Bond Strength to Dentin of Primary Teeth Irradiated With Varying Er:YAG Laser Energies and SEM Examination of the Surface Morphology

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Background and Objectives: This study aimed to assess in vitro the influence of Er:YAG laser energy on the shear bond strength of a total-etch adhesive to lased dentin of primary teeth, and observe under SEM the morphological appearance of laser-ablated dentin surfaces.
Study Design/Materials and Methods: For SBS, specimens were irradiated with the energies 60 mJ/2 Hz (G2), 80 mJ/2 Hz (G3), 100 mJ/2 Hz (G4). Control (G1) was not irradiated. Dentin surfaces were acid-etched, Single Bond® adhesive (3 M) was applied, and resin cylinders were fabricated from Z250® resin (3 M). Bond strength was tested in shear (0.5 mm/min). For morphological analysis, specimens were irradiated using the same energies, either or not acid-etched, and observed by SEM.
Results and Conclusions: SBS means, in MPa, were: G1-17.89(±4.75); G2-12.34(±4.85); G3-10.30(±3.67); G4-10.41(±4.20). Overall, Er:YAG laser irradiation of primary teeth dentin, prior to the adhesive protocol, adversely affected bond strength. Furthermore, it was noticed that even though there was no significant difference among the Er:YAG laser-treated groups, the increase of laser energy resulted in increasingly cratered surfaces, regardless of acid-etching association. Lasers Surg. Med. 34:254–259, 2004. © 2004 Wiley-Liss, Inc.

Key words: dentin; Er:YAG laser; primary teeth; scanning electron microscopy; adhesion

INTRODUCTION

The use of alternative technologies in dentistry, such as air abrasion and laser irradiation, has been increasingly widespread for the treatment of enamel and dentin, in an attempt to optimize acid conditioning, seeking the achievement of a microretentive structure that is advantageous for bonding procedures, without causing any damage to the dental substrate [1].

Amongst laser wavelengths currently used in dentistry, the Er:YAG laser has proved to be a promising system that presents a notorious ability to remove dental hard tissues [2], with minimal injury to the pulp and without causing severe thermal side-effects, such as melting or charring, to the remaining tooth structure and/or surrounding tissues [3]. The Er:YAG laser emits a 2.94-μm wavelength that coincides with the absorption peak of water and is also well absorbed by OH^- groups in hydroxyapatite [4–6]. Due to the great water content in its composition, dentin substrate is a target tissue with a strong interaction with Er:YAG laser beam. During irradiation, the incident energy is readily and highly absorbed by water molecules present in dentin crystalline structures and organic components, mainly the intratubular fluid and collagen network, thus causing sudden heating and water vaporization. The resulting high-stream pressure within the irradiated tissue leads to the occurrence of multiple microexplosions, which constitute the major principle of Er:YAG laser ablation and provide a non-uniform destruction of tooth structure and the ejection of both organic and inorganic tissue particles [3,4].

Scanning electron microscopy examinations of permanent teeth dentin reveal a scaly, rough surface, with no evidence of severe thermal injuries [3,7–9], lack of smear layer, opened dentinal tubules [10–13], and ultrastructurally modified intertubular dentin [8,9]. When laser irradiation is followed by acid etching, dentin surface appears less irregular, with a greater number of exposed and preserved dentinal tubules and evident peritubular dentin [14,15].

The outcomes of some studies have demonstrated that, when different adhesive systems were bonded to non-etched Er:YAG laser-irradiated dental structure, the bond

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strengths recorded were similar or higher than those reached with acid etching only [14,16,17], whereas other authors [18,19] found notably lower bond strengths for the groups in which laser ablation was not followed by acid etching. When both methods were associated, Gimbel et al. (1994) [7] and Saraceni (1998) [1] observed higher bond strengths than acid conditioning alone. On the other hand, it was reported that conventional acid etching yielded markedly higher tensile [9,20,21] and shear [19] bond strengths than the association of Er:YAG laser irradiation and acid conditioning.

Indeed, the discussion on the real benefits and possible disadvantages of Er:YAG laser on bonding to dentin is not conclusive and the reliability of the adhesion obtained remains a controversial subject. Moreover, in view of the widespread appeal for adhesive dentistry and taking into account the remarkably scarce number of published researches focusing on the applicability of laser approach in pediatric dentistry, it seems of paramount relevance to investigate the features involved in the use of Er:YAG laser technology in primary enamel and dentin substrates and to establish the most appropriate laser parameters for primary teeth.

