

Thermal Alteration and Morphological Changes of Sound and Demineralized Primary Dentin After Er:YAG Laser Ablation

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ABSTRACT The purpose of this study was to assess the influence of Er:YAG laser pulse repetition rate on the thermal alterations occurring during laser ablation of sound and demineralized primary dentin. The morphological changes at the lased areas were examined by scanning electronic microscopy (SEM). To this end, 60 fragments of 30 sound primary molars were selected and randomly assigned to two groups ($n = 30$); namely A sound dentin (control) and B demineralized dentin. Each group was divided into three subgroups ($n = 10$) according to the employed laser frequencies: I–4 Hz, II–6 Hz, and III–10 Hz. Specimens in group B were submitted to a pH-cycling regimen for 21 consecutive days. The irradiation was performed with a 250 mJ pulse energy in the noncontact and focused mode, in the presence of a fine water mist at 1.5 mL/min, for 15 s. The measured temperature was recorded by type K thermocouples adapted to the dentin wall relative to the pulp chamber. Three samples of each group were analyzed by SEM. The data were submitted to the nonparametric Kruskal-Wallis test and to qualitative SEM analysis. The results revealed that the temperature increase did not promote any damage to the dental structure. Data analysis demonstrated that in group A, there was a statistically significant difference among all the subgroups and the temperature rise was directly proportional to the increase in frequency. In group B, there was no difference between subgroup I and II in terms of temperature. The superficial dentin observed by SEM displayed irregularities that augmented with rising frequency, both in sound and demineralized tissues. In conclusion, temperature rise and morphological alterations are directly related to frequency increment in both demineralized and sound dentin. *Microsc. Res. Tech.* 75:126–132, 2012. © 2011 Wiley Periodicals, Inc.

INTRODUCTION

New technologies have been developed to replace the conventional restorative procedure, to obtain a more selective and conservative therapy (Hibst and Keller, 1989; Keller and Hibst, 1995; Raucci-Neto et al., in press; Yazici et al., 2010) that is also more comfortable to the patient, and often eliminate the need for anesthesia (Chaiyavej et al., 2000). The reported advantages of laser technology compared to conventional high-speed rotatory instrumentation include, apart from the possibility of treatment without local anesthesia, elimination of stress factors such as vibration, pressure, and noise, which cause anxiety in patients, especially children (Chaiyavej et al., 2000; Keller and Hibst, 1997; Liu et al., 2006a).

Many researchers have investigated the efficiency of the Er:YAG laser for dental applications, such as cavity preparation (Attrill et al., 2004; Bertrand et al., 2006; Chinelatti et al., 2006; Contente et al., in press; Dostalová et al., 1996; Gimbel, 2000; Raucci-Neto et al., 2007; Takamori et al., 2003), periodontal treatment (Crespi et al., 2006), and superficial treatment for caries prevention (Correa-Afonso et al., 2010; Liu et al., 2006b). This laser has an active yttrium-aluminum-

garnet crystal doped with erbium ions and emits energy of 2.94 μm wavelength, which coincides with the maximum water and OH⁻ absorption peak in tooth hard tissues. Dentin ablation by Er:YAG laser occurs when the organic components of the tissues absorb the radiated energy, causing water and OH⁻ to vaporize (Hibst and Keller, 1989). A small portion of this energy is not consumed in the ablation process, causing heating of the dental structures (Hibst and Keller, 1989). Among Er:YAG laser parameters, pulse frequency deserves attention because it is directly related to temperature increment in tissues and dental pulp (Armengol et al., 2000; Attrill et al., 2004; Dostalová et al., 1998; Raucci-Neto et al., 2007; Takamori et al., 2003). According to Zach and Cohen (1965), temperature rises above 5.5°C for 1 min may promote irreversible pulp damage. However, thermal

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variations may be minimal if the Er:YAG laser parameters are adjusted to the ideal cooling system (Correa-Afonso et al., 2008; Hibst and Keller, 1989; Matsumoto et al., 1996; Raucci-Neto et al., 2007).

