Dentin Microhardness and Subsurface Morphology After Er:YAG Laser Cavity Preparation Using Different Parameters

Aline Evangelista Souza–Gabriel, DDS, MSc Michelle Alexandra Chinelatti, DDS, PhD Jesus Djalma Pecora, DDS, PhD Regina Guenka Palma–Dibb, DDS, PhD Silmara Aparecida Milori Corona, DDS, PhD

ABSTRACT

Purpose: The purpose of this study was to evaluate the influence of the Er:YAG laser, using different parameters, on dentin microhardness and subsurface morphology.

Methods: One hundred thirty dentin fragments were randomly assigned to 13 groups: 12 received laser irradiation with different energies (200, 250, 300, or 350 mJ) and pulse repetition rates (2, 3, or 4 Hz); and 1 (control) was prepared using a carbide bur. Specimens were bisected. One hemisection was fixed with the subsurface face up and polished. The other one was prepared for scanning electron microscopy. The microhardness test was performed at 5 depths (30, 60, 90, 120, and 150 μ m) and 7 points (6 in the cavity edges and 1 in a nonirradiated area). Data were tested by analysis of variance and Tukey test.

Results: The highest microhardness values were recorded for lased-irradiated groups with 250 mJ/4 Hz and 350 mJ/4 Hz, only in the deep region of the cavity and until 60 μ m. The parameters 300 mJ/3 Hz, 350 mJ/3 Hz, and 200 mJ/4 Hz changed the morphology until 10 μ m; and 250 mJ/4 Hz, 300 mJ/4 Hz, and 350 mJ/4 Hz until 30 μ m (*P*=0.0328). The bur-prepared group displayed the lowest microhardness values, being statistically similar to 200 mJ/2 Hz (*P*=0.1824), and the subsurface did not exhibit morphological alterations. **Conclusions:** The Er:YAG laser with 250 mJ/4 Hz and 350 mJ/4 Hz increased dentin microhardness in the deepest area of the cavity until 60 μ m. Use of the lower parameters (200 mJ/2 Hz, 250 mJ/2 Hz, or 300 mJ/2 Hz) to prepare dentin with the Er:YAG laser produced results similar to those for bur-prepared cavities. **(J Dent Child 2009;76:58-66)**

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Correspond with Dr. Souza-Gabriel at aline.gabriel@gmail.com

Since the discovery of the ruby laser by Mainman in 1960, lasers have been widely used in Dentistry.¹⁻³ Among the laser systems currently available, the erbium:yttrium-aluminium garnet (Er:YAG) laser has been advocated as a viable approach for caries removal and cavity preparation with minimal effect on sound tooth structure and surrounding tissues.³⁻⁹ Compared to rotary cutting instrumentals, Er:YAG laser cavity preparation takes more

Drs. Souza-Gabriel is doctoral student, Dr. Chinelatti is research fellow, Dr. Pecora is professor of endodontics, and Drs. Palma-Dibb and Corona are professors of restorative dentistry, all in the Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, São Paulo, Brazil.

time,^{3,6,10} but its advantages include low noise and vibration^{6,9,11} and elimination of the need for local anesthesia,^{9,11} particularly in pediatric dentistry.

The Er:YAG laser acts on dentin via a thermomechanical ablation interaction. During irradiation, the incident energy is 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.^{2,4,5,12} The resulting high-stream pressure within the irradiated tissue leads to the occurrence of successive microexplosions, resulting in the ejection of both organic and inorganic particles.^{4,5,13} This process successfully occurs due to the Er:YAG laser wavelength of 2.94 μ m that is coincident to the absorption spectrum of water and OH groups in hydroxyapatite (-3.0 μ m).^{1,4,5}

The amount of tissue removed by the Er:YAG laser and the impact upon tissue temperature measurements are dependent on various parameters, such as the irradiation time, output energy, pulse repetition rate, emission mode, tissue water cooling, and the distance between the laser device and tooth surface.¹⁴⁻¹⁸ Regarding the laser settings advised for dental treatment, the most important parameters are energy and pulse repetition rate because they are directly related to the laser's ablation ability^{14,17} and to the deposition of residual heat on dental substrates.¹⁹

