickness of the smear layer could be affected by various factors such as the type of bur, use of water spray, and speed of rotation.<sup>42</sup> It can be speculated that the smear layer obtained in our samples was less favorable to the self-etch mode adhesion than others, thus explaining the better results obtained with diamond bur and the 3-step etch-and-rinse adhesives.

The characteristics of the substrate created by the excavation procedure and the adhesive employed, however, are not the only factors affecting bond strength. An additional fundamental factor affecting adhesive interfaces is time; thus, in the present study the efficacy and longevity of adhesive bonding was also considered for both laser and diamond bur-treated specimens.

The present study showed that aging in artificial saliva at 37°C for six months decreased the bond strength in all specimens, regardless of the treatment and the adhesive employed. The literature contains several studies showing a bond strength reduction with Er:YAG laser-treated dentin, with which the results of the present study are in agreement. The quality of composite restorations and the bond strength decrease over time may be associated with the water sorption, microleakage, and dissolution of the adhesive resin.<sup>7</sup>

Two patterns of hybrid layer degradation have been described: the disorganization of collagen fibrils and the hydrolysis and leaching of the adhesive resin from the interfibrillar spaces.<sup>15</sup> Hydrolysis is considered the primary reason for resin degradation within the hybrid layer;<sup>14</sup> it occurs only in the presence of water and is a chemical reaction capable of breaking covalent bonds between polymers, causing loss of the resin mass.<sup>40</sup>

Dentin is a naturally moist substrate and therefore intrinsically hydrophilic. Hence, contemporary adhesives contain hydrophilic resin monomers, such as 2-hydroxyethyl methacrylate (HEMA) in diluents and organic solvents. However, due to these resin monomers, adhesives become high hydrophilic, which causes high water sorption by the adhesives and generates a hybrid layer that behaves as a permeable membrane after polymerization, permitting water movement throughout the bonded interface.<sup>38</sup> This susceptibility to fluid movement was correlated with variable degrees of incomplete polymerization that was found with several adhesives, irrespective of the number of steps required for their application.<sup>6</sup>

Nanoleakage patterns simulate the path of water movement within the adhesive-dentin interface. This water movement may extract unconverted monomers from adhesive resins over time leading to a decrease in bond strength.<sup>39</sup> Nanoleakage is considered an important indicator of the sealing ability and bond efficacy of adhesives.<sup>43</sup>

Tay et al<sup>39</sup> developed a 50% ammoniacal silver nitrate solution to detect and analyze nanoleakage, because the diameter of silver ions is so small (0.059 nm) that they can easily infiltrate micro- or even nano-gaps, showing water uptake pathways at the adhesive-dentin interface. After a reduction reaction, nanoleakage was displayed by silver precipitation within water-filled channels and the interaction between diamine silver ions and acidic/ hydrophilic resin components, which cannot be eliminated by cutting or washing.<sup>39</sup>

In the present study, nanoleakage analysis confirmed the immediate  $\mu$ TBS results, thus showing a significant difference between laser-treated and bur-treated specimens, with a higher infiltration score for the laser-treated dentin. However, the 6-month results, despite a better outcome for the bur cut groups, did not demonstrate a significant increase in nanoleakage infiltration score and thus did not reflect the bond strength in all groups. These results could be due to the short storage time, which may not have been sufficient to create a detectable increase of infiltration, but was enough to produce a general bond strength reduction.

## CONCLUSIONS

Bond strengths for the bur-prepared groups were statistically higher when compared with the Er:YAG groups. Etch-and-rinse adhesives demonstrated better performance when used in combination with a diamond bur. However, the results of the study suggest that self-etch adhesives reached similar  $\mu$ TBS in laser- and bur-treated dentin, thus presenting clinicians with a possible solution to problems related to decreased bond strength on laser-treated dental surfaces. Regardless of surface treatment and adhesive application, aging affected  $\mu$ TBS in all groups, showing a general decrease over time. Further studies are needed to clarify the influence of dentin treatment in combination with different adhesive protocols.

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**Clinical relevance:** Traditional diamond burs and Er:YAG laser could both be used to remove carious tissue. However, different adhesives and protocols behave differently on dentinal substrate treated with traditional diamond burs and Er:YAG laser.