

The Influence of Dentin Demineralization on Morphological Features of Cavities Using Er:YAG Laser

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Abstract

Objective: The purpose of this study was to evaluate the influence of erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser parameters and different degrees of demineralization on morphological features, diameter, and depth of prepared cavities. **Background data:** Minimally invasive dentin caries removal has been recommended. Ablation of deep caries lesions using Er:YAG laser should preserve remaining demineralized dentin; however, the influence of the degree of mineralization of this substrate had not been entirely described. **Materials and methods:** A randomized, factorial design was used to study the effects of two factors. Laser parameter was tested at two levels (250 mJ/4 Hz vs. 200 mJ/2 Hz) and degree of demineralization was tested at four levels (control, two-four-eight cycles). Twelve slabs of human dentin were divided into four groups according to the number of cycles induced by pH-cycling: G1, zero cycles; G2, two cycles, G3, four cycles, and G4, eight cycles. An Er:YAG laser was used at an output energy of 250 mJ/4 Hz and 200 mJ/2 Hz for all groups, for 10 sec at 12 mm distance focus/object. Circumference and depth of the cavities were measured on scanning electron microscopy (SEM) images using image analysis software. The mean values were subjected to two way analysis of variance (ANOVA) and Tukey tests. **Results:** When using 250 mJ/4 Hz, the mean values of circumferential area increased significantly in relation to control ($503.54 \mu\text{m}^2$) with increasing demineralization level (eight cycles) ($555.45 \mu\text{m}^2$). Regardless of the demineralization level, there was also significant statistical difference in the studied measurements of the cavities when 250 mJ/4 Hz and 200 mJ/2 Hz were used. SEM also showed that laser cavity preparations left no smear layer, and the dentinal tubules were clear. **Conclusions:** The circumferential area and depth measurements were affected by laser parameter and demineralization level (eight cycles). Energy level output represents a relevant factor for increased circumferential area and depth measurements. High demineralized artificially caries-affected dentin may also imply higher ablation. Appropriated parameter of laser pulse frequency/power density for demineralized dentin should be used for effective less-invasive caries treatment.

Introduction

DURING THE PAST FEW DECADES, scientific developments in cariology have improved because of an evolution in caries management via a minimally invasive approach. When eliminating a carious lesion, the goal should be to achieve the minimum possible invasion into the crown of the tooth.¹ The carious dentin removal is an important step in a restorative conservative approach, and the benefits of complete caries removal have been questioned.^{2,3} These lesions have been described as consisting of two distinct layers with different ultrastructural and chemical characteristics.⁴ The outer layer is contaminated with bacteria and should be completely removed during the carious dentin removal. The inner layer is partially demineralized and should ideally be preserved, as it

is possible for it to be remineralized because there is only limited collagen degradation.⁵ This particularly layer is less mineralized, and its volume is replaced by water. Therefore, the water content of carious lesions should increase in proportion to demineralization.⁶ This demineralized dentin substrate created at the end of cavity preparation may play a significant role in conservative methods for cavity preparations such as laser irradiation and, subsequently, in the bonding of the adhesive restorative materials to tooth structure.⁷

More conservative approaches, often known as minimally invasive treatments, for deep caries lesions have been adopted, and are becoming more widely accepted in efforts to address a contemporary conservative concept.³

Dental lasers for cavity preparation are a conservative approach with some advantages over mechanical drilling

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instruments, such as non-irritation of the sensitive fibers of the pulp, thereby reducing the pain reaction significantly, and ablation free of vibration or pressure that may trigger pain sensation.⁸

Among the laser systems currently available, the erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser is a widely used dental hard laser, and is considered a safe and effective tool for cavity preparation.⁹ Its wavelengths are absorbed by collagen, hydroxyapatite, and water.⁷ Erbium laser radiation has a great affinity for the water molecule, which is present in quantity in biological hard tissues, especially in carious tissue, where the water content is greater.⁹ The Er:YAG laser irradiation interacts with water to vaporize, resulting in high steam pressure within the lasered tissue. As a consequence, multiple microexplosions occur, which constitute the major principle of Er:YAG laser ablation.¹⁰ Clinically, the louder characteristic sounds of laser-tissue interaction are intended to alert the dentist when a carious substrate is reached.¹¹

Deep demineralized dentin is one of most relevant substrates in clinical practice. Therefore, there is a substantial need for studies pertaining to the influence of Er:YAG lasers for cavity preparation on this substrate. Some studies have pointed out that there is increased ablation of demineralized

dentin, as it has high permeability and therefore is moister than the sound dentin.^{12,13} However, we are not certain about the suggested greater ablation on demineralized content of laser-irradiated tissue.