Morphological examinations of surfaces treated by the Er:YAG laser revealed characteristic microirregularities and the absence of a smear layer.^{15,18,20-25} Dentinal tubules are opened and the intertubular dentin is ablated to a greater extent than peritubular dentin, due to the former's high water and hydroxyapatite content.^{15,23,25} Its real effect on

Table 1. Means and Standard Deviations of DentinMicrohardness (KHN) Based on the Cavity PreparationDevice

Cavity preparation device	mJ	Hz	Mean±(SD)*
Er:YAG laser	200	2	65.62±3.42 ^b
		3	66.62±4.25 ^{ab}
		4	67.16±5.41 ^{ab}
		2	65.92±2.42 ^{ab}
	250	3	66.98±4.28 ^{ab}
		4	67.70±4.42ª
	300	2	66.12±3.56a ^b
		3	66.57±4.01 ^{ab}
		4	67.40±4.38 ^{ab}
	350	2	66.44±3.28 ^{ab}
		3	66.15±6.18 ^{ab}
		4	68.06±4.89ª
High-speed turbine	No. 330 ca	rbide bur	65.64±2.31 ^b

* Same letters indicate statistical similarity.

irradiated tissue has not yet been clarified, however, and it remains unclear whether the structural alterations extend to the subsurface.

An earlier investigation²⁶ showed that cavities prepared with the erbium lasers display an acceleration of demineralization compared to conventionally prepared cavities. Hossain et al²⁷ verified that the Er:YAG laser device produces minimal thermal-induced changes on dental hard tissue compositions, and Knoop hardness of the lased cavity was nearly similar to the bur cavities. Conversely, Delbem et al²⁸ indicated a tendency toward increased caries resistance following erbium laser irradiation. Others^{16,17,20,21} also have reported the presence of melting and recrystalization areas, leaving the surface hypermimeralized and less permeable,^{13,21} which can hamper the restorative material's penetration. ^{13,22-24,29}

Nevertheless, the aforementioned studies utilized different parameters and are insufficient to describe the modifications of cavities prepared by the Er:YAG laser. Indeed, the available dental literature did not correlate microhardness changes provided by the Er:YAG laser irradiation with the morphological aspects of subsurfaces.

Considering these facts, this study's purpose was to assess in vitro the influence of the Er:YAG laser, using different parameter settings, on microhardness and subsurface morphology of dentin cavity walls.

METHODS

Sound human third molars, extracted within a 6-month period and stored in a 0.9% saline solution at 4°C, were cleaned with a scaler and water/pumice slurry in dental prophylactic cups and examined under a X20 magnifier to discard those with structural defects. Thirty-three teeth were selected for the study and stored in 0.5% chloramine solution at 4°C for 1 week prior to the experiment.

SPECIMENS PREPARATION

Roots were sectioned 2 mm below the cementoenamel junction with a water-cooled diamond saw (Struers A/S, Copenhagen, Denmark). Next, crowns were fixed with wax in Plexglass plates and bisected longitudinally in both mesiodistal and buccolingual directions using a double-faced diamond disk (KG Sorensen, Barueri, São Paulo, Brazil) mounted in a low-speed handpiece (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil), thus providing 132 fragments. One hundred thirty fragments were randomly selected and individually stored in plastic containers filled with distilled water at 4°C.

Each specimen was individually fixed in a cylindrical Teflon abutment with wax and ground in a water-cooled polishing machine (Struers A/S, Copenhagen, Denmark) with no. 600-grit silicon carbide (SiC) paper (Buehler, Lake Bluff, Ill) until superficial dentin was exposed. Additional polishing was accomplished with no. 1200-grit SiC paper for 20 seconds to produce a smooth, standardized surface.

Table 2. Means and Standard Deviations of Dentin Micro- hardness (KHN) Based on Point			Table 3. Means and Standard Deviations of Dentin Micro- hardness (KHN) Based on Depth		
Point	Mean±(SD)*		Depth (µm)	Mean±(SD)*	
А	64.02±2.92b		30	71 05+3 25a	
В	65.16±3.27b		60	66 27±4 71b	
С	68.26±4.24a		00	00.2/±4./10	
D	69.43±5.12a		90	65.4/±6.12c	
F	67 91+4 13a		120	65.16±5.21c	
F	65.38±4.91b		150	65.26±4.97c	

* Same letters indicate statistical similarity.