The ablation rate depends on the dental tissues' water content (Armengol et al., 1999; Colucci et al., 2009; Hibst and Keller, 1989; Hossain et al., 1999; Jelinková et al., 1996; Mercer et al., 2003) therefore, demineralized dentin ablation is greater than sound dentin ablation and varies according to the tissue's water content (Meister et al., 2006).

Several studies (Corona et al., 2007; Dostalová et al., 1998; Hibst and Keller, 1989; Hossain et al., 1999; Paghdiwala et al., 1993; Palma-Dibb et al., 2002) have confirmed the Er:YAG laser efficiency during cavity preparation in the case of permanent teeth. Studies on deciduous teeth are still scarce, and the methodologies and parameters employed in this case are largely diverse, which prevents researchers from reaching conclusions about cavity uniformity (Kato et al., 2003; Kornblite et al., 2008; Krause et al., 2008; Monghini et al., 2004), and the thermal effect produced by this type of laser (Castillo et al., 2007; Contente et al., in press). There are morphological and structural differences between primary and permanent tooth dentin, which may interfere with both the ablation intensity and the temperature reached after irradiation. Among these differences thickness, permeability, numerical tubular density, and available area of intertubular dentin should be highlighted (Hirayama et al., 1986; Sumikawa et al., 1999).

In this context, the aim of this *in vitro* study was to assess the influence of Er:YAG laser pulse repetition rate on the thermal alterations occurring during laser ablation of sound and demineralized primary dentin. The morphological changes at the lased areas were analyzed by scanning electronic microscopy (SEM).

MATERIALS AND METHODS

This *in vitro* study was approved by the Research and Ethics Committee of the Ribeirão Preto School of Dentistry, University of São Paulo.

Experimental Design

The factors under study were the irradiated primary dentin substrate at two levels; namely sound and demineralized dentin, and the Er:YAG laser pulse repetition rates used for irradiation at three levels: 4, 6, and 10 Hz. The experimental units consisted of 60 dentin blocks obtained from the deciduous molars. The specimens were randomly assigned to two groups, according to the irradiated substrate, and each group was divided into three subgroups ($n = 10$), depending on the employed frequency. The response variables were temperature alteration ($^{\circ}\text{C}$) and morphological analysis through SEM.

Sample Preparation

The 30 sound deciduous molars used in this study were selected from the Ribeirão Preto Dentistry School Human Tooth Bank. The teeth were thoroughly cleaned with a hand scaler and rubber cup/pumice prophylaxis and were stored in a 0.4% sodium azide solution at 4°C until use.

When necessary, the roots were sectioned 2 mm below the cemento-enamel junction by means of a water-cooled diamond saw, using a sectioning machine (Minitom, Struers A/S, Copenhagen, DK-2610, Denmark). Next, crowns were fixed with wax in Plexiglass[®] plates and bisected longitudinally in a mesiodistal direction using a double-faced diamond disk (KG Sorensen, 7015, Barueri-SP, Brasil) mounted on a low-speed handpiece, which provided 60 fragments with standard dimensions of 4 mm height \times 4 mm width. Afterward, the fragments were individually fixed with wax in a cylindrical Plexiglass[®] and taken to a polishing machine (Politriz DP-9U2, Panambi/Struers, A/S Copenhagen, DK-2610, Denmark) for grinding with silicon carbide paper disks of decreasing granulation (# 280 - # 1200, Norton/Saint-Gobain Abrasives, Guarulhos-SP, 07111-150, Brazil), under refrigeration. This procedure was accomplished until dentin with flat surfaces was achieved. The dentin surface was exposed using a standard 2.0 mm thickness.

Demineralized dentin group ($n = 30$) sample were submitted to an *in vitro* pH cycling model as proposed by Serra and Cury (1992), but the experimental period was modified based on a pilot study. The fragments were covered with acid-resistant nail varnish, so that only 9 mm² area dentin remained exposed. The square area was bound using a tape of predetermined size. The samples were individually immersed in 150 mL of demineralizing solution (DES) (2 mmol/L calcium, 2 mmol/L phosphate, and 75 mmol/L acetate at pH 4.6) for 16 h. After this period, they were removed and washed with deionized water for 10 s and slightly dried with paper towels. Then, the samples were immersed for 8 h in the same amount of remineralizing solution (RE) (1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L potassium chloride, and 20 mmol/L cacodylate buffer at pH 7.0), which presents a mineral saturation degree similar to that of saliva and similar to the one proposed by Ten Cate and Duijsters (1982). The entire procedure was performed in an oven at 37°C . The DES and RE solutions were replaced daily during a 21-day experimental period, so it was possible to obtain demineralization depth of ~ 1.0 mm.