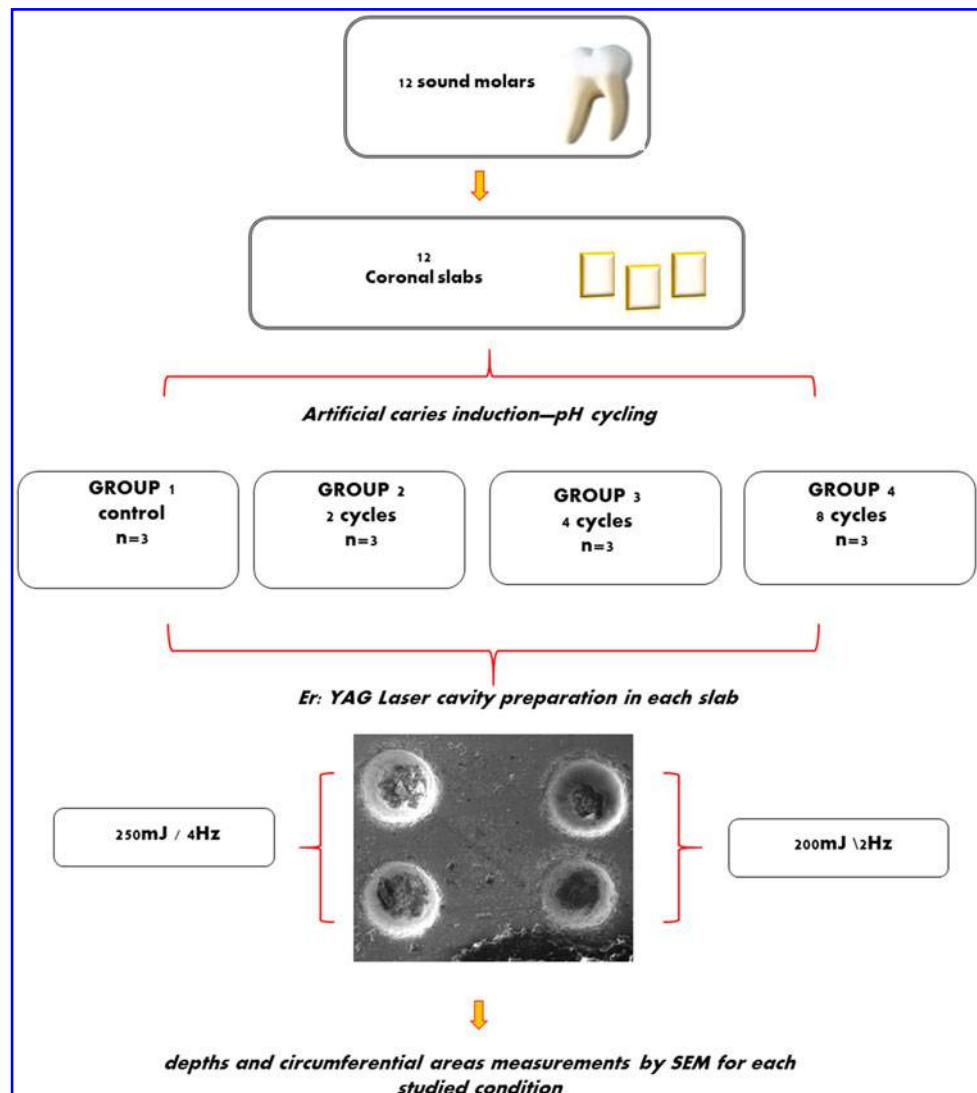
Therefore, the aim of this *in vitro* study was to evaluate the influence of Er:YAG laser parameters and different degrees of demineralization on morphological features, diameter, and depth of prepared cavities. The null hypothesis was that degrees of demineralization on dentin tissue or laser parameters would have no effect on the morphological features, diameter, and depth of these cavities.

Materials and Methods

Experimental design and specimen preparation

This study involved a two by four factorial design. Treatments involved two levels of laser parameters and four levels of degree of demineralization (control, two-four-eight cycles). The variable degrees of demineralization were here obtained by exposing the samples to different numbers of pH cycling. The variable laser parameter (sound and carious dentin) and variable degrees of demineralization were used, because clinically different areas of dentin within the lesion may present

FIG. 1. Experimental design and analysis realized in this study.



different degrees of demineralization. Figure 1 illustrates the experimental design and analysis realized in this study.