The specimens were removed from the cylindrical abutment, cleaned, and reimmersed in distilled water at 4°C for 24 hours to rehumidify the substrate.

After this period, the fragments were individually fixed on Plexglass plates with wax, using a parallelometer (EL Quip, São Carlos, São Paulo, Brazil) to ensure that the surface was kept parallel to the horizontal plan. To demarcate the 3-mm-diameter ablation dentin site, a piece of insulating tape with a central hole made by means of a modified Ainsworth rubber-dam punch, was attached to the specimen surface.

The fragments were randomly assigned into 13 groups of equal size. One group (control) was prepared using a no. 330 carbide bur (KG Sorensen) with a high-speed turbine. In the 12 other groups (experimental), the Er:YAG laser was irradiated using parameters most commonly advised for cavity preparation: 200 mJ/2 Hz, 200 mJ/3 Hz, 200 mJ/4 Hz, 250 mJ/2 Hz, 250 mJ/3 Hz, 250 mJ/4 Hz, 300 mJ/2 Hz, 300 mJ/3 Hz, 300 mJ/4 Hz, 350 mJ/2 Hz, 350 mJ/3 Hz, and 350 mJ/4 Hz.

The Er:YAG laser device used was a Kavo Key Laser 2 (Kavo Dental GmbH & Co, Biberach, Germany) The laser beam was delivered on noncontact, focused mode, with a fine water mist at 1.5 mL/minute for 1 minute. The laser beam spot size was 0.63 mm, and a 2051 handpiece (Kavo Dental GmbH & Co, Biberach, Germany) with a removable tip attached to a flexible fiber delivery system was used. The irradiation distance was standardized by using a custom-designed apparatus consisting of 2 parts:

- a holder to fix the laser handpiece in such a way that the laser beam was delivered perpendicular to the specimen surface, at a constant distance of 12 mm (focused mode) from the target site; and
- 2. a semi-adjustable base, on which the Plexglass plate with the fragment attached to it was firmly fixed with wax.

Two operators manipulated the apparatus' micrometer screws so that the semi-adjustable base was alternately moved in both right-to-left and forward-to-back directions, thus allowing the laser beam to provide an accurate ablation of the entire dentin site. The irradiation distance for every sample was checked with a ruler.



Figure 1. Schematic illustration of specimen preparation and details about the methodology. (a) Tooth section. (b) Standardization of specimen dimensions. (c) Immersion in distilled water. (d) Specimen fixation. (e) Polishing machine. (f) Dentin surface exposed. (g) Fixation in Plexglass plates. (h) Site delimitation. (i) Er:YAG laser or high–speed turbine preparation. (j) Immersion in distilled water. (k) Specimen section in half. (l) Hemisection for the microhardness test. (m) Microhardness test. (n) Hemisection for the morphological analysis. (o) Immersion in glutaraldehyde solution. (p) Dehydration process. (q) Metallization to scanning electron microscopy evaluation.

Once the irradiation was performed, the fragments were bisected and removed from the Plexglass plates. One hemisection of each specimen was fixed, using the parallelometer, in the cylindrical Teflon abutment with the subsurfaces upfaced facing up and grounded in the polishing machine with no. 600 to 2,000-grit SiC paper. The other hemisection was used for morphological analysis.

MICROHARDNESS TEST

The polished hemisections were placed into a microhardness apparatus (HMV-2000, Shimadzu, Kyoto, Japan). The equipment was adjusted with a Knoop hardness indentor, with 25-g load, for 10 seconds. A schematic illustration of specimen preparation and details about the microhardness test are presented in Figure 1.

The microhardness test was performed at 5 depths (30, 60, 90, 120, and 150 μ m) and 7 points (6 on the edges of the cavity preparation and 1 in a nonirradiated area). The indentations were situated in 7 points, named from A to F. Each point was formed by 15 indentations positioned in 5 rows. The interval between each indentation was 30 μ m (vertical) and 100 μ m (horizontal). Thus, the demarcation in each row represented the average of 3 indentations. Since obtaining a Knoop hardness number of the cavity surface was impossible, the first row was positioned 30 μ m from the cavosurface margin, and the others followed the same distance until 150 μ m.