Cavity Preparation and Temperature Measurement

During the study, the specimens were kept in distilled water at 4°C and removed from water 2 h before laser irradiation. Before cavity preparation and temperature measurements, one small orifice was made with a #1/2 low-speed carbide bur on the opposed surface corresponding to the roof of the pulp chamber, to which the thermocouple was attached.

All measurements were performed in a temperature/humidity-controlled room, at a constant temperature of 21°C and 30% relative humidity. The fragments were divided into three subgroups ($n = 10$) according to the laser repetition rate (4, 6, or 10 Hz). The cavities were prepared with a 2.94- μm wavelength laser (KaVo Key 2, KaVo Dental GmbH, KG, Bismarckring 39, 88396, Biberach, Germany) using the noncontact and focused mode for 15 s, in the presence of a fine water mist at 1.5 mL/min. The laser parameters are summarized in Table 1 (Ortolan et al., 2009). The irradiation distance of 12 mm was standardized using a custom-made appa-

TABLE 1. Laser parameters

	4 Hz	6 Hz	10 Hz
Energy per Pulse	250 mJ	250 mJ	250 mJ
Energy Density	88.4 J/cm ²	88.4 J/cm ²	88.4 J/cm ²
Average Power Output	1 W	1.5 W	2.5 W
Total energy	15 J	22.5 J	37.5 J

ratus consisting of a holder that positioned the hand-piece in such a way that the laser beam was delivered perpendicular to the specimen surface at a constant working distance from the target site, and a semi adjustable base, to which the specimen was fixed with wax. One previously trained operator handled the apparatus micrometer screws in such a way that the semi adjustable base with the specimen was alternately moved right-to-left and forward-to-backward, thereby allowing the laser beam to provide accurate irradiation of the entire enamel site.

Before the beginning of the procedure, the type K thermocouple attached to the dentin was connected to a portable USB-based data acquisition module (NI USB-9211A, National Instruments, Austin, TX) with four channels of 24-bit resolution. After temperature stabilization within the set, the initial temperature was recorded. For each specimen, the temperature was registered from the start of the laser irradiation until the end of cavity preparation or temperature stabilization. The equipment was calibrated with 0.2°C accuracy and 0.6 s response time. The “Measurement and Automation Software and VI Logger Lites” connected to a computer configured for temperature reading was employed during data acquisition.

Morphological Characterization

After the temperature measurement, three specimens of each group were selected for morphological cavity characterization by SEM. Specimen dehydration was performed in increasing series of ethanol at 25, 50, and 75% for 20 min each, 95% for 30 min, and 100% for 60 min. Next, the specimens were immersed in HMDS solution (hexamethyldisilazane) for 10 s. Each group had its samples fixed on stubs with the treated surface turned to the upper surface for analysis. A Scanning Electronic Microscope (Phillips, XL30 FEGm Eindhoven, Holland) operating at 20 KV was employed. The entire surface was scanned to characterize the resulting cavity.

Data Analysis

For statistical analysis of temperature measurements, means and standard deviations were calculated, and data were analyzed by the nonparametric Kruskal-Wallis test using a factorial design with pulse repetition rate and substrate as independent factors. The Mann-Whitney test was employed for means comparison ($P < 0.05$). SEM analysis findings were not statistically analyzed, because the objective of this analysis was to perform a visual and qualitative comparison of the different experimental conditions proposed in the study.