The use of human teeth in this investigation was in conformity with the norms of the local Research and Ethics Committee Medical School (protocol # 14/2012). Initially, 12 extracted human third molars that had more than two thirds of the formed roots stored in 0.01% (w/v) thymol solution at 4°C were used.

From these teeth, 12 coronal dentin slabs (5×5×2 mm) were obtained using a water-cooled diamond saw and a cutting machine (Isomet™ Low Speed Saw, Buehler, Lake Bluff, IL). The slabs were subsequently finished with water-cooled abrasive discs in series (320, 600, and 1200 grit Al₂O₃ papers; Buehler, Lake Bluff, IL) and polished with felt paper and diamond spray (1 μm; Buehler, Lake Bluff, IL). Only the occlusal dentin face was used, the remaining surfaces of the slabs were protected with resin epoxy adhesive Araldite Hobby 10 min (Brascola, São Bernardo do Campo, SP, Brazil) according to a previous study.¹⁴ A 25 mm² dentin surface area was exposed to pH cycling for producing caries-like lesions. The specimens were stored at 4°C with 95% relative humidity throughout the study period in order to avoid dryness of the dentin surface.

To select dentin samples with similar features, the surface microhardness was determined using Knoop hardness numbers (parameters: Knoop diamond, 25 g, 5 sec) (FM 100, Future Tech, Tokyo, Japan). Samples with means values ranging from 48.15±4.81 were randomly divided according to a computed aleatorization process into four groups (*n*=3): G1, control, G2, two cycles, G3, four cycles, and G4, eight cycles.

Artificial caries induction: pH cycling

Artificial dentin carious lesions were created by a pH cycling procedure, modified from a previously described protocol.¹⁵ The dentin slabs were fixed onto the lids of glass container vessels with plastic wire and kept immersed in sterile distilled water. Artificial caries was induced by immersing nine slabs (groups G2–G4) in demineralizing and remineralizing solutions. These slabs were submitted to demineralizing and remineralizing cycles according each protocol group. Each cycle consisted of 4 h in the demineralizing solution followed by 20 h in the remineralizing solution. The samples were washed before and after immersion in demineralization solution. The solutions were renewed before the fifth cycle began. The demineralizing solution was composed of acetic acid buffer with 2.0 mmol/L calcium (CaCl₂H₂O), 2.0 mmol/L phosphate (KH₂PO₄), 0.75 mol/L acetic acid, and pH 4.6. The chemical composition of the remineralizing solution was 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 0.05 ppm fluoride, 0.15 mol potassium chloride and 0.1 mol/L Tris buffer, pH 7.4.^{16,17} This procedure was performed at 37°C without agitation.

Laser treatment

A short-pulsed Er:YAG laser (Key Laser 2, KaVo, Joinville, SC, Brazil) emitting a wavelength of 2940 μm was used. The output power and repetition rate of this equipment ranges from 60 to 500 mJ and from 1 to 15 Hz, respectively. The beam diameter at the focal area for handpiece #2051 (non-contact) was 0.63 mm. Handpiece #2051 was indicated to be used for both soft tissue procedures and removal/treatment of

mineralized hard tissues.¹⁸ To ensure consistent spot size, the laser handpiece was fixed in a supported position perpendicular to the dentin surface. A millimeter ruler was used to measure the distance of 12 mm from the surface to handpiece.

Cavities were performed using the energy depicted on the equipment display and corresponded to the energy delivered by the handpiece (250 mJ/4 Hz and 200 mJ/2 Hz) under abundant water spray coolant for 10 sec (~1.5 mL/min). These parameters were recommended by manufacturer for ablation of sound dentin (250 mJ/4 Hz) and primary carious lesions in dentin near to the pulp (200 mJ/2 Hz), respectively.¹⁹ The energies described refer to the energy delivered at the end of the tip, taking into account the transmitting factor (1.0) for this handpiece. The transmitting factor is given by the manufacturer and is depicted in the user manual of the equipment. A power meter Lasermate (Coherent Inc, Santa Clara, CA) was used to measure the maximum output power.