Figure 2. Dentin photomicrographs. (a, b, and c) Bottom of the Er:YAG laser cavity preparations at 200 mJ/2 Hz, 250 mJ/2 Hz, and 300 mJ/2 Hz, respectively (X1,500). The subsurfaces did not present morphological alterations (asterisks). (d) Bottom of the Er:YAG laser cavity preparation with 350 mJ/2 Hz (X1,500). The cavity preparation boundary was more irregular than in the other parameters at the same pulse repetition rate (arrows). (e) Bottom of high-speed turbine cavity preparation (X1,500). Note the subsurface appearance without morphological alterations (asterisks). (f) Lateral region of high-speed turbine cavity preparation (X1,500). Notice the topographical aspect without modifications (arrows).

Point A was placed 0.5 mm from the irradiated site, representing the control point (nonirradiated area). Point B was positioned at the beginning of the irradiated area. Point C was placed between B and D. Point D was positioned at the medium point between A and F, always being distant 1.5 mm from B, once the irradiated area was standardized in a 3-mm diameter. Points E and F were positioned the same manner as points B and C, however, they were situated in the opposite region of cavity preparation.

Dentin microhardness averages and standard deviations were calculated and data were analyzed by 3-way analysis of variance (ANOVA) using a factorial design, with the cavity preparation device, depths, and regions as independent variables. Multiple comparisons were done using Tukey statistical test (α =0.05)

MORPHOLOGICAL ANALYSIS

This analysis was performed with each specimen's nonpolished hemisection. The specimens were not polished for the purpose of detecting possible morphological alterations in the subsurfaces. To prepare for analysis under scanning electron microscopy (SEM), each specimen was cleaned with an ultrasound apparatus (Odontobras, Ribeirão Preto, São Paulo, Brazil) for 10 minutes and immersed in 2.5%



Figure 3. Dentin photomicrographs. (a and b) Bottom of the Er:YAG laser cavity preparations at 200 mJ/3 Hz and 250 mJ/3 Hz, respectively (X1,500). Note the irregular subsurface pattern without significant morphological alterations (asterisks). (c and d) Bottom of the Er:YAG laser cavity preparation at 300 mJ/3 Hz and 350 mJ/3 Hz, respectively (X1,500). Note the modified regions characterized as dense areas without dentinal tubules until approximately 10 µm (arrows). (e) Bottom of the Er:YAG laser cavity preparation at 200 mJ/3 Hz, respectively (X1,500). Note the subsurface aspect without morphological alterations. (f) Lateral region of the high-speed turbine cavity preparation (X1,500). Note the topographical appearance without modifications (arrows).



Figure 4. Dentin photomicrographs. (a) Bottom of the Er:YAG laser cavity preparation at 200 mJ/4 Hz (X1,500). Note the morphological alterations until 10 µm (asterisks). (b, c, and d) Bottom of the Er:YAG laser cavity preparations at 250 mJ/4 Hz, 300 mJ/4 Hz and 300 mJ/4 Hz, respectively (X1,500). Note the morphological modifications until 30 µm (arrows). (e) Lateral region off the Er:YAG laser cavity preparation at 250 mJ/4 Hz (X1,500): The dentin subsurface did not exhibit morphological changes (arrows). (f) The Er:YAG laser cavity preparation at 350 mJ/4 Hz (X150). Note the irregular aspect of the boundary and cavity preparation interior.

glutaraldehyde (Merck KGaA, Darmstadt, Germany) in 0.1 M sodium cacodylate buffer at pH 7.4 (Merck KGaA, Darmstadt, Germany) for 12 hours at 4°C.

After fixation, the samples were: rinsed with a 0.1 M sodium cacodylate buffer several times; sequentially dehydrated in an ascending ethanol (Labsynth, Diadema, São Paulo, Brazil) series (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 90% for 30 minutes, 100% for 60 minutes); immersed in examethyldisizilane (HMDS; Merck KGaA, Darmstadt, Germany) for 10 minutes; placed on absorbing paper inside glass plates; and left to dry in an exhaust system. Specimens were mounted on stubs with their treated surfaces facing up, sputter-coated with gold (Bal-Tec AG, Balzers, Liechtenstein), and examined with a scanning electron microscope (Philips XL30 FEG, Eindhoven, The Netherlands) operating at 15 kV. During the analysis, a standardized series of photomicrographs were taken in the most representative area of each group, using different magnifications.