RESULTS

The means, standard deviations, and medians of each group, as well as their interaction are listed in

TABLE 2. Means, standard deviations, and medians of temperature rise during cavity preparation with Er:YAG laser in sound and demineralized deciduous dentin (°C)

Substrate Frequency	Group A Sound Dentin	Group B Demineralized Dentin
I–4 Hz	$-0.036 \pm 0.11/-0.049$ a	$-0.060 \pm 0.87/-0.056$ A
II–6 Hz	$0.071 \pm 0.80/0.049$ b	$0.012 \pm 0.14/0.008$ A
III–10 Hz	$0.979 \pm 0.92/0.968$ c	$0.458 \pm 0.48/0.354$ B

Same letters equal statistical similarity—comparison in the column.

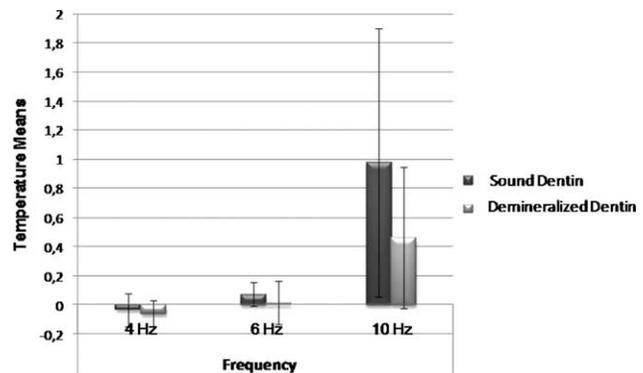


Fig. 1. Median of temperature values (°C) of each group and subgroup.

Table 2. Data analysis demonstrated that there was statistically significant difference between the frequency variation factor and the temperature mean values. Overall, the 10 Hz frequency resulted in higher mean temperature compared to other frequencies (Fig. 1). In case of the sound substrate, there were differences among all the frequencies; however, no difference was found between the frequencies 4 and 6 Hz for the demineralized substrate (Table 2).

SEM analysis revealed dentin ablation with nonselective pattern, which culminate in irregular topography with open dentinal tubules and the absence of smear layer (Figs. 2–4). Irregularities in the laser-prepared cavities increased proportionally with rising repetition rate, mainly because greater intertubular dentin ablation occurred, which led to greater evidence of peritubular dentin protusions. Small cracks were also detected on the surface (Figs. 2 and 3).

The groups irradiated with 10 Hz frequency exhibited decreased dentinal tubules lumen as well as more fissures and cracks, in addition to an amorphous pattern of irradiated dentin (Fig. 4). The sound and demineralized dentin irradiated with 10 Hz laser were similar, probably due to the complete removal of demineralized dentin achieved by using this frequency.

DISCUSSION

Laser has emerged in Dentistry as a promising tool for cavity preparation and selective carious tissue removal. However, its major inconvenience is lased tissue heating during ablation, which can cause morphological and microstructural changes as well as irreversible pulp damage (Hibst and Keller, 1989).