Scanning electron microscopy (SEM)

All specimens were immersed in modified Karnovsky's fixative solution containing 2.5% cold glutaraldehyde in 0.1 mol/L cacodylate buffer at a pH of 7.4 for 8 h. The specimens were then dehydrated in a graded series of aqueous ethanol solutions (70%, 85%, 95%, and 100% ethanol) for 10 min each. The samples were dried overnight at room temperature. They were then mounted on aluminum stubs, sputter-coated with gold/palladium, and examined by a TESCAN SEM (Model VEGA \ XMU, Brno, Czech Republic) with different magnifications at an accelerating voltage of 20 kV. A set of 4 images from each sample was chosen, yielding 12 images for each condition. To evaluate the surface morphological alterations, circumferential and depth measurements of each microcavity were taken. The irradiated area was cross-sectioned perpendicular to the direction of occlusal surface. The depths and circumferential areas were measured using Quantax 800 software (Bruker AXS, Karlsruhe, Germany).

Statistical analysis

Mean values of measurements were submitted to a two way analysis of variance (ANOVA), and post-hoc Tukey tests were used to compare the effects of the parameter (200 mJ/2 Hz vs. 250 mJ/4 Hz) and demineralization level (sound vs. artificially created caries-affected dentin substrates). The software BioStat 2007 Professional (Analyst Soft Robust business solutions company, Vancouver, British Columbia, Canada) was used.

Results

Measurements of the cavities

Two way ANOVA revealed that both laser parameter and demineralization level demonstrated a significant effect on the circumferential area of the cavities. The mean values increased with increasing of demineralization cycles of samples in both parameters used, as summarized in Table 1. Figure 2 showed SEM images at 150× magnification of circumferential measurements of microcavities irradiated with 200 mJ/2 Hz and 250 mJ/4 Hz in groups G1, G2, G3, and G4, respectively. When comparing only the variable laser parameter at 200 mJ/2 Hz subjected to all four substrates, no statistically significant difference among the groups was

TABLE 1. MEAN (\pm STANDARD DEVIATION) OF CIRCUMFERENTIAL AREA AND DEPTH MEASUREMENTS FOR ALL SAMPLES INVESTIGATED WITH THE SCANNING ELECTRON MICROSCOPE AFTER ER:YAG LASER IRRADIATION

Parameters	(G1) Sound dentin	(G2) Carious dentin (2 cycles)	(G3) Carious dentin (4 cycles)	(G4) Carious dentin (8 cycles)
<i>Circumferential measurements (μm^2)</i>				
250 mJ/4 Hz/10 sec	503,543.25 ^{b,B} ($\pm 10,101.77$)	426,095.60 ^{a,B} ($\pm 30,029.87$)	448,331.20 ^{a,B} ($\pm 26,738.12$)	555,459.70 ^{a,A} ($\pm 48,447.31$)
200 mJ/2 Hz/10 sec	416,344.53 ^{a,A} ($\pm 42,433.26$)	391,254.67 ^{b,A} ($\pm 19,507.65$)	405,664.88 ^{b,A} (± 7812.25)	463,488.65 ^{b,A} ($\pm 40,789.58$)
<i>Depth measurements (μm)</i>				
250 mJ/4 Hz/10 sec	1218.83 ^{a,A} (± 13.23)	1083.35 ^{a,A} (± 86.64)	1216.99 ^{a,A} (± 21.33)	1204.34 ^{a,A} (± 16.99)
200 mJ/2 Hz/10 sec	599.61 ^{b,B} (± 2.51)	515.59 ^{b,B} (± 10.00)	691.03 ^{b,B} (± 3.14)	630.35 ^{b,B} (± 13.25)

The letters next to the values represent statistical analysis performed to all conditions. Different upper case letters in rows indicate significant differences among the groups. Similar lower case letters within columns indicate no statistically significant difference between the parameters.

found ($p > 0.05$). For variable parameter (250 mJ/4 Hz), a statistically significant difference was found, in which G4 differed from all the other groups.

In relation to depth measurements, there were no statistically significant differences ($p > 0.05$) among all groups when the similar parameters were used; however, a statistically significant difference was found when considering the variable laser parameter (200 mJ/2 Hz \times 250 mJ/4 Hz) in all groups. The depth measurements in all groups at 80 \sim 120 \times magnification can be observed in Fig. 3.