Therefore, the aims of this study were to assess in vitro the influence of different Er:YAG laser energies on the shear bond strength of a single-bottle total-etch adhesive system to laser-irradiated dentin of primary teeth, and to observe, under scanning electron microscopy, the morphological appearance of laser-ablated dentin surfaces.

MATERIALS AND METHODS

Sound primary canines exfoliated/extracted within a 6-month period were cleaned with scaler and water/pumice slurry in dental prophylactic cups and examined under ×20 magnifier to discard those with structural defects. Sixty-nine teeth were selected for the study and stored in 0.9% saline solution with 0.4% sodium azide at 4°C.

Shear Bond Strength Test

For tensile bond strength tests, 48 canines were used. When necessary, roots were sectioned 2 mm below the amelocemental junction with a water-cooled diamond saw in a sectioning machine (Minitron, Struers A/S, Copenhagen, DK-2610, Denmark). Crowns were individually embedded in polyester resin using PVC cylinders (2.1 cm diameter; 1.1 cm high) and, after polymerization, specimens were ground under water cooling in a polishing machine (Politriz, Struers A/S, Copenhagen, DK-2610, Denmark) using #320- to #400-grit silicon carbide paper to remove the overlying enamel and expose flattened, smooth middle dentin surface. Additional grinding was accomplished with #600-grit SiC paper for 30 seconds to produce a standardized smear layer.

To delimit the dentin-bonding site, a piece of insulating tape with a 3-mm-diameter central hole, made by means of a modified Ainsworth rubber-dam punch, was attached to the specimens' surface. The delimitation of the bonding site had a double aim: to define a fixed test surface, so that the bond strengths recorded would be solely related to the evaluated area; and to warrant that the resin composite cylinder could be further adhered precisely to the treated dentin surface, thus avoiding accidental adhesion to the surrounding enamel or to non-lased dentin (for Er:YAG lased specimens).

The specimens were randomly assigned to four groups of equal size (n = 12), according to the superficial treatment. The groups and treatments accomplished are listed on Table 1.

TABLE 1. Bond Strength Means (MPa) to Dentin for the Experimental Groups

Groups	Treatment	Means (SD)
1	35% phosphoric acid	17.89 (±4.75)a
2	Er:YAG laser 60 mJ/2 Hz + acid	12.34 (±4.85)b
3	Er:YAG laser 80 mJ/2 Hz + acid	10.30 (±3.67)b
4	Er:YAG Laser 100 mJ/2 Hz + acid	10.41 (±4.20)b

Same letters indicate statistical similarity.

For Group 1 (control), the conventional bonding protocol, advised by the manufacturers, was performed. Dentin surface was etched with a 35% phosphoric acid gel (Etching gel, 3 M/ESPE, St. Paul, MN, 55144) for 10 seconds, rinsed thoroughly for 20 seconds and gently dried with absorbing paper to remove excess water and keep tooth surface moist. Two consecutive coats of Single Bond® were applied to the etched surface, slightly thinned with a mild oil-free air stream, and light-cured for 20 seconds with a visible light curing-unit with a 450 mW/cm^2 output (XL 3000, 3 M/ESPE). For all groups, the adhesive system was carefully applied with microbrush® disposable tips to avoid excess and pooling of adhesive along the edges of the insulating tape, what could compromise the distribution of tensions during the tensile test and hence the validity of results.

For Groups 2, 3, and 4, the dentin surface was previously irradiated by an Er:YAG laser device emitted at a 2.94 µm wavelength (Kavo Key Laser 2, Kavo Co., Biberach Germany), using 60, 80, and 100 mJ energies, respectively, and 2 Hz frequency. Afterwards, the conventional bonding protocol was accomplished, following the manufactures' instructions, as mentioned above. The irradiation was performed on non-contact, defocused mode, at a distance of 17 mm, with fine water mist at 5 ml/min. The 2051 handpiece, attached to flexible fiber delivery system, was used. The irradiation distance was standardized using a custom designed apparatus consisting of a holder that positioned the handpiece in such way that the laser beam was delivered perpendicular to the specimen surface, at a constant working distance of 17 mm from the target site; and a semi-adjustable base, in which the specimen was fixed with wax. Two previously gauged operators manipulated the apparatus' micrometer screws, in such way that the semi-adjustable base with the specimen was alternately moved in right-to-left and forward-to-back directions, thereby allowing the laser beam to provide a more accurate irradiation of the entire dentin site. The length of irradiation was, in average, 35 seconds.