RESULTS

MICROHARDNESS MEASUREMENTS ASSESSMENT

The data analysis revealed that, overall, there was a statistically significant difference (P<.05) among the microhardness averages for all the variables investigated (cavity preparation device, depth, and point).

Regarding the cavity preparation device, the highest microhardness values were found when the Er:YAG laser was irradiated at 250 mJ/4 Hz and 350 mJ/4 Hz. These parameters are only different from 200 mJ/2 Hz and the high-speed turbine, which presented the lowest averages (Table 1).

There were statistically significant differences (P<.01) when points were compared (Table 2). The higher microhardness values were recorded in D (deep area of the cavity preparation), and the statistically nonsignificant differences (P<.05) in microhardness were observed between points A (nonirradiated area), B, and F (cavity preparation edges).

When depth was analyzed, an increase in microhardness of all laser-irradiated groups was verified until 60 μ m, however, the highest hardness values were observed at 30 μ m (Table 3).

Considering the factors' interactions, 3-way ANOVA showed a statistically significant difference for cavity preparation device x depth and point x depth. At 30 μ m, the laser parameters 300 mJ/4 Hz and 350 mJ/4 Hz increased microhardness averages at the bottom of the dentin cavities. The bur-prepared group (control) displayed the lowest values, and no significant differences (*P*<.05) were observed among the investigated depths.

ANALYSIS OF SUBSURFACE MORPHOLOGY UNDER SEM

Analysis of the subsurfaces' morphological aspect revealed that cavities prepared with the Er:YAG laser at 200 mJ/2 Hz, 250 mJ/2 Hz, 300 mJ/2 Hz, 350 mJ/2 Hz, 200 mJ/3 Hz, and 250 mJ/3 Hz did not exhibit significant alterations (Figures 2a-d and 3a-b). An increase in subsurface irregularities was observed when dentin was irradiated at 350 mJ/2 Hz, 200 mJ/3 Hz, 250 mJ/3 Hz (Figures 2D, 3A, 3B and 3E).

Er:YAG laser irradiation modified the subsurface aspect until: 10 μ m with the parameter settings 300 mJ/3 Hz, 350 mJ/3 Hz, and 200 mJ/4 Hz (Figures 3c-d and 4a); and 30 μ m with 250 mJ/4 Hz, 300 mJ/4 Hz, and 350 mJ/4 Hz (Figure 4b-d). Subsurface modified regions can be characterized as dense areas without evident dentinal tubules. In general, an increase of energy with the same pulse repetition rate intensifies the irregularities on the edges of cavities preparations.

Regarding the laser-prepared cavities' deep regions, the lateral portions did not exhibit evident morphological alterations, regardless of the parameters, but were more irregular than those conventionally prepared (Figures 3f and 4e-f). The bur-prepared group did not present topographical alterations on the cavity preparation's subsurface (Figure 2e-f).

DISCUSSION

This study revealed that higher microhardness values were observed with Er:YAG laser irradiation at a pulse repetition rate of 4 Hz (250 mJ/4 Hz and 350 mJ/4 Hz). This is because an increase in the pulse repetition rate inherently leads to a greater number of microexplosions at the same time interval. Therefore, laser interaction with the tissue is higher.^{5,14,17} Indeed, the pulse repetition rate is described as the most important parameter in the heat deposition on lased-irradiated tissue.^{5,19} Although the heating caused by laser irradiation does not propagate into pulp tissue, its photothermal effect has been reported as causing structural^{13,23-25,27} and chemical ^{8,25,28} alterations on the dental surface.

It is possible to correlate the microhardness data with the morphological analysis, since irradiation at 4 Hz formed modified areas in a greater extension than those observed by other parameters. However, although dentin microhardness increased until 60 µm, the visible topographical alterations did not exceed 30 um. The subsurfaces' modified regions are dense areas without evident dentinal tubules, probably due to the recrystallization that occurred after superficial heating. Ceballos et al²³ showed that the superficial part of the subsurface irradiated by the Er:YAG laser consisted of electron-dense flakes that exhibited a rippled appearance separated by microcracks, while the basal part consisted of fused areas and denatured collagen fibrils. Martinez-Insua et al²² also described the occurrence of dentin morphological alterations when the Er:YAG laser was applied at 160 mJ/4 Hz. Schein et al²⁴ disclosed that Er:YAG laser irradiation at 250 mJ/4 Hz created a surface aspect unfavorable to the restorative material diffusion.