Morphological findings

The cavities found were round and of very similar depth, with regular and clean-cut margins. According to Lizarelli and Bagnato,²⁰ microcavities are divided into three zones: Z1, center of the microcavity; Z2, an intermediate zone that may or may not exist; Z3, area unaffected by the irradiation. Intermediate zone was broadly and clearly defined in images

from G4 for both laser parameters (Fig. 4A). This may suggest a higher susceptibility of demineralized dentin substrate to laser ablation rate.

When the magnification is 1500 \times (Fig. 4B), there are virtually no highlighted findings among the samples irradiated with Er:YAG laser at 250 mJ/4 Hz and 200 mJ/2 Hz. A typical crater-like surface or scaly roughened surface, accompanied by good definition of the exposed orifices of the dentinal tubules, was observed. Vaporization of intertubular dentin is greater than that of peritubular dentin, showing a protrusion of the dentinal tubules with a cuff-like appearance, which was typically noticed.

Discussion

Cavity preparations and carious dentin removal with Er:YAG lasers are consistent with the principles of minimally invasive preparation. In these processes, the ablative effect of erbium lasers on dental hard tissues can vary according to

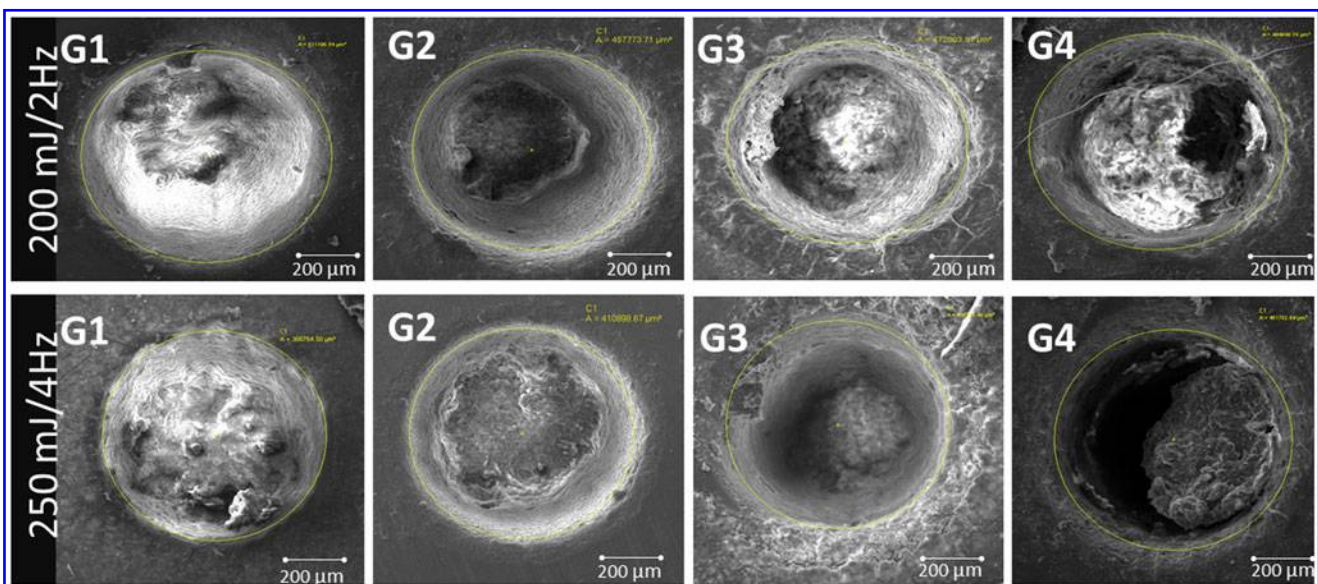


FIG. 2. Circumferential measurements of microcavities irradiated with 200 mJ/2 Hz and 250 mJ/4 Hz in groups G1, G2, G3, and G4, respectively.