Once the bonding protocols were completed, the specimens were individually fixed in a metallic clamping device (developed by Houston Biomaterial Research Center, Brazil) keeping dentin surface parallel to a flat base. A split bisected Teflon® matrix was positioned over the tooth/resin block, thus providing a cylindrical cavity with the smaller diameter coincident to the delimited bonding site (Ø 3 mm). A hybrid light-curing composite resin (Filtek Z250, 3 M/ESPE) was inserted into the matrix in three increments, polymerized for 40 seconds each. As the matrix cavity was completely filled, the specimen was removed from the clamping device and the matrix was opened and separated, leaving a resin composite cylinder adhered to the limited dentin surface.

The specimens were stored in distilled water at 37°C for 48 hours and subjected to a thermocycling regimen of 500 cycles between 5 ± 2°C and 55 ± 2°C waterbaths. Dwell time was 1 minute, with a 3-second transfer time between baths. After thermocycling, the specimens were stored distilled water for 14 hours at 37°C and, afterwards, the cylinder-shaped composite/polyester resin blocks were loaded in shear bond in a Universal Testing Machine (Mod. MEM 2000, EMIC Ltda., SJ Pinhais-PR, Brazil), at a crosshead speed of 0.5 mm/min and using a 50 Kgf load cell until fracture. Shear bond strength values were recorded in Kgf and converted into MPa. Means and standard deviation were calculated. Data were analyzed for sample distribution and homogeneity. Since the distribution was not homogenous, Kruskal–Wallis non-parametric test was used to determine intergroup differences.

Fractured specimens were observed with a 40× stereomicroscope to assess the failure modes, which were classified as adhesive, cohesive, or mixed.

Scanning Electron Microscopy Analysis

The remaining 21 teeth were used in this part of the study. Roots were sectioned 2 mm below the amelocemental junction with a water-cooled diamond saw in the sectioning machine. Specimens were ground under water cooling in a polishing machine (Politriz, Struers A/S, Copenhagen, DK-2610, Denmark) using #320- to #400-grit silicon carbide paper to remove the overlying enamel and expose flat, smooth middle dentin surface. Additional grinding was accomplished with #600-grit SiC paper for 30 seconds to produce a standardized smear layer. Afterwards, the specimens were randomly assigned to seven groups of equal size ($n = 3$), as follows: one acid-etched control group and six experimental groups corresponding to laser energies used (60, 80, and 100 mJ), with and without further acid etching.

The specimens were irradiated following the same Er:YAG laser parameters (i.e., 2 Hz frequency, non-contact, defocused mode) stated for shear bond strength test. The working distance (17 mm) was standardized using the same custom designed apparatus previously described. For the groups in which subsequent acid etcing was performed, after laser irradiation, dentin surface was conditioned with 35% phosphoric acid gel (Etching gel, 3 M/ESPE) for 10 seconds. The specimens were prepared for analysis under SEM, according to the following protocol: immediately after the superficial treatment, the specimens were immersed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4, for 12 hours at 4°C. After fixation, the specimens were washed with 10 ml of 0.1 M sodium cacodylate buffer at pH 7.4 for 1 hour and, immediately, washed with distilled water for 1 minute. The dehydration was carried out in an increasing series of ethanol (25, 50, 75, 95, and 100%, respectively, for 20, 20, 20, 30, and 60 minutes). Soon after, the specimens were immersed in hexamethyldisilazane (HMDS) for 10 minutes, so that the collagen fibres could return to the normal tonus, lost during dehydration.

Specimens were mounted on stubs with their treated surfaces up-faced, sputter-coated with gold, and then examined with a JSM T330 scanning electron microscope (JEOL, Osaka, Japan). A standardized series of photomicrographs were taken from the most representative areas, using increasing magnifications.

The morphological appearance of Er:YAG laser-irradiated dentin surfaces, submitted or not to adjuntive acid etching, was compared. The findings were not statistically analyzed, since the objective of the analysis was to perform a visual and qualitative comparison of the different experimental conditions proposed in the study.

RESULTS

Acid-etched control group (G1) yielded shear bond strength means statistically higher ($P < 0.01$) than those recorded for the other experimental groups. Comparing the Er:YAG laser-treated groups (G2, G3, G4), gradual but not significant ($P > 0.01$) decline in bond strength was observed, and the means recorded showed statistically similarity among them (Table 1).

The analysis of the bonding sites after shear bond strength test revealed that, for non-lased fractured specimens a cohesive-failure mode was predominantly observed whereas adhesive failures mostly occurred for the Er:YAG laser-ablated specimens.