In this study, the turbine handpiece group displayed low microhardness values, similar to the laser parameter 200 mJ/2 Hz. This result mirrors the results obtained by Hossain et al,²⁷ who verified that dentin subsurface microhardness prepared with the Er:YAG laser (200 mJ/2 Hz) and conventional bur are the same. Morphologically, the lower tested parameters (200 mJ/2 Hz, 250 mJ/2 Hz, and 300 mJ/2 Hz) were close to the control group because cavity margins were nearly regular and no significant topographical alterations were verified in these specimens' subsurfaces.

Regarding the different regions where microhardness was measured, it was found that the most evident raise occurred in point D (the cavity preparation's deep area). Microhardness on the edges of the cavity preparation (points B and F) were similar to the nonirradiated area (point A). This speculation is justified due to the laser beam's profile.^{2,5,30} Er:YAG laser irradiation acts intensively at the bottom of the cavity, and the external margins are exposed only to the laser beam's lateral portion. Besides, the increase in dentin tissue temperature depends on the dentinal tubules' direction.^{1,7} Dentinal tubules running parallel to the surface prevent significant heat penetration, whereas those running in a transverse direction to the surface (parallel to the laser beam) support the penetration of heat.¹

Concerning the analyses depths, Er:YAG laser irradiation was shown to increase microhardness until 60 μ m, however, the higher value was founded at 30 μ m. These findings disagree with those reported by Aoki et al⁶, who observed an increase in dentin microhardness until 25 μ m, when the Er:YAG laser was pulsed at 180 mJ/10 Hz. The microscopic findings showed subsurfaces alterations until 30 μ m, when the laser was used at 4 Hz (250 mJ/4 Hz, 300 mJ/4 Hz and 350 mJ/4 Hz) were utilized. Sasaki et al⁸ corroborated that the topographical alterations caused by the Er:YAG extended to 30 μ m. Nevertheless, this layer was formed by a superficial portion with intensive changes and for a deep portion with less alterations. On the other hand, Kataumi et al²⁰ described intense irregularities until 20 μ m on dentin treated by the Er:YAG laser (126 mJ/10 Hz).

By varying the irradiation conditions, the Er:YAG laser can induce different modifications. Some chemical and morphological alterations probably occur due to the surface liquefaction.^{21,23,25} These modifications included the reduction of carbonate content and the formation of more stable and less soluble components.²⁶⁻²⁸ Microhardness measurements of the cavity preparation can confirm these facts. The clinical consequence for these modifications is that a dentin cavity prepared by laser device might be more resistant to secondary caries attack than cavities prepared by conventional handpiece turbine.^{21,26,28} The excessive irregular surface and fissured subsurface combined with a dense substrate, however, might be adverse for the bonding process.^{13,20,22-24}

Based on this study's results, it seems appropriate to recommend lower parameter settings (200 mJ/2 Hz, 250 mJ/ 2 Hz or 300 mJ/2 Hz) when the Er:YAG laser is used to prepare dentin cavity walls. This is because the microhardness of lased cavities with these dosimetries were similar to the microhardness of bur-prepared cavities and no expressive differences in subsurface morphology were verified. The lack of published studies investigating the Er:YAG laser's effects on dental microhardness and the differences in parameter settings and methodologies hinders the settlement of reliable comparisons.

The Er:YAG laser is recognized as a device of outstanding applicability in operative dentistry and appears to be a promising alternative to the turbine handpiece. Nevertheless, long-term clinical data are required before general application of any new method used in routine patient treatment. Further in vitro studies are needed to demonstrate improvement in laser technology.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. The Er:YAG laser with 250 mJ/4 Hz and 350 mJ/4 Hz increased dentin microhardness in the deepest area of the cavity until 60 μ m.

2. Use of lower parameters (200 mJ/2 Hz, 250 mJ/2 Hz, or 300 mJ/2 Hz) to prepare dentin with the Er:YAG laser produced results similar to those for bur-prepared cavities.

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