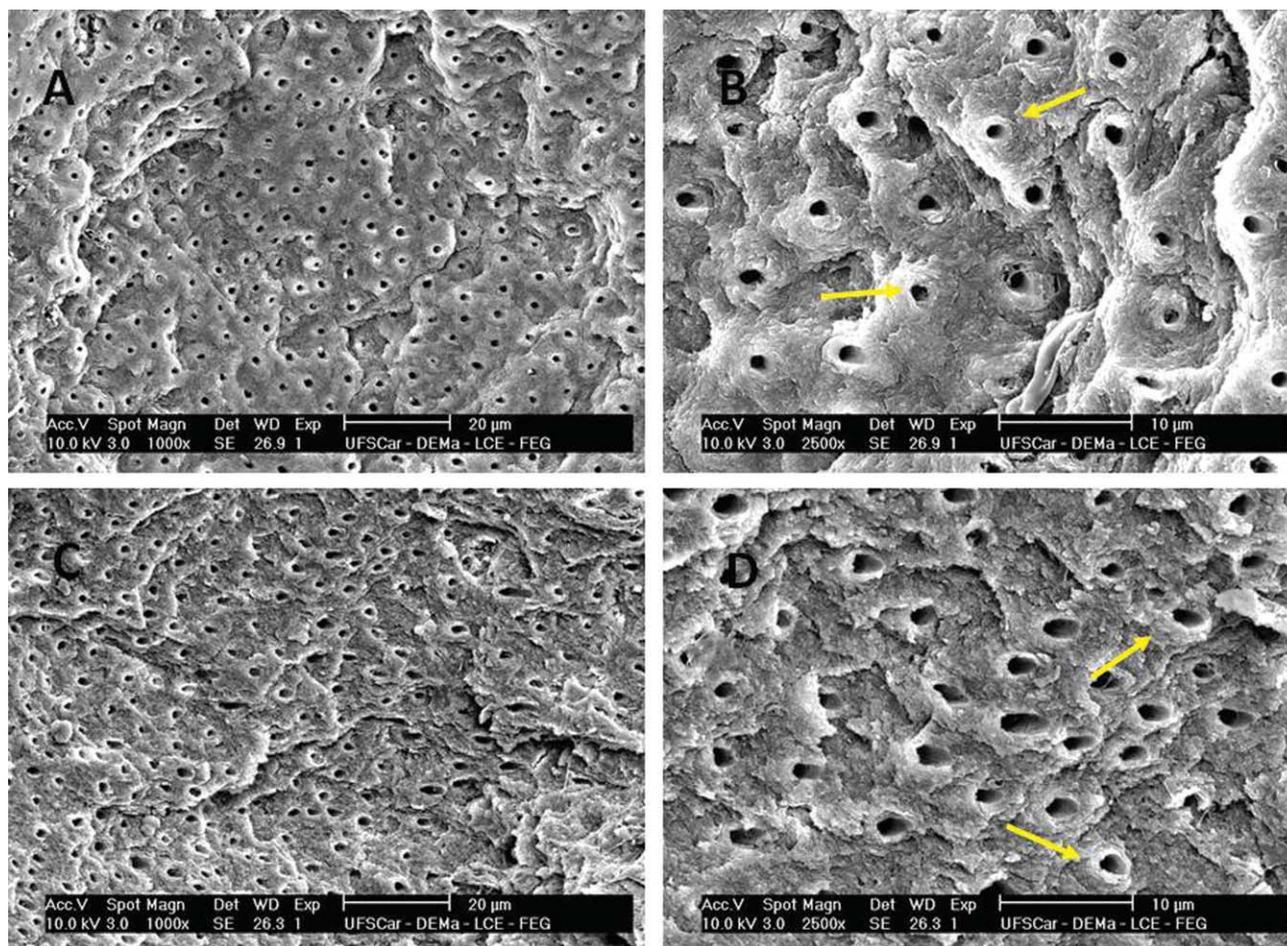


Fig. 2. Irradiated primary dentin surface using 4 Hz of frequency. Sound dentin: (A) $\times 1000$ and (B) $\times 2500$; Irregular topography with open dentinal tubules, absence of smear layer, and greater evidence of peritubular dentin. Demineralized dentin: (C) $\times 1000$ and (D) $\times 2500$;

Nonselective pattern of dentin ablation, open dentinal tubules, homogeneous, and flat topography without cracks. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Studies evaluating Er:YAG laser irradiation on primary dentin and relating possible temperature rise with morphological alterations on the substrate surface are scarce in the literature, which makes comparison of the methodologies and results of this work difficult. Moreover, the morphological and structural characteristics of deciduous teeth differ from those of permanent teeth and may interfere in the ablation mechanism, thereby resulting in increased or decreased temperature upon Er:YAG laser irradiation (Castilho et al., 2007).

Considering these differences, Hirayama et al. (1986) and Ruschel et al. (1996) have reported that the peritubular dentin thickness is ~ 2 – 5 times thicker in primary compared to permanent teeth. Sumikawa et al. (1999) have emphasized that the numerical density of dentinal tubules is greater in deciduous teeth, so there is a decrease in the available intertubular dentin area. These differences in the composition of each tooth type and the major organic characteristics of deciduous teeth influence the parameters employed for primary and permanent teeth irradiation (Borsatto et al., 2006; Celiberti et al., 2006; Monghini et al., 2004; Wanderley

et al., 2005) and may result in different temperature behaviors in the surrounding tissues after irradiation.