The analysis of the SEM micrographs revealed that acid-etched primary dentin surfaces showed an even topography with desmineralized peritubular dentin, and open and widened tubules' entrances (Fig. 1). In the Er:YAG laser irradiated specimens, dentin surface appeared extremely rough and scaly, exhibiting a disordered pattern of craters and grooves. The intertubular dentin was further more affected, and the existence of areas that were not reached by the laser beam was noticed throughout.

Laser irradiation with 60 mJ/2 Hz parameters yielded the formation of uneven dentin surfaces, exhibiting some areas with open dentinal tubules and some other with smear layer oblitering the tubules' openings (Fig. 2). When 35% phosphoric acid etching was associated, dentin morphology still appeared uneven, but exhibited open dentinal tubules, absence of smear layer, cratered intertubular dentin, and apparently intact peritubular dentin (Fig. 3).

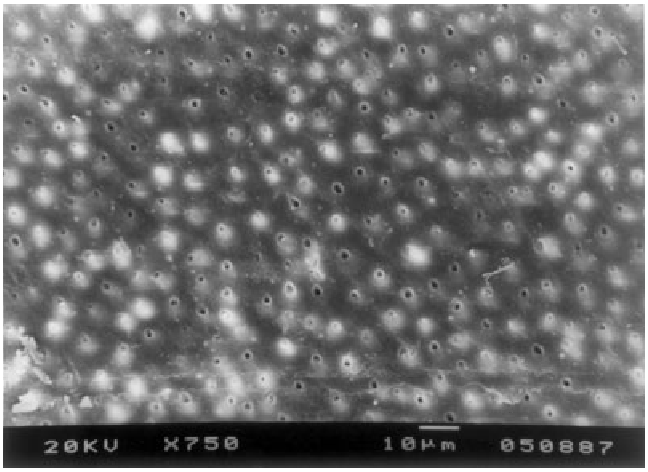

Fig. 1. Scanning electron micrograph of dentin surface etched with a 35% phosphoric acid for 10 seconds (original magnification ×750).

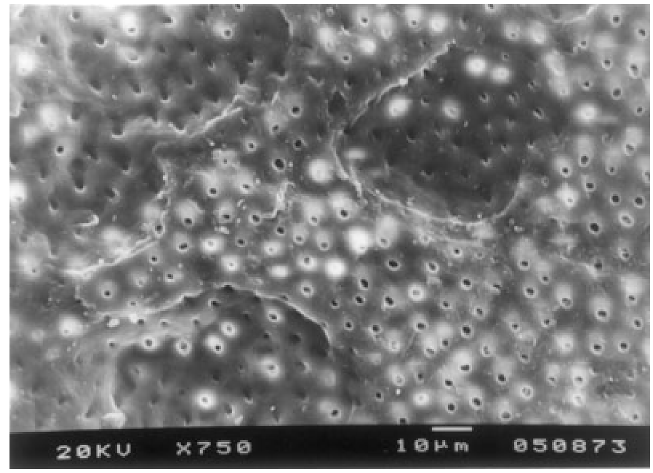

Fig. 3. Scanning electron micrograph of dentin surface treated with Er:YAG laser (60 mJ/2 Hz) + phosphoric acid (10″) (original magnification ×750).

Laser ablation with 80 mJ/2 Hz parameters provided an irregular surface, with craters, fissures, and cracks. In some regions, the dentinal tubules were opened while and in other, smear layer was present (Fig. 4). For those specimens in which the lased dentin surface was also acid-etched, a less irregular, scaly surface was observed, with the widening of dentinal tubules's entrances (Fig. 5).

Primary dentin surfaces irradiated with 100 mJ/2 Hz parameters appeared uneven and scaly, with the presence of fissures and cracks, and open dentinal tubules (Fig. 6). Acid etching following laser ablation resulted in irregular, flaky surfaces, with the presence of cracks throughout, open dentinary tubules, evident peritubular dentin, and irregular intertubular dentin (Fig. 7).

DISCUSSION

Laser irradiation has been suggested for modifying the enamel and dentin surfaces in an attempt to improve the adhesion of resin materials to those substrates. The use of some types of lasers, such as ruby, neodymium:yttrium-aluminum garnet (Nd:YAG), and carbon dioxide (CO_2) was proposed. Nevertheless, the first results with these lasers were not encouraging because a great deal of thermally induced injuries to the surrounding structures and tissues, including pulpal damage, was frequently associated with their use [3,8].