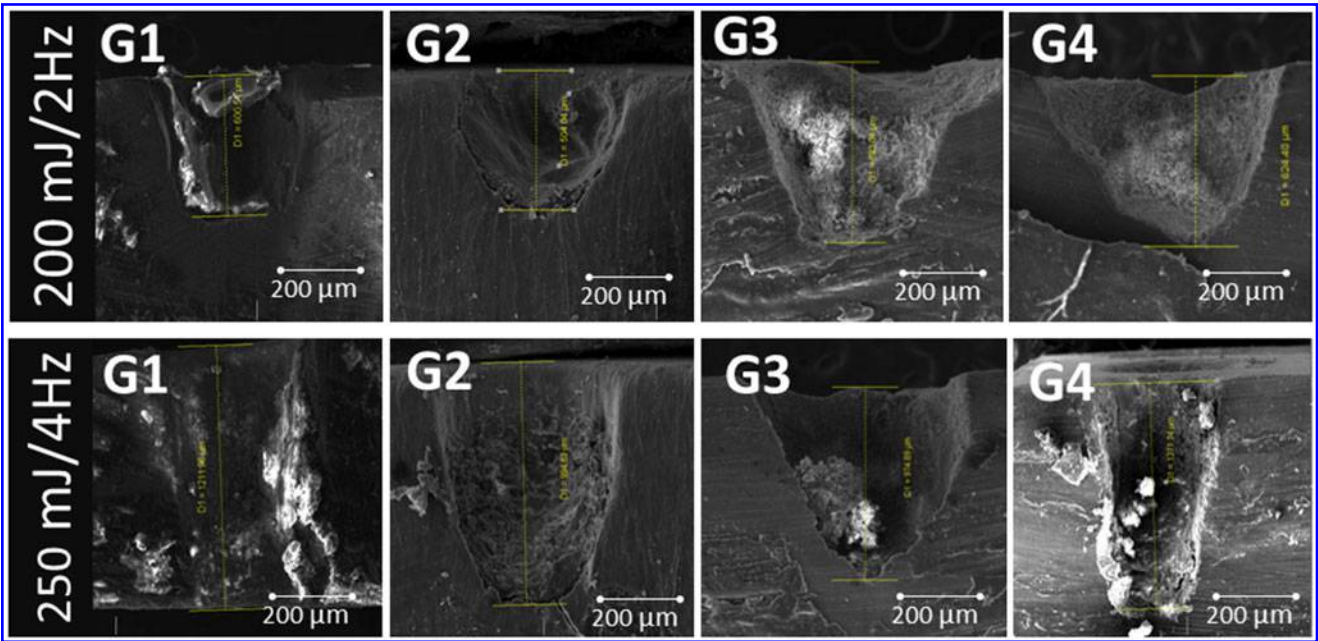


FIG. 3. Longitudinal profiles of microcavities irradiated using 200 mJ/2 Hz and 250 mJ/4 Hz in groups G1, G2, G3, and G4, respectively.

the tissue composition and the water concentration.^{21,22} Many morphological studies related to Er:YAG laser and human sound dentin have been published. Nevertheless, not a single study comparing the effects of Er:YAG at different degrees of dentin demineralization has been reported. Results from this study suggest that variation in the water and mineral contents of dentin promoted by demineralization were sufficiently significant to affect the circumferential dimensions of microcavities. The results also expressed the relevant role of laser parameters to demineralized dentin, leading to the rejection of the null hypothesis.

Concerning the dentin demineralized substrate, a pH cycling model was selected to promote distinctive degrees of dentin demineralization.²³ The proposed laboratory model was de-

signed to mimic the dynamics of mineral loss and gain involved in caries formation, and has been shown to demonstrate accuracy equivalent to that for animal caries.²⁴ Other benefits include the high level of scientific control and the resulting lower variability intrinsic to *in vitro* models, as well as the smaller sample size required.²⁵ A recent report by Melo et al.¹⁵ using this model demonstrated that different numbers of cycles produce dissimilar degrees of demineralization, which was evaluated by cross-sectional hardness testing (CSH) measurements.

The increasing means values for circumferential dimensions with increased demineralized dentin may be explained by a more intense ablation caused by the higher water content of dentin. In our results, when under the same parameter, the slight and moderate demineralization promoted by two and four