The results of this study have demonstrated that there are significant differences in terms of temperature changes during ablation of both substrates, sound, and demineralized, only at 10 Hz frequency. These findings corroborate those of several studies reporting that increasing thermal alterations to the irradiated substrate are related to rising of pulse repetition rates (Armengol et al., 2000; Geraldo-Martins et al., 2005; Hibst and Keller, 1989; Keller and Hibst, 1989; Li et al., 1992; Mehl et al., 1997). The temperature rise in demineralized dentin showed a tendency of lower values than sound tissue, which probably be influenced by carious dentin features. In spite of this study used in vitro simulation by pH cycling model for demineralized dentin, which does not have the same morphological characteristics as the carious dentin, since in the latest contaminated and affected tissue can be observed, the in vitro demineralized dentin tissue presents demineralization, disorganization, and that also contains a high volume of water. In theory, the ablation rate is directly related to the amount of water present

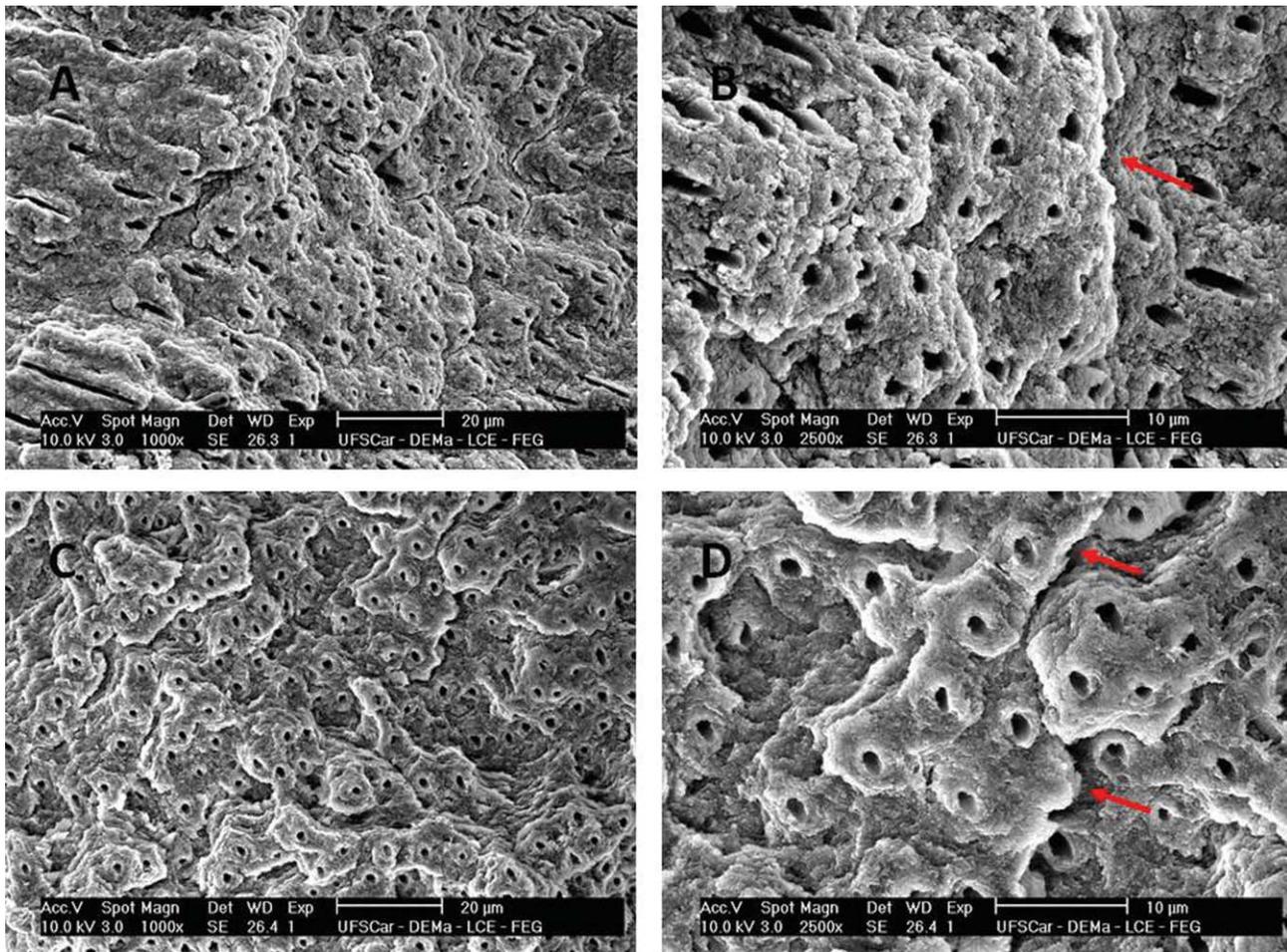


Fig. 3. Irradiated primary dentin surface using 6 Hz of frequency. Sound dentin: (A) $\times 1000$ and (B) $\times 2500$; Small cracks, greater superficial irregularities and evidence of peritubular dentin than the groups using 4 Hz. Demineralized dentin: (C) $\times 1000$ and (D) $\times 2500$;

Small cracks, some occluded dentinal tubules and superficial irregularities. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the substrate (Meister et al., 2006). Bearing this in mind, the carious tissue irradiation may consume most of the energy from the ablation process, so that just a small portion of the energy dissipates and produces temperature rise. This in turn, can cause lower heating of adjacent tissues compared to sound dentin irradiation (Armengol et al., 2000). These characteristics of sound and carious dentin from primary teeth warn of the need for determining appropriate parameters for utilization of Er:YAG laser on different substrates.