The results of the reported research disclosed that, overall, Er:YAG laser ablation of primary teeth dentin,

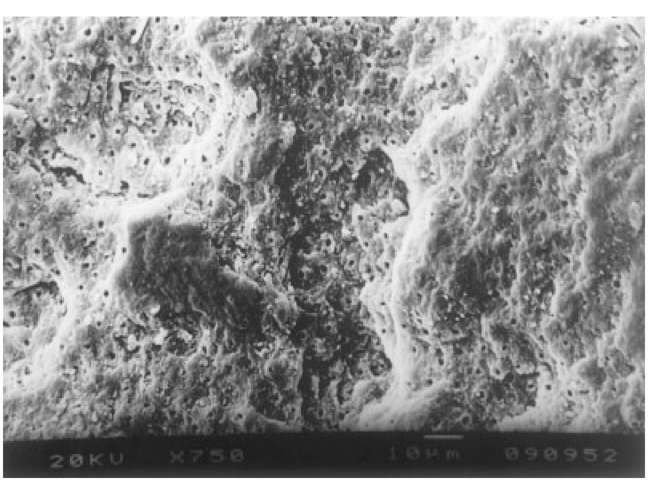

Fig. 2. Scanning electron micrograph of dentin surface treated with Er:YAG laser (60 mJ/2 Hz) (original magnification ×750).

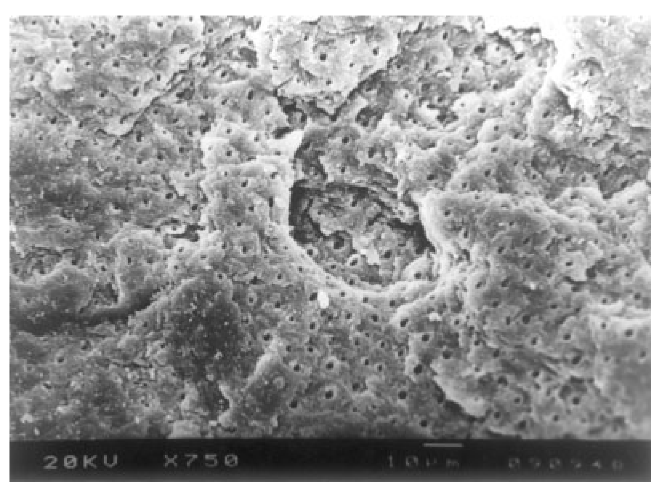

Fig. 4. Scanning electron micrograph of dentin surface treated with Er:YAG laser (80 mJ/2 Hz) (original magnification ×750).

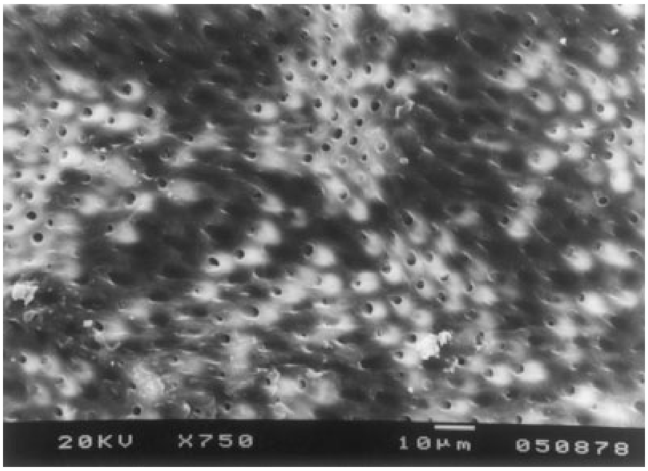

Fig. 5. Scanning electron micrograph of dentin surface treated with Er:YAG laser (80 mJ/2 Hz) + phosphoric acid (original magnification ×750).

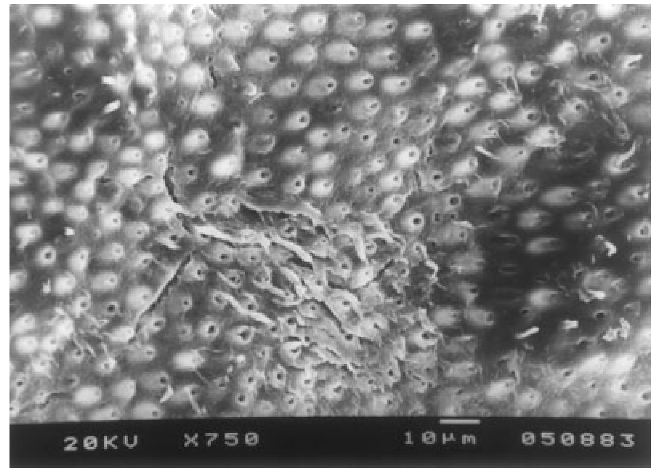

Fig. 7. Scanning electron micrograph of dentin surface treated with Er:YAG laser (100 mJ/2 Hz) + phosphoric acid (original magnification ×750).

prior to the adhesive protocol, adversely affected bond strength, since notably higher bond strengths were recorded for the acid-etched control group. Similar results in permanent teeth were reported by Palma Dibb (2002) [9], Ceballos et al. (2002) [19], Chimello et al. (2001) [20], and Ramos et al. (2002) [21].

The findings of the scanning electron microscopy analyses revealed that the morphological appearance of Er:YAG lased dentin strongly differed from that of the conventionally acid-etched substrate. This morphological aspect derives from Er:YAG laser's microablasive process. Er:YAG laser radiation initially vaporizes the water and other hidrated organic components of the tissues, which results in an increase of the internal pressure what inherently leads to the rupture (ejection) of dental structure [22]. Similar results in permanent teeth were found by Hossain et al. (1999) [13], who also reported a gradual removal of smear layer with the increase of laser energy. Likewise, it was observed in the conducted research the presence of fissures and cracks that increased in size with the rise of energy. An earlier investigation with permanent teeth [9] reached similar findings. Tanji et al. (1997) [12] advocated that Er:YAG laser with an energy level of 100 mJ was effective in the removal of smear layer and exposure of dentinal tubules. Previous studies that used Er:YAG laser energies higher than 100 mJ reported the formation of extremely scaly dentin surfaces [8,14,19].

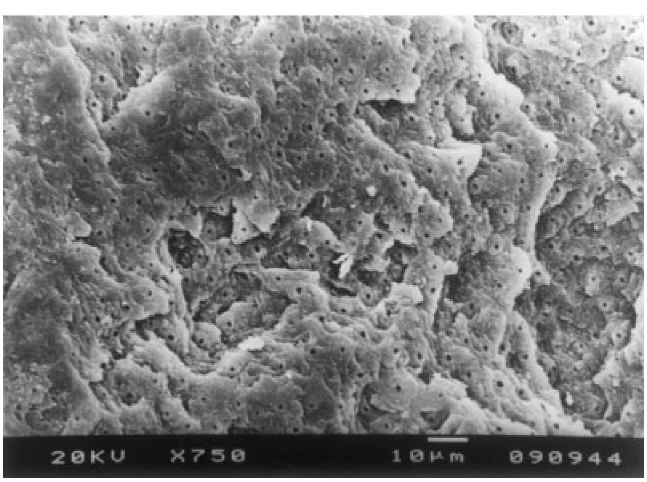

Fig. 6. Scanning electron micrograph of dentin surface treated with Er:YAG laser (100 mJ/2 Hz) (original magnification ×750).

In the current study, when Er:YAG laser ablation was associated with acid etching, the intertubular dentin exhibited a less cratered superficial appearance. These results were corroborated by those obtained by Kataumi et al. (1998) [15], which reported that the use of Er:YAG laser with an energy level of 126 mJ followed by acid conditioning, resulted in widened dentinal tubules and preserved peritubular dentin. This is probably ascribed to the fact that, since intertubular dentin contains greater amount of organic constituents (mostly collagen fibrils) and hence greater amount of water, it is highly more ablated than the peritubular dentin [14].

The results of this study, as regards shear bond strength and SEM examination of the surface morphology, were confirmed by the analysis of the types of failure. Although no statistically significant difference could be found, it was noticed that the increase of energy resulted in decrease of bond strength, and therefore adhesive failures were predominant.

It is important to highlight that the lack of studies focusing on the use of Er:YAG laser on primary teeth was definitely a hindrance to state a comparison with the findings of the conducted research.

The outcomes reached in the current study revealed that even though dentin surface treated with Er:YAG laser appeared adequate for the adhesive procedures, the results of shear bond strength test showed that, regardless of the energy level, conventional acid-etching of dentin was most benefitial for adhesion. Nevertheless, it is mandatory that further studies be developed with the aim of investigating the structural alterations that Er:YAG laser ablation produces on primary teeth substrates, testing other energy and frequency parameters, as well as different adhesive systems, to widen the scopes and establish the basis for rational applicability of this technology in Pediatric Dentistry.

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