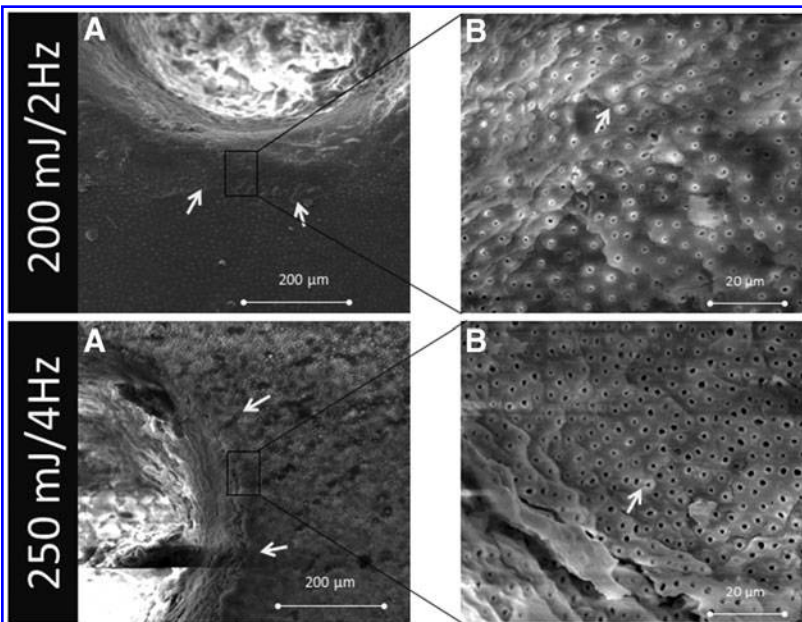


FIG. 4. Scanning electron micrographs of the border of the cavity treated with both parameters used in group G4.

cycles, respectively, were not able to promote significant alterations in the diametral and depth measurements, showing similar values to those of the control group. This may suggest that deeper lesions (eight cycles), such as those in natural carious dentin, have a much higher water content that may improve the ablation of the tissue. The scarcity of published studies dealing with Er:YAG laser on demineralized dentin substrates makes it difficult to compare the results; however, some previous reports supported these findings for deeply demineralized dentin because of the greater permeability of this substrate compared with sound dentin.^{26–28} Given the exploratory aspect of our study, the sample sizes available for this initial investigation were small, and further investigations using a larger sample size are needed to confirm these results.

The different power and pulse repetition rate applied to demineralized dentin revealed the stronger influence of this parameter on the Er:YAG laser's ablation ability. Although previous studies^{29–31} have attempted to determine Er:YAG laser parameters for carious dentin, the literature is scarce and inconclusive about this. Katleen et al.³² claimed that energy output ≤ 200 mJ should be applied using the Er:YAG laser for caries-affected dentin. In the present study, the variable laser parameters based on the manufacturer's recommendations revealed a greater influence than a variable degree of demineralization. The depth and cavities measurements were increased under 250 mJ/4 Hz/10 sec. This also confirms the findings of previous investigations that similar effects of laser parameters were also demonstrated.^{29,33} The selected pulse frequency has been also considered the most influential parameter during dentin ablation.³⁴ Previous studies by Raucchi-Neto^{35,36} showed that the increase of Er:YAG laser frequency provided greater dentin caries removal, in which a larger amount of tissue was removed during irradiation of demineralized substrate, creating a deeper cavity. The use of appropriated parameters of laser pulse frequency/power density for carious substrate should be applied when approaching areas of higher demineralized substrate, such as the inner layers of deep dentin lesions. A nanosecond pulsed laser with a wavelength of 5.8 μm also has been suggested for selective removal of demineralized dentin, with minimal damage to sound dentin.³⁷

In relation to morphological features, our findings are in agreement with reports in the literature.^{38,39} Open and protruding dentinal tubules were found in human dentinal samples irradiated at 150 mJ.⁴⁰ According to study by Kohara et al.⁴¹ using Er:YAG laser and CO₂, respectively, protruding dentinal tubules are the result of greater ablation of the intertubular dentin as opposed to the peritubular dentin. The intensity and extension of the effects will depend upon the laser parameters: fluence, frequency, and wavelength, as well as the tissue optical and thermal characteristics resulting in specific alterations on the surface according to the type of laser applied and the dental tissue irradiated.⁴² In addition, there was no evidence of charring or cracking of dentin using the selected densities. These findings are supported by previous studies of Er:YAG laser irradiation, emphasizing that minimal thermal damage to the surrounding tissues can be expected when a continuous water spray is utilized.^{43,44}

Conclusions

Within the limits imposed in this study, energy level output represents a relevant factor for increased circumfer-

ential area and depth measurements of the ablated dentin. High demineralized artificially caries-affected dentin may also imply higher ablation. Appropriated parameters of laser pulse frequency/power density for caries-affected substrate should be used when approaching areas of higher demineralized substrate as a deep layer of dentin lesions. Therefore, further investigations should be undertaken to determine which parameter/protocol should be preferred to yield an optimal conservative outcome for these substrates. Moreover, additional studies should evaluate the bonding performance of current dental materials to different demineralized laser-treated dentin surfaces.

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Author Disclosure Statement

No competing financial interests exist.

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