Pulse frequency is the parameter that most influences the temperature rise during ablation. Because of the increase in the laser pulse repetition rate, more energy is deposited in dental tissue per time unit, thus providing greater number of micro explosions, and consequently removing more substrate (Corona et al., 2007; Hibst and Keller, 1989). In deciduous teeth, 250 mJ/4 Hz parameters are considered effective for sound dentin tissue ablation (Ortolan et al., 2009). The energy applied in this study for demineralized and sound dentin removal was 250 mJ with variable fre-

quency rates of 4, 6, and 10 Hz, using the focused mode and 12 mm distance. The results showed that there was a greater temperature enhancement in the frequency case of 10 Hz for both substrates, and that there were no dentin demineralization differences between 4 and 6 Hz. Therefore, it is possible to indicate with certainty that cavity preparations can be performed 250 J/4 Hz parameters in primary teeth, since sound and demineralized tissue will be ablated without temperature elevation to critical levels.

The results obtained from the groups irradiated with 10 Hz in both substrate groups revealed greater thermal alteration, but the temperature rise did not reach 1.0°C , which is well below the critical value of 5.5°C recommended by Zach and Cohen (1965). However, the morphological analysis performed in this study showed that these irradiated groups presented greater irregularities compared to the other groups, along with an amorphous pattern for the irradiated dentin various dentinal tubules obstruction. Moreover, the both substrate groups presented similar morphological changes. These findings lead to the assumption that

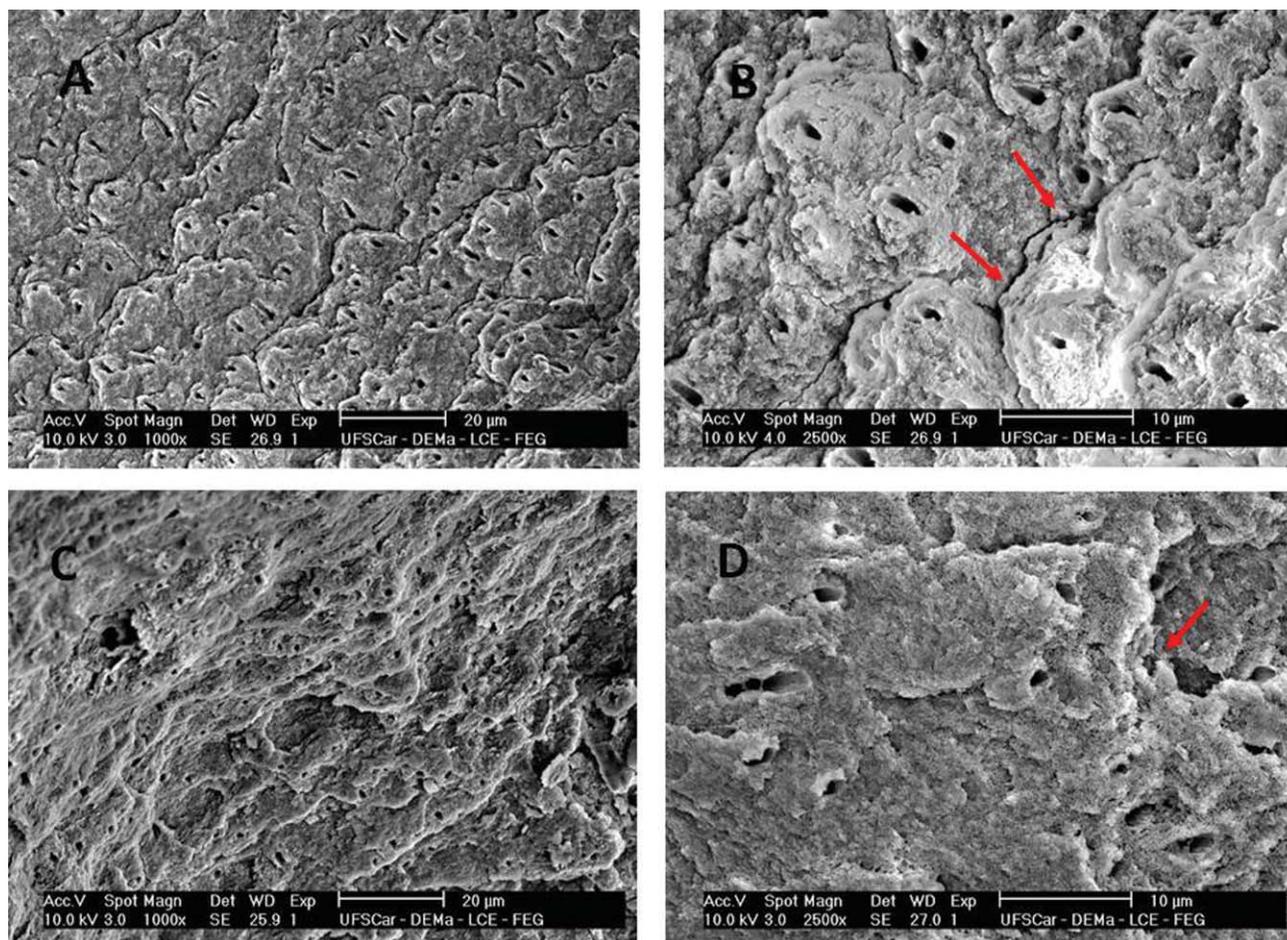


Fig. 4. Irradiated primary dentin surface using 10 Hz of frequency. Sound dentin: (A) $\times 1000$ and (B) $\times 2500$; Irregular surface, cracks presence and considerable reduction of dentin tubules diameter. Demineralized dentin: (C) $\times 1000$ and (D) $\times 2500$; Presence of cracks and amorphous aspect of irradiated dentin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

there is complete carious tissue removal at a 10 Hz frequency, and possible sound tissue removal.

The morphological pattern found in the dentin after irradiation with Er:YAG laser is characterized by an irregular surface secondary to the increased intertubular dentin ablation rate. This resulted in protrusion of peritubular dentin. Increased intertubular dentin ablation is probably a consequence of its higher percent organic constituents, collagen fibers, and water content. It also can be observed that as the pulse repetition rates increased from 4 to 6 Hz, these morphological changes became more evident. These results are consistent with those of permanent teeth found in the literature (Bertrand et al., 2006; Corona et al., 2007; Hosain et al., 2003; Monghini et al., 2004; Raucci-Neto et al., 2007; Souza-Gabriel et al., 2009).

The results of this study indicate that the use of Er:YAG laser using the evaluated parameters does not significantly change the temperature, once the highest mean value achieved for temperature rise was around 1°C , which is far from the critical value of 5.5°C . However, for this laser to be applied in the clinical setting, further studies should be performed. Assessment of the

adhesive procedures on these surfaces modified by laser must be accomplished, so that new technology can be finally added as an extra feature in dental offices